

Phylogeny and biogeography of *Coenonympha* butterflies (Nymphalidae: Satyrinae) – patterns of colonization in the Holarctic

ULLASA KODANDARAMAIAH¹ and NIKLAS WAHLBERG^{1,2}

¹Department of Zoology, Stockholm University, Stockholm, Sweden and ²Laboratory of Genetics, Department of Biology, University of Turku, Turku, Finland

Abstract. We studied the historical biogeography of a group of butterflies in the Holarctic region belonging to the genus *Coenonympha* (Nymphalidae: Satyrinae: Coenonymphina), based on a phylogenetic hypothesis estimated from three genes. The genus is distributed mainly in the Palaearctic region, with two species extending into the Nearctic region. The tree is generally well supported and shows that *Coenonympha* is paraphyletic with respect to *Lyela* (**syn.n.**) and *Triphysa* (**syn.n.**), and we hence synonymize the latter two with *Coenonympha*. Within *Coenonympha* we identify three species groups, the *tullia*, *glycerion* and *hero* groups. The North American *tullia* exemplars are not sister to the Eurasian ones. A DIVA analysis indicates that the ancestor of the group was present in the Central Palaearctic or Central Palaearctic + Western Palaearctic or Central Palaearctic + Eastern Palaearctic. We conclude that the most likely origin of extant members of *Coenonympha* was in the Central Asian mountains. The *tullia* and *hero* groups started diverging in Europe following dispersal into the region. There have been two independent colonizations into Africa. The drying up of the Mediterranean during the Messinian period probably played an important role, allowing colonization into the Mediterranean islands and Africa.

Introduction

The inference of the origins and spatio-temporal dynamics of endemic radiations is a fascinating part of historical biogeography. Among butterflies, tropical groups form the vast majority of such studies: examples include the genus *Delias* in the Australasian region (Pieridae; Braby & Pierce, 2007), the subtribe Mycalesina in Africa (Nymphalidae; Torres *et al.*, 2001), the subtribe Phyciodina in the Neotropical region (Nymphalidae; Wahlberg & Freitas, 2007), the pantropical genus *Junonia* (Nymphalidae; Kodandaramaiah & Wahlberg, 2007) and the Neotropical genus *Heliconia* (Nymphalidae; Brower, 1996). Relatively few such studies, however, have been undertaken on temperate groups:

examples include *Euphydryas* (Nymphalidae; Zimmermann *et al.*, 2000), *Pararge* (Nymphalidae; Weingartner *et al.*, 2006) and Parnassiinae (Papilionidae; Nazari *et al.*, 2007).

The diversity of butterflies in the Holarctic is well documented (Scott, 1986; Tolman & Lewington, 1997; Tuzov, 2000), and the butterflies of this region have arguably been the most important in terms of their contribution as model-organisms in evolutionary biology and ecology (Boggs *et al.*, 2003). Although the bulk of such research has historically been carried out in Europe and North America, little is known of the origins and patterns of diversification of the butterfly fauna in these regions. The regions per se are interesting from the point of view of butterfly biogeography. The butterfly fauna of the Nearctic is an admixture of Palaearctic and Neotropical descendant groups, with a few endemic radiations. The Palaearctic, in contrast, is host to many endemic groups of butterflies (e.g. *Melitaea*, a large number of satyrine genera, *Agrodiaetus*, etc.), perhaps as a result of the presence of the Himalayas in

Correspondence: Ullasa Kodandaramaiah, Department of Zoology, Stockholm University, 106 91 Stockholm, Sweden. Tel.: +46 8 164 398; fax: +46 8 167 715; e-mail: ullasa.kodandaramaiah@zoologi.su.se

Asia and the Sahara in Africa. It has three potential sources of colonization – the Nearctic, and the African and Oriental regions – and the relative contributions of these three sources to the diversity of the butterfly fauna in the Palaearctic are unknown, mainly owing to a lack of information about the phylogenetic relationships of butterflies in the region. Several groups of butterflies potentially could shed more light on the biogeographical processes that have shaped the extant distribution of the butterfly diversity in the region.

One such group is the subtribe *Coenonymphina* Miller, 1968 (Nymphalidae: Satyrinae: Satyrini). The subtribe *Coenonymphina* (hereafter *coenonymphines*) was until recently considered to consist of four genera: *Coenonympha* Hübner, 1819 with 30–31 species (Bozano, 2002); *Triphysa* Zeller, 1850 (two spp.); *Lyela* Swinhoe, 1908 (three spp.); and the monobasic *Sinonympha* Lee, 1974. Bozano (2002) removed *Lyela* from the group, whereas Peña *et al.* (2006) added 13 more genera within *Coenonymphina* – all genera hitherto classified under *Hypocystina* (sensu Miller, 1968; Indo-Australian), *Oressinoma* (previously *Euptychiina*: Neotropical) and *Orsotriaena* (previously *Mycalesina*: Indo-Australian). However, their study included *Coenonympha* as the sole representative from the Holarctic region. *Coenonympha* has a Holarctic distribution with perhaps two species in North America, *C. tullia* and *C. haydeni* (the specific status of *C. haydeni* has been contested – it has sometimes been considered a subspecies of *C. tullia*), 26 in Europe and temperate parts of Asia, and four in Northern Africa (Bozano, 2002). Several species are endemic to the islands in the Mediterranean Basin. *Triphysa*, *Lyela* and *Sinonympha* are restricted to the Eastern Palaearctic. In this study we aim to gain an understanding of the biogeographical history of *Coenonympha* based on a phylogenetic hypothesis derived from three genes. We include samples of most species of *Coenonympha* from across its range and exemplars from *Triphysa* and *Lyela*.

