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

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**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 \textit{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 *Heliconius* (Nymphalidae; Brower, 1996). Relatively few such studies, however, have been undertaken on temperate groups: examples include *Euphydryas* (Nymphalidae; Zimmermann \textit{et al.}, 2000), *Pararge* (Nymphalidae; Weingartner \textit{et al.}, 2006) and Parnassiinae (Papilionidae; Nazari \textit{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 \textit{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
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 Palearctic 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 Coenonympha – all genera hitherto classified under Hypocystina (sensu Miller, 1968; Indo-Australian), Oressinoma (previously Euptychina: 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 Palearctic. 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 Palearctic 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-1α (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-1α, namely Starsky–Lake, Cho–Verdi and EF51–EF54 (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–Lake 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
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

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**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.

<table>
<thead>
<tr>
<th>Species</th>
<th>Voucher code</th>
<th>Collection locality</th>
<th>GenBank accession numbers</th>
</tr>
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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 Mycalesis was omitted, as preliminary analyses suggested long-branch artefacts in the results (i.e. Mycalesis 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. thys- sis (Crete), C. tullia (Holarctic), C. amaryllyis (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. amaryllyis were nested between them. This grouping received strong support with no conflict among the genes. The Cretan endemic, C. thysis, 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. vaucherti (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
of the Coenonympha + Lyela + Triphysa clade in the Central Palaearctic or Central + Eastern Palaearctic. Figure 3b depicts the ancestral distributions reconstructed in the analysis. The first clade to diverge was Lyela + C. nolckeni in the Central Palaearctic, followed by the Triphysa + C. oedippus clade, whose ancestral distribution is unclear. Following this, C. saadi diverged in the Central Palaearctic. 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 Palaearctic, possibly following dispersal from the Central Palaearctic. The ancestor of the glycerion group was found either in the Central Palaearctic or spanned both the Central and Western Palaearctic regions. Africa has been subject to two independent dispersal events from the Western Palaearctic, 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. thyrsis 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 Palaearctic is robust to alternative positions of the species on the tree. Indeed, it is quite likely that increasing the number of characters will
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 Palaeartic *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

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*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.
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 Palaeartic 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 *Systematic Entomology*, 34, 315–323).

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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.
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 Palearctic 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 (Hsu 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 (Hsu 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 Palearctic regions (Enghoff, 1995; Sanmartin et al., 2001). The Palearctic and Nearctic underwent episodes of connection and separation. The multiple ephemeral connections were through either trans-Atlantic or trans-Beringian bridges (Sanmartin 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 allopatic distribution (Wiley, 1988). However, recent studies question the centrality of vicariance in allopatic 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 allopatic speciation. However, the number of ancestral areas was constrained such that only the most likely areas were reconstructed in the DnA analysis. DnA 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.

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