

Phylogeny and classification of the *Phengaris*–*Maculinea* clade (Lepidoptera: Lycaenidae): total evidence and phylogenetic species concepts

ZDENĚK FRIC^{1,2}, NIKLAS WAHLBERG^{3,4}, PAVEL PECH¹
and JAN ZRZAVÝ^{1,2}

¹Department of Zoology, University of South Bohemia, České Budějovice, Czech Republic

²Institute of Entomology, Academy of Sciences of the Czech Republic, České Budějovice, Czech Republic

³Department of Zoology, Stockholm University, Stockholm, Sweden

⁴Department of Biology, Laboratory of Genetics, University of Turku, Turku, Finland

Abstract. Total evidence analysis, based on a combination of morphological and ecological characters with two mitochondrial sequences (cytochrome *c* oxidase subunits I and II) and one nuclear (elongation factor-1 α) sequence, provides a new phylogeny of the uniquely obligate ant parasitic *Phengaris*–*Maculinea* butterflies. The clade, including all species of *Maculinea* and *Phengaris*, is very stable and well supported. However, various analyses suggest that either *Phengaris* or *Maculinea* is not monophyletic with regard to the other, which necessitates generic reclassification of the clade. Application of the diagnostic and monophyletic ‘phylogenetic’ species concepts leads to species-level reclassification, including ten species (*P. alcon* **comb.n.** including ‘*P. rebeli*’, *P. daitozana*, *P. albida*, *P. atroguttata*, *P. kurentzovi* **comb.n.**, *P. nausithous* **comb.n.**, *P. teleius* **comb.n.**, *P. arion* **comb.n.**, *P. arionides* **comb.n.**, *P. takamukui* **comb.n.**) and one unresolved metaspecies (‘*P. cyanecula*’ **comb.n.**) in four monophyletic species groups. The existence of further or additional cryptic species is possible within *P. nausithous* and *P. teleius*. *Maculinea* Van Eecke, 1915 **syn.n.** is considered a junior synonym of *Phengaris* Doherty, 1891.

Introduction

The large blues of *Maculinea* Van Eecke, 1915 (Lepidoptera: Lycaenidae) belong to the most intensively studied butterflies in Eurasia (Thomas *et al.*, 1998; Van Swaay & Warren, 1999; Maes & Van Dyck, 2005). In contrast to a vast majority of myrmecophilous lycaenid butterflies that receive only protection against their natural enemies from the ants, an intricate nest parasitism has evolved in *Maculinea* (Fiedler, 1998; Pierce *et al.*, 2002; Stadler *et al.*, 2003). Their early larval instars are mono- or oligophagous feeders on flowers and developing seeds of herbs, but later they parasitize nests of the ant genera *Myrmica* Latreille, 1804 and *Aphaenogaster* Mayr, 1853 (Fiedler, 1991; Elmes & Thomas, 1992; Wardlaw *et al.*, 2000). In the ant nests, the larval feeding may either be trophallactic

[the ants feed caterpillars via regurgitation; termed ‘cuckoo’ by Hochberg *et al.* (1994)] or predatory (the caterpillars prey on ant brood). For a detailed description of the biology and ecology of *Maculinea* see Thomas *et al.* (1998), Als *et al.* (2004) and Pech *et al.* (2004). Conservation biologists have also studied the *Maculinea* blues because their specialized habits make them vulnerable to habitat alteration. They have suffered severe declines in Europe (Wynhoff, 1998; Van Swaay & Warren, 1999); indeed, it was the extinction of *M. arion* (Linnaeus, 1758) in Britain in the 1970s (Thomas, 1980) that sparked the interest in butterfly conservation and habitat restoration (Elmes & Thomas, 1992; Hochberg *et al.*, 1994; Wynhoff, 1998). Five species of this genus were listed in the IUCN *Red List of Threatened Species* as globally ‘near threatened’, *M. rebeli* as ‘vulnerable’. Three taxa were included in Annexes II and IV of the European Habitat Directive, and all European taxa are being monitored under the European Union network project, MacMan (‘*Maculinea* Management’).

Correspondence: Z. Fric, Department of Zoology, University of South Bohemia, CZ-370 05 České Budějovice, Czech Republic.
E-mail: fric@entu.cas.cz.

Recently, two phylogenetic analyses of *Maculinea* and related taxa have been published, based on morphology and ecology (Pech *et al.*, 2004) and on molecular sequence data (Als *et al.*, 2004). Their results agree that both the *M. alcon* group ('cuckoos') and the *M. arion* and *teleius* groups ('predatory') are sister clades. However, the two papers differ in the detailed position of East Asian *Phengaris* [a paraphyletic stem lineage of *Maculinea* s.str. according to Als *et al.* (2004) or the most derived subclade of the *M. arion* group according to Pech *et al.* (2004)]. Knowledge of the phylogenetic position of *Phengaris* is essential for the reconstruction of the evolution of myrmecophily in this group of butterflies. These phylogenetic studies caused some controversy concerning the taxonomical status of individual taxa, including those with a high conservation priority. This applies predominantly to *M. alcon* (Denis & Schiffermüller, 1775) and *M. rebeli* (Hirschke 1904), two threatened European taxa spending the early phases of their development on plants belonging to Gentianaceae (Sibatani *et al.*, 1994).

To investigate further the species- and genus-level taxonomical questions, we provide here a combined ('total evidence') analysis of the phylogeny of *Maculinea* and related blue butterflies, and an interpretation of the results from the point of view of the pattern-based phylogenetic species concept. There are two basically different 'phylogenetic' species concepts, namely nontree-based 'diagnostic species' (see Eldredge & Cracraft, 1980; Nixon & Wheeler, 1990; Wheeler & Platnick, 2000) and tree-based 'monophyletic (autapomorphy-based) species' (Mishler & Donoghue, 1982; Mishler & Theriot, 2000; Brooks & McLennan, 2002; but see Kizirian & Donnelly, 2004). According to the former, a species is the smallest *diagnosable* cluster of individuals within which there is a parental pattern of ancestry and descent; according to the latter, a species is the least inclusive monophyletic group definable by at least one *autapomorphy*. It is evident that each approach, however 'phylogenetic', may provide a different solution (or outcome). The diagnostic character, which is present uniformly within and absent outside a species (regarding individuals of similar sex and age), can sometimes be a uniquely preserved plesiomorphy. By contrast, the autapomorphy of a species cannot necessarily be uniformly present in all of the species' subunits as it can secondarily be lost or modified in some derived populations. In both concepts, the diagnostic and autapomorphic characters can be of whatever nature, i.e. structural, behavioural, genetic, and so on. As the various approaches can sometimes give conflicting results, we prefer to combine both phylogenetic species concepts (i.e. to count both synapomorphies and diagnostic characters) in suggesting the species-level reclassification of the *Maculinea*–*Phengaris* clade.