As part of the study, we seek also to clarify the relationships among *Coenonympha*, *Lyela* and *Triphysa*, because of their bearing on the biogeography of *Coenonympha*. Peña & Wahlberg (2008) inferred that *Coenonympha* diverged from its sister group ca. 24 million years ago (Ma), based on a fossil-calibrated molecular dating estimate. Although the confidence intervals are quite wide, this age would preclude a direct Gondwanan ancestry. If this is indeed the case, from where did the ancestor or ancestors of the Palaearctic *coenonymphines* colonize the region? *Coenonympha* has four species endemic to Northern Africa, making Africa a potential source of colonization, as has been shown for *Pararge* (Weingartner *et al.*, 2006). If this is the case, one or more of these African endemics would be recovered as sister of the remaining species of *Coenonympha*. Alternatively, as *Coenonympha* has been shown to be related to both Indo-Australian and Neotropical genera (*Oressinoma*), colonization could have been from either of those regions. Finally, *Triphysa*, *Lyela* and *Sinonympha* are restricted to temperate Asia, and an origin in Asia of *Coenonympha* is a good working hypothesis.

Materials and methods

Data collection

Specimens of 24 of the 31 species of *Coenonympha*, representing all major regions of its current distribution, were studied (Table 1). Samples from one species each of *Lyela* and *Triphysa* were studied, but we were unable to obtain samples of *Sinonympha*. Specimens were preserved by desiccation. In some cases, the DNA was preserved by placing two of the legs in alcohol.

DNA was extracted from two legs using the DNEasy extraction kit (QIAGEN; Hilden, Germany). DNA was amplified from three gene regions – COI (mitochondrial cytochrome oxidase subunit I), and two nuclear genes, EF-1 α (elongation factor 1-alpha) and *wingless*. The phylogenetic utility of the combination of these three genes at the level of the genus is well established (Wahlberg *et al.*, 2003; Wahlberg *et al.*, 2005; Brower *et al.*, 2006; Peña *et al.*, 2006; Weingartner *et al.*, 2006; Kodandaramaiah & Wahlberg, 2007; Wahlberg & Freitas, 2007). COI was amplified using the primer pairs LCO–HCO and Jerry–Pat (primers given in Wahlberg & Freitas, 2007). Three primer pairs were used for EF-1 α , namely Starsky–Luke, Cho–Verdi and EF51.9–EFrcM4 (primers given in Peña *et al.*, 2006), and LepWing1 and LepWing2 (Brower & DeSalle, 1998) were used for *wingless*. The polymerase chain reaction (PCR) protocol used for Starsky–Luke was as follows: 95°C for 7 min; 40 cycles of 95°C for 30 s, 55°C for 30 s and 72°C for 1 min; followed by a final extension period of 72°C for 10 min. For the rest of the primer pairs we used the following protocol: 95°C for 7 min; 40 cycles of 95°C for 30 s, 50°C for 30 s and 72°C for 1 min; followed by a final extension period of 72°C for 10 min. Successfully amplified PCR products were sequenced using a Beckmann–Coulter CEQ8000 automated sequencer. The resulting chromatograms were visualized in the software BioEDIT ver. 7.0.5.3 (Hall, 1999) and aligned by eye. Outgroup taxa were chosen based on the most recent phylogenetic hypothesis of Satyrinae (Peña *et al.*, 2006). The tree was rooted with *Mycalesis* (*Mycalesina*), and other *coenonymphine* exemplars were used to test whether *Triphysa*, *Lyela* and *Coenonympha* were monophyletic.

Phylogenetic analysis

The combined dataset was analysed under the maximum parsimony criterion using the software TNT ver. 1.1 (Goloboff *et al.*, 2004). Equal-weighted heuristic searches involving traditional tree bisection–reconnection (TBR) branch swapping procedures and new technology searches were performed on 1000 random addition replicates. Support for respective clades was estimated using bootstrapping (Felsenstein, 1985) and Bremer support values (Bremer, 1994). The bootstrap values were calculated on 1000 pseudo-replicates with 10 random replicates each. To assess the relative support of each of the three genes to the

Table 1. List of samples used in the study with their collection localities and GenBank accession numbers. NA indicates that the gene region was not amplifiable.

