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# Phylogeny of *Polygonia*, *Nymphalis* and related butterflies (Lepidoptera: Nymphalidae): a total-evidence analysis

SÖREN NYLIN[1]*, KLAS NYBLOM[1], FREDRIK RONQUIST[2], NIKLAS JANZ[1], JOSEPH BELICEK[3] and MARI KÄLLERSJÖ[4]

[1]*Department of Zoology, Stockholm University, S-106 91 Stockholm, Sweden;*
[2]*Department of Systematic Zoology, Evolutionary Biology Centre, Uppsala University, Sweden;*
[3]*15004-96 Avenue, Edmonton, Alberta, Canada;*
[4]*Molecular Systematics Laboratory, Natural History Museum, Stockholm, Sweden*

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We investigated the phylogeny of butterflies in the tribe Nymphalini *sensu* Harvey 1991, comprising the genera *Vanessa*, *Cynthia*, *Bassaris*, *Aglais*, *Inachis*, *Nymphalis*, *Polygonia*, *Kaniska*, *Antanartia*, *Hypanartia*, *Symbrenthia*, *Mynes* and *Araschnia*. Evidence from the mitochondrial gene nd1, the nuclear gene 'wingless' and from morphology/ecology/behaviour were used separately and combined to analyse relationships. Phylogenies based on the different types of data agreed in many aspects of basic topology. We show that an analysis of only wing pattern characters (based on Nijhout's homology system) results in a topology broadly similar to the one resulting from analysis of the complete matrix. We found support for a monophyletic Nymphalini, where *Hypanartia* may be the sister clade to all other genera. *Mynes*, *Symbrenthia* and *Araschnia* together seem to form another basal clade. Evidence presented gives only moderate support for a monophyletic *Vanessa* in the wide sense, including also *Cynthia* and *Bassaris*, but strong support for the monophyly of the largely holarctic clade *Aglais* + *Inachis* + *Nymphalis* + *Polygonia* + *Kaniska* + *Roddia*. Within the latter group there is strong support for a clade consisting of *Aglais* + *Inachis* and for a second clade which includes *Nymphalis*, *Polygonia* (and its sister clade, the monotypic *Kaniska*) as well as *Roddia l-album* (=*Nymphalis vaualbum*). As a consequence of this topology, *Aglais* is recognized as a taxon separate from *Nymphalis*. We present a hypothesis of species relationships within the focal group of genera. We also analyse and discuss the implications of excluding or including ecological data in phylogenetic tree construction, when the tree is to be used for studies in phylogenetic ecology. 

ADDITIONAL KEY WORDS: taxonomy – systematics – cladistics – mitochondrial DNA – nuclear DNA – phylogenetic ecology – Nymphalini.

## INTRODUCTION

The phylogeny of nymphaline butterflies is of considerable interest not only for taxonomists but also for evolutionary biologists in general. The diversity among these butterflies (e.g. wing patterns, behaviour and host–plant associations) is intriguing, and the subfamily includes several important model groups for ecological and evolutionary studies, such as *Euphydryas*, *Precis* and *Polygonia*. A step towards an improved understanding of nymphaline phylogeny is taken here by investigating the relationships within a subset of the Nymphalinae, the tribe Nymphalini. The remaining tribes in the Nymphalinae are generally taken to be Melitaeini and Kallimini.

Nymphalini was suggested by Harvey (1991) to include the genera *Vanessa* Fabricius, *Cynthia* Fabricius, *Bassaris* Hübner, *Aglais* Dalman, *Inachis* Hübner, *Nymphalis* Kluk, *Polygonia* Hübner, *Kaniska* Moore, *Antanartia* Rothschild & Jordan, *Hypanartia* Hübner, *Symbrenthia* Hübner, *Mynes* Boisduval and *Araschnia* Hübner. He also suggested that the most likely sister group is either the Melitaeini (including e.g. *Melitea* and *Euphydryas*) or Melitaeini + his Kallimini (including e.g., *Kallima* and *Precis*). In contrast, Ackery (1988) included in his Nymphalinae the tribe Coloburini, as well as a wider Nymphalini corresponding to Harvey's

* Corresponding author. E-mail: Soren.Nylin@zoologi.su.se



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Nymphalini + Kallimini, but not the Melitaeini. Harvey (1991) placed the Coloburini in a different subfamily, the Limenitidinae, which he, however, did not believe to be monophyletic. Hence, there is a controversy regarding both the monophyly of Harvey's Nymphalini and which are the closest relatives of this group, if it is indeed a natural grouping of genera.

Within the Nymphalini, systematics has been in a great deal of flux, although species circumscriptions have remained fairly stable. The genera *Cynthia*, *Bassaris*, *Aglais*, *Inachis* and *Kaniska* have sometimes been recognized and sometimes been included in other genera. *Cynthia* (type species *C. cardui* (L.)) and *Bassaris* (type species *B. itea* (Fabricius)) are often included in *Vanessa* (type species *V. atalanta* (L.)), although the revision of Field (1971) resurrected the former two genera. The species of *Aglais* and *Inachis* have sometimes been included in *Nymphalis* or *Vanessa*. For instance, Layberry, Hall & Lafontaine (1998) recently argued in favour of including the two widely recognized species of *Aglais* (*urticae* (L.) and *milberti* (Godart)) in *Nymphalis*, because "*Aglais* might be a highly modified (derived) group within *Nymphalis*". In contrast, Niculescu (1965) and others have argued that *Nymphalis* in the strict sense (excluding *Aglais*) is in fact more closely related to *Polygonia* than either of these genera is to *Aglais*. The monotypic *Kaniska canace* (L.) is sometimes treated as a *Polygonia*. We will investigate which of these classifications is likely to reflect phylogeny. We will also attempt to resolve the controversy regarding the Holarctic species or species-group *Roddia l-album* (Korshunov). This species is most often referred to in the literature as either *Nymphalis vaualbum* (e.g., Higgins, 1975) or *Polygonia l-album* (e.g., Niculescu, 1965). As pointed out by Kocak (1981), *Nymphalis vau-album* Denis & Schiffermueller, 1775 = *vaualbum* is a *nomen nudum*, and the species name *l-album* of Esper (1780) is the correct one. Recently this taxon has been placed in a new monotypic genus; *Roddia* Korshunov, 1996 (Korshunov & Gorbunov, 1995; Korshunov, 1996).

In this study we take a total-evidence approach to resolving phylogenetic relationships. We combine available data on morphology and ecology from literature with our own observations on all developmental stages, particularly adult wing patterns, and with molecular data from both mitochondrial (nd1) and nuclear ('wingless') genes. In a separate study we analysed only the wing pattern data. This was done because we have made extensive use of the recently available system for suggesting homologies between patterns in different taxa (Nijhout, 1991). We found it of interest to investigate whether there is a phylogenetic signal in this type of readily available data which is congruent with other kinds of evidence, or whether wing patterns are too affected by selection for, e.g., mimicry, creating homoplasy.

The phylogeny obtained in this study is intended to be used in studies on the evolution of host plant utilization in the Nymphalini. For this reason we also studied more closely the effects of host plant data on the resulting phylogenies.

## MATERIAL AND METHODS

### INGROUP

We strived to include all species in the focal group of genera: *Polygonia*, *Kaniska*, *Nymphalis*, *Aglais* and *Inachis*. This group is predominantly holarctic. The number of species of *Polygonia* (in the narrow sense) recognized varies, especially in North America, where some relatively distinct populations are seen as either species in their own right or as subspecies of *P. faunus* (Edwards): *hylas*, *smythi*, *silvius*; *P. gracilis* (Grote & Robinson): *zephyrus*; or *P. progne* (Cramer): *oreas*, *nigrozephyrus*. The populations sampled by us represent all of the nearctic species recognized by Scott (1986). In addition, there is probably at least one good species south of the US, *P. haroldii* Dewitz, from which we did not manage to obtain material. Of the four palearctic species we included three: *P. c-album* (L.), *P. egea* (Cramer) and *P. c-aureum* (L.). The fourth species that was not studied, *P. gigantea* (Leech), is found in China. The genus *Kaniska* holds a single species, *K. canace*, which is often placed in *Polygonia*. The same is true for *Roddia l-album*. Both of these species were studied, *l-album* from both the Nearctic and the Palearctic. However, as nd1 sequence difference between the two populations was found to be negligible, and we saw no diagnostic differences in morphology, we included only the Eurasian sample in the final analyses. The same is true for the Holarctic species *Nymphalis antiopa* (L.), where also samples from both the Nearctic and Palearctic were initially studied.

There are five clear species of *Nymphalis*, four of which were studied: *N. polychloros* (L.), *N. antiopa*, *N. xanthomelas* (Denis & Schiffermüller) and *N. californica* (Boisduval). The fifth, *N. cyanomelas* (Doubleday), is only very rarely found in the highlands of Mexico, Guatemala and El Salvador (De la Maza E. & White Lopez, 1986) and was not sampled for DNA. The immature stages of this species are not known. It was included in the morphological study on the basis of a few adult traits, in order that we would be able to suggest a probable phylogenetic position.

We studied the nearctic *Aglais milberti* (often placed in *Nymphalis*) and the palearctic *A. urticae*. The other species of *Aglais* described from Asia, *A. kashmirensis* (Kollar) and *A. ladakensis* (Moore), in all probability represent races of *urticae*, or at the very least closely

related species, such that their phylogenetic position can be inferred from that of *urticae*. *Inachis* holds a single species, *I. io* (L.), which was studied.

Within the Nymphalini, but outside of the focal group, each genus was represented by one or more species. *Vanessa* was represented by *V. atalanta*. *Cynthia* was represented by *C. cardui* and *V. virginiensis* (Drury). *Bassaris* was represented by *B. gonerilla* (Fabricius), *Antanartia* by *A. schaeneia* (Trimen) and *Hypanartia* by *H. lethe* (Fabricius), but we were unsuccessful in extracting DNA from dry samples of this species, so *Hypanartia* DNA in this study originated from *H. lindigii* (Felder). *Symbrenthia* was represented by *S. hypselis* Godart (DNA, however, from *S. hypatia* Wallace). *Mynes* was represented by *M. geoffroyi* (Guérin-Méneville) and finally *Araschnia* by *A. levana* (L.).

### OUTGROUP EXEMPLARS

We included in the outgroup members of the tribe Argynniti in the Heliconiinae, a subfamily well outside of the Nymphalinae, in order to investigate monophyly of the Nymphalini in the initial analyses. *Argynnis paphia* (L.) of the Argynniti was used for the nuclear gene 'wingless', morphology, ecology and behaviour, but DNA had to be taken from *Issoria lathonia* (L.) in the case of nd1.

As explained in the Introduction, the tribes Melitaeini, Kallimini and Coloburini are more likely to be closely related to the Nymphalini, and in all the main analyses we also included two species from the Kallimini in the outgroup: *Hypolimnas bolina* (L.) and *Precis coenia* (Hübner). *Colobura dirce* (L.) from Coloburini was included in one analysis (see below).

### SPECIMENS STUDIED

#### *Morphology*

The following taxa were followed throughout their life cycle in the laboratory and the traits of immature stages were investigated: *Precis coenia*, *Araschnia levana*, *Vanessa atalanta*, *V. indica*, *Cynthia cardui*, *C. virginiensis*, *Bassaris gonerilla*, *B. itea*, *Aglais urticae*, *A. milberti*, *Inachis io*, *Nymphalis polychloros*, *N. antiopa*, *N. xanthomelas*, *Kaniska canace*, *Polygonia c-album*, *P. faunus*, *P. egea*, *P. c-aureum*, *P. satyrus*, *P. gracilis zephyrus*, *P. interrogationis* and *P. comma*. Last-instar larvae, as well as pupae, of *Mynes geoffroyi* preserved in alcohol were kindly provided by Darrel Kemp. Traits of immature stages for the remaining species included in the study, as well as some additional traits of the entire set of species, were found in the literature (references given below and in the character list, Appendix 1).

External adult traits were also investigated in museum specimens of *Argynnis paphia*, *Hypolimnas bolina*, *Nymphalis californica*, *Polygonia progne*, *Antanartia schaeneia*, *Hypanartia lethe*, *Symbrenthia hypselis* and *Mynes geoffroyi* deposited in the Department of Zoology and Natural History Museum, Stockholm. Mounted adults of *N. cyanomelas* were studied at the Natural History Museum, London. Most novel adult traits concern wing shape and especially wing patterns, where we suggest possible homologies based on the homology system presented by Nijhout (1991). Several of these traits are depicted in Figures 6–8.

#### *DNA*

Butterflies for DNA analysis were collected by the authors or obtained from colleagues (see Table 1 for source populations). In most cases we used adult, pupal or larval specimens which were killed by freezing and stored at $-70°C$ until analysis. In a few cases we successfully used dry adult specimens from collections, but most attempts did not yield a useful DNA extract. Voucher specimens are stored at the Department of Zoology, Stockholm, except for *Symbrenthia hypatia* (wings retained by K. Fiedler) and *Hypanartia lindigii* (extracted DNA kindly sent by A. V. Z. Brower). DNA sequences will be uploaded to GenBank and are available from K. Nyblom upon request.

### SOURCES OF CHARACTER INFORMATION (LITERATURE)

Data on internal adult morphology and ecology, as well as several other traits, were taken from the literature when we could not directly study them. Sources of data for some particular traits are given in the character list, but a few general sources can be mentioned here. World butterflies: Harvey (1991); North America: Scott (1986); South America: DeVries (1987); Europe: Niculescu (1965); Australia: Common & Waterhouse (1972); Asia: Johnston & Johnston (1980); Nakanishi (1988); Shirozu (1960); Shirozu & Hara (1960); Teshirogi (1990); Africa: Larsen (1991). In addition, some sources were of special importance for specific taxa. *Mynes*: Hawkswood (1990); *Vanessa*, *Cynthia* and *Bassaris*: Field (1971).

