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Phylogeny of the Nymphalidae (Lepidoptera)

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Abstract.—A generic-level phylogeny for the butterfly family Nymphalidae was produced by cladistic analysis of 234 characters from all life stages. The 95 species in the matrix (selected from the 213 studied) represent all important recognized lineages within this family. The analysis showed the taxa grouping into six main lineages. The basal branch is the Libytheinae, with the Danainae and Ithomiinae on the next branch. The remaining lineages are grouped into two main branches: the Heliconiinae-Nymphalinae, primarily flower-visitors (but including the fruit-attracted Coeini); and the Limenitidinae (*sensu strictu*), Biblidinae, and the satyroid lineage (Apaturinae, Charaxinae, Biinae, Calinaginae, Morphinae, Brassolinae, and Satyrinae), primarily fruit-attracted. Data partitions showed that the two data sets (immatures and adults) are very different, and a partitioned Bremer support analysis showed that the adult characters are the main source of conflict in the nodes of the combined analysis tree. This phylogeny includes the widest taxon coverage of any morphological study on Nymphalid butterflies to date, and supports the monophyly and relationships of most presently recognized subgroups, providing strong evidence for the presently accepted phylogenetic scheme. [Adults; combined data; eggs; juveniles; larvae; morphology; Nymphalidae; phylogeny; pupae.]

The cosmopolitan butterfly family Nymphalidae (Lepidoptera) includes about 7200 species occurring in all habitats and continents except Antarctica (DeVries, 1987; Shields, 1989; Heppner, 1991). The systematic relationships among its many different subfamilies and tribes are still poorly understood, however (Harvey, 1991); most subfamilies are vaguely defined or supported by few characters. Even the widespread and well-studied subfamily Nymphalinae (*sensu* Harvey, 1991) has been considered an unnatural assemblage (Harvey, 1991; De Jong et al., 1996). Nevertheless, some main subgroups of the Nymphalidae (such as Acraeinae, Heliconiinae, Brassolinae, Morphinae, Satyrinae, and Danainae) have been recognized by many authors since they were first defined by Müller (1886).

The phylogeny of this family has been frequently discussed, with the relationships among the taxonomic categories below the family level varying with the sample and the author (Clark, 1949; Ehrlich, 1958; DeVries, 1987; Harvey, 1991). In historical perspective, the work of Müller (1886) in southern Brazil, with his access to and preferential use of characters from early stages to identify the proposed lineages of Nymphalidae, provided one of the best foundations for the infrafamilial classification of the Nymphalidae. A reevaluation of butterfly classification by Ehrlich (1958), including over 300 species and using characters from early stages and adults, gave a first attempt at a coherent classification of all butterfly groups, including the subdivisions of Nymphalidae. Ehrlich and Ehrlich (1967) then proposed a complete phenetic scheme for butterflies, based on numerical methods of analysis. Ackery (1984, 1988) attempted to define the main groups within the butterflies; the results were quite conservative, especially for the subfamilies of Nymphalidae, and there was no definition of the relationships within the different subfamilies. The classification of Harvey (1991) was partly based on the larval characters of Müller (1886) and some other authors according to the subgroup (especially Ehrlich, 1958; Ehrlich and Ehrlich, 1967; Miller, 1968; Ackery and Vane-Wright, 1984; Ackery, 1988). This classification became popular for its lists of genera for each group, although the relationships within and among the subgroups were not fully resolved.

Several additional morphological studies also contributed to Nymphalidae systematics (Clark, 1947, 1949; Stelkonikov, 1967; Kristensen, 1976; Scott, 1985; De Jong et al., 1996), but these were broad and did not focus on the subdivisions of the family.

Recently, studies using molecular data and phylogenetic methods were added to this list (Martin and Pashley, 1992; Weller et al., 1996), but these had limited taxon coverage and did not add much to the resolution of the Nymphalidae phylogeny. The recent paper of Brower (2000) using the *wingless* gene, and Wahlberg et al. (2003) using one mitochondrial (COI) and two nuclear (EF-1α and wingless) genes, both including good taxonomic coverage of the Nymphalidae (Calinaginae not represented in Brower's paper), showed that many of the traditional subgroups are monophyletic.