Species	Voucher code	Collection locality	GenBank accession numbers		
			COI	EF-1 α	<i>Wingless</i>
<i>Triphysa phryne</i>	CP16-21	SW Siberia, Orenburg reg., Russia	EU920739	EU920773	EU920804
<i>Triphysa phryne</i>	UK2-12	Donskoe env., Orenburg reg., Russia	EU920740	NA	NA
<i>Lyela myops</i>	CP16-22	Golestan, Kalaleh, Iran	EU920741	EU920774	EU920805
<i>Coenonympha austauti</i>	UK2-19	Atlas Tellien, Morocco	EU920742	EU920775	NA
<i>Coenonympha amaryllis</i>	UK2-21	W. Dulan, Qinghai, China	EU920743	EU920776	EU920806
<i>Coenonympha arcania</i>	EW7-6	Öland, Sweden	EU920744	EU920777	EU920807
<i>Coenonympha arcaniodes</i>	UK2-15	NE Bab-Taza, Rocc, Morocco	EU920764	EU920778	EU920808
<i>Coenonympha corinna</i>	UK2-14	Marte Tormeri, Sardegna, Italy	EU920745	EU920779	EU920809
<i>Coenonympha dorus</i>	UK2-23	Las Tours, France	EU920746	EU920780	EU920810
<i>Coenonympha gardetta</i>	UK4-1	Passo Campolongo, Switzerland	EU920747	EU920781	EU920811
<i>Coenonympha elbana</i>	UK2-10	Verolonia, Italy	EU920748	EU920782	NA
<i>Coenonympha glycerion</i>	EW5-18	Nummela, Finland	EU920749	EU920783	EU920812
<i>Coenonympha hero</i>	EW3-14	Sweden	EU920750	EU920784	EU920813
<i>Coenonympha iphioides</i>	UK2-13	Cerdania, Catalonia, Spain	EU920751	EU920785	EU920814
<i>Coenonympha leander</i>	UK2-2	Iran	EU920752	NA	NA
<i>Coenonympha mahometana</i>	UK2-9	E Terskei Ala Too Mt. Range, Kirgiztan	EU920753	EU920786	EU920815
<i>Coenonympha nolkeni</i>	UK2-6	Trans Alai Mt. Range, Kirgiztan	EU920754	EU920787	EU920816
<i>Coenonympha oedippus</i>	UK1-24	Obluchye, Russia	EU920755	EU920788	EU920817
<i>Coenonympha pamphilus</i>	EW7-3	Öland, Sweden	DQ338777	DQ338920	DQ338637
<i>Coenonympha rhodopensis</i>	UK4 3	Schar Mountains, Macedonia	EU920756	EU920789	EU920818
<i>Coenonympha semenovi</i>	UK2-7	Minshan Mts, N. Sichuan, China	EU920757	EU920790	NA
<i>Coenonympha saadi</i>	NW150-14	Armavir marz, Vanand, Armenia	EU920758	EU920791	EU920819
<i>Coenonympha saadi</i>	UK4-5	Ishafan, Zagros Mountains, Iran	EU920759	EU920792	EU920820
<i>Coenonympha subbecca</i>	UK2-4	S Terskei Ala Too Mts., Kirgiztan	EU920760	EU920793	EU920821
<i>Coenonympha thyrsis</i>	UK4 2	Psyloritis Moutains, Greece	EU920761	EU920794	EU920822
<i>Coenonympha tullia</i>	EW5-11	Öland, Sweden	EU920762	EU920795	NA
<i>Coenonympha tullia</i>	EW5-15	Öland, Sweden	EU920765	EU920797	EU920824
<i>Coenonympha tullia</i>	EW5-16	Öland, Sweden	EU920766	EU920798	EU920825
<i>Coenonympha tullia</i>	FS-b-984	California, U.S.A.	AF170860	AF173399	DQ351126
<i>Coenonympha tullia</i>	EW8-5	McDonald State Forest, Oregon, U.S.A.	EU920767	EU920799	NA
<i>Coenonympha tullia</i>	EW8-7	McDonald State Forest, Oregon, U.S.A.	EU920768	EU920800	EU920826
<i>Coenonympha arcania</i>	EW18-5	Åkersberga, Sweden	EU920769	EU920801	EU920827
<i>Coenonympha vaucheri</i>	UK2-20	Lac de Tislit, N Imilchil, Morocco	EU920763	EU920796	EU920823
<i>Mycalesis terminus</i>	EW 18-8	Australia	DQ338765	DQ338905	DQ338632
<i>Heteronympha merope</i>	UK1-8	Australia	EU920736	EU920770	EU920802
<i>Oressinoma typha</i>	CP07-71	La Solitaria-Quebrada Siete Jeringas, Peru	DQ338802	DQ338949	DQ338666
<i>Oressinoma sorata</i>	DNA99-065	Pichincha, Ecuador	AY508561	AY509087	NA
<i>Oressinoma sorata</i>	PE6-1	Cuzco, Peru	NA	NA	AF246602
<i>Altiapa decolor</i>	NW136-13	Simbu Prov., Kegsugle, Papua New Guinea	EU920737	EU920771	EU920803
<i>Hypocysta adiante</i>	KB339	Kimberley, Australia	EU920738	EU920772	NA

combined dataset, we calculated partitioned Bremer support (PBS; Baker & DeSalle, 1997) values for each of the nodes. A positive PBS value indicates support for the given clade by the partition, and a negative value indicates conflict (Gatesy *et al.*, 1999) between that partition and others that support it.