Concerning the male genitalia, we noted a controversy regarding terminology and homology of structures (see Niculescu (1985), between Niculescu (1965) and Higgins (1975). Without substantially adding to the scope of this study it was not possible for us to study these traits independently. For this reason we followed one author (Niculescu, 1965), reasoning that homologies are more likely to be consistent within a single author's work, and Niculescu has the most

**Table 1.** Origin of butterflies from which DNA was extracted

| | |
|---|---|
| Ingroup taxa: | |
| *Polygonia c-album* | Stockholm, Sweden |
| *Polygonia faunus* | Salmon River, Idaho, USA |
| *Polygonia progne progne* | Forest Co., Wisconsin, USA |
| *Polygonia gracilis zephyrus* | Vale Mount., B.C., Canada |
| *Polygonia satyrus* | Blue Mountains, Washington, USA |
| *Polygonia c-aureum* | Japan |
| *Polygonia interrogationis* | Fayette Co., Tennessee, USA |
| *Polygonia comma* | Shelby Co., Tennessee, USA |
| *Polygonia egea* | Greece |
| *Kaniska canace* | Nagano, Japan |
| *Roddia l-album ('Nymphalis vaualbum')* | Manitoba, Canada |
| *Roddia l-album ('Nymphalis vaualbum')* | Ussuriysk Dist., Siberia, Russia |
| *Nymphalis antiopa* | Stockholm, Sweden |
| *Nymphalis antiopa* | Blue Mountains, Washington, USA |
| *Nymphalis polychloros* | Öland, Sweden |
| *Nymphalis xanthomelas* | Kirgisia, USSR |
| *Nymphalis californica* | Jeff. Co., Colorado, USA |
| *Aglais milberti* | Blue Mountains, Washington, USA |
| *Aglais urticae* | Stockholm, Sweden |
| *Inachis io* | Stockholm, Sweden |
| *Araschnia levana* | Estonia |
| *Antanartia schaeneia* | Cameroon |
| *Hypanartia lindigii* | South America (DNA from A.V.Z. Brower) |
| *Vanessa atalanta* | Stockholm, Sweden |
| *Bassaris gonerilla* | New Zealand |
| *Cynthia cardui* | DeSoto Co., Mississippi, USA |
| *Cynthia virginiensis* | Shelby Co., Tennessee, USA |
| *Symbrenthia hypatia* | W. Malaysia (wings retained by K. Fiedler) |
| *Mynes geoffroyi* | Queensland, Australia |
| Inner outgroup taxa: | |
| *Precis coenia* | Shelby Co., Tennessee, USA |
| *Hypolimnas bolina* | W. Malaysia |
| Outer outgroup taxa: | |
| *Colobura dirce* | Costa Rica |
| *Issoria lathonia* | Stockholm, Sweden |
| *Argynnis paphia* | Stockholm, Sweden |

complete treatment of our study species. For the same reason we have treated data as missing for non-European species except when homology was evident from illustrations, as in the case of penis shape.

## MOLECULAR ANALYSES

### *General*

We initially intended to study only the mitochondrial nd1 gene, which has been used in some earlier studies of butterfly relationships (Aubert *et al.*, 1996; Weller, Pashley & Martin, 1996). A pilot study involving only a few species also gave promising results; species of the same genus ended up together in the phylogenetic analyses and there seemed to be a reasonable resolution of relationships. However, as more species were added much of this resolution was lost, and it now seems that nd1 provides relatively little information on relationships. This is despite the fact that we have sequenced a considerably longer segment than in previous studies. For this reason we added the 'wingless' gene to the analysis. During the course of the nd1 work, this gene had emerged as a good candidate for providing phylogenetic information at the level of species and genera in butterflies (Brower & Egan, 1997; Brower & DeSalle, 1998).

The Extraction/PCR/Sequencing work was done over a period of several years, so the procedures/protocols has varied somewhat over this period. The methods described here are the ones used most recently.

*DNA extraction*

Total DNA was extracted from adults using the abdomen (and later a single leg) or parts of larvae/pupae using Qiagen's QIAamp tissue kit (Qiagen GmbH, Hilden, Germany) with the standard insect protocol. The extracted DNA was stored at −20°C. Vouchers have been stored at the Department of Zoology, Stockholm University.

Extractions were further purified with Qiagen's QIAquick Spin PCR Purification Kit (Qiagen GmbH, Hilden, Germany) before amplification. This improved PCR efficiency.

*PCR*

PCR was performed on a Perkin Elmer Gene Amp 2400 using Amersham Pharmacia Biotech's (Uppsala, Sweden) Ready·To·Go PCR Beads (with 1 μl of each primer (at 10 μmol/μl) together with 3 μl template DNA and 20 μl sterile distilled water). A typical cycling profile for both nd1 and wingless was 95°C for 5 min then 30 × (95° for 30 s, 52° for 30 s, 72° for 1 min) – hold at 4°. Some taxa needed variations to these conditions. The amplified products were purified with the QIAquick Spin PCR Purification kit (Qiagen GmbH, 1993) and stored at −20°C until sequencing.

*ND1*

The mitochondrial nd1 gene codes for an NADH subunit and is located between the 16S rRNA and cytochrome b genes in the insect mitochondrion (Clary & Wolstenholme, 1985). The 16S primer (5′-TTCAAACCGGTGTAAGCCAGG-3′) of Weller *et al.* (1994) was used in conjunction with a primer located in the tRNA for Serine (5′-AAGCATTTGTTTTGAAAACTTAAG-3′) downstream from nd1. These two primers amplify an 1155 bp section of mtDNA that contains all of nd1.

*Wingless*

The wingless (wg) protein is a member of the wnt gene family and is expressed at the wing margin in imaginal discs in Drosophila playing a role in adult wing pattern formation (Sidow, 1992; Carroll *et al.*, 1994; Neumann & Cohen, 1996).

PCR was performed using the primers of Brower & DeSalle (1998): (LepWG1 (5′-GARTGYAARTGYCAYGGYATGTCTGG-3′), LepWG2 (5′-ACTICGCARCACCARTGGAATGTRCA-3′). These primers amplify a 400 bp stretch of nuclear DNA in lepidopterans.

*Sequencing*

The double-stranded PCR-products were sequenced using (Cy-5) labelled PCR-primers as well as internal sequencing primers (Table 2) via the cyclic dideoxy chain termination method using the Thermo Sequenase Fluorescent Sequencing kit from Amersham Pharmacia Biotech AB, Uppsala, Sweden. A Corbett thermal cycler was used with the following cycling profile for nd1: 95° for 2 min – (95° for 30 s, 42° for 30 s, 70° for 1 min) × 30. And for wingless: 95° for 2 min – (95° for 30 s, 55° for 30 s, 70° for 1 min) × 30. The reactions were electrophoresed on 6% Long Ranger gels on the Pharmacia ALF-Express automated sequencer. Both strands of the two genes were sequenced (except for parts of nd1, due to sequencing difficulties). The sequences were aligned using the MacVector/AssemblyLIGN software/hardware package (International Biotechnologies, 1989). Almost no indels and low base-pair divergence made alignment uncomplicated.

## PHYLOGENETIC ANALYSES

*Monophyly of Nymphalini*

In order to study the monophyly of Nymphalini with respect to the close outgroup Kallimini a more distant outgroup, Argynniti, was included in the analysis. Subsequently we tested the effect of removing Argynniti, to control for effects of a too distant outgroup.

Representatives of the Melitaeini were not successfully sequenced, and *Colobura dirce* only for the 'wingless' gene. We performed an additional analysis of 'wingless' which included also this species, using only *Argynnis paphia* as outgroup.

*The data sets*

We used three main data sets, which were analysed separately and together. The first data set, henceforth referred to as the MEB data set (Appendices 1, 2), contains 97 characters consisting of traits of morphology (including wing patterns), ecology and behaviour (including host plants). Ninety-six of these traits are phylogenetically informative with respect to the included taxa. The second data set consists of 695 mitochondrial nd1 characters (157 of which are phylogenetically informative) and the third data set, the nuclear wingless matrix, consists of 379 characters (100 of which are phylogenetically informative). The complete total evidence matrix consists of 31 taxa and 1171 characters, 353 of which are phylogenetically informative. All characters are unweighted and unordered except for some characters from the MEB data set which are ordered (see list of characters in Appendix 1). Gaps in molecular data were treated as a 'fifth base', because indels were very rare and we wanted to

**Table 2.** Primers employed

| Gene | Primer name | Primer sequence (5′-3′) | PCR-primer (p)/internal sequencing primer (i) |
|---|---|---|---|
| nd1 | 16s | TTCAAACCGGTGTAAGCCAGG | p |
| nd1 | nd1c | TAGAATTAGAAGATCAACCAGC | i |
| nd1 | TrsLep2 | AAGCATTTGTTTTGAAAACTTAAG | p |
| nd1 | nd440 | CAAACTATTTCTTATGAAGT | i |
| nd1 | ndr640 | TCAGCAAAATCATAAGGAGT | i |
| wingless | LepWG1 | GARTGYAARTGYCAYGGYATGTCTGG | p |
| wingless | LepWG2 | ACTICGCARCACCARTGGAATGTRCA | p |
| wingless | LepWG3 | ACIGIIAARACYTGYTGGATGAG | i |
| wingless | LepWG5 | CGCARCACATRAGRTCGCARCCGTC | i |

distinguish them from uncertain identification of bases, coded as missing data.

*Search options*

The different data sets were analysed using PAUP* v 4b2 (Swofford, 1998). Most parsimonious trees were constructed by heuristic searches using the TBR branch swapping option and 10 000 random addition sequences for the separate data sets and 100 000 for the total evidence data set. All analyses were made with parsimony-uninformative characters excluded.

*Support*

Support for branches was investigated using decay and bootstrap analysis. Decay indices were computed with Autodecay 3.0.3 (Eriksson & Wikström, 1996) using the PAUP search parameters: *addseq = random, nreps = 1000, rseed = 1*.

Bootstraps were performed on Paup4b3a set to 5000 full heuristic (TBR) replicates with 10 random addition sequences per bootstrap replicate. A rearrangement limit of 10 000 000 rearrangements per addition sequence was used for the wingless data set as some bootstrap samples took too long to compute otherwise. The rearrangement limit was not hit, however, for all addition sequences in any single bootstrap replicate.

*Wing pattern and host plant data*

For reasons explained in the Introduction, we performed a separate analysis of the data on adult wing pattern (Wp 1-38 in Appendices 1, 2).

We were also interested in studying the degree of dependence of the results on host plant data. For this reason we performed an additional analysis of the 'total evidence' data set, with host plant data removed.

## RESULTS

### GENERAL

Decay indices and bootstrap values are given in Figures 1–4 to show support for specific branching patterns. Parsimony analysis of the data set based on morphology, ecology and behaviour (including host plants; Table 2; the MEB data set) resulted in eight most parsimonious trees. A strict consensus tree is shown in Figure 1.

Parsimony analysis of the mitochondrial data set (nd1) resulted in one most parsimonious tree, shown in Figure 2. Note the weak phylogenetic signal in this data set, as indicated by low decay values and bootstrap support, little resolution and low consistency index (0.365).

Parsimony analysis of the 'wingless' data set resulted in 72 most parsimonious trees, a strict consensus tree is shown in Figure 3. The consistency index (but not retention index) of this data set (0.530) is higher than for either MEB (0.431) or nd1 (0.365).

Figure 4 shows the strict consensus tree for the two most parsimonious trees found by analysis of total evidence. These trees have a consistency index of 0.402.

### MONOPHYLY OF NYMPHALINI

Nymphalini did not appear as a monophyletic group in the analysis based on the MEB data set (Fig. 1). In this analysis *Mynes* and *Symbrenthia* ended up with members of the Kallimini in the outgroup. However, nd1 data (Fig. 2) as well as 'wingless' data (Fig. 3) support a monophyletic Nymphalini, and so does total evidence (Fig. 4).

Removing the distantly related Argynniti from the outgroup did not change this result, and had very little effect on branching patterns (*Antanartia* moves from its position with the *Vanessa*-type genera (Fig. 4) to being the sister taxon to the focal group,

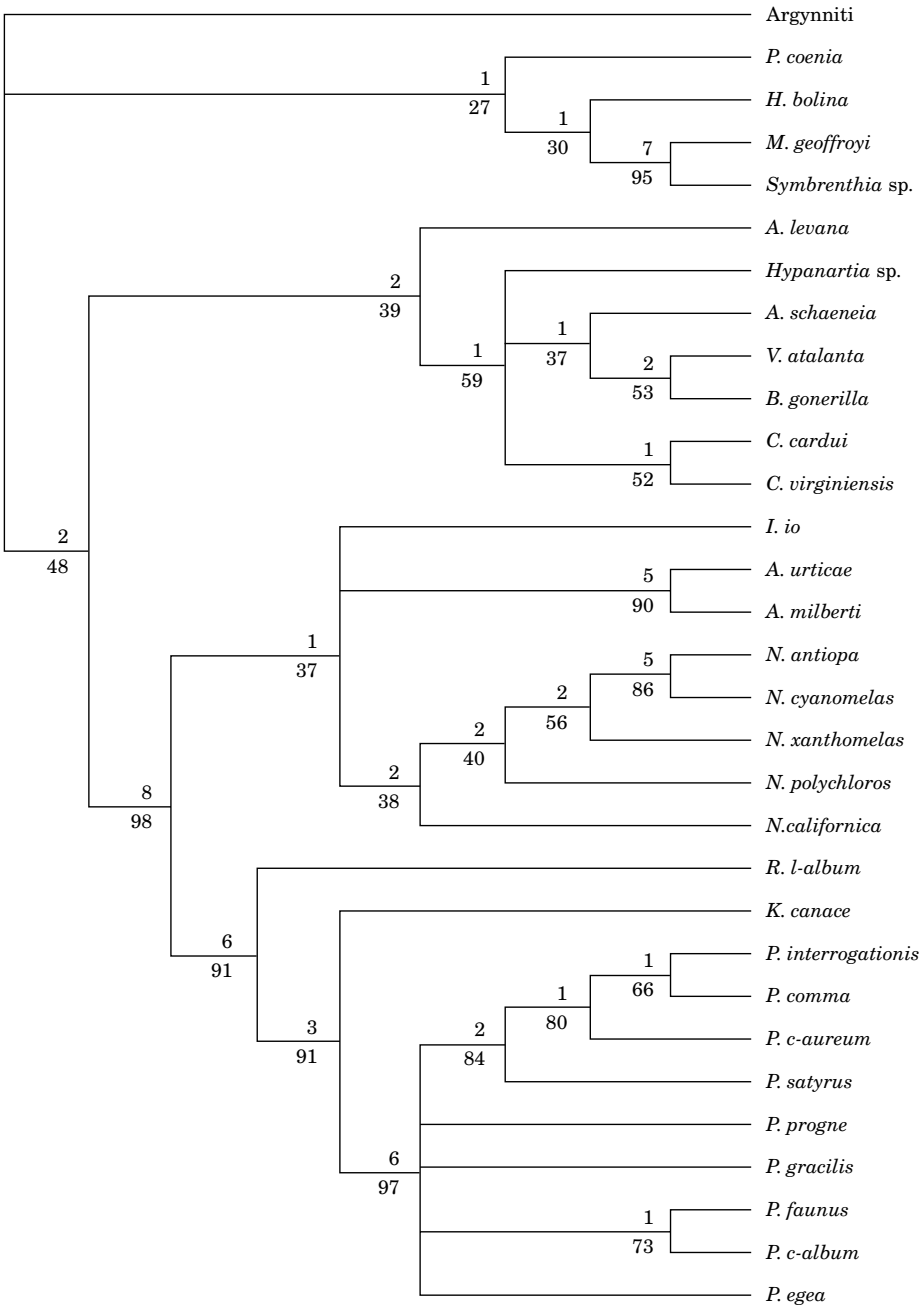


**Figure 1.** Results from cladistic analysis of data on morphology, ecology and behaviour (the MEB database). Shown is a strict consensus tree of eight most parsimonious trees (311 steps) with consistency index (CI) 0.431 and retention index (RI) 0.747. Decay indices (top) and boot strap values (bottom) on branches show level of support.