Materials and methods

Taxa and character combinations

Our analysis was based on the combination of a morphological data matrix from Pech *et al.* (2004) and two-gene

alignment from Als *et al.* (2004). The dataset of Pech *et al.* (2004) included 20 taxa of *Maculinea*, three *Phengaris* and eleven outgroups; that of Als *et al.* (2004) included 32 samples of *Maculinea*, three *Phengaris* and 13 outgroups. In total, the combined molecular data matrix included 3172 base pairs (bp), of which 2001 bp are from the mitochondrial cytochrome *c* oxidase subunits I and II (COI and COII) and 1171 bp are from the nuclear elongation factor-1 α (EF-1 α). Of these, 325 characters were cladistically informative, plus 91 informative morphological and ecological characters for 50 terminal taxa (including six outgroup terminals).

Nineteen terminal taxa (six outgroups, three *Phengaris*, ten *Maculinea*) occurred in both original datasets. For every ingroup species that occurred in both data matrices, we chose one sequence per species (with an exception of European *M. alcon* whose monophyly was tested by using two terminals) and combined it with the morphological data. With regard to the three Japanese subspecies of *M. teleius*, no data on subspecific assignment of the sequenced voucher specimens are available, and therefore we chose the subspecies with the most complete morphological representation (there were only three minor morphological differences between the subspecies).

Phylogenetic analyses

The different data partitions to be combined in the simultaneous analyses cover different species spectra. Thus, we performed a combined analysis of all morphological characters and sequences of all 50 taxa, introducing missing values for the absent partitions (= 'all-species strategy'). We undertook a combined analysis of all character partitions and the 19 taxa whose morphological, mitochondrial, or EF-1 α characters are complete (= 'complete-species strategy'). We did not perform separate analyses of the datasets as this has already been done in Pech *et al.* (2004) for morphology and Als *et al.* (2004) for DNA data.

We used the maximum parsimony program NONA version 2.0 (Goloboff, 1999), with heuristic search strategy option 'hold 100000; mult*100; hold/100; mult*; max*', to find the shortest trees. Tree support was calculated with the bootstrap (NONA: 1000 replications, option 'mult*100; hold/100') and Bremer support (NONA: 'bsupport 10000'). To determine the effects of individual data partitions on the total evidence topology, the incongruence length difference (ILD; Farris *et al.*, 1994) and partitioned Bremer support were calculated for the 19 'complete-species' data matrix.

We also analysed the 'complete-species' combined data matrix using Bayesian phylogenetic methods in the program MRBAYES 3.1 (Ronquist & Huelsenbeck, 2003). The molecular data partitions were analysed with mixed models, i.e. parameters for the GTR + Γ + I model were estimated separately for the two regions using the 'unlink' command in MRBAYES. The morphological data were subjected to a rate variable model (Lewis, 2001). The analyses were run for 10 million generations with the chain being sampled every 1000 generations. The point of convergence was determined

visually by plotting the log likelihood of the data against the number of generations and then the 'burn-in' period was discarded. The posterior probabilities for clades were summarized through a majority rule consensus tree of the sampled trees.

Species concept

The taxonomical validity of all traditional species of the *Phengaris*–*Maculinea* clade was investigated according to the diagnostic and monophyletic 'phylogenetic' species concepts. A species was regarded as phylogenetically valid if either: (i) there is at least one diagnostic character, either morphological or molecular, that is present in all terminal taxa of the species and absent in all other terminal taxa within the *Phengaris*–*Maculinea* clade; or (ii) there is at least one autapomorphy making the species monophyletic; or (iii) the taxon is a sister group of a clade consisting of two or more diagnosable and/or monophyletic species.

Results

Phylogeny

From the combined 'all-species' morphological–molecular matrix, we obtained 120 equally parsimonious trees (length 1461, consistency index = 0.64, retention index = 0.80) (Fig. 1). The maximum parsimony analyses showed that three species of *Phengaris* form a clade that is nested within *Maculinea*, between the 'cuckoo' clade (*M. alcon* group) and the rest of *Maculinea* species (the 'predatory' clade, consisting of *M. arion* and *M. teleius* groups).

In the total evidence analysis limited to the 19 taxa represented well by all three data partitions (mitochondrial, EF-1 α , morphological), two trees (length 1301, consistency index = 0.55, retention index = 0.64) with topologies congruent to the 'all-species' analyses are recovered (Fig. 2). Combining the three data partitions produced 20 extra steps (ILD 0.0212). This data partition conflict is based predominantly on the conflict between morphology and EF-1 α (ILD 0.0398), whereas the incongruence between morphological–mitochondrial data and between both molecular partitions is lower (ILD 0.0188 and 0.0035, respectively).

Partitioned Bremer analysis (PBS) shows that all data partitions support the monophyly of: (i) the *Phengaris*–*Maculinea* complex; (ii) the *alcon* group; (iii) *Phengaris* s.str.; (iv) the *P. albida*–*P. atroguttata* clade; (v) the *teleius* group, and (vi) the *arion* group. On the other hand, morphology conflicts with the total evidence topology because the monophyly of neither the *arionides* nor the *arion*–*teleius* clade is supported morphologically (because of *arionides*–*Phengaris* attraction). Mitochondrial sequences do not support the monophyly of *M. arion* s.l. The EF-1 α sequence is in conflict with the combined topology in the *Phengaris* s.str.–*teleius*–*arion* clade, evidently because EF-1 α supports the basal position and paraphyly of *Phengaris* s.str. The

paraphyly of *Phengaris* is found only with the EF-1 α sequences, whereas both mitochondrial and morphological characters support the monophyly of *Phengaris* as well as its position within *Maculinea*.

The combined 19-taxon tree has the same topology as the mitochondrial tree. The sum of PBS scores, which is a measure of dataset influence in combined analysis (Gatesy *et al.*, 1999), shows that COI + COII is the most influential data partition (Σ PBS 155.8), whereas morphology and EF-1 α are comparably (and far less) influential partitions (Σ PBS 36.9 and 35.3, respectively).

All the important internal branches of the combined tree are relatively well supported (in Fig. 2: Bremer > 10, bootstrap > 95%) with the single exception of the moderately supported *Phengaris*–*teleius*–*arion* clade (Bremer 6, bootstrap 90%). The Bayesian analysis showed results very similar to those of Als *et al.* (2004) and identical to the maximum parsimony tree derived from EF-1 α data only: *P. daitozana* is found to be the sister species to the rest of the group, which is further split into the *P. albida*–*atroguttata* subclade and *Maculinea* s.str. (Fig. 3). Branch lengths at the base of the *Phengaris*–*Maculinea* clade are very short and have low posterior probabilities.