Additional analyses were performed in TNT to test whether long-branch attraction (Felsenstein, 1978) was responsible for unexpected hypotheses of relationship that emerged from the analyses. The (potential) 'rogue' taxa were removed successively from the dataset, initially one at a time, and then together in all possible combinations. The logic

behind such analyses is that, if the grouping of two taxa was the result of long-branch attraction, the removal of one of the taxa would lead to the placement of the other in its true position (Siddall & Whiting, 1999; Bergsten, 2005). Thus, changes in implied relationships of the remaining taxa that arise as a result of the removal of taxa indicate possible long-branch attraction.

Bayesian inference of phylogeny and estimation of times of divergence was performed using the program BEAST ver. 1.4.6 (Drummond & Rambaut, 2007). The dataset was analysed under the GTR + G model with a relaxed clock allowing branch lengths to vary according to an

uncorrelated lognormal distribution (Drummond *et al.*, 2006). The data were partitioned into the mitochondrial and nuclear genes, with parameter values being estimated independently for each partition. The tree prior was set to the Yule process, and all other priors were left to the defaults in BEAST. In order to estimate times of divergences, the node leading to *Coenonympha* and *Hypocysta* was calibrated at 24 Ma (with a standard deviation of 4 Ma), according to results in Peña & Wahlberg (2008). For this analysis, the relatively distant *Mycalasis* was omitted, as preliminary analyses suggested long-branch artefacts in the results (i.e. *Mycalasis* coming out as sister to *Oressinoma*). Parameters were estimated using two independent runs of 10 million generations each (with a pre-run burn-in of 100 000 generations), and sampled every 1000 generations. Convergence was checked in the TRACER ver. 1.4.6 program, and summary trees were generated using TREEANNOTATOR ver. 1.4.6, both part of the BEAST package.

Biogeographical analysis

A Dispersal Vicariance Analysis (DIVA; Ronquist, 1997) was performed to determine the most likely ancestral state reconstruction of distributions. We used the tree from the parsimony analysis in this exercise. The program DIVA assigns a cost of one for dispersal and extinction and a zero cost for vicariance and within-area speciation. The least-cost ancestral reconstruction is derived based on this cost matrix (Ronquist, 1997). We divided the Holarctic into five broad areas in order to identify the part of the Holarctic where *Coenonympha* was most likely to have started evolving: the Eastern Palaearctic (eastern and northern Asia), Central Palaearctic (Central Asia), Western Palaearctic (Europe), North Africa and the Nearctic. Nodes subtending taxa with the same distributions were collapsed into a single taxon. In cases where the species were diphyletic, they were divided into two terminals. We analysed the dataset by constraining the maximum ancestral areas to two using the 'maxareas' option. This option improves the resolution of the analysis and estimates the most likely ancestral distribution of the nodes (Ronquist, 1997).

Results

Characteristics of the dataset

The combined dataset consisted of 3075 base pairs (bp), of which 717 were parsimony-informative. The heuristic searches in TNT based on the maximum parsimony criterion yielded two equally most parsimonious trees, the strict consensus of which is shown in Fig. 1. The Bayesian analysis in BEAST resulted in the phylogeny shown in Fig. 2. The two trees were congruent with respect to specific-level relationships except for two nodes, indicated in the trees by shaded circles. In reporting the results, bootstrap support values from 50 to 70% are referred to as weak, those from 71

to 90% as moderately good, and anything higher as strong. Similarly, Bayesian posterior probability (PP) values from 0.5 to 0.75 are referred to as weak, those from 0.76 to 0.95 as moderately good, and those >0.95 as strong. Conflict between the three genes is assessed from PBS values.

General phylogenetic patterns

Coenonympha along with *Lyela* and *Triphysa* were recovered as a monophyletic unit with strong bootstrap and PP support, and positive PBS values for all three genes. The genus *Coenonympha* was paraphyletic with respect to *Lyela* and *Triphysa*. *Lyela* was sister to a Central Palaearctic species, *C. nolckeni*, with moderately good bootstrap and strong PP values. *Triphysa* was sister to *C. oedippus* with weak bootstrap and moderately good PP values, and there was no conflict among the genes. The *Triphysa* + *C. oedippus* clade was sister to the remaining *Coenonympha* species (excluding *C. nolckeni*). Taxon removal analyses were performed to check whether the surprising groupings of *C. nolckeni* and *C. oedippus* with *Lyela* and *Triphysa* respectively were artefacts of long-branch attraction. Removal of the taxa, either individually or in groups of two or three, did not affect the relationships of the remaining taxa.

There were three well-defined and well-supported clades within the remaining *Coenonympha* species. We refer to these as the 'tullia', 'glycerion' and 'hero' groups (Figs 1, 2). The 'tullia' group included *C. austauti* (African), *C. dorus* (West Palaearctic), *C. pamphilus* (pan-Palaearctic), *C. thyraxis* (Crete), *C. tullia* (Holarctic), *C. amaryllis* (Eastern and Central Palaearctic) and *C. rhodopensis* (West and Central Palaearctic). The Palaearctic and Nearctic exemplars of *C. tullia* formed respective clades, but these two clades were not sister groups in either analysis, as *C. rhodopensis* and *C. amaryllis* were nested between them. This grouping received strong support with no conflict among the genes. The Cretan endemic, *C. thyraxis*, was sister to the widespread *C. pamphilus*.