*Polygonia*, *Kaniska*, *Nymphalis*, *Roddia*, *Aglais* and *Inachis*).

In the separate analysis of 'wingless' data, which also included *C. dirce*, this representative of the Coloburini ended up together with the outgroup, *A. paphia*. Nymphalini was again monophyletic, with the Kallimini as sister group. Note, however, that the tribe Melitaeini was not represented.

## MAJOR CLADES IN NYMPHALINI

It should be noted that there is some agreement between phylogenies resulting from the three data sets regarding several aspects of the basic topology (Figs 1–3). *Hypanartia* is given a basal position by both nd1 and 'wingless' data (Figs 2, 3), and this is the position favoured by total evidence (Fig. 4), although MEB data

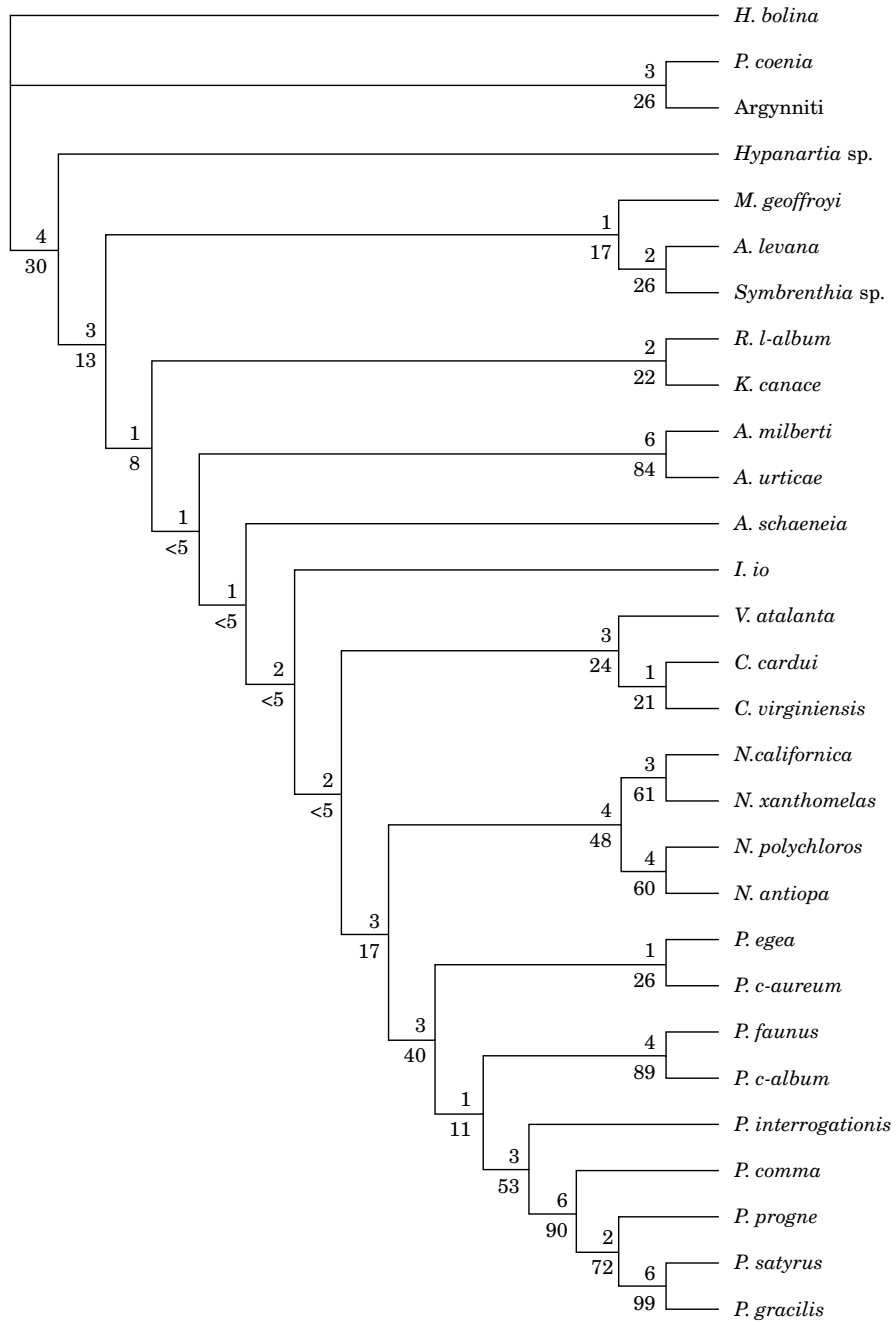


**Figure 2.** Results from cladistic analysis of data from the nd1 gene. Shown is a single most parsimonious tree (631 steps) with CI 0.365 and RI 0.417. Decay indices (top) and boot strap values (bottom) on branches show level of support.

suggest a position with the *Vanessa*-type genera and *Antanartia* (Fig. 1).

Another basal clade supported by total evidence consists of *Mynes*, *Symbrenthia* and *Araschnia*, the first two being sister genera (Fig. 4). This arrangement is also seen after analysis of the 'wingless' data alone (Fig. 3), whereas MEB data joins *Mynes* and *Symbrenthia* but not *Araschnia* (Fig. 1) and nd1 data joins the three genera but in a different arrangement (Fig. 2). The support for placing these three genera outside of the *Vanessa*-type genera and the focal group is rather weak (Figs 1–4), but the consistency between the types of data speak in favour of it.

Approaching the focal group, there is moderate support, in the analysis of total evidence, for a clade corresponding to *Vanessa* in the wider sense (*Vanessa* + *Cynthia* + *Bassaris*) and weak support for also placing *Antanartia* on this branch (Fig. 4). MEB data

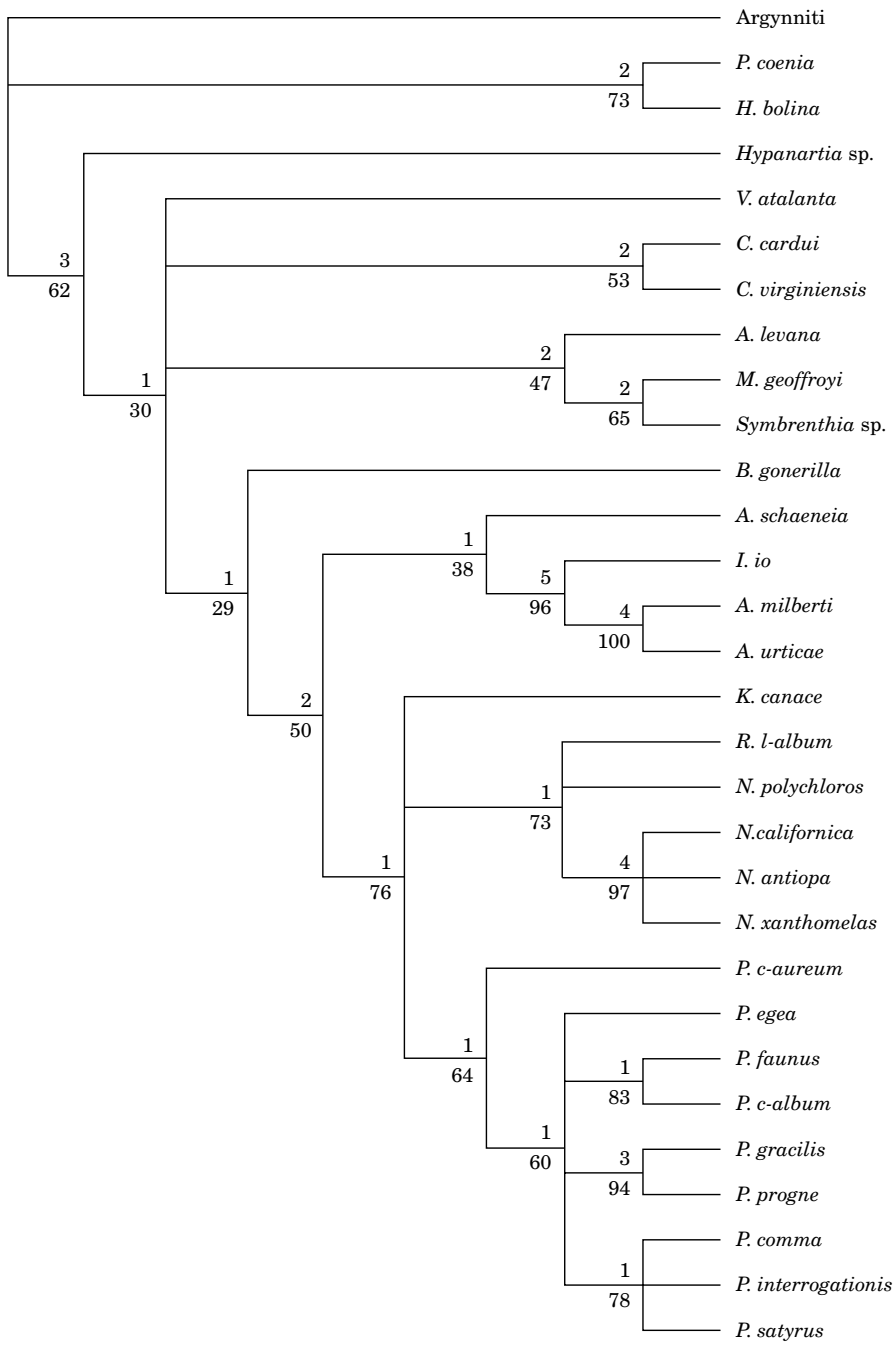


**Figure 3.** Results from cladistic analysis of data from the 'wingless' gene. Shown is a strict consensus tree of 72 most parsimonious trees (270 steps) with CI 0.530 and RI 0.601. Decay indices (top) and boot strap values (bottom) on branches show level of support.

alone suggest a larger clade including also *Hypanartia* and *Araschnia* (Fig. 1). The phylogeny suggested by nd1 data joins *Vanessa* with *Cynthia* but is otherwise unresolved (Fig. 2; *Bassaris* was not successfully sequenced) and 'wingless' gives even less information (Fig. 3).

There is strong support by total evidence for a predominantly holarctic major clade corresponding to the focal group, consisting of *Polygonia, Kaniska, Nymphalis, Roddia, Aglais* and *Inachis* (Fig. 4). This clade has strong support in the MEB data analysis (Fig. 1), and is suggested also by 'wingless' data (although

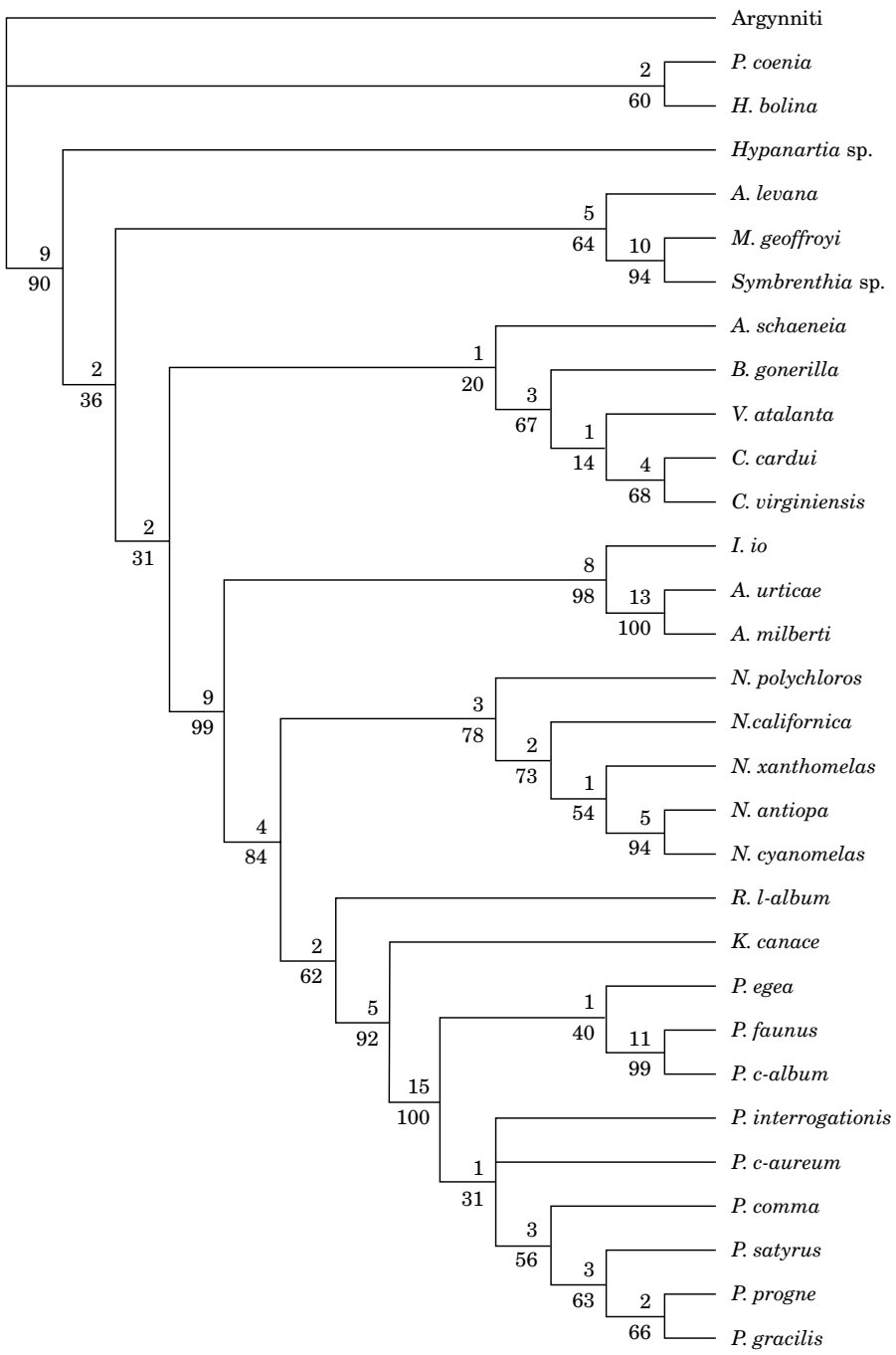


**Figure 4.** Results from cladistic analysis of total evidence. Shown is a strict consensus tree of two most parsimonious trees (1272 steps) with CI 0.402 and RI 0.553. Decay indices (top) and boot strap values (bottom) on branches show level of support.

invaded by *Antanartia*; Fig. 3). Nd1 data give little evidence for or against this clade (Fig. 2).

### GENERIC RELATIONSHIPS WITHIN THE FOCAL GROUP

Apparently there are two major clades in the focal group, one consisting of *Aglais* + *Inachis*, the other of the remaining genera. A close relationship between *Aglais* and *Inachis* is supported by 'wingless' data (Fig. 3) and by analysis of total evidence (Fig. 4). Once again, nd1 contributes little information (Fig. 2) but it should be noted that this is another data set that gives no support to the common practice of including *Aglais* in *Nymphalis*. MEB data weakly joins *Aglais*

and *Inachis* with *Nymphalis* (Fig. 1) but a position outside of the other genera in the focal group is suggested by 'wingless' data (Fig. 3) and partly by nd1 data (Fig. 2). This position is favoured with moderate support by total evidence (Fig. 4).