Although the higher level phylogeny of Nymphalidae is still partly unresolved, relationships among certain subgroups are widely accepted (Danainae with Ithomiinae, Acraeini with Heliconiini, and Satyrinae with Morphinae-Amathusinae-Brassolinae; Ehrlich, 1958; Ackery, 1984, 1988; Scott, 1985; De Jong et al., 1996). The position of Libytheinae as the basal group of Nymphalidae has also been accepted by many recent authors (Ehrlich and Ehrlich, 1967; Ackery and Vane-Wright, 1984; Scott, 1985; Harvey, 1991; De Jong et al., 1996). However, several questions remain in the Nymphalidae, such as the positions of Calinaginae and Tellervinae and the status and relationships within Nymphalinae (*sensu latu*).

Studies with Butterfly Immatures

Wilhelm Müller (1886) was the pioneer in the use of morphology of early stages for nymphalid classification,

but he never proposed taxonomic changes (Harvey, 1991: 255–257). The many studies describing immature stages of butterflies that have been published since then form a vast body of information available for systematic analysis (see Freitas, 1999). The concerted effort to gather this information allowed comparative studies like those of Chapman (1893, 1895), Packard (1895), Mosher (1916), Moss (1920, 1949), D'Almeida (1922), and Hinton (1946), all of which are landmarks in morphological studies of immature Lepidoptera. Besides simple descriptions, fine scale morphological studies have also been published using chaetotaxy of first instar larvae, some of them with a phylogenetic approach (Hinton, 1946; Fleming, 1960; DeVries et al., 1985; Nakanishi, 1988; Motta, 1998, 2003). In recent years, techniques of scanning electron microscropy (SEM) have been used successfully to obtain characters of first instar larvae and chorionic structure of eggs (Kitching, 1985; Motta, 1989; Tyler et al., 1994; Sourakov, 1996, 1997; Sourakov and Emmel, 1997a, 1997b). The morphology and biology of immature tropical nymphalids, the main focus of this study, are fairly well known, but information is still lacking for several important groups.

The use of early stage characters is increasing in importance for the study of butterfly systematics, because they can offer answers to questions that remain unsolved with the characters of adults only (Freitas, 1999). The higher level relationships within Morphinae and Satyrinae (DeVries et al., 1985); the phylogeny of Danainae (Kitching, 1985), Ithomiinae (Motta, 1989, 1998, 2003; Brown and Freitas, 1994), Papilionidae (Tyler et al., 1994), and Heliconiini (Penz, 1999); the relationships of some genera of Biblidinae (Freitas et al., 1997); and the overall higher classification of Nymphalidae (Harvey, 1991) are examples of recent studies in which characters of immatures provided important information for systematic research.

Even with this recognition of the importance of characters from immature stages, they have been used only rarely in the higher classification of butterflies. This may be a result of the lack of adequate material for comparison in museums (especially for tropical groups), little interest of lepidopterists, and the difficulties in field work (Freitas, 1999).

Although various studies have been undertaken to solve the question of Nymphalidae classification, the characters used were almost always the same, taken from adults. Instead of multiplying the number of adult characters, different data sets need to be obtained (Freitas, 1999; Vane-Wright, 2003). Molecular characters and those drawn from immature stages are the most promising at the present (as discussed by De Jong et al., 1996; Ackery et al., 1999; Brower, 2000; Wahlberg et al., 2003).

In this study, we use morphology of all life stages to resolve the higher-level phylogeny of the Nymphalidae. To address this point, many species of Nymphalidae were reared and preserved over the last 16 years by AVLF, complementing data gathered by KSB in the last 35 years (especially on Heliconiini and Ithomiinae) to form a data bank broad enough to permit analysis. Some of these data have already been published in previous papers (Freitas, 1991, 1993, 1996, 2002, 2003, 2004a, 2004b; Freitas and Oliveira, 1992; Brown and Freitas, 1994; Freitas et al., 1997, 2001, 2002, 2003; Freitas and Brown, 2002). The proposed phylogeny of Nymphalidae in this work is based on characters from all life stages, with a preponderant contribution (more than half of the total) of characters from immatures, resulting in a data set different from all those published previously, and presenting a well-resolved phylogeny of the family.

Material and Methods

Taxon Sampling

Immature stages (eggs, larvae, and pupae) and adults of Nymphalidae were collected in more than 200 localities in Brazil (see list in Freitas, 1999). To help cover all Nymphalidae subgroups, material from other regions was provided by many investigators or examined in museums.