The 'glycerion' group included *C. sunbecca* (Central Palaearctic), *C. mahometana* (Central Palaearctic), *C. glycerion* (West Palaearctic) and *C. iphiodes* (Iberian Peninsula). *Coenonympha iphiodes*, which is sometimes considered a subspecies of *C. glycerion* (Tolman & Lewington, 1997), was not sister to the latter. The 'hero' group consisted of *C. corinna* (Sardinia & Corsica), *C. elbana* (Elba), *C. arcaniodes* (North Africa), *C. vaucheri* (North Africa), *C. arcania* (Europe and Central Palaearctic), *C. gardetta* (Europe), *C. semenovi* (Eastern Palaearctic), *C. hero* (Palaearctic) and *C. leander* (West and Central Palaearctic). *Coenonympha corinna* (Sardinia & Corsica) and *C. elbana* (Elba) were sister species.

Biogeographical patterns and times of divergences

For the areas defined in the DIVA analysis (Fig. 3a), the optimized ancestral state reconstruction indicates an origin

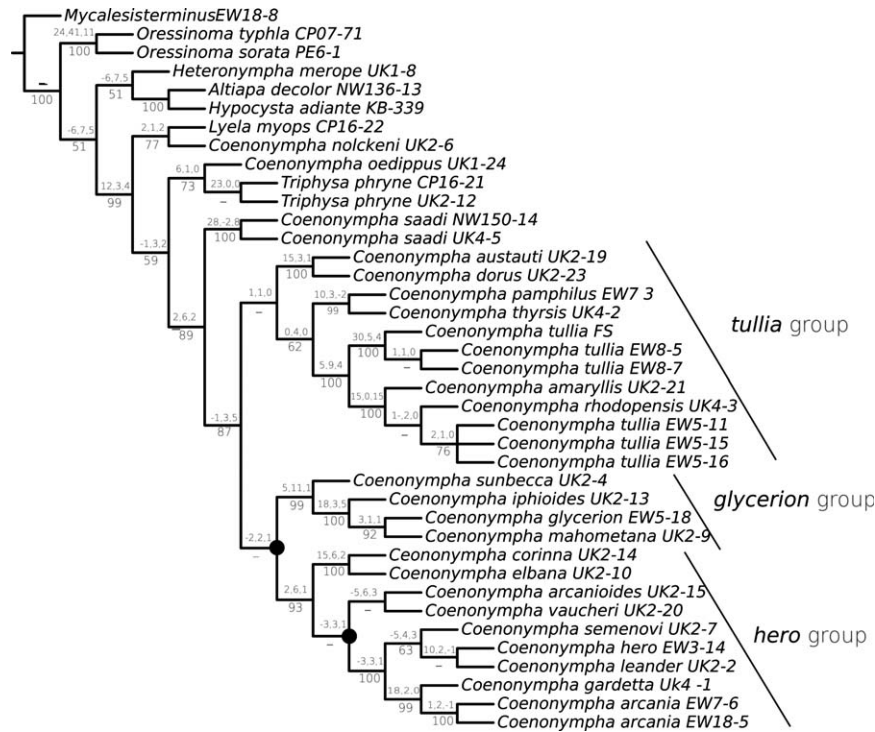


Fig. 1. Summary of the strict consensus of the two equally parsimonious trees derived from the analysis of the combined dataset ($L = 3179$, $RI = 0.54$, $CI = 0.48$). Numbers above are partitioned Bremer support values for COI, EF-1 α and *wingless*, respectively. Numbers below are bootstrap support values. The grey circles indicate the nodes that conflicted with the Bayesian tree.

of the *Coenonympha* + *Lyela* + *Triphysa* clade in the Central Palaeartic or Central Palaeartic + Western Palaeartic or Central Palaeartic + Eastern Palaeartic. Figure 3b depicts the ancestral distributions reconstructed in the analysis. The first clade to diverge was *Lyela* + *C. nolckeni* in the Central Palaeartic, followed by the *Triphysa* + *C. oedippus* clade, whose ancestral distribution is unclear. Following this, *C. saadi* diverged in the Central Palaeartic. The ancestor of the *tullia*, *glycerion* and *hero* groups diverged next. Within the larger clade, the *tullia* and *hero* groups started evolving in the Western Palaeartic, possibly following dispersal from the Central Palaeartic. The ancestor of the *glycerion* group was found either in the Central Palaeartic or spanned both the Central and Western Palaeartic regions. Africa has been subject to two independent dispersal events from the Western Palaeartic, one by an ancestor from the *tullia* group leading to *C. austauti* and the other by an ancestor from the *hero* group leading to the *C. vaucheri* + *C. arcanioides* clade.

Divergence time estimates in BEAST (Fig. 2) indicate that the ancestor of *Coenonympha* + *Triphysa* + *Lyela* began diverging in the mid-Miocene, with most divergences taking place in the late Miocene, between 13 and 5 Ma. The divergence of the *C. vaucheri* + *C. arcanioides* clade (Africa) from its sister group is timed to the Messinian period (ca. 7–5.3 Ma). Two divergences – those of *C. thyraxis* and *C. austauti* from their sister clades – are estimated to have

happened ca. 4 Ma. The split between the *C. elbana* (Elba) + *C. corinna* (Sardinia & Corsica) clade and its sister is timed at ca. 2 Ma.