The bulk of the species in the remaining genera belongs to either *Nymphalis* or *Polygonia* in the strictest sense. *Kaniska*, the only species of which (*canace*) is often placed in *Polygonia*, is most probably the sister taxon to this genus. This is suggested by total evidence (Fig. 4), due mostly to MEB data (Fig. 1). Molecular data do not resolve relationships among genera in this group (Figs 2, 3).

Regarding relationships between genera, the most problematic is the position of the monotypic *Roddia* (a.k.a. '*Nymphalis vaualbum*'). The only species in this genus has been placed in either *Nymphalis* or *Polygonia* by various authors. MEB data rather strongly suggest a position with *Kaniska* and *Polygonia* (Fig. 1), and this position is favoured by total evidence as well (Fig. 4). Nd1 data weakly join *Roddia* with *Kaniska*, but outside of both *Nymphalis* and *Polygonia* (Fig. 2). 'Wingless' data weakly put *Roddia* with *Nymphalis* (Fig. 3).

### SPECIES RELATIONSHIPS IN THE FOCAL GROUP

Species relationships within *Nymphalis* and *Polygonia* were not resolved in this study. Within *Nymphalis*, *antiopa* and *cyanomelas* are evidently sister taxa, but this is based only on a subset of the MEB data matrix (Fig. 1) and no molecular data are present for *N. cyanomelas*. The three data sets conflict regarding the relative position of the remaining species. Total evidence favours the ladder arrangement seen in Figure 4, with *N. polychloros* as the basal species, but only with moderate support. The basal position for *N. polychloros* is the result of 'wingless' data (Fig. 3), conflicting arrangements have weaker support from other kinds of data (Figs 1, 2).

Within *Polygonia* the only strongly supported relationship is that *P. c-album* and *P. faunus* are sister taxa (Fig. 4). This clade appears in the separate analyses of all three data sets (Figs 1–3). Total evidence weakly supports association of the western palearctic *P. egea* with this clade, and a major clade consisting of the remaining nearctic species and the eastern palearctic *P. c-aureum* (Fig. 4). Within the latter group there is moderate support for a clade consisting of *P. comma, P. satyrus, P. gracilis* and *P. progne* (Fig. 4).

The phylogeny based on MEB data is unresolved concerning these relationships (Fig. 1). Nd1 data show weak support for the last-mentioned clade of four species and strongly support a close relationship between *P. satyrus* and *P. gracilis*, with *P. progne* as the closest associate (Fig. 2). In contrast, 'wingless' data join *P. gracilis* with *P. progne*, and as this arrangement is also supported by some of the individual trees behind the consensus tree in Figure 1, it is the one that occurs after analysis of total evidence (Fig. 4). The two most parsimonious trees obtained in the analysis of total evidence conflict only regarding the position of *P. c-aureum*, inside or outside of *P. interrogationis*. The outside position is the one favoured after successive weighting (Fig. 4).

### WING PATTERN DATA

We performed a separate analysis with only the wing pattern data, which resulted in 180 most parsimonious trees, the strict consensus of which is seen in Figure 5. The consistency index was as high as 0.515. Note that the topology found is broadly similar to that supported by molecular data (Figs 2, 3) and total evidence (Fig. 4). *Mynes* and *Symbrentia* are joined in a basal position, *Hypanartia* is found outside of the other *Vanessa*-like genera (which are joined), the focal group and the genus *Polygonia* in the narrow sense are monophyletic, and the relationships within *Polygonia* are relatively similar.

### EFFECTS OF HOST PLANT DATA

The analysis of total evidence without the host plant data character (ec2) resulted in two most parsimonious trees with a consistency index of 0.398. The consensus tree was identical to the one obtained previously from total evidence (Fig. 4).

## DISCUSSION

### MONOPHYLY AND POSITION OF NYMPHALINI

Harvey (1991) recognizes 13 subfamilies of the Nymphalidae, among them the Nymphalinae. This subfamily is further divided into the three tribes Nymphalini, Melitaeini and Kallimini. In contrast, Ackery (1988) recognized a larger Nymphalini, corresponding to Harvey's Nymphalini + Kallimini, and also the tribe Coloburini as members of the Nymphalinae, but not the Melitaeini. Harvey notes that Kallimini and Melitaeni are united by one larval trait, the presence of filiform seta on the sclerotized base of the scolus on A9. On the other hand, Nymphalini and Melitaeini are united by the presence of filiform setae on A1,2. Coloburini lack both of these traits, according to Harvey (1991). These traits were not studied by us. Overall, taking also host plants into account, Harvey favoured the hypothesis of a sister-group relationship between Kallimini and Melitaeini, Nymphalini being the sister-group to this clade.

The synapomorphies used by Harvey (1991) to define

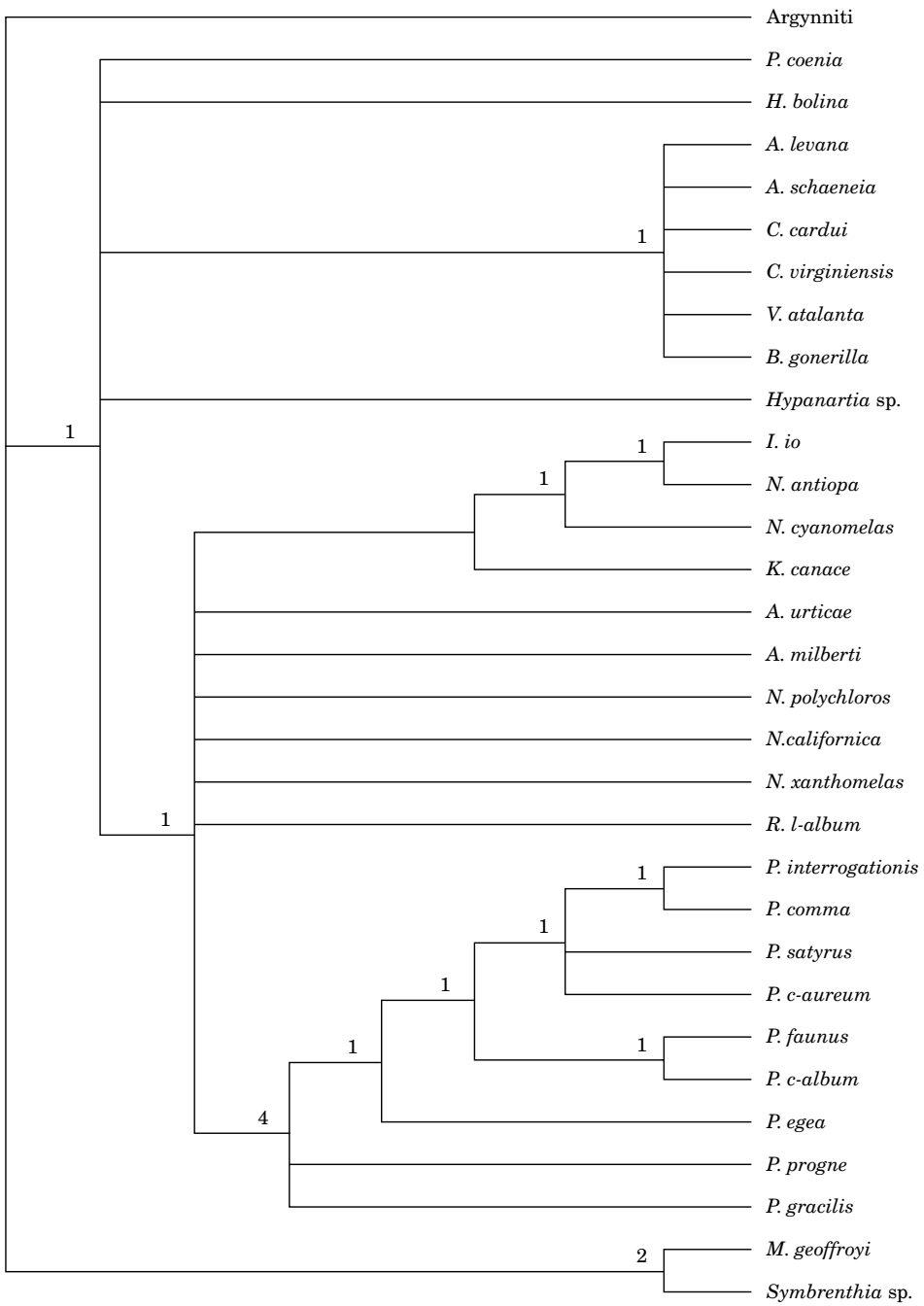


**Figure 5.** Results from cladistic analysis of only wing pattern traits (38 characters). Shown is a strict consensus tree of 180 most parsimonious trees (103 steps) with CI 0.515 and RI 0.811.

the Nymphalini as distinct from the Kallimini and Melitaeini are the hairy eyes (our character ey1) and the stiff, projecting bristlelike scales on the palpi. He noted that the latter trait is absent in *Mynes* and that projecting, flat scales are present in some Kallimini. There was no further description of this trait, and we failed to see a clear difference between members of Nymphalini and Kallimini. Our traits lp1-4 deal with the morphology of palpi. We found eyes to be hairy in all studied species of the ingroup, and not in any of the outgroup species.

Our analysis was not specifically designed to answer most of the questions regarding relationships among tribes (this will be done in a companion study), but our results suggest monophyly of Nymphalini *sensu* (Harvey, 1991), relative to the Kallimini and Coloburini

(the latter conclusion based on the analysis of 'wingless' data). Melitaeini was not included in the analysis, but this is a uniform tribe very likely to be monophyletic and hence it is unlikely that members of this tribe would intrude in the Nymphalini.

### PHYLOGENY OF NYMPHALINI AT THE GENUS LEVEL

We found some support for a basal position of the predominantly neotropical genus *Hypanartia*. This conflicts with the present view of a close relationship between this genus and the African *Antanartia* (DeVries, 1987). We are not aware of any proposed synapomorphies joining these two genera, and our results suggest that the similarities between them may be plesiomorphic. A position for *Hypanartia* together with *Antanartia* results in a tree which is four steps longer, a position inside of the *Mynes* + *Symbrenthia* + *Araschnia* clade three steps longer, and all other positions (including instead moving *Antanartia* to the *Hypanartia* branch) result in considerably longer trees.

The position of *Hypanartia* should be considered as tentative, as it is not supported by any uniquely derived traits in our matrix. Seven traits in the MEB data change along the branch leading to the remaining Nymphalini, but none of them unequivocally, and they all change again higher in the tree. Seven molecular traits change unequivocally along this branch, but all of them change again. However, we did not have access to the juvenile stages of *Hypanartia*. It should be noted that Müller (1886) on the basis of juvenile morphology divided his 'Vanessinae' into two groups; one group consisting of only *Hypanartia*, the other of his '*Pyrameis*', '*Vanessa*' and '*Grapta*'. Scrutiny of the studied species reveals that he considered members of modern *Cynthia*, *Vanessa*, *Araschnia*, *Aglais*, *Inachis*, *Nymphalis* and *Polygonia* to all belong to the second group! He states that it is especially the smooth, compressed pupa that sets *Hypanartia* apart from the other genera.

Also unexpectedly, we found consistent support for another basal clade consisting of *Mynes*, *Symbrenthia* and *Araschnia*, with strong support for a sister group relationship between the two former genera. A basal position for *Araschnia* relative to the *Vanessa*- and *Nymphalis*-type species has been suggested by Niculescu (1965, 1985) and Teshirogi (1990). These authors studied only palearctic genera. One synapomorphy for this clade of three genera is apparently the absence of sclerotized spines on larval abdominal segment 9 (the narrow penultimate segment), where two subdorsal spines occur in last-instar larvae of the remaining genera studied. Teshirogi (1990) noted the absence of spines on this segment in *Araschnia levana*, *A. burejana* and *Symbrenthia hippoclus*, and we found them to be absent in *Mynes geoffroyi* as well. Müller (1886) and Niculescu (1965) note the presence of spines on all abdominal segments in *A. levana*, apparently mistakenly (like Teshirogi, (1990), we found them to be absent on this segment, also in a European stock). A remaining uncertainty concerns the state of this trait in *Hypanartia*, as we could not study larval material of this species ourselves.

Another potential synapomorphy for the clade is the presence, in first-instar larvae, of several short secondary setae on the small pinaculae on which P1 and Sp1 arise on abdominal segment 10. These were noted by Nakanishi (1988) to occur in *Symbrenthia* and *Araschnia* and to be absent in all other genera in Nymphalini and Kallimini studied by him (which led him to suggest a sister-group relationship). However, first instar larvae of *Mynes* do not seem to have been studied, and we did not have access to early-instar larval material. In addition, in the molecular data there are three synapomorphies, i.e., traits changing uniquely and unequivocally along the branch leading to these three genera. Moving *Araschnia* to the clade of *Vanessa*-type genera, as might be suggested by the MEB data (wing pattern similarity), results in a tree that is seven steps longer.

The position for this clade of three genera outside of both the *Vanessa*-type genera and the focal group is only weakly supported (Fig. 4). One character in support of this arrangement is the hibernating stage (ec1), which is the adult stage in both the *Vanessa*-group and in the focal group (some taxa lack hibernation diapause), whereas a juvenile stage (egg, larva or pupa) is used in those temperate genera placed outside by total evidence. Adult hibernation is rare in butterflies, and this potential synapomorphy should be given some consideration.

A close relationship between *Mynes* and *Symbrenthia* has to our knowledge not been suggested previously. Support for this clade was high from the MEB data (Fig. 1) but there was no synapomorphy without homoplasy in this data set. Two synapomorphies were suggested by molecular data, one of them an unequivocal change along this branch. The alternative arrangement with *Mynes* outside of the other two genera, suggested by nd1 data (Fig. 2) results in a tree which is no less than 11 steps longer.

As expected, we found some support for a close relationship between the genera *Vanessa*, *Cynthia* and *Bassaris*, *sensu* Field (1971). Many subsequent authors have not followed Field's division into separate genera. With the limited number of species included in this analysis our results have no bearing on the question of whether the three genera can be defended on cladistic principles, i.e. whether they are all monophyletic. Interestingly, the association between them is not so strongly supported by the analysis as could have been

expected. We found no good candidates for synapomorphies in the molecular data or in the MEB data, despite the obvious similarities. The apparently basal position of *Hypanartia*, often considered to be another *Vanessa*-type genus, suggests the possibility that most of the similarities are in fact plesiomorphic. As Field (1971) did find diagnostic differences between the genera, it may be prudent to recognize them as separate, at least until there is better evidence that they really are a closely related monophyletic group.