Immature stages of 213 species of Nymphalidae were studied, most of them (182) collected in the field. Data for a few species were obtained from the literature and unpublished descriptions (especially immatures of *Calinaga buddha*). Of these 213, 95 species in 94 genera were selected as sufficient taxa to represent the Nymphalidae (Table 1), and were included in the data matrix for the phylogenetic analysis (available as nexus file at http://systematicbiology.org). The taxa were selected to give coverage of all widely recognized subfamilies and tribes of Nymphalidae. In the cladograms, only the generic name was used to represent the species (except for *Callicore*, with two species in the matrix).

Eggs were collected in the field or from females confined in plastic bags. In some cases, fertilized eggs were obtained by pressing the end of the abdomen. This procedure usually resulted in a single fertile egg, and was used in species that did not oviposit in the laboratory. Larvae were reared in plastic pots with parts of the host plants. Individuals of each instar were preserved whenever possible, and detailed notes were recorded for all species reared.

Cladistic Analysis

The character states were polarized in relation to several Pieridae and Papilionidae, together with some Lycaenidae and Hesperiidae. Then, to simplify the picture, a hypothetical outgroup with all characters set to 0 (zero) was added to the matrix (as in Livezey, 1996, and De Jong et al., 1996). According to Livezey (1996), this method facilitates rooting of trees without digressions into relationships among outgroups. Multistate characters were mostly ordered, except for characters 1, 9, and 14, which are unordered. Characters and states not comparable were coded with [−], and characters without available information were coded with [?] in the matrix. Seven uninformative (autapomorphic) characters were maintained in the matrix, but not used in the analyses (character numbers 46, 47, 62, 112, 161, 191, 199), because

TABLE 1. List of Nymphalidae taxa (*sensu* Harvey, 1991) used in cladistic analysis and the main study sites for each species. Localities presented as "country, state: municipalities."