Different combinations of individual data partitions provide two alternative tree topologies of the *Phengaris*–*Maculinea* complex (exemplified by Figs 2 and 3). The strict consensus of these two topologies shows three distinct clades, which are well supported in all analyses: (i) *P. albida*–*atroguttata*; (ii) the *M. alcon* group; (iii) *M. arion*–*teleius* groups. The two topologies differ exclusively in the position of the root (either between the *alcon* group and the rest of the complex, or within *Phengaris* s.str.). In the morphological tree, *Phengaris* nests deeply within the *arion* group (Pech *et al.*, 2004). However, all molecular and combined trees support the monophyly of both the *arion* group and its *arion*–*arionides* subclade, so that the morphology-based position of *Phengaris* should be regarded as artefactual, derived from homoplastic similarities, mostly in wing colour patterns.

If an unrooted tree topology is inspected (Fig. 4), all molecular and combined trees provide a single set of relationships: the *alcon* group is close to *Phengaris*, and the *arion* and *teleius* groups are closely related. The branch connecting the *Maculinea* clade to the *Phengaris* s.str. clade is very short, indicating that the basal subclades diverged in quick succession. Interestingly, the relatively long branch of the sister lineage in our analyses, *Pseudophilotes*, attaches to the long branch leading to the *M. alcon* clade in the parsimony analysis, and to the long branch leading to *P. daitozana* in the Bayesian analysis. The true position of the root may thus be unknowable with the current data.

Phylogenetic species

The results of the species analysis are summarized in Tables 1 and 2. From the ten traditional species of the *Phengaris*–*Maculinea* complex, four (*M. nausithous*, *M. teleius*, *P. daitozana* and *P. atroguttata*) are diagnosable by several characters, both molecular and morphological.

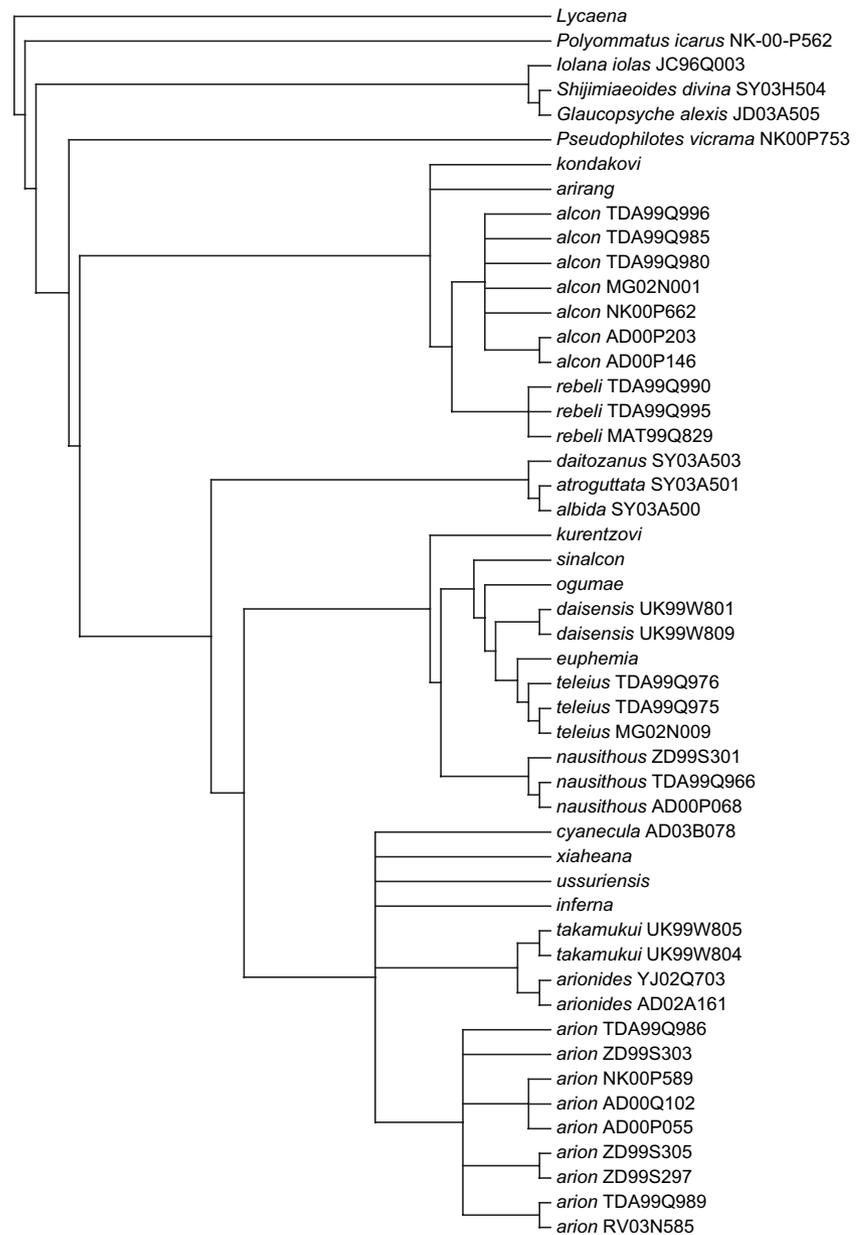


Fig. 1. Strict consensus of 120 trees found for the 'all-species' analysis.

Maculinea alcon, *M. arionides* and *P. albida* are marginally diagnosable by a single character (morphological in *M. alcon*); *M. rebeli*, *M. arion* and *M. kurentzovi* are not diagnosable at all. Consequently, there are two problematic complexes – the 'cuckoo' clade (*M. alcon* and *M. rebeli*) and the *M. arion*–*arionides* clade. Moreover, both *M. arion* and *M. arionides* can be further split into diagnosable units, whereas *M. alcon* and *M. rebeli* cannot. Most large blue species are monophyletic in the combined tree, with two exceptions (paraphyletic *M. alcon* and unresolved *M. arion*). The monophyly of *M. rebeli* and *M. arion* is contradicted by both molecular partitions, which, in contrast, support the monophyly of *M. arionides*, contradicted by morphology.

Four species that are represented by several taxa in the present analysis (see also Als *et al.*, 2004) show a well-supported basal tree-like branching pattern, which indicates that several cryptic species can be present. They include *M. arionides*, *M. arion*, *M. nausithous* and *M. teleius*. Indeed, the two sister 'subspecies' within *M. arionides* are both diagnosable and monophyletic, which qualify them to be separate phylogenetic species. Within *M. arion* s.l., there is a well-supported and diagnosable clade of *M. arion arion*, and an unresolved assemblage of little known populations. *Maculinea nausithous* and *M. teleius* seem to be divisible into several cryptic species; however, current information is insufficient to make a taxonomical decision.