We believe that we can settle with some confidence a couple of long-standing controversies regarding the taxonomy of butterflies in the Nymphalini. The first regards the position of the palearctic *A. urticae* and the nearctic *A. milberti*. The former has long been placed in the genus *Aglais* by Eurasian authors, but the latter is often placed in *Nymphalis* by American and Canadian authors (e.g., Scott, 1986; Layberry *et al.*, 1998). However, it has long been recognized that the two species are closely related (Scudder, 1889; Seitz, 1914; Miller & Miller, 1990), as confirmed by the present study. We show here that the practice of placing these species in *Nymphalis* cannot be defended from cladistic principles, as *Aglais* is in fact most closely related to *Inachis*, and *Nymphalis* is more closely related to *Polygonia* and relatives. The same conclusion was reached by Niculescu (1965) and Teshirogi (1990). The similarity between *Aglais* and *Nymphalis* is plesiomorphic and superficial, as demonstrated also by the fact that the male genitalia are very different (Niculescu, 1965).

*Aglais* and *Inachis* are united by their shared habit of laying very large batches of eggs, in many layers, on the underside of nettle (*Urtica*) leaves. In the MEB data set this complex character was split into several traits (batch size, site and shape, host plant) in order to maximize the number of potentially informative characters, and none of them change uniquely and unequivocally along the branch leading to the two genera. However, no less than five molecular traits do so. Three of them consists of adjacent bases in the 'wingless' data which are found to be missing in both (and only) *I. io*, *A. urticae* and *A. milberti* when aligning the sequences. This might be considered a single trait, but as gaps are very infrequent in the studied sequences we chose to give this extraordinary evolutionary event added weight by treating it as three separate traits in the analyses.

The position of *Aglais* + *Inachis* outside of the remaining genera in the focal group has moderately strong support (Fig. 4). There are no traits in the MEB database uniquely uniting the other genera, but one change in the molecular data does so. The alternative position with best support is as the sister clade to *Nymphalis*; however, this tree is four steps longer.

The second controversy has regarded the position of *R. l-album* and *K. canace*, which are shown here to most probably belong in the *Polygonia* clade. The proposed position of *R. l-album* with *Polygonia*, suggested earlier by e.g. Niculescu (1965, 1985), is supported by total evidence (Fig. 4), albeit not very strongly. Niculescu relied partly on the presence of larval head horns in *R. l-album* and *Polygonia* (absent in *Nymphalis*) but such horns are absent in *K. canace* as well (our trait L2). There are four other potential synapomorphies in the MEB database joining *Roddia*, *Kaniska* and *Polygonia*. These are: lp1, the palpi have a distinctly set off apical segment; ws2, the posterior part of the fore wing is already more incised in *R. l-album* than in *Nymphalis*, approaching the state in *canace* and *Polygonia*; ws5, the outer margin of the fore wing is deeply incised; and wp3, the presence of an angled white spot on the underside of the hind wings which has given the 'commas' (*Polygonia*) their common name, and (ironically) the epithet 'The false comma' to *R. l-album*. The alternative position, with *Nymphalis*, also has supporting traits, e.g. the slender shape of the antennal club (a1) and the yellowish colour of the eggs (e5); however, this tree is three steps longer. Teshirogi (1990) arrived at a similar conclusion, favouring a closer relationship with *Polygonia* but noting the remaining uncertainty. He also did not resolve the relationship between *R. l-album*, *K. canace* and *Polygonia* in the narrow sense, whereas we see the closer relationship between the latter two taxa as relatively certain. There is relatively strong support for this clade (Fig. 4) and there are two synapomorphies in the MEB database (ws2, the deeply incised posterior part of the fore wing; and L7, the particular coloration seen in larva) and one in the molecular data.

*Polygonia* in the narrow sense forms a very well-defined clade with high support (Fig. 4). This is probably one reason for the reluctance by many authors to include the two controversial taxa in *Polygonia*. This is particularly true in the case of *R. l-album*, which in many respects resembles the species in *Nymphalis* more than it resembles the species in *Polygonia s.s.*. This similarity is evidently plesiomorphic and if so cannot form the basis for taxonomic groupings. However, a taxonomy could be adopted which recognizes the many traits which distinguish *Polygonia s.s.*, as well as the conflicting evidence regarding the position of *R. l-album*, which leaves this species as something of an 'evolutionary link' between *Nymphalis* and *Polygonia*. The differences between the narrow sense *Polygonia* and *K. canace* in e.g., adult coloration and larval host plants are certainly impressive enough to warrant use of the genus *Kaniska* for the latter. If so, *R. l-album* (which is even more distantly related to the narrow sense *Polygonia*) must also be placed in a genus of its own. The appropriate name for this genus would

seem to be *Roddia*, recently suggested by Korshunov (Korshunov & Gorbunov, 1995; Korshunov, 1996).

### PHYLOGENY OF THE *NYMPHALIS* GROUP AT THE SPECIES LEVEL

Relationships within *Nymphalis* remain uncertain, except for the well-supported clade *N. antiopa* + *N. cyanomelas*, suggested earlier by Scudder (1889) and Miller & Miller (1990). The high support may partly be the result of lack of conflicting evidence, due to absence of molecular data and juvenile traits for *N. cyanomelas*.

Miller & Miller (1990) suggested the clade *N. californica* + *N. xanthomelas*, based on similarity of male genitalia. This is worth noting because the clade is supported also by our nd1 data (Fig. 2). However, we failed to see any unique similarities between the genitalia of the two species from the illustrations in Miller & Miller (1990), and there was no description given of the alleged similarity. In the absence of synapomorphies, the 'similarity' (and possibly the nd1 results) is equally well explained by the ladder arrangement in Figure 4, and hence we provisionally favour the resolution supported by total evidence.

The basal position for *N. polychloros* is relatively well supported by 'wingless' data (Fig. 3), including one synapomorphy for the remaining species (*N. cyanomelas* not sequenced).

### PHYLOGENY OF *POLYGONIA* AT THE SPECIES LEVEL

As noted in the Results section, the resolution within *Polygonia* at the species level supported by total evidence (Fig. 4) must be considered as tentative (except for *faunus* + *c-album*), although it is the best hypothesis presently available. The sister-group relationship between *P. faunus* and *P. c-album* is supported by e.g. larval coloration (L6) and two synapomorphies in the molecular data. Adults are also very similar, and larvae have similar polyphagous habits. These traits were not well captured by the particular coding that we employed, but nevertheless, support for this clade was one of the highest in the phylogeny.

The nearctic clade *P. comma* + *P. progne* + *P. gracilis* + *P. satyrus* is not supported by any synapomorphies. One molecular synapomorphy supports monophyly of the latter three genera. In our earlier analyses (with less data) *P. comma* grouped instead with *P. interrogationis*, and more data are needed before we can conclusively choose between these alternatives. The same is true for the relationships between *progne*, *gracilis* and *satyrus*, where nd1 shows very strong similarity between the last two species, conflicting with total evidence.

### WING PATTERN DATA

Analysis of only wing pattern characters resulted in a topology broadly similar to the one resulting from analysis of the complete matrix or only molecular data (Fig. 5, cf. Figs 1–4). This suggests that wing pattern data can be very useful in analysing butterfly relationships, a fact that may be of importance considering that such characters can be collected easily and non-destructively from museum material, and if necessary even from good illustrations.

There are pitfalls, however, illustrated by the clearly artificial grouping of *Nymphalis antiopa* (and *cyanomelas*) with *I. io* and *K. canace*. These species evidently have lost much of their ancestral pattern (the one seen in *Aglais*, *Roddia*, *Polygonia* and other *Nymphalis*), and for this reason lack the specific synapomorphies joining other species. Consequently they tend to be pulled together by superficial similarities. Another example is the position of *Araschnia* with the *Vanessa*-type genera (Fig. 5), which is only supported by wing pattern similarities that may well be plesiomorphic for the entire tribe. Clearly, other kinds of data will often be needed as well, in order to obtain a reliable hypothesis of phylogeny.

### PHYLOGENETIC ECOLOGY – INCLUDE THE STUDY TRAITS OR NOT?

Phylogenies are used in many areas of evolutionary biology, e.g. ecology and ethology. This is either because a detailed historical reconstruction is necessary to test or suggest theories about the evolutionary process, or in order to control for the phylogenetic interdependence of species characteristics in statistical tests (Wanntorp *et al.*, 1990; Brooks & McLennan, 1991; Harvey & Pagel, 1991; Miller & Wenzel, 1995). When the use of such methods first became popular in the early 1990s, it was suggested that it was important that the traits under study, e.g. the 'ecological' characters were not used to construct the phylogeny in the first place (Brooks & McLennan, 1991). This is because this procedure would induce some circularity; the number of transformations in the studied traits would be underestimated because branches in the phylogeny with the same state would more often be placed together in the search for the most parsimonious arrangement of the taxa.

This conclusion has, however, been debated (see Miller & Wenzel, 1995 for additional references) and recently Zrzavy (1997) argued strongly that all available characters should be included in the data matrix. He reasoned that problems of circularity should in fact be less if, for example, ecological traits are included rather than excluded, because the null hypothesis should be that the ecological traits are historically contingent and so an adaptive explanation is not

needed for each state in each taxon. The null hypothesis is equally strong or stronger when ecological traits are included, so this procedure is more conservative and does not overestimate adaptive change.

We agree in principle with this reasoning, and so have included host plant data in the data matrix, although we intend to use the phylogeny to study butterfly–plant relationships (Janz, Nyblom & Nylin, 2001). However, some of the uses that we intend for the phylogeny have particular problems in this respect. These problems arise when the question under study is not the typical one in phylogenetic ecology (whether some environmental variable has affected a biological characteristic, or one biological trait has affected another during evolution) but instead the question concerns the transformation rates themselves. For instance: is host plant use in butterflies phylogenetically constrained and, if so, in what ways (Janz & Nylin, 1998)? Including host plants in an otherwise small data matrix will tend to result in a reconstruction showing a conservative host plant utilization, whether this is the real situation or not.

In our opinion, whenever the transformation rates themselves are of interest it is necessary to include the traits under study in the data matrix, but also to show that the resulting tree is stable enough to show essentially the same topology if the study traits are subsequently excluded. Phylogenetic ecologists will typically not go to the trouble of testing whether the phylogenetic hypothesis from the literature that they are using would change if their study traits were included in the data matrix, but chances are that it would, judging from our experiences in the present study. We found that our results were very dependent on inclusion of host plant data, even with a large morphological and ecological data matrix, as well as when we added data from the nd1 gene. Not until we added also data from the 'wingless' gene did we have a large enough data matrix to achieve a tree stable against inclusion/exclusion of host plant data. Moreover, the chances of finding the tree conforming to the 'true' phylogeny will be better with more data. Using a published tree based on, for example, a single gene, without combining these data with the researcher's database on ecological traits will often amount to using a phylogeny which is not the best hypothesis available.

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## APPENDIX 1: CHARACTER LIST

### EXTERNAL MORPHOLOGY EXCEPT WING SHAPE AND PATTERN

1. (a1) Shape of antennal club: (0) thick, short; (1) intermediate; (2) thin, elongate. Ordered 012.
2. (a2) Colour of antennal club: (0) dark with bright apical spot; (1) uniformly dark without bright apical spot.
3. (ey1) Interfacetal hairs: (0) short and sparsely set; (1) long and densely set.
4. (ey2) Radial connections between central and side pupils: (0) absent; (1) weak; (2) strong. Ordered 012. Data from Yagi & Koyama (1963).
5. (lp1) Apical segment of labial palp: (0) not distinctly set off from subapical segment; (1) distinctly set off from subapical segment.
6. (lp2) Colour of labial palp ventrally: (0) entirely white; (1) with a black median stripe.
7. (lp3) Pubescence ventrally on labial palp: (0) hairs absent; (1) uniformly dark, thin hairs; (2) dark, thick bristles with whitish tip. Ordered 012.
8. (lp4) Dark hairs laterally on labial palp: (0) absent; (1) present.
9. (le1) Colour of fore legs: (0) light; (1) light with dark ventral band; (2) entirely dark. Ordered 012.
10. (le2) Position of secondary tooth of claws on hind leg: (0) distinctly basad primary tooth; (1) laterad or apicad primary tooth.

### WING SHAPE AND PATTERN

See Figures 6–8 for position of some traits. Wing vein terminology follows Niculescu (1965) and Scott (1986).

11. (ws1) Shape of median part of anterior margin of hind wing: (0) straight or rounded, not incised; (1) distinctly incised.
12. (ws2) Shape of posterior margin of fore wing: (0) straight or convex, not incised; (1) slightly incised; (2) strongly incised. Ordered 012.
13. (ws3) Process from outer margin of hind wing close to $M_3$: (0) absent; (1) small; (2) large. Ordered 012.
14. (ws4) Shape of outer margin of hind wing: (0) not expanded posterior to $M_3$; (1) distinctly expanded posterior to $M_3$.
15. (ws5) Shape of outer margin of fore wing: (0) not or only slightly incised; (1) deeply incised.
16. (ws6) Process from outer margin of fore wing between $M_1$ and $M_2$: (0) absent; (1) present.
17. (ws7) Process from outer margin of fore wing close to $Cu_2$: (0) absent; (1) present.
18. (ws8) Shape of outer margin of fore wing: (0) more or less even; (1) distinctly jagged.
19. (ws9) Hairs anteriorly on ventral side of fore wing: (0) absent or only present basally; (1) extending considerable distance from wing base.
20. (ws10) Vein $R_2$ of fore wing: (0) issuing from cell separately from $R_3+R_4+R_5$; (1) separating from $R_3+R_4+R_5$ distal to cell.
21. (ws11) Wing span: (0) small, <50 mm; (1) large, >= 50 mm.
22. (ws12) Shape of anterior margin of hind wing: (0) rounded distally, smoothly continuing in outer margin; (1) slightly incised distally; (2) strongly incised distally. Ordered 012.
23. (ws13) Hairs basally on ventral side of hind wing: (0) short and thin, hairlike; (1) long and thick, bristlelike.
24. (wp1) Colour pattern on ventral side of hind wing: (0) with contrasting bands and spots; (1) more uniformly coloured with part basad outer band of central symmetry system darker than rest of hind wing.
25. (wp2) Shape of outer band of central symmetry system on ventral side of hind wing: (0) more regular, running outside discal spot; (1) irregular, distinctly incised medially, touching discal spot.
26. (wp3) Posterior part of lightly coloured discal spot on ventral side of hind wing: (0) absent or not distinguishable; (1) rounded or occasionally elongate, not angled; (2) elongate and angled, v-, u- or l-shaped. Unordered.
27. (wp4) Ripple pattern on ventral side of fore and hind wing: (0) absent; (1) present.
28. (wp5) Colour of wing veins basally on ventral side

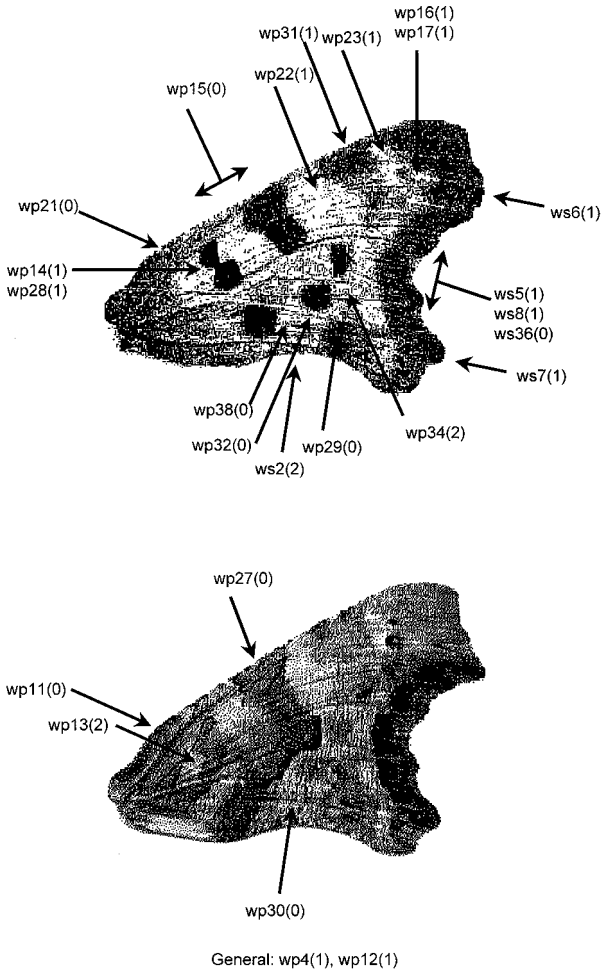


**Figure 6.** *Polygonia c-album*: fore wing wing shape and wing pattern characters. Arrows point to positions of traits discussed in the text and numbers in parentheses show the illustrated state of the character, conforming to states in Appendices 1 & 2. Top half: dorsal side. Bottom half: ventral side.