Discussion

Systematic and taxonomic implications

The phylogeny of the group is well supported, with very little conflict between the Bayesian and parsimony trees. The paraphyly of *Coenonympha* was a surprising result of the study. The *C. nolckeni* - *Lyela* clade had moderately good support values, and the tests for long-branch attraction indicated this was not an artefact. Thus we believe that the relationship will remain stable with increased taxon/character sampling. The *C. oedippus* - *T. phryne* clade had weaker support, although *C. oedippus* (the type species of the genus *Coenonympha*) retained its position in the tree when *Triphysa* was pruned from the analysis. The addition of data from more samples may affect its position in the tree. Nevertheless, given the distributions of the three clades that branch off first (the two mentioned above as well as *C. saadi*), the broad biogeographical interpretation that the group began evolving in the Central Palaeartic is robust to alternative positions of the species on the tree. Indeed, it is quite likely that increasing the number of characters will

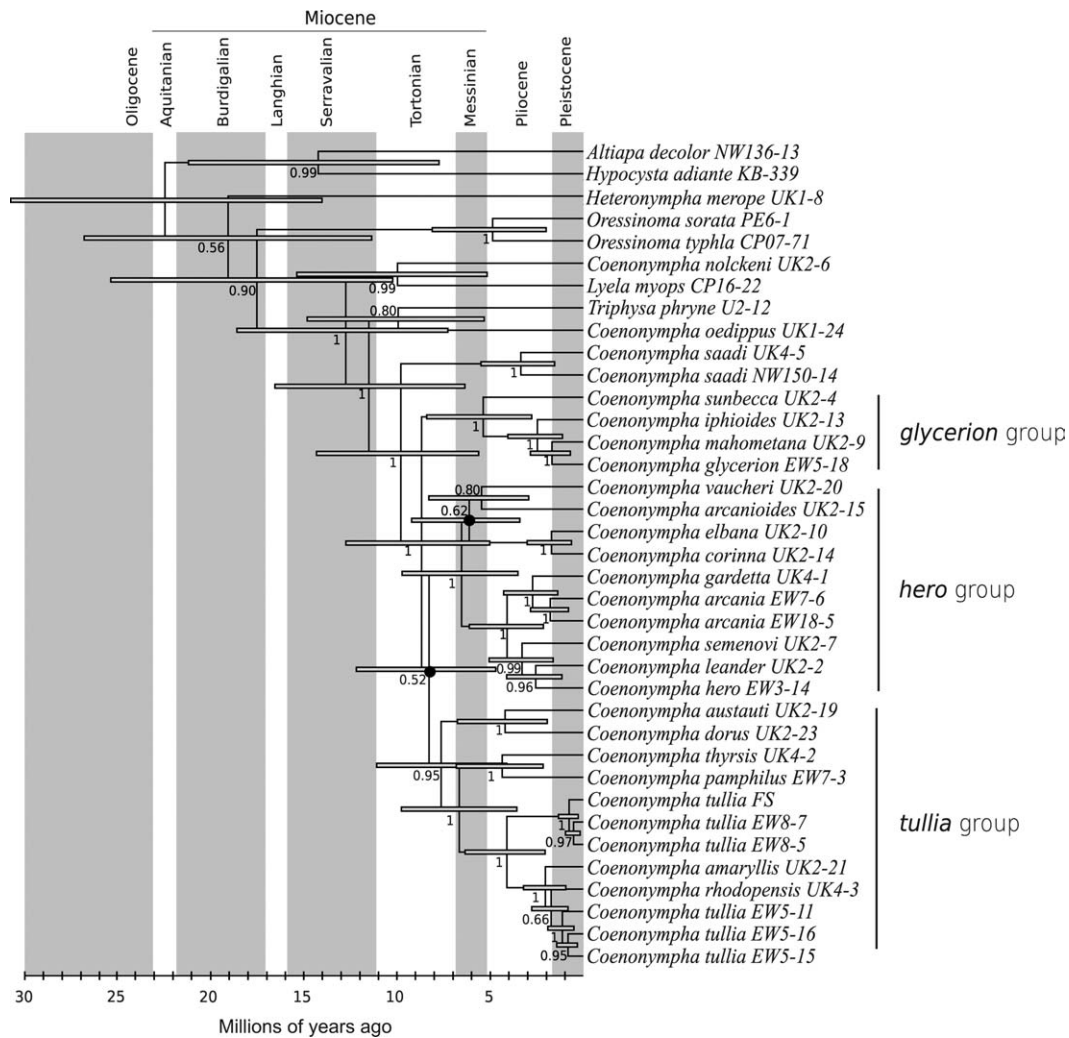


Fig. 2. Ultrametric tree resulting from the Bayesian inference of the combined dataset in BEAST. The numbers are the posterior probabilities of the respective nodes. The grey circles indicate the nodes that conflicted with the parsimony tree.

never recover a monophyletic *Coenonympha* with regard to *Lyela* (**syn.nov.**) and *Triphysa* (**syn.nov.**), and hence we synonymize these genera with *Coenonympha*. The relationships among *Coenonympha* and other members of the subtribe are weakly supported and need to be studied in more detail.