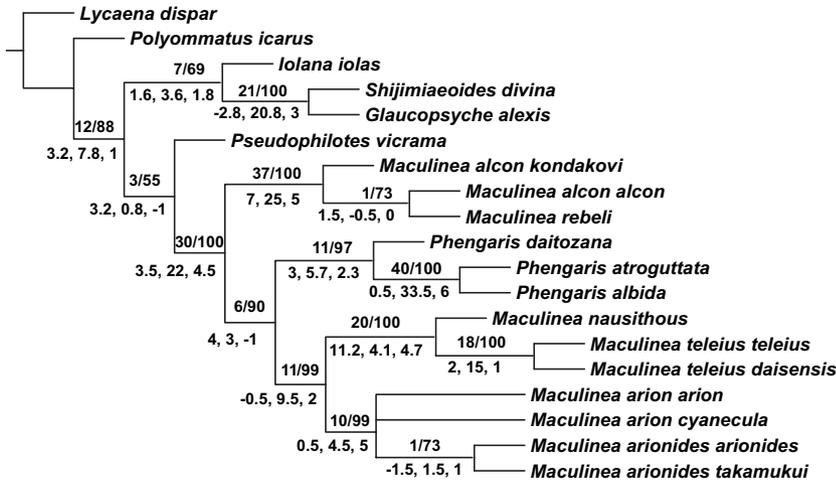


Fig. 2. Strict consensus of two trees found for the ‘complete-species’ analysis using maximum parsimony. The numbers above the branches are Bremer support/bootstrap support for nodes to the right of the numbers; the numbers below the branches give the partitioned Bremer support (morphology, cytochrome *c* oxidase I and elongation factor-1 α , respectively) for the same node.

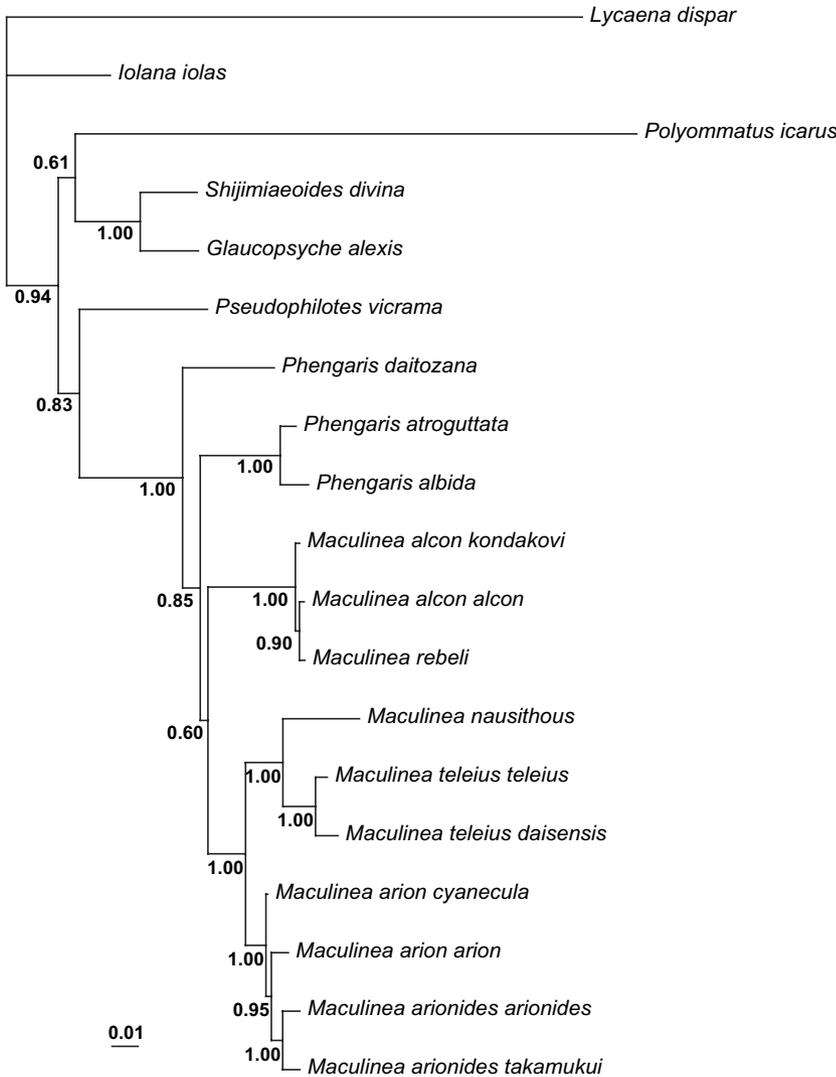


Fig. 3. Tree derived from Bayesian analysis of morphology, cytochrome *c* oxidase I and elongation factor-1 α sequences. The numbers to the right of each node are posterior probabilities of that node.

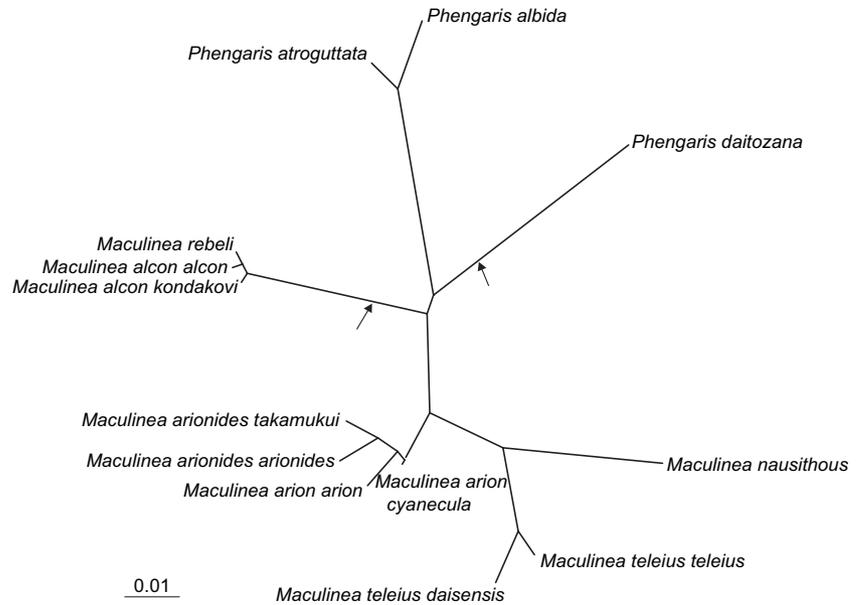


Fig. 4. An unrooted network of the ingroup species. Arrows indicate the alternative positions of the root in the two analyses (see text for details).

Discussion

Genus-level taxonomy and suppression of *Maculinea*

Our results show that the genera *Maculinea* and *Phengaris* are phylogenetically very closely related. Indeed, both parsimony and Bayesian analyses suggest that either one is not monophyletic with regard to the other (Figs 1–3).