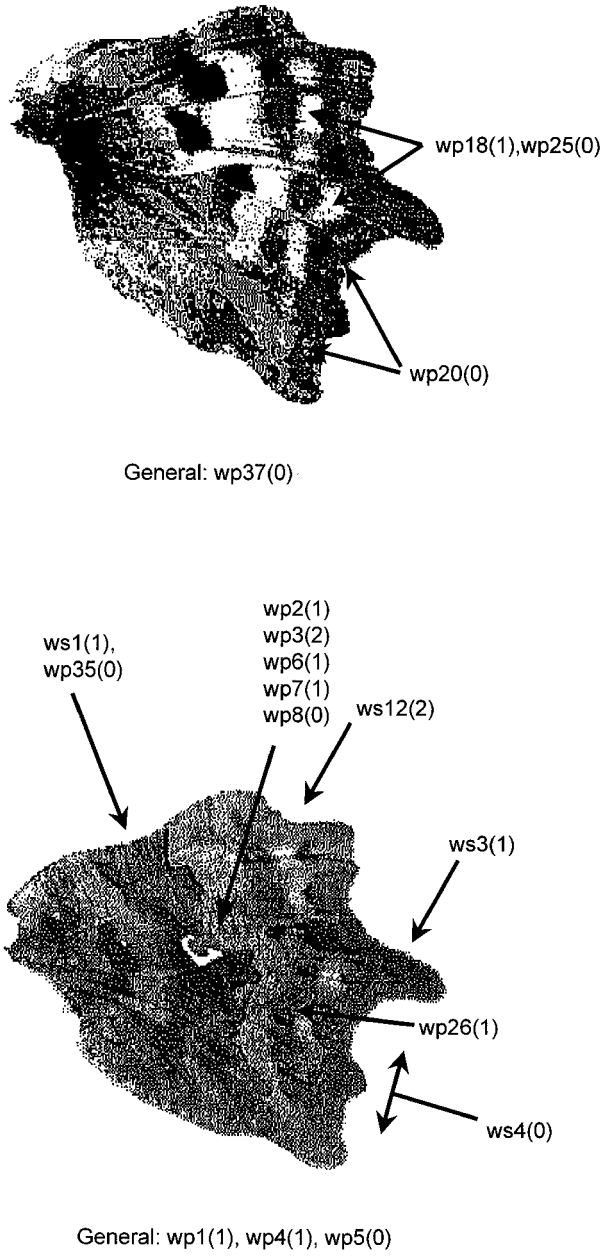


**Figure 7.** *Polygonia c-album*: hind wing wing shape and wing pattern characters. Arrows point to positions of traits discussed in the text and numbers in parentheses show the illustrated state of the character, conforming to states in Appendices 1 & 2. Top half: dorsal side. Bottom half: ventral side.

of hind wing: (0) dark; (1) white or yellow, contrasting with surrounding darker spots.

29. (wp6) (Subdivision of wp3:2) Shape of elongate and angled posterior part of discal spot: (0) sharply angled, v-shaped; (1) more roundedly angled, u-shaped.
30. (wp7) Margin of discal spot: (0) indistinct, spot not set off by narrow dark line; (1) distinct, spot at least partly set off by a narrow dark line.
31. (wp8) Colour of discal spot: (0) white or yellow, not metallic; (1) metallic silver or gold.
32. (wp9) Colour of ventral side of fore wing, between $M_1$ and $M_2$: (0) light or intermediate, not contrasting with surrounding areas; (1) dark, contrasting with surrounding areas.
33. (wp10) Large white eyespots on ventral side of fore wing: (0) absent; (1) present, at least the spot between $M_2$ and $M_3$.
34. (wp11) Colour pattern along basal part of anterior margin of ventral fore wing: (0) uniform or with more or less irregular pattern of dark and light areas; (1) with regular, contrasting white and dark lines.
35. (wp12) Colour pattern on ventral side of fore wing: (0) different from ventral side of hind wing; (1) similar to ventral side of hind wing.
36. (wp13) Shape of basal symmetry system on ventral side of fore wing: (0) more or less evenly curved; (1) weakly and roundedly bent medially; (2) strongly and sharply bent medially. Ordered 012.
37. (wp14) Anterior part of basal symmetry system on dorsal side of fore wing: (0) represented by continuous dark band or subcontiguous, square, dark spots; (1) represented by well separated, rounded spots.
38. (wp15) Colour pattern along anterior margin of fore wing: (0) dark areas broken by at least one band of lighter colour; (1) uniformly dark.
39. (wp16) Median eye spots on dorsal side of fore wing: (0) present as contrasting white or red spots, at least one of the spots between $M_1$ and $M_3$; (1) absent.
40. (wp17) Row of bright yellow-orange or blue, distinctly wedge-shaped spots immediately distad position of eyespots on dorsal side of fore wing: (0) absent; (1) present.
41. (wp18) Row of contrasting bright yellow-orange or blue spots or blue-green band immediately distad position of eyespots on dorsal side of hind wing: (0) absent; (1) present.
42. (wp19) Eye spots on ventral side of hind wing: (0) small and simple or absent; (1) at least one eyespot large, consisting of several concentric rings, but no eyespot circular and completely closed; (2) at least two eyespots circular and closed. Ordered 012.
43. (wp20) Blue band or series of blue spots immediately outside parafocal elements on dorsal side of hind wing: (0) absent; (1) present only posteriorly; (2) percurrent. Unordered.
44. (wp21) Colour pattern on field along basal part of anterior margin of dorsal fore wing: (0) uniformly coloured or with weak stripes or mozaic pattern of light and dark areas; (1) with distinct white and black stripes.
45. (wp22) Colour of anterior spot or band immediately distad central symmetry system on dorsal side of fore wing: (0) white; (1) yellow to orange; (2) blue. Unordered.
46. (wp23) Colour of anterior spot immediately distad the g-system on dorsal side of fore wing: (0) white; (1) yellow to red; (2) green. Unordered.
47. (wp24) Anterior part of discal spot on ventral hind wing: (0) absent or indistinguishable; (1) dark, contrasting, well-defined elongate spot.

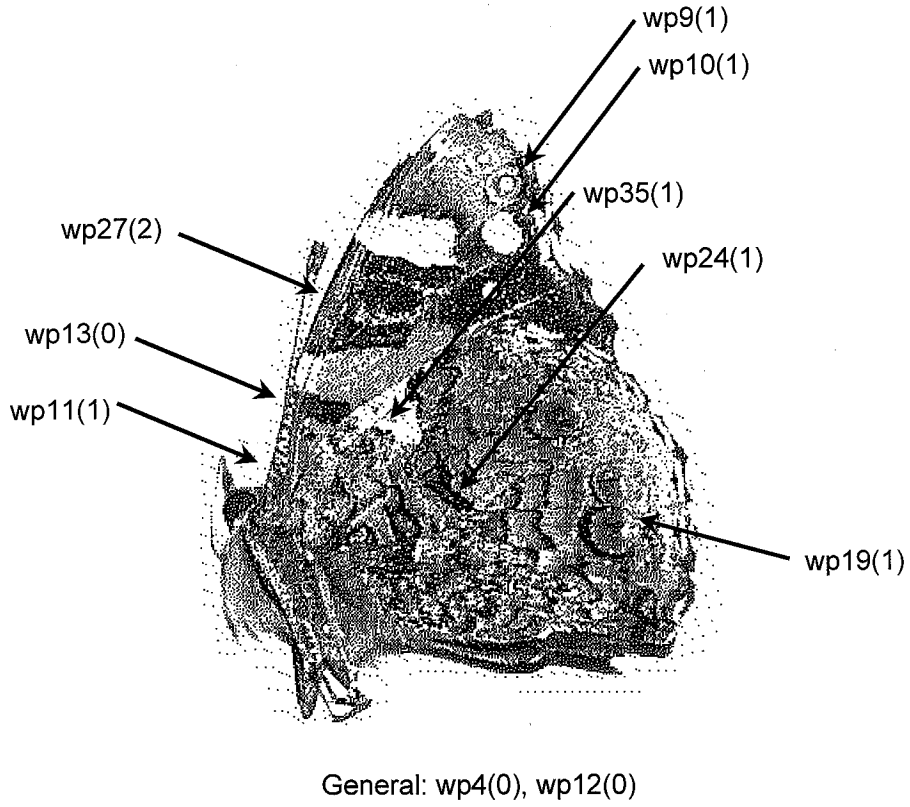


**Figure 8.** *Vanessa atalanta*: fore wing and hind wing shape and pattern characters. Arrows point to positions of traits discussed in the text and numbers in parentheses show the illustrated state of the character, conforming to states in Appendices 1 & 2.

48. (wp25) (Subdivision of wp18) Colour of contrasting spots or band immediately distad position of eyespots on dorsal side of hind wing: (0) yellow-orange; (1) blue or blue-green.
49. (wp26) Colour of eyespot or area around eyespot between $Cu_1$ and $Cu_2$ on ventral side of hind wing: (0) brown or orange, occasionally with some blue; (1) yellow-green; (2) olive-green. Unordered.
50. (wp27) Colour of anterior part of central symmetry system on ventral side of fore wing: (0) uniformly dark or with large light and dark areas; (1) dark, broken by narrow light lines of background colour; (2) dark, broken by blue lines. Ordered 012.
51. (wp28) Colour of wing cell on dorsal fore wing: (0) uniformly dark; (1) light, broken by vertical dark bands or spots; (2) uniformly light, not broken by dark spots. Ordered 012.
52. (wp29) Dark spot subapically between $Cu_2$ and $A_2$ on dorsal side of fore wing: (0) present; (1) absent.
53. (wp30) Dark band along posterior margin on ventral side of fore wing, reaching or almost reaching outer wing margin: (0) absent; (1) present.
54. (wp31) Colour of anterior, marginal dark spot corresponding to element g on dorsal side of fore wing: (0) as dark as anterior, marginal dark spot corresponding to element f; (1) lighter than dark spot corresponding to element f.
55. (wp32) Dark spot between $Cu_1$ and $Cu_2$ corresponding to element g on dorsal side of fore wing: (0) present; (1) absent (or shifted out of this area).
56. (wp33). Seasonal polyphenism in wing colour: (0) absent or not evident; (1) lightly coloured (dominated by orange) dorsally in spring after hibernation, dark (dominated by brown to black with white band) in summer; (2) darkly coloured (grey to black) ventrally in spring after hibernation, light (yellow to light brown) ventrally in summer; (3) lightly coloured (orange) dorsally in spring after hibernation, black areas present at least on posterior part of hindwing in summer. Unordered.
57. (wp34) Background colour outside central symmetry system in posterior part of dorsal side of fore wing: (0) blue; (1) white; (2) yellow to orange; (3) red; (4) red-brown; (5) dark brown. Ordered 012345.
58. (wp35) Colour of anterior part of central symmetry system on ventral side of hind wing: (0) dark; (1) bright.
59. (wp36) Colour pattern along outer margin of dorsal side of fore wing: (0) uniformly dark, or indistinct darker and lighter areas; (1) distinct, alternating white and black areas.
60. (wp37) Colour of dorsal side of hind wing basad outer margin of central symmetry system, posterior part: (0) bright or dark, not contrasting with areas outside central symmetry system; (1) dark, contrasting with brighter areas outside central symmetry system.
61. (wp38) Colour just distad posterior part of central symmetry system on dorsal side of fore wing: (0) not

contrasting yellow-orange to brown; (1) contrasting, bright yellow.

### INTERNAL MORPHOLOGY

Data and terminology mainly from Niculescu (1965).

62. (mg1) Shape of uncus: (0) with single point; (1) with two points; (2) with many points. Unordered.
63. (mg2) Processus inferior and processus superior of valva: (0) absent; (1) present.
64. (mg3) Fultura superior: (0) absent; (1) present.
65. (mg4) Shape of penis: (0) broad-based, distinctly constricted distally, with narrow opening; (1) tube-shaped, not constricted distally, with wide opening. Argynniti was coded as having the character non-applicable because the penis shape differs markedly from the two states found in the ingroup.

### EGG MORPHOLOGY AND ECOLOGY

Data from general sources (see Material and Methods) and our own investigations.

66. (e1) Number of vertical ribs on egg surface: (0) more than ten; (1) ten or less.
67. (e2) Number of eggs in clutch: (0) one; (1) more than two but less than twenty; (2) more than twenty. Ordered 012.
68. (e3) Oviposition site: (0) leaves; (1) twigs.
69. (e4) Shape of egg clutch: (0) string; (1) heap; (2) single layer. Unordered.
70. (e5) Colour of eggs: (0) yellow to brown; (1) green.

### LARVAL MORPHOLOGY AND ECOLOGY

Data from general sources (see Material and Methods) and our own investigations.