The taxonomy of *C. tullia* has been controversial. Bozano (2002) divided the species into several subspecies, whereas other authors (e.g. Tuzov, 2000) elevated these subspecies to the level of species. The question of whether the American *Coenonympha* species are the result of a single colonization event (in which case *C. haydenii* would be sister to the American *C. tullia* samples) needs to be investigated. Given that there are several morphologically distinct groups of 'tullia' in North America and that we have only three samples from the region, it is not clear whether the Palearctic *C. tullia* extends into the Nearctic. A more detailed taxonomic sampling of *Coenonympha* from North America is essential in order to resolve the taxonomy of the species,

and we refrain from making any taxonomic changes to the North American 'tullia' in this study.

The specific status of *C. iphioides*, which has been considered a subspecies of *C. glycerion*, needs to be ascertained with better sampling. Our preliminary results indicate that *C. iphioides* is not sister to *C. glycerion*, and thus its putative subspecific status within *C. glycerion* is not valid.

Biogeography and dating estimates

The results from the DIVA analysis with broadly defined geographic areas clearly indicate that Central Asia was important in the early evolution of the group and clearly reject the Nearctic and the African origin hypotheses. Although the distribution of the hypothetical ancestor is not clear, all three ancestral reconstructions in DIVA include this region. Four of the six major lineages are almost

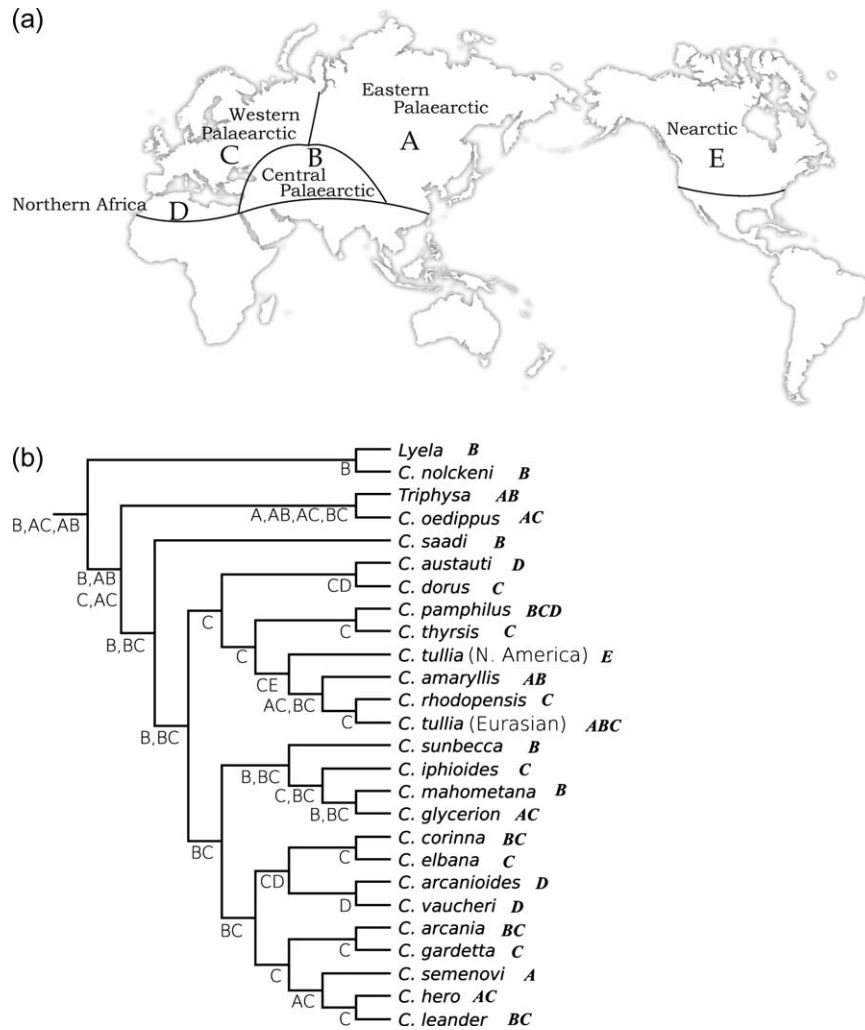


Fig. 3. Results of the DIVA analysis. (a) Map showing the areas defined in the DIVA analysis. (b) Reconstruction of ancestral distributions with maxareas = 2.

restricted to the Central Asian mountains. Furthermore, *Sinonympha* also is endemic to these mountains. Although the phylogenetic position of *Sinonympha* is not known, it is morphologically most similar to *Coenonympha* among all the coenonymphine genera. We hence think that it is likely to be sister to *Coenonympha* or within *Coenonympha*, as in the case of the former *Triphysa* and *Lyela*. We thus conclude that *Coenonympha* probably began diverging in the Central Asian mountains.