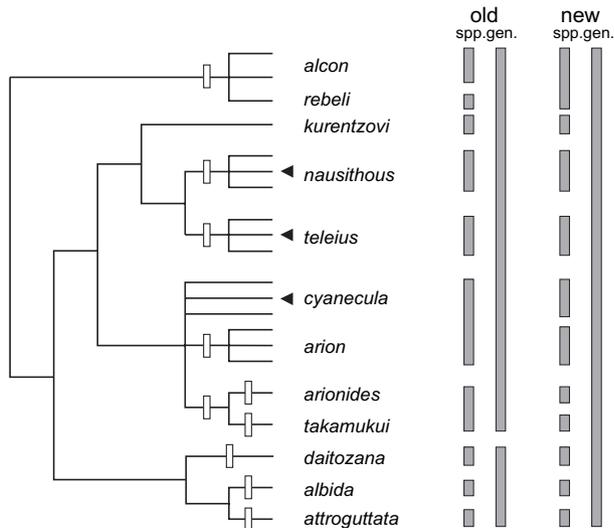


Fig. 5. Simplified phylogeny of the *Phengaris*–*Maculinea* clade with superimposed traditional ('old') and proposed ('new') genus- and species-level classification. White rectangles indicate diagnosable species, arrows indicate species that will possibly be split into several cryptic units.

Inspection of the unrooted tree (Fig. 4) suggests that the ancestral populations of *Maculinea* + *Phengaris* diversified rapidly to form two distinct lineages in *Phengaris* and two lineages in *Maculinea*. With the current available data from two genes and morphology, we cannot verify the monophyly of both genera, although we can verify that the two genera are very closely related (Fig. 5). The clade including all *Maculinea* and *Phengaris* species is characterized by the unique obligate ant parasitism (Als *et al.*, 2004; Pech *et al.*, 2004), is stable (present in all trees derived from individual data partitions and their combinations) and well supported (Bremer 30, bootstrap 100%, Bayesian posterior probability 1.00). Given this evidence, we conclude that all species in these two genera should be placed in one genus. Because the name *Phengaris* Doherty, 1891 is older than *Maculinea* Van Eecke, 1915, the generic name *Maculinea* must be treated as a junior synonym (**syn.nov.**). There is no way to suppress the name *Phengaris* as a senior synonym according to Article 23.9 of ICZN (1999), because the name *Phengaris* is used in the present-day literature, namely, for the South-East Asian species (see Io, 1994; Igarashi & Fukuda, 2000). As stated above, the various European species of '*Maculinea*' are highly endangered, and some legal and conservation problems would possibly arise as the result of changing the nomenclature. However, we believe that the nomenclatorial principle of priority should be broken only on exceptional occasions. Moreover, even if the seniority of '*Phengaris*' was suppressed as a name for the genus, the name would persist further for the subgenus including '*M.* *daitozana*', '*M.* *albida*' and '*M.* *atroguttata*'. Furthermore, the erroneous synonymy of *Maculinea* with *Glaucopsyche* by Nässig (1995) had no impact on the conservation value of the species.

Table 1. Analysis of species boundaries according to two phylogenetic species concepts (monophyletic species vs. diagnosable species).

	Monophyletic? Number of apomorphies					Diagnosable? Number of characters				Species? Y/N
	mt	EF-1 α	MOL	morph	comb	mt	EF-1 α	morph	sum	
<i>alcon</i> s.l.	26	8	33	4	6	24	8	15	47	Y
<i>alcon</i> s.str.	0	0	0	0	0	0	0	1	1	N
<i>rebeli</i>	0	0	0	2	2	0	0	0	0	N
<i>kurentzovi</i>	X	X	X	X	X	X	X	0	0	Y
<i>nausithous</i>	20	3	22	7	26	13	1	2	16	Y
<i>teleius</i>	14	?	16	4	4	9	1	4	14	Y
<i>arion</i> s.l.	0	0	0	3	?	0	0	0	0	N
<i>a. arion</i>	0	6	6	1	7	0	4	0	4	Y
<i>cyanecula</i>	X	X	X	X	X	0	0	0	0	Y?
<i>arionides</i> s.l.	1	1	6	0	7	0	1	0	1	Y
<i>a. arionides</i>	3	?	3	6	5	2	0	0	2	?
<i>takamukui</i>	9	?	11	1	11	2	0	0	2	?
<i>daitozana</i>	X	X	X	X	X	7	1	0	8	Y
<i>albida</i>	X	X	X	X	X	1	0	0	1	Y
<i>atroguttata</i>	X	X	X	X	X	2	0	1	3	Y

monophyletic? = number of autapomorphies in a tree; diagnosable? = number of diagnostic characters in a matrix; mt = mitochondrial DNA sequences/tree; EF-1 α = elongation factor-1 α matrix/tree; MOL = combined molecular matrix/tree; morph = morphological matrix/tree; comb = combined morphological-molecular tree; X = inapplicable.

How many species in *Phengaris* s.l.?

Phengaris alcon and *Phengaris rebeli*. In no analysis of any data combinations do *P. alcon* and *P. rebeli* form monophyletic sister groups, as *P. rebeli* is always nested within *P. alcon*. Moreover, they are not diagnosable genetically and hardly by morphology. Hirschke (1905), the author of its original description, described *P. rebeli* only as 'die hochalpine Form der *Lycaena alcon*' based on wing

coloration, without any note on its habitat and larval host-plant use (see also Kudrna & Belicek, 2005). Larval instars of *P. alcon* and *P. rebeli* are indistinguishable, although Munguira (1989) claimed to have found a few minor morphological differences in larvae of the Spanish populations (size of head, length of dorsal setae in the final instar). The present convention that *P. rebeli* deserves a full-species status is based on differences in host-plant use and host-ant species. The presumed difference in host-ant range (Thomas *et al.*, 1989: *Myrmica schencki* for *P. rebeli*; *M. rubra*, *M. ruginodis* and *M. scabronodis* for *P. alcon*) was based on insufficient data and is untenable at present because many more host-ant species have been found recently in both 'cuckoo' *Phengaris* species (Sielezniew & Stankiewicz, 2002; Steiner *et al.*, 2003; Tartally, 2005). The only reliable distinguishing character is host-plant use – *Gentiana cruciata* (rarely *G. lutea*) for *P. rebeli* and *G. pneumonanthe*, *G. germanica* and *G. asclepiadea* for *P. alcon* (Krisman, 2000; Als *et al.*, 2004; Sielezniew & Stankiewicz, 2004; Berezki *et al.*, 2005). However, the situation seems to be more complicated and less clear, as, at least in Poland, *P. alcon* also lays eggs on *G. cruciata* (Sielezniew & Stankiewicz, 2004). All the reported differences between '*P. rebeli*' and the rest of *P. alcon* populations are probably caused by various local microhabitat adaptations ('alcon' to marshy and humid meadows, 'rebeli' to dry swards and mountain pastures; Berezki *et al.*, 2005; references therein), which is no older than a few hundred thousand years [the age of differentiation of the whole Recent *alcon* group is estimated as 0.77 Myr by Als *et al.* (2004)]. The phylogenetic analyses show that the 'alcon phenotype' is ancestral (Als *et al.*, 2004; Pech *et al.*, 2004; present study) and that the 'rebeli phenotype' is polyphyletic (Als *et al.*, 2004; Berezki *et al.*, 2005; Pecsénye

Table 2. Analysis of the possible presence of more cryptic species.