71. (L1) Larval nests: (0) none; (1) nest made out of leaf or leaves; (2) communal web. Unordered.
72. (L2) Spiny clubs on larval head: (0) absent; (1) present.
73. (L3) Middorsal spine on abdomen segment II-III: (0) present (odd total number of spines per segment); (1) absent (even total number).
74. (L4). Lightly coloured, contrasting, longitudinal stripes on the larval abdomen between the mid-dorsal and subdorsal spines. (0) present; (1) absent.
75. (L5). Lightly coloured contrasting pattern in the shape of an inverted 'V' on the face of the larval head. (0) absent; (1) present.
76. (L6). Large white patch or patches dorsally on posterior section of larval abdomen, contrasting with orange anterior section. (0) absent; (1) present.
77. (L7). Alternating light and dark wedge-shaped spots middorsally on larval abdomen: (0) absent; (1) present.
78. (L8) (Subdivision of L3:0) Colour of middorsal spine anteriorly: (0) black; (1) yellow-orange; (2) greenish. Unordered.
79. (L9) Colour of subdorsal spines: (0) black; (1) black on thorax (t2) otherwise light; (2) light. Ordered 012.
80. (L10) Light, contrasting, lateral band on larva: (0) present; (1) absent.
81. (L11) Secondary setae on the pinaculum on which L1 arises: (0) absent; (1) present. Data from Nakanishi (1988).
82. (L12) Relative position of D1 and D2 on abdominal segment 9: (0) D1 situated anteriorly to D2; (1) D2 situated anteriorly to D1. Data from Nakanishi (1988).
83. (L13) Relative position of D2 and SD1 on abdominal segment 9: (0) D2 dorsal to SD1; (1) SD1 dorsal to D2. Data from Nakanishi (1988).
84. (L14) Secondary setae on abdominal segment 10, on pinaculae from which P1 and Sp1 arises: (0) absent; (1) present. Data from Nakanishi (1988).
85. (L15) Microspines on larval setae: (0) absent; (1) present. Data from Nakanishi (1988).
86. (L16) Ornamentation of minute spines on larval abdominal segment 10: (0) much; (1) restricted; (2) absent. Ordered 012. Data from Nakanishi (1988).
87. (L17) Colour of silk spin: (0) white; (1) pinkish.
88. (L18) Position of filiform setae on abdominal segment 9 of larva: (0) arising from larval body surface; (1) arising from the sclerotized base of the scolus. Data from Harvey (1991).
89. (L19) Filiform setae on abdominal segments 1 and 2 of larva: (0) absent; (1) present. Data from Harvey (1991).
90. L20. Subdorsal spines on abdominal segment 9 (penultimate segment) of last-instar larva: (0) two spines present; (1) no spines present. Data from Teshirogi (1990) and our own observations.

### PUPAL MORPHOLOGY

Data from general sources (see Material and Methods) and our own investigations.

91. (p1). Metal spots around base of dorsal spines and in saddle of pupa: (0) present; (1) absent.
92. (p2). Dorsal projection on mesothorax of pupa: (0) not or very slightly projecting; (1) raised into a sharp point, (2) raised into a keeled projection. Unordered.
93. (p3). Subdorsal spines on abdominal segments of pupa: (0) absent or very slightly projecting; (1) present, distinctly projecting.
94. (p4) Shape of pupal anterior projections: (0) very slight projections; (1) projecting straight forwards and to the sides, inner sides forming a straight line; (2) projecting forwards, anterior section of inner sides bending inwards, forming a curve. Ordered 012.
95. (p5) (Subdivision of p3:1) Length of subdorsal spines on abdominal segment IV of pupa: (0) about as long as spines on other segments; (1) distinctly longer.

### ECOLOGY

96. (ec1). Hibernating developmental stage: (0) juvenile; (1) adult. Data from general sources (see Material and Methods).
97. (ec2). Host plant family: (0) Urticaceae; (1) Ulmaceae; (2) Cannabidaceae; (3) Salicaceae; (4) Grossulariaceae (*Ribes*); (5) Betulaceae; (6) Ericaceae; (7) Asteraceae; (8) Malvaceae; (A) Acanthaceae; (B) Convolvulaceae; (C) Schrophulariaceae; (D) Boraginaceae; (E) Verbenaceae; (F) Fabaceae; (G) Rosaceae; (H) Violaceae.

Data from general sources (see Material and Methods) and from a database compiled by N. Janz and S. Nylin from the literature (see Janz & Nylin (1998) for sources) and unpublished laboratory observations (Janz, Nyblom & Nylin, 2001).

APPENDIX 2: DATA MATRIX ON MORPHOLOGY, ECOLOGY AND BEHAVIOUR

| 1 | | 1<br>A1 s | 2<br>A2 c | 3<br>ey1 | 4<br>ey2 | 5<br>Lp1 | 6<br>Lp2 | 7<br>Lp3 | 8<br>Lp4 | 9<br>Le 1 | 10<br>le2 s | 11<br>ws1 | 12<br>ws2 | 13<br>ws3 | 14<br>ws4 | 15<br>ws5 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Argynniti | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 2 | *P. coenia* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | *H. bolina* | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | *M. geoffroyi* | 2 | 1 | 1 | ? | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 2 | 1 | 0 |
| 5 | *Symbrentia* sp. | 2 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 1/2 | 1 | 0 |
| 6 | *A. levana* | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| 7 | *Hypanartia* sp. | 0 | 0 | 1 | ? | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 2 | 1 | 0 |
| 8 | *A. schaeneia* | 0 | 0 | 1 | ? | ? | 0 | 1 | 1 | 1 | ? | 0 | 0 | 2 | 1 | 0 |
| 9 | *C. cardui* | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| 10 | *C. virginiensis* | 0 | 0 | 1 | ? | ? | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 11 | *V. atalanta* | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 12 | *B. gonerilla* | 0 | 0 | 1 | ? | ? | 0 | 1 | 1 | 1 | ? | 0 | 0 | 1 | 0 | 0 |
| 13 | *I. io* | 0 | 0 | 1 | 2 | 0 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 2 | 0 | 0 |
| 14 | *A. urticae* | 0 | 0 | 1 | 2 | 0 | 1 | 1 | 1 | 2 | 0 | 0 | 0 | 2 | 0 | 0 |
| 15 | *N. milberti* | 0 | 0 | 1 | ? | 0 | 1 | 1 | 1 | 2 | 0 | 0 | 0 | 2 | 0 | 0 |
| 16 | *N. antiopa* | 1 | 0 | 1 | ? | 0 | 1 | 2 | 1 | 2 | 0 | 0 | 0 | 2 | 0 | 0 |
| 17 | *N. cyanomelas* | 1 | ? | 1 | ? | ? | 1 | ? | ? | 2 | ? | 0 | 0 | 2 | 0 | 0 |
| 18 | *N. polychloros* | 1 | 0 | 1 | ? | 0 | 1 | 2 | 1 | 2 | 0 | 0 | 0 | 2 | 0 | 0 |
| 19 | *N. californica* | 1 | 0 | 1 | ? | 0 | 1 | 2 | 1 | 1 | 0 | 0 | 0 | 2 | 0 | 0 |
| 20 | *N. xanthomelas* | 1 | 0 | 1 | 2 | 0 | 1 | 2 | 1 | 2 | 0 | 0 | 0 | 2 | 0 | 0 |
| 21 | *R. lalbum* | 1 | 0 | 1 | ? | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 2 | 0 | 1 |
| 22 | *K. canace* | 0 | 0 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 2 | 2 | 0 | 1 |
| 23 | *P. interrogationis* | 0 | 0 | 1 | ? | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 2 | 2 | 0 | 1 |
| 24 | *P. comma* | 0 | 0 | 1 | ? | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 2 | 2 | 0 | 1 |
| 25 | *P. progne* | 0 | 0 | 1 | ? | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 2 | 2 | 0 | 1 |
| 26 | *P. satyrus* | 0 | 0 | 1 | ? | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 2 | 2 | 0 | 1 |
| 27 | *P. gracilis* | 0 | 0 | 1 | ? | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 2 | 2 | 0 | 1 |
| 28 | *P. faunus* | 0 | 0 | 1 | ? | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 0 | 1 |
| 29 | *P. calbum* | 0 | 0 | 1 | 2 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 0 | 1 |
| 30 | *P. caureum* | 0 | 0 | 1 | 2 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 2 | 2 | 0 | 1 |
| 31 | *P. egea* | 0 | 0 | 1 | ? | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 2 | 2 | 0 | 1 |

*continued*

APPENDIX 2 – *continued*

| 2 | | 16 ws6 | 17 ws7 | 18 ws8 | 19 ws9 | 20 ws10 | 21 ws11 | 22 ws12 | 23 ws13 | 24 wp1 | 25 wp2 | 26 wp3 | 27 wp4 | 28 wp5 | 29 wp6 | 30 wp7 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Argynniti | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | – | – |
| 2 | *P. coenia* | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | – | – |
| 3 | *H. bolina* | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | – | – |
| 4 | *M. geoffroyi* | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | – | – |
| 5 | *Symbrentia* sp. | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | – | – |
| 6 | *A. levana* | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | – | – |
| 7 | *Hypanartia* sp. | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | – | – |
| 8 | *A. schaeneia* | 1 | 0 | 0 | 0 | ? | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | – | – |
| 9 | *C. cardui* | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | – | – |
| 10 | *C. virginiensis* | 1 | 0 | 0 | 0 | ? | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | – | – |
| 11 | *V. atalanta* | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | – | – |
| 12 | *B. gonerilla* | 1 | 0 | 0 | 0 | ? | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | – | – |
| 13 | *I. io* | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 1 | 0 | – | 0 |
| 14 | *A. urticae* | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | – | 0 |
| 15 | *N. milberti* | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | – | 0 |
| 16 | *N. antiopa* | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | – | 0 |
| 17 | *N. cyanomelas* | 1 | 1 | 0 | ? | ? | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | – | 0 |
| 18 | *N. polychloros* | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | – | 0 |
| 19 | *N. californica* | 1 | 0 | 1 | 1 | ? | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | – | 0 |
| 20 | *N. xanthomelas* | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | – | 0 |
| 21 | *R. lalbum* | 1 | 1 | 1 | 1 | 0 | 1 | 2 | 0 | 1 | 0 | 2 | 1 | 0 | 0 | 0 |
| 22 | *K. canace* | 1 | 1 | 1 | 1 | 0 | 1 | 2 | 0 | 1 | 0 | 2 | 1 | 0 | 0 | 0 |
| 23 | *P. interrogationis* | 1 | 1 | 0 | 1 | 0 | 1 | 2 | 0 | 1 | 1 | 2 | 1 | 0 | 1 | 1 |
| 24 | *P. comma* | 1 | 1 | 1 | 1 | 0 | 0 | 2 | 0 | 1 | 1 | 2 | 1 | 0 | 1 | 1 |
| 25 | *P. progne* | 1 | 1 | 1 | 1 | ? | 0 | 2 | 0 | 1 | 1 | 2 | 1 | 0 | 0 | 1 |
| 26 | *P. satyrus* | 1 | 1 | 1 | 1 | ? | 0 | 2 | 0 | 1 | 1 | 2 | 1 | 0 | 1 | 1 |
| 27 | *P. gracilis* | 1 | 1 | 1 | 1 | ? | 0 | 2 | 0 | 1 | 1 | 2 | 1 | 0 | 0 | 1 |
| 28 | *P. faunus* | 1 | 1 | 1 | 1 | ? | 0 | 2 | 0 | 1 | 1 | 2 | 1 | 0 | 1 | 1 |
| 29 | *P. calbum* | 1 | 1 | 1 | 1 | 0 | 0 | 2 | 0 | 1 | 1 | 2 | 1 | 0 | 1 | 1 |
| 30 | *P. caureum* | 1 | 1 | 1 | 1 | ? | 1 | 2 | 0 | 1 | 1 | 2 | 1 | 0 | 1 | 1 |
| 31 | *P. egea* | 1 | 1 | 1 | 1 | 0 | 0 | 2 | 0 | 1 | 1 | 2 | 1 | 0 | 0 | 1 |

*continued*

APPENDIX 2 – *continued*

| 3 | | 31 wp8 | 32 wp9 | 33 wp10 | 34 wp11 | 35 wp12 | 36 wp13 | 37 wp14 | 38 wp15 | 39 wp16 | 40 wp17 | 41 wp18 | 42 wp19 | 43 wp20 | 44 wp21 | 45 wp22 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Argynniti | – | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| 2 | *P. coenia* | – | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0/1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3 | *H. bolina* | – | 0 | 1 | 0 | 1 | 0 | – | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 | *M. geoffroyi* | – | 0 | 0 | 0 | 0 | – | – | 1 | 1 | 0 | 0 | 0 | 0 | 0 | – |
| 5 | *Symbrentia* sp. | – | 0 | 0 | 0 | 0 | 0 | – | 1 | 1 | 0 | 0 | 1 | 0 | 0 | – |
| 6 | *A. levana* | – | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 1 |
| 7 | *Hypanartia* sp. | – | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| 8 | *A. schaeneia* | – | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 9 | *C. cardui* | – | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 0 |
| 10 | *C. virginiensis* | – | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 0 | 1 |
| 11 | *V. atalanta* | – | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| 12 | *B. gonerilla* | – | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 |
| 13 | *I. io* | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 |
| 14 | *A. urticae* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 1 |
| 15 | *N. milberti* | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 1 |
| 16 | *N. antiopa* | 0 | 0 | 0 | 0 | 1 | 2 | – | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 |
| 17 | *N. cyanomelas* | 0 | 0 | 0 | 0 | 1 | 2 | – | 0 | 1 | 0 | 1 | 0 | 2 | 0 | – |
| 18 | *N. polychloros* | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 1 | 0 | 1 | 0 | 2 | 0 | 1 |
| 19 | *N. californica* | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 1 |
| 20 | *N. xanthomelas* | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 1 |
| 21 | *R. lalbum* | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 1 |
| 22 | *K. canace* | 0 | 0 | 0 | 0 | 1 | 2 | – | 0 | 1 | 0 | 1 | 0 | 2 | 0 | 2 |
| 23 | *P. interrogationis* | 1 | 0 | 0 | 0 | 1 | 2 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| 24 | *P. comma* | 1 | 0 | 0 | 0 | 1 | 2 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| 25 | *P. progne* | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| 26 | *P. satyrus* | 1 | 0 | 0 | 0 | 1 | 2 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| 27 | *P. gracilis* | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| 28 | *P. faunus* | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| 29 | *P. calbum* | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| 30 | *P. caureum* | 1 | 0 | 0 | 0 | 1 | 2 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 |
| 31 | *P. egea* | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 0 | 1 |