Coenonympha is most diverse in terms of species in Asia. Higher species diversity in temperate Asia in comparison with the rest of the Holarctic has been documented in various groups of flora and fauna (Sanmartín *et al.*, 2001, and references therein). This disparity is striking even if the greater expanse of the Palearctic is taken into account. Tiffney (1985) proposed the refugium hypothesis to explain this parochial distribution of richness. It is known that the Pleistocene glaciations were more severe and advanced further south in North America than in Asia, whereas

Europe was covered almost completely by the ice-sheets (Pielou, 1979; Sanmartín *et al.*, 2001). Tiffney suggested that this resulted in a greater number of refugia in Asia, whereas the glaciations led to higher rates of extinction in North America and Europe. Although the specific role of extinctions in the evolution of *Coenonympha* is difficult to judge, it is likely that the genus has been affected by them. Species endemic to this region are restricted mainly to the Central Asian highlands, and isolation in glacial refugia is likely to have played a role in speciation.

Where did the ancestor of the subtribe come from? The sister grouping of *Coenonympha* (including *Lyela* and *Triphysa*) and *Oressinoma* (Neotropical) in the Bayesian tree is not corroborated by the parsimony tree, in which *Coenonympha* is sister to the Australasian clade *Altiapa* + *Hypocysta* + *Heteronympha*. Indeed, the placement of *Coenonympha* relative to other coenonymphines has been extremely unstable (see Peña *et al.*, 2006; Peña & Wahlberg, 2008) and remains under study (Kodandaramaiah *et al.*, in

preparation). The possibility of early extinctions in the Nearctic is another confounding factor. Assuming that extinctions have not obliterated traces of the early evolution of the group completely, the best explanation is that the ancestor colonized the Palaearctic from the Oriental region.

The colonization of Africa and the Mediterranean islands from Europe happened relatively recently. During the Messinian period (7–5.3 Ma) the Mediterranean Sea dried up partially or completely as a result of the closure of its connection with the Atlantic Ocean (Hsü *et al.*, 1973; Krijgsman, 2002; Duggen *et al.*, 2003; Rouchy & Caruso, 2006), and this has been termed the Messinian Salinity Crisis. The precise geological causes and timing of this closure are under debate. The re-opening of the connection to the Atlantic at the end of the Messinian (5.3 Ma) led to the refilling of the Mediterranean (Hsü *et al.*, 1977; Krijgsman *et al.*, 1999; Duggen *et al.*, 2003). The broad confidence intervals on the divergence times make it difficult to conclude whether the refilling of the Mediterranean resulted in vicariant speciation in Africa and the islands, but we surmise that the Messinian period was quite probably important in allowing colonizations into Africa and the Mediterranean islands.

Studies on other groups of animals and plants in the Holarctic have demonstrated vicariance patterns between the Nearctic and the Palaearctic regions (Enghoff, 1995; Sanmartín *et al.*, 2001). The Palaearctic and Nearctic underwent episodes of connection and separation. The multiple ephemeral connections were through either trans-Atlantic or trans-Beringian bridges (Sanmartín *et al.*, 2001). Newly formed bridges allowed taxa to disperse across the two regions, and this was followed by vicariance when the bridges disappeared. The ancestor of the North American ‘*tullia*’ probably colonized North America quite recently (ca. 4 Ma) through one of these bridges.

The dynamics and relative importance of dispersal vis-a-vis vicariance in the speciation and evolution of biotas is a topic of much debate (Zink *et al.*, 2000). Although the earliest historical biogeographers, in the absence of knowledge of plate tectonics, invoked dispersal to explain much of the diversity on Earth (Udvardy, 1969; Platnick & Nelson, 1978) subsequently more weight has been given to vicariance (Humphries & Parenti, 1999; Ebach & Humphries, 2003). Indeed, vicariance is usually invoked as the null hypothesis to explain patterns of allopatric distribution (Wiley, 1988). However, recent studies question the centrality of vicariance in allopatric speciation, suggesting that dispersal is at least as important as vicariance (de Queiroz, 2005; Yoder & Nowak, 2006; Kodandaramaiah & Wahlberg, 2007). The history of *Coenonympha* seems to favour dispersal over vicariance as an explanation for allopatric speciation. However, the number of ancestral areas was constrained such that only the most likely areas were reconstructed in the DIVA analysis. DIVA also tries to reconstruct areas implying the minimum number of extinction events, thus underestimating the role of vicariance. Hence we conclude that, although dispersal has been important for speciation in the group, the relative importance of vicariance cannot be determined.

Summary and conclusions

We have shown that *Coenonympha* is paraphyletic with regard to *Lyela* and *Triphysa*, and synonymize the latter two with *Coenonympha*. We have identified three species groups within *Coenonympha*: the *tullia*, *hero* and *glycerion* groups. The ancestor of the extant members of *Coenonympha* probably started diverging in the Central Asian mountains. Dispersal has been important in the evolution of the group at a broader geographic scale. The divergence-time estimates suggest that the drying up of the Mediterranean during the Messinian age allowed colonizations into Africa and the Mediterranean islands.

Acknowledgements

This study was funded by a grant to Niklas Wahlberg from Vetenskapsrådet (Swedish Research Council) and the Academy of Finland (grant number 118369). We thank Roger Vila, Ian Kitching, Andy Brower and an anonymous referee for critical comments on the manuscript. Elisabet Weingartner kindly provided some of the sequences used in this study. We also thank Bertil Borg for useful suggestions for improving the manuscript.

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Accepted 24 October 2008

First published online 22 January 2009