	Divisible? Y/N	Hierarchical? Y/N				More species? Y/N
		mt	EF-1 α	morph	comb	
<i>alcon</i> s.l.	N	N	Y	Y	Y	N
<i>alcon</i> s.str.	N	N	N	N	N	X
<i>rebeli</i>	N	N	N	Y	N	X
<i>nausithous</i>	Y	Y	Y	Y	Y	Y?
<i>teleius</i>	Y	Y	N	Y	Y	Y
<i>arion</i> s.l.	Y	N	Y	Y	Y	Y
<i>a. arion</i>	?	N	Y	Y	Y	?
<i>cyanecula</i>	X	X	X	N	N	?
<i>arionides</i> s.l.	Y	Y	N	N	Y	Y
<i>a. arionides</i>	X	X	X	X	X	N
<i>takamukui</i>	X	X	X	X	X	N

divisible? = presence/absence of diagnosable units within a taxon; hierarchical? = presence of well-resolved hierarchical phylogenetic structure within a taxon; mt = mitochondrial DNA sequences/tree; EF-1 α = elongation factor-1 α matrix/tree; morph = morphological matrix/tree; comb = combined morphological-molecular tree; X = inapplicable.

et al., 2007). In conclusion, there is no real argument for retaining *P. rebeli* as a separate species. The East Palearctic populations of *P. alcon* (*P. a. kondakovi* and *P. a. arirang*) have yet to be studied by molecular methods, and their taxonomical status remains uncertain.

Phengaris arion and *Phengaris cyanecula*. The status of *P. cyanecula* as a separate species is not fully resolved. In the Bayesian analysis (Fig. 3), it is situated as the sister taxon to the *arion* group, a placement corresponding to that in the ‘all-species’ parsimony tree (together with *xiaheana*, *inferna* and *ussuriensis* taxa). However, *P. arion* s.l. (including *cyanecula*) is neither monophyletic nor diagnosable in any molecular tree (see Als *et al.*, 2004). In the mitochondrial tree, *P. arion* is paraphyletic in respect of *cyanecula* and *arionides*; in both EF-1 α and the combined molecular trees, *P. cyanecula* seems to be a sister species of *P. arionides*. Although *P. cyanecula* has no diagnosable characters, it provides a unique combination of characters and should be provisionally classified as a species. Consequently, the status of the East Asian ‘*arion*’ taxa then becomes problematic: the three subspecies (*xiaheana*, *inferna* and *ussuriensis*) are not diagnosable from *P. cyanecula* and do not form a clearly delimited clade; on the contrary, all four taxa are unique in the *arion* group in having less developed blue scales on the dorsal side of wings (probably ancestral character state, shared with the *alcon* group, *P. teleius*, *P. kurentzovi*, and some outgroups). At present, *P. cyanecula*–*xiaheana*–*inferna*–*ussuriensis* appears as a mono- or paraphyletic complex of basal East Asian populations without evident synapomorphies, which corresponds to the concept of metasppecies *sensu* Archibald (1994) [for critical comments concerning the application of the criterion of reciprocal monophyly to decisions about species boundaries see Kizirian & Donnelly (2004)].

Phengaris arionides and *Phengaris takamukai*. Pech *et al.* (2004) proposed that *P. arionides* might be paraphyletic as the *P. daitozana*–*P. albida*–*P. atroguttata* clade seemed to group with *P. arionides arionides*, with *P. arionides takamukai* as a sister group. However, in all molecular and total evidence analyses, both *P. arionides* subspecies form a clade. Both taxa are monophyletic, morphologically distinct, diagnosable (in mitochondrial sequences) and allopatric. Reclassification of ‘*P. arionides* s.l.’ as two separate species, East Asian *P. arionides* (Staudinger, 1887) and Japanese *P. takamukai* (Matsumura, 1919), is then a realistic possibility, even if the paraphyly of ‘*P. arionides* s.l.’ was most probably artefactual.

Phengaris kurentzovi. Although no molecular data of *P. kurentzovi* are available, its species-level status is not disputable. In all analyses, *P. kurentzovi* groups as a sister species of the whole *teleius* group and, consequently, deserves to be treated as a separate species. It has no single diagnostic morphological character, but is well characterized by a unique combination of structural features.

Even more cryptic species? Two species of *Phengaris*, namely *P. nausithous* and *P. teleius*, seem to be composed

of several further diagnosable units. In *P. nausithous*, which has never been formally split into subspecies, molecular analyses suggest unexpectedly deep diversification [estimated as 1.44 Myr old by Als *et al.* (2004)] even in a relatively local geographical scale (eastern Europe). However, as yet the species cannot be split into more phylogenetic species, and a comprehensive phylogeographical analysis is needed urgently.

In *P. teleius*, molecular analysis suggests that European and Japanese populations are deeply different, which accords to traditional taxonomical views. Unfortunately, the subspecific status of the sample specimens (*kazamoto*, *dai-sensis*, *hosonoi*) was not determined (Als *et al.*, 2004). Both populations, European and Japanese, seem to be diagnosable molecularly (one diagnostic character of the European populations, three of the Japanese one). The combined phylogeny suggests that East Palearctic *P. teleius euphemia* is closely related to West Palearctic *P. t. teleius*, followed by the Japanese population(s), whereas the northern Chinese *P. t. sinalcon* and Sakhalin-Kurile *P. t. ogumae* are the successive sister groups of all other populations. This hierarchy is, naturally, affected by the absence of any molecular information on *euphemia*, *sinalcon* and *ogumae* blues. Historically, the northern Chinese and Sakhalin populations of *P. teleius* have been classified occasionally as separate species (‘*P. sinalcon*’ and ‘*P. ogumae*’; cf. Tuzov *et al.*, 2000); the present data corroborate, in addition, the species rank for the Japanese populations (‘*P. kazamoto*’?). Nevertheless, the complicated relationships among the little-known East Palearctic populations preclude any final taxonomical solution.

Classification of the *Phengaris*–*Maculinea* complex

Based on the above phylogenetic and species-concept analyses, we propose the reclassification of *Phengaris* s.l. into four species groups and eleven species, out of which ‘*P. cyanecula*’, *P. nausithous* and *P. teleius* are the most problematic taxonomically and deserve further study (Fig. 5).