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APPENDIX 2 – *continued*

| 4 | | 46 wp23 | 47 wp24 | 48 wp25 | 49 wp26 | 50 wp27 | 51 wp28 | 52 wp29 | 53 wp30 | 54 wp31 | 55 wp32 | 56 wp33 | 57 wp34 | 58 wp35 | 59 wp36 | 60 wp37 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Argynniti | 1 | 0 | – | 2 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 |
| 2 | *P. coenia* | 0 | 0 | – | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| 3 | *H. bolina* | 0 | 0 | – | 0 | 0 | 0 | – | 0 | 0 | – | 0 | 5 | 0 | 0 | 0 |
| 4 | *M. geoffroyi* | – | 0 | – | 2 | 0 | 2 | – | 0 | 0 | – | 0 | 1 | 0 | 0 | 0 |
| 5 | *Symbrentia* sp. | – | 0 | – | 2 | 1 | 2 | – | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| 6 | *A. levana* | 0 | 0 | – | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 2 | 1 | 1 | 0 |
| 7 | *Hypanartia* sp. | 1 | 1 | – | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 2 | 1 | 0 | 0 |
| 8 | *A. schaeneia* | 0 | 1 | – | 0 | 2 | 1 | – | 1 | 0 | 1 | 0 | 2 | 1 | 1 | 0 |
| 9 | *C. cardui* | 0 | 0 | – | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 0 | 2 | 1 | 1 | 0 |
| 10 | *C. virginiensis* | 0 | 0 | – | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 1 | 1 | 0 |
| 11 | *V. atalanta* | 0 | 1 | – | 0 | 2 | 1 | – | 1 | 0 | 1 | 0 | 3 | 1 | 1 | 0 |
| 12 | *B. gonerilla* | 0 | 1 | – | 0 | 2 | 1 | 0 | 1 | 0 | – | 0 | 3 | 1 | 1 | 0 |
| 13 | *I. io* | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 4 | 0 | 0 | 0 |
| 14 | *A. urticae* | 0 | 0 | – | 0 | 0 | 1 | 1 | 0 | 0 | 0/1 | 0 | 2 | 0 | 0 | 1 |
| 15 | *N. milberti* | 0 | 0 | – | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 2 | 0 | 0 | 1 |
| 16 | *N. antiopa* | 0 | 0 | 1 | 0 | 0 | 0 | – | 0 | 0 | – | 0 | 5 | 0 | 0 | 0 |
| 17 | *N. cyanomelas* | 2 | 0 | 1 | 0 | 0 | 0 | – | 0 | 0 | – | ? | 5 | 0 | 0 | 0 |
| 18 | *N. polychloros* | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| 19 | *N. californica* | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| 20 | *N. xanthomelas* | 0 | 0 | – | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| 21 | *R. lalbum* | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| 22 | *K. canace* | 0 | 0 | 1 | 1 | 0 | 0 | – | 0 | 0 | – | 0 | 0 | 0 | 0 | 0 |
| 23 | *P. interrogationis* | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 3 | 2 | 0 | 0 | 0 |
| 24 | *P. comma* | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 3 | 2 | 0 | 0 | 0 |
| 25 | *P. progne* | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 |
| 26 | *P. satyrus* | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 2 | 2 | 0 | 0 | 0 |
| 27 | *P. gracilis* | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 |
| 28 | *P. faunus* | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 0 |
| 29 | *P. calbum* | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 1 | 0 | 2 | 2 | 0 | 0 | 0 |
| 30 | *P. caureum* | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 2 | 2 | 0 | 0 | 0 |
| 31 | *P. egea* | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 2 | 2 | 0 | 0 | 0 |

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APPENDIX 2 – *continued*

| 5 | | 61 wp38 | 62 mg1 | 63 mg2 | 64 mg3 | 65 mg4 | 66 e1 e | 67 e2 n | 68 e3 e | 69 e4 st | 70 e5 e | 71 L1 la | 72 L2 s | 73 L3 m | 74 L4 tv | 75 L5 in |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Argynniti | 0 | 2 | 1 | 0 | – | 0 | 0 | – | – | 0 | 0 | 0 | 1 | 0 | 0 |
| 2 | *P. coenia* | 0 | ? | ? | ? | ? | 1 | 0 | 0 | – | 1 | 0 | 1 | 0 | 0 | 0 |
| 3 | *H. bolina* | 0 | ? | ? | ? | ? | 0 | 0 | 0 | – | 0 | ? | 1 | 1 | 1 | 0 |
| 4 | *M. geoffroyi* | 0 | ? | ? | ? | ? | 0 | 2 | 0 | 2 | 0 | ? | 1 | 0 | 1 | 0 |
| 5 | *Symbrentia* sp. | 0 | ? | ? | ? | ? | 0 | 2 | 0 | 2 | ? | ? | 1 | ? | ? | ? |
| 6 | *A. levana* | 0 | 1 | 1 | 0 | 1 | 0 | 2 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 |
| 7 | *Hypanartia* sp. | 0 | ? | ? | ? | ? | ? | 0 | 0 | – | 0 | 1 | 0 | ? | ? | ? |
| 8 | *A. schaeneia* | 0 | ? | ? | ? | ? | ? | 0 | ? | – | ? | 1 | ? | ? | ? | ? |
| 9 | *C. cardui* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | – | 1 | 1 | 0 | 0 | 0 | 0 |
| 10 | *C. virginiensis* | 0 | ? | ? | ? | ? | 0 | 0 | 0 | – | 1 | 1 | 0 | ? | ? | ? |
| 11 | *V. atalanta* | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | – | 1 | 1 | 0 | 0 | 1 | 0 |
| 12 | *B. gonerilla* | 0 | 0 | ? | ? | 0 | 1 | 0 | 0 | – | 1 | 1 | 0 | 0 | 1 | ? |
| 13 | *I. io* | 0 | 1 | 1 | 1 | 0 | 1 | 2 | 0 | 1 | 1 | 2 | 0 | 1 | 1 | 0 |
| 14 | *A. urticae* | 1 | 0 | 1 | 0 | 1 | 1 | 2 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 0 |
| 15 | *N. milberti* | 1 | ? | ? | 1 | 1 | 1 | 2 | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 0 |
| 16 | *N. antiopa* | 0 | 0 | 1 | 1 | 0 | 1 | 2 | 1 | 2 | 0 | 2 | 0 | 1 | 1 | 0 |
| 17 | *N. cyanomelas* | 0 | ? | ? | 1 | 0 | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? |
| 18 | *N. polychloros* | 0 | 0 | 1 | 1 | 0 | 1 | 2 | 1 | 2 | 0 | 2 | 0 | 0 | 0 | ? |
| 19 | *N. californica* | 0 | ? | ? | 1 | 0 | ? | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | ? |
| 20 | *N. xanthomelas* | 0 | 0 | 1 | 1 | 0 | 1 | 2 | 1 | 2 | 0 | ? | 0 | 1 | 0 | 0 |
| 21 | *R. lalbum* | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 0 |
| 22 | *K. canace* | 0 | 0 | 1 | ? | 0 | 1 | 0 | 0 | – | 1 | ? | 0 | 0 | 1 | 1 |
| 23 | *P. interrogationis* | 0 | 0 | ? | ? | ? | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 |
| 24 | *P. comma* | 0 | 0 | ? | ? | ? | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 |
| 25 | *P. progne* | 0 | 0 | ? | ? | ? | 1 | 0 | 0 | – | 1 | 0 | 1 | 0 | 1 | 1 |
| 26 | *P. satyrus* | 0 | 0 | ? | ? | ? | ? | 0/1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 |
| 27 | *P. gracilis* | 0 | 0 | ? | ? | ? | 1 | 0/1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 |
| 28 | *P. faunus* | 0 | 0 | ? | ? | ? | 0 | 0 | 0 | – | 1 | 0 | 1 | 0 | 1 | 1 |
| 29 | *P. calbum* | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | – | 1 | 0 | 1 | 0 | 1 | 1 |
| 30 | *P. caureum* | 0 | 0 | ? | ? | 0 | 1 | 0 | 0 | – | 1 | ? | 1 | 0 | 1 | 1 |
| 31 | *P. egea* | 0 | 0 | 1 | 1 | 0 | 1 | 0 | 0 | – | 1 | 0 | 1 | 0 | 1 | – |

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APPENDIX 2 – *continued*

| 6 | | 76 L6 p | 77 L7 ″ | 78 L8 c | 79 L9 s | 80 L10 | 81 L11 | 82 L12 | 83 L13 | 84 L14 | 85 L15 | 86 L16 | 87 L17 | 88 L18 | 89 L19 | 90 L20 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Argynniti | 0 | 0 | – | 2 | 0 | ? | ? | ? | ? | ? | ? | ? | 0 | 0 | 0 |
| 2 | *P. coenia* | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 |
| 3 | *H. bolina* | 0 | 0 | – | 2 | 0 | ? | ? | ? | ? | ? | ? | ? | 1 | 0 | 0 |
| 4 | *M. geoffroyi* | 0 | 0 | 0 | 0 | 1 | ? | ? | ? | ? | ? | ? | ? | ? | ? | 1 |
| 5 | *Symbrentia* sp. | ? | ? | ? | ? | ? | 0 | 1 | 0 | 1 | 1 | 1 | ? | ? | ? | 1 |
| 6 | *A. levana* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | ? | ? | 1 |
| 7 | *Hypanartia* sp. | 0 | ? | ? | 0 | ? | ? | ? | ? | ? | ? | ? | ? | 0 | 1 | – |
| 8 | *A. schaeneia* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | – |
| 9 | *C. cardui* | 0 | 0 | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 0 |
| 10 | *C. virginiensis* | 0 | ? | ? | ? | 0 | ? | ? | ? | ? | ? | ? | ? | 0 | 1 | 0 |
| 11 | *V. atalanta* | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 2 | 0 | 0 | 1 | 0 |
| 12 | *B. gonerilla* | 0 | 0 | 2 | 2 | 0 | ? | ? | ? | ? | ? | ? | ? | 0 | 1 | 0 |
| 13 | *I. io* | 0 | 0 | – | 0 | 1 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 0 | 1 | 0 |
| 14 | *A. urticae* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 2 | 0 | 0 | 1 | 0 |
| 15 | *N. milberti* | 0 | 0 | 0 | 0 | 0 | ? | ? | ? | ? | ? | ? | 0 | 0 | 1 | 0 |
| 16 | *N. antiopa* | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 2 | 0 | 0 | 1 | 0 |
| 17 | *N. cyanomelas* | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | ? | – |
| 18 | *N. polychloros* | 0 | 0 | 1 | 2 | 0 | ? | ? | ? | ? | ? | ? | 1 | 0 | 1 | 0 |
| 19 | *N. californica* | 0 | 0 | 1 | ? | 0 | ? | ? | ? | ? | ? | ? | ? | 0 | 1 | 0 |
| 20 | *N. xanthomelas* | 0 | 0 | – | 0 | 1 | 0 | 0 | 1 | 0 | 1 | 2 | ? | ? | ? | 0 |
| 21 | *R. lalbum* | 0 | 0 | 0 | 0 | 0 | 1 | ? | 0 | 0 | 0 | 0 | ? | ? | ? | 0 |
| 22 | *K. canace* | 0 | 1 | 1 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | ? | 0 | 1 | 0 |
| 23 | *P. interrogationis* | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| 24 | *P. comma* | 0 | 1 | 1 | 2 | 0 | ? | ? | ? | ? | ? | ? | 1 | 0 | 1 | 0 |
| 25 | *P. progne* | 0 | 1 | 1 | 1 | 0 | 1 | ? | ? | ? | ? | ? | 1 | 0 | 1 | 0 |
| 26 | *P. satyrus* | 0 | 1 | 2 | 2 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 0 |
| 27 | *P. gracilis* | 0/1 | 1 | 1 | 2 | 0 | 1 | ? | ? | ? | ? | ? | 1 | 0 | 1 | 0 |
| 28 | *P. faunus* | 1 | 1 | 1 | 2 | 0 | ? | ? | ? | ? | ? | ? | 1 | 0 | 1 | 0 |
| 29 | *P. calbum* | 1 | 1 | 1 | 2 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 30 | *P. caureum* | 0 | 1 | 1 | 2 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | ? | 0 | 1 | 0 |
| 31 | *P. egea* | 0 | 1 | 1 | 2 | 0 | ? | ? | ? | ? | ? | ? | 0 | 0 | 1 | 0 |

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APPENDIX 2 – *continued*

| 7 | | 91 p1 m | 92 p2 d | 93 p3 a | 94 p4 p | 95 p5 p | 96 ec1 | 97 ec2 |
|---|---|---|---|---|---|---|---|---|
| 1 | Argynniti | 0 | 1 | 1 | 1 | 1 | 0 | H |
| 2 | *P. coenia* | 1 | 0 | 0 | 0 | – | 0 | A&C&E |
| 3 | *H. bolina* | 1 | 1 | 1 | 0 | 0 | – | 0&8&A&B |
| 4 | *M. geoffroyi* | 0 | 1 | 1 | 0 | 1 | – | 0 |
| 5 | *Symbrentia* sp. | ? | ? | ? | ? | ? | – | 0 |
| 6 | *A. levana* | 0 | 1 | 1 | 1 | 1 | 0 | 0 |
| 7 | *Hypanartia* sp. | 0 | 0 | 0 | 0 | – | – | 0&1 |
| 8 | *A. schaeneia* | ? | 3 | 1 | 2 | 1 | – | 0 |
| 9 | *C. cardui* | 0 | 1 | 0 | 0 | – | – | 0&7&8&B&D&E&F |
| 10 | *C. virginiensis* | ? | ? | 0 | 0 | – | 1 | 0&7&8&C&D&F |
| 11 | *V. atalanta* | 0 | 1 | 1 | 0 | 0 | 1 | 0 |
| 12 | *B. gonerilla* | 0 | 1 | 1 | 0 | 0 | 1 | 0 |
| 13 | *I. io* | 1 | 1 | 1 | 1 | 0 | 1 | 0&2 |
| 14 | *A. urticae* | 0/1 | 1 | 1 | 1 | 0 | 1 | 0 |
| 15 | *N. milberti* | 1 | 1 | 1 | 1 | 0 | 1 | 0 |
| 16 | *N. antiopa* | 1 | 1 | 1 | 1 | 0/1 | 1 | 1&3&5&F&G |
| 17 | *N. cyanomelas* | ? | ? | ? | ? | ? | – | ? |
| 18 | *N. polychloros* | 0 | 1 | 1 | 1 | 1 | 1 | 1&3&G |
| 19 | *N. californica* | 1 | 1 | 1 | 1 | 0/1 | 1 | J |
| 20 | *N. xanthomelas* | 1 | 1 | 1 | 1 | 1 | 1 | 1&3 |
| 21 | *R. lalbum* | 0 | 1 | 1 | 1 | 0 | 1 | 1&3&5 |
| 22 | *K. canace* | 0 | 1 | 1 | 2 | 1 | 1 | K |
| 23 | *P. interrogationis* | 0 | 2 | 1 | 1 | 1 | 1 | 0&1&2 |
| 24 | *P. comma* | 0 | 2 | 1 | 1 | 1 | 1 | 0&1&2 |
| 25 | *P. progne* | 0 | 2 | 1 | ? | 0 | 1 | 4&5&6 |
| 26 | *P. satyrus* | 0 | 2 | 1 | 1 | 1 | 1 | 0&2&3 |
| 27 | *P. gracilis* | 0 | 2 | 1 | 2 | 0 | 1 | 4&5&6 |
| 28 | *P. faunus* | 0 | 2 | 1 | 2 | 1 | 1 | 3&4&5&6 |
| 29 | *P. calbum* | 0 | 2 | 1 | 2 | 1 | 1 | 0&1&2&3&4&5 |
| 30 | *P. caureum* | 0 | 2 | 1 | 1 | 1 | 1 | 2 |
| 31 | *P. egea* | 1 | 2 | 1 | 1 | 0 | 1 | 0 |