Subfamily	Tribe	Species	Localities
Libytheinae		*Libytheana carinenta* (Cramer, 1777)	Brazil, São Paulo: Campinas, Castilho, Jundiaí, São Vicente
Tellervinae		*Tellervo zoilus* (Fabricius, 1775)	Australia, Queensland: Cairns
Danainae	Danaini	*Danaus plexippus erippus* (Cramer, 1775)	Brazil, São Paulo: Campinas, Castilho, Cubatão, Jundiaí, São Vicente
		Amauris niavius (Linaneus, 1758)	Ackery and Vane-Wright, 1984
	Euploeini	*Lycorea cleobaea halia* (Hübner, 1823)	Brazil, São Paulo: Cubatão, Jundiaí, São Sebastião, São Vicente
		Anetia briarea (Godart, 1819)	Brower et al.,1992
Ithomiinae	Tithoreini	*Tithorea harmonia* (Cramer, 1777)	Brazil, São Paulo: Campinas, Mogi Guaçu, Serra Negra
	Melinaeini	*Melinaea ludovica* (Cramer, 1780)	Brazil, São Paulo: Peruíbe, São Vicente, Ubatuba
	Methonini	*Methona themisto* (Hübner, 1819)	Brazil, São Paulo: Campinas, Santos, São Vicente
	Mechanitini	*Mechanitis lysimnia* (Fabricius, 1793)	Brazil, São Paulo: Campinas, Jundiaí, São Bernardo, São Vicente
	Napeogenini	*Hypothyris ninonia daeta* (Boisduval, 1836)	Brazil, São Paulo: Campinas, Jundiaí, São Vicente
	Ithomiini	*Ithomia drymo* (Hübner, 1816)	Brazil, São Paulo: Campinas, Jundiaí, São Vicente
	Dircennini	*Dircenna dero celtina* (Burmeister, 1878)	Brazil, São Paulo: Campinas, Cubatão, Jundiaí, São Vicente
	Godyridini	*Heterosais edessa* (Hewitson, 1854)	Brazil, São Paulo: Mongaguá, São Vicente
Charaxinae	Charaxini	*Charaxes varanes* (Cramer, 1764)	Van Son, 1979
	Preponini	*Archaeoprepona chalciope* (Hübner, 1825)	Brazil, São Paulo: Campinas, Jundiaí
	Anaeini	*Zaretis itys strigosa* (Gmelin, 1788)	Brazil, São Paulo: Campinas, Jundiaí
		Siderone marthesia (Cramer, 1777)	Brazil, São Paulo: Cubatão; Santa Catarina: Joinville
		Hypna clytemnestra (Butler, 1866)	Brazil, São Paulo: Campinas, Jundiaí
		Consul fabius (Cramer, 1775)	Brazil, São Paulo: São Vicente; Santa Catarina: Joinville; Acre: Marechal Thaumaturgo
		Memphis ryphea phidile (Geyer, 1834)	Brazil, São Paulo: Campinas, Jundiaí
Apaturinae		*Doxocopa agathina vacuna* (Godart, 1824)	Brazil, São Paulo: São Vicente
		Asterocampa argus (Bates, 1864)	Mexico, Oaxaca: unknown locality
Calinaginae		*Calinaga buddha formosana* (Fruhstorfer, 1908)	Ashizawa and Muroya, 1967; Lee and Chang, 1989; M. Teshirogi, unpublished figures
Morphinae	Morphini	*Morpho achilles achillaena* (Hübner, 1819)	Brazil, São Paulo: São Vicente; Santa Catarina: Joinville
	Antirrheini	*Antirrhea archaea* (Hübner, 1822)	Brazil, São Paulo: São Vicente, Campinas
		Caerois chorinaeus (Fabricius, 1775)	Brazil, Acre: Marechal Thaumaturgo
	Amathusiini	*Taenaris onolaus* (Kirsch, 1944)	Material sent by Stephen Hall from a butterfly house
Brassolinae	Brassolini	*Brassolis sophorae* (Linnaeus, 1758)	Brazil, São Paulo: Campinas, São Vicente
		Dynastor darius (Fabricius, 1775)	Brazil, São Paulo: Campinas, Jaú, Ubatuba; Espírito Santo: Linhares
		Opsiphanes invirae (Hübner, 1808)	Brazil, São Paulo: Campinas, São Vicente
		Dasyophthalma creusa (Hübner, 1822)	Brazil, São Paulo: Cotia, Jundiaí, São Vicente
		Eryphanis reevesi (Doubleday, 1849)	Brazil, São Paulo: Campinas
		Caligo beltrao (Illiger, 1801)	Brazil, Santa Catarina: Joinville
Satyrinae	Haeterini	*Haetera diaphana* (Lucas, 1857)	Brazil, Espírito Santo: Linhares
		Pierella lamia (Sulzer, 1776)	Brazil, Espírito Santo: Linhares
	Biini	*Bia actorion* (Linnaeus, 1763)	Brazil, Mato Grosso: Alta Floresta; Acre: Marechal Thaumaturgo
	Melanitini	*Melanitis leda* (Linnaeus, 1758)	Shirozu and Hara, 1974
	Zetherini	*Penthema formosana* (Rothschild, 1898)	Lee and Chang, 1988; Lee and Wang, 1995 (pp. 145–147); Wolfe, 1996
	Pronophilini	*Eteona tisiphone* (Boisduval, 1836)	Brazil, São Paulo: Campinas, São Bernardo; Minas Gerais: Poços de Caldas
		Parapedaliodes parepa (Hewitson, 1861)	Pelz, 1997
	Euptychiini	*Taygetis laches* (Fabricius, 1793)	Brazil, São Paulo: Campinas
		Paryphthimoides phronius (Godart, 1823)	Brazil, São Paulo: Campinas, São Vicente
		Pareuptychia interjecta (D'Almeida, 1952)	Brazil, São Paulo: Campinas, São Vicente
		Godartiana muscosa (Butler, 1870)	Brazil, São Paulo: Capão Bonito, Jundiaí
Limenitidinae	Cyrestini	*Cyrestis thyodamas* Boisduval, 1846	Lee and Chang, 1988; Fukuda et al., 1972; Shirôzu and Hara, 1974
		Marpesia petreus (Cramer, 1778)	Brazil, São Paulo: São Vicente
	Limenitidini	*Adelpha syma* (Godart, 1823)	Brazil, São Paulo: Jundiaí, São Bernardo
		Neptis laeta (Overlaet, 1955)	Material sent by Stephen Hall from a butterfly house; Van Son, 1963
		Hamanumida daedalus (Fabricius, 1775)	Van Son, 1979
Biblidinae	Biblidini	*Biblis hyperia* (Cramer, 1779)	Brazil, São Paulo: Campinas, Jundiaí
	Eurytelini	*Eurytela dryope angulata* (Aurivillius, 1898)	Kenya: Kilifi
		Mestra hypermestra Staudinger, 1888	Brazil, São Paulo: Campinas; Mato Grosso: Diamantino

TABLE 1