Phengaris Doherty, 1891

1. *P. alcon* group (= *Maculinea* van Eecke, 1915)
 - 1.1. *Phengaris alcon* (Denis & Schiffermüller, 1775) **comb.n.**
2. *P. atroguttata* group (= *Phengaris* s.str.)
 - 2.1. *Phengaris daitozana* Wileman, 1908
 - 2.2. *Phengaris albida* Leech, 1893
 - 2.3. *Phengaris atroguttata* (Oberthür, 1876)
3. *P. teleius* group
 - 3.1. *Phengaris kurentzovi* (Sibatani *et al.*, 1994) **comb.n.**
 - 3.2. *Phengaris nausithous* (Bergsträsser *et al.*, [1779]) **comb.n.** (several spp.?)
 - 3.3. *Phengaris teleius* (Bergsträsser *et al.*, [1779]) **comb.n.** (several spp.?)
4. *P. arion* group
 - 4.1. ‘*Phengaris cyanecula*’ (Eversmann, 1848) **comb.n.** (metasp., several spp.?)
 - 4.2. *Phengaris arion* (Linnaeus, 1758) **comb.n.**
 - 4.3. *Phengaris arionides* (Staudinger, 1887) **comb.n.**
 - 4.4. *Phengaris takamukai* (Matsumura, 1919) **comb.n.**

Evolutionary and conservation units

Species and subspecies are often used as proxies for units of conservation, which requires their evolutionary independence. Even in the birds, whose taxonomy is seemingly well based, mitochondrial DNA sequence data reveal that no less than two-thirds of the continentally distributed subspecies lack the population genetic structure indicative of a distinct evolutionary unit. Although sequence data show that avian species include 1.9 historically significant units on average, these units are usually not reflected by current nomenclature (Zink, 2004; Phillimore & Owens, 2006). These unnamed and, in general, usually unknown units should guide conservation efforts and identify biological diversity, not the formally named taxa. In entomology, the situation is evidently even worse (for instance, the European Union Habitat Directive lists the beetle *Osmoderma eremita*, which in reality is a complex of two to four distinct species; see Ranius *et al.*, 2005), and in butterflies, a group burdened by a tradition of amateur collectors' quasi-taxonomy, a massive reorganization of the classifications is required so that the lowest ranks reflect evolutionary diversity (see Vila *et al.*, 2006).

The use of phylogenetic species concepts often leads to recognition of a far greater number of much less inclusive units. Agapow *et al.* (2004) have shown that species surveys based on the phylogenetic species concepts include more species (48%), with an associated decrease in the species' population size and geographical range and serious consequences for conservation. In the present analysis, the number of proposed phylogenetic species is not higher than that of the traditional species (eleven vs. ten to eleven). However, future phylogeographical analyses of *P. nausithous*, *P. teleius* and '*P. cyanecula*' will probably lead to a significant elevation of the number of evolutionary units, with all its serious consequences for conservation policy.

Acknowledgements

For valuable comments and help with obtaining hard accessible literature resources we are obliged to M. Konvička, O. Kudrna and M. Munguira. We would like to thank Ian Kitching, Frank Krell, Pavel Štys and an anonymous referee for discussing the manuscript. Our study was supported by the Czech Science Foundation (206/03/H034), Czech Ministry of Education (MSM6007665801, LC06073 and FRVS21-2926) and Grant Agency of CAS (KJB600070601).

References

Agapow, P.M., Bininda-Emonds, O.R.P., Crandall, K.A., Gittleman, J.L., Mace, G.M., Marshall, J.C. & Purvis, A. (2004) The impact of species concept on biodiversity studies. *Quarterly Review of Biology*, **79**, 161–179.

Als, T.D., Vila, R., Kandul, N.P., Nash, D.R., Yen, S.H., Hsu, Y.F., Mignault, A.A., Boomsma, J.J. & Pierce, N.E. (2004) The

evolution of alternative parasitic life histories in large blue butterflies. *Nature*, **432**, 386–390.

Archibald, J.D. (1994) Metataxon concepts and assessing possible ancestry using phylogenetic systematics. *Systematic Biology*, **43**, 27–40.

Berezcki, I., Pecsénye, K., Peregovits, L. & Varga, Z. (2005) Patterns of genetic differentiation in the *Maculinea alcon* species group (Lepidoptera, Lycaenidae) in Central Europe. *Journal of Zoological Systematics and Evolutionary Research*, **43**, 157–165.

Brooks, D.R. & McLennan, D.A. (2002) *The Nature of Diversity: an Evolutionary Voyage of Discovery*. University of Chicago Press, Chicago.

Eldredge, N. & Cracraft, J. (1980) *Phylogenetic Patterns and the Evolutionary Process*. Columbia University Press, New York.

Elmes, G.W. & Thomas, J.A. (1992) Complexity of species conservation in managed habitats: interaction between *Maculinea* butterflies and their ant hosts. *Biodiversity and Conservation*, **1**, 155–169.

Farris, J.S., Källersjö, M., Kluge, A.G. & Bult, C. (1994) Testing significance of incongruence. *Cladistics*, **10**, 315–319.

Fiedler, K. (1991) Systematic, evolutionary, and ecological implications of myrmecophily within the Lycaenidae [Insecta: Lepidoptera: Papilionoidea]. *Bonner Zoologische Monographien*, **31**, 1–210.

Fiedler, K. (1998) Lycaenid–ant interactions of the *Maculinea* type: tracing their historical roots in a comparative framework. *Journal of Insect Conservation*, **2**, 3–14.

Gatesy, J., O'Grady, P. & Baker, R.H. (1999) Corroboration among data sets in simultaneous analysis: hidden support for phylogenetic relationships among higher level artiodactyl taxa. *Cladistics*, **15**, 271–313.

Goloboff, P. (1999) *NONA (NO NAME)*, Version 2.0. Published by the author, Tucumán, Argentina (<http://www.cladistics.com/aboutNona.htm>).

Hirschke, H. (1905) Eine neue hochalpine Form der *Lycaena alcon* F. aus den steirischen Alpen. *Jahres-Bericht des Wiener Entomologischen Vereines*, **1904** (15), 9–11.

Hochberg, M.E., Clarke, R.T., Elmes, G.W. & Thomas, J.A. (1994) Population dynamic consequences of direct and indirect interactions involving a large blue butterfly and its plant and red hosts. *Journal of Animal Ecology*, **63**, 375–391.

ICZN (1999) *International Code of Zoological Nomenclature*, 4th edn. The International Trust for Zoological Nomenclature, London.

Igarashi, S. & Fukuda, H. (2000) *The Life Histories of Asian Butterflies*, Vol. 2. Tokai Daigaku Shuppankai, Tokyo.

Io, C. (1994) *Monographia Rhopalocerorum Sinensium*. Henan Scientific and Technological Publishing House, Zhengzhou.

Kizirian, D. & Donnelly, M.A. (2004) The criterion of reciprocal monophyly and classification of nested diversity at the species level. *Molecular Phylogenetics and Evolution*, **32**, 1072–1076.

Krismann, A. (2000) Zum Eiablageverhalten von *Maculinea alcon* ([D. & S.], 1775) an *Gentiana pneumonanthe* und *Gentiana asclepiadea*. *UFZ-Bericht*, **2000** (2), 103–110.

Kudrna, O. & Belicek, J. (2005) On the 'Wiener Verzeichnis', its authorship and the butterflies named therein. *Oedippus*, **23**, 1–32.

Lewis, P.O. (2001) A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology*, **50**, 913–925.

Maes, D. & Van Dyck, H. (2005) Habitat quality and biodiversity indicator performances of a threatened butterfly versus a

- multispecies group for wet heathlands in Belgium. *Biological Conservation*, **123**, 177–187.
- Mishler, B.D. & Donoghue, M.J. (1982) Species concepts: a case for pluralism. *Systematic Zoology*, **31**, 491–503.
- Mishler, B.D. & Theriot, E.C. (2000) The phylogenetic species concept (*sensu* Mishler and Theriot): monophyly, apomorphy, and phylogenetic species concepts. *Species Concepts and Phylogenetic Theory: a Debate* (ed. by Q.D. Wheeler and R. Meier), pp. 44–69. Columbia University Press, New York.
- Munguira, M.L. (1989) *Biología y Biogeografía de Los Licénidos Ibéricos en Peligro de Extinción (Lepidoptera, Lycaenidae)*. PhD Thesis, Ediciones Universidad Autónoma de Madrid.
- Nässig, W. (1995) Die Tagfalter der Bundesrepublik Deutschland: Vorschlag für ein modernes, phylogenetisch orientiertes Artenverzeichnis (kommentierte Checkliste). *Entomologische Nachrichten und Berichte*, **39**, 1–28.
- Nixon, K.C. & Wheeler, Q.D. (1990) An amplification of the phylogenetic species concept. *Cladistics*, **6**, 211–223.
- Pech, P., Fric, Z., Konvička, M. & Zrzavý, J. (2004) Phylogeny of *Maculinea* blues (Lepidoptera: Lycaenidae) based on morphological and ecological characters: evolution of parasitic myrmecophily. *Cladistics*, **20**, 362–375.
- Pecsenye, K., Bereczki, J., Tihanyi, B., Tóth, A., Peregovits, L. & Varga, Z. (2007) Genetic differentiation among the *Maculinea* species (Lepidoptera: Lycaenidae) in eastern Central Europe. *Biological Journal of the Linnean Society*, **91**, 11–21.
- Phillimore, A.B. & Owens, I.P.F. (2006) Are subspecies useful in evolutionary and conservation biology? *Proceedings of the Royal Society B*, **273**, 1049–1053.
- Pierce, N.E., Braby, M.F., Heath, A., Lohman, D.J., Mathew, J., Rand, D.B. & Travassos, M.A. (2002) The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). *Annual Review of Entomology*, **47**, 733–771.
- Ranius, T., Aguado, L.O., Antonsson, K., *et al.* (2005) *Osmoderma eremita* (Coleoptera, Scarabaeidae, Cetoniinae) in Europe. *Animal Biodiversity and Conservation*, **28**, 1–44.
- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.
- Sibatani, A., Saigusa, T. & Hirowatari, T. (1994) The genus *Maculinea* van Eecke, 1915 (Lepidoptera: Lycaenidae) from the East Palaearctic Region. *Tyo to Ga*, **44**, 157–220.
- Sielezniew, M. & Stankiewicz, A. (2002) First data on host-ant specificity of parasitic butterfly *Maculineaalcon* (Den. & Schiff.) (Lepidoptera: Lycaenidae) in Poland and eastern Europe. *Fragmenta Faunistica*, **45**, 123–130.
- Sielezniew, M. & Stankiewicz, A. (2004) *Gentiana cruciata* as an additional host plant of *Maculineaalcon* on a site in Eastern Poland. *Nota Lepidopterologica*, **27**, 91–93.
- Stadler, B., Kindlmann, P., Šmilauer, P. & Fiedler, K. (2003) A comparative analysis of morphological and ecological characters of European aphids and lycaenids in relation to ant attendance. *Oecologia*, **135**, 422–430.
- Steiner, F.M., Sielezniew, M., Schlick-Steiner, B.C., Höttinger, H., Stankiewicz, A. & Górnicki, A. (2003) Host specificity revisited: new data on *Myrmica* host ants of the lycaenid butterfly *Maculinea rebeli*. *Journal of Insects Conservation*, **7**, 1–6.
- Tartally, A. (2005) *Myrmica salina* (Hymenoptera: Formicidae) as a host of *Maculineaalcon* (Lepidoptera: Lycaenidae). *Sociobiology*, **46**, 39–43.
- Thomas, J.A. (1980) Why did the large blue become extinct in Britain? *Oryx*, **15**, 243–247.
- Thomas, J.A., Elmes, G.W., Wardlaw, J.C. & Woyciechowski, M. (1989) Host specificity among *Maculinea* butterflies in *Myrmica* ant nests. *Oecologia*, **79**, 452–457.
- Thomas, J.A., Simcox, D.J., Wardlaw, J.C., Elmes, G.W., Hochberg, M.E. & Clarke, R.T. (1998) Effects of latitude, altitude and climate on the habitat and conservation of the endangered butterfly *Maculinea arion* and its *Myrmica* ant hosts. *Journal of Insect Conservation*, **2**, 39–46.
- Tuzov, V.K., Bogdanov, P.V., Churkin, S.V., *et al.* (2000) *Guide to the Butterflies of Russia and Adjacent Territories (Lepidoptera, Rhopalocera)*, Vol. 2. Pensoft, Sofia.
- Van Swaay, C.A.M. & Warren, M.S. (1999). *Red Data Book of European Butterflies (Rhopalocera)*. Nature and Environment Series no. 99. Council of Europe, Strasbourg.
- Vila, M., Lundhagen, A.C., Thuman, K.A., Stone, J.R. & Bjorklund, M. (2006) A new conservation unit in the butterfly *Erebia triaria* (Nymphalidae) as revealed by nuclear and mitochondrial markers. *Annales Zoologici Fennici*, **43**, 72–79.
- Wardlaw, J.C., Thomas, J.A. & Elmes, G.W. (2000) Do *Maculinea rebeli* caterpillars provide vestigial mutualistic benefits to ants when living as social parasites inside *Myrmica* ant nests? *Entomologia Experimentalis et Applicata*, **95**, 97–103.
- Wheeler, Q.D. & Platnick, N.I. (2000) The phylogenetic species concept (*sensu* Wheeler and Platnick). *Species Concepts and Phylogenetic Theory: a Debate* (ed. by Q.D. Wheeler and R. Meier), pp. 55–69. Columbia University Press, New York.
- Wynhoff, I. (1998) Lessons from the reintroduction of *Maculinea teleius* and *M. nausithous* in the Netherlands. *Journal of Insect Conservation*, **2**, 47–57.
- Zink, R.M. (2004) The role of subspecies in obscuring avian biological diversity and misleading conservation policy. *Proceedings of the Royal Society of London B*, **271**, 561–564.

Accepted 14 February 2007

First published online 14 May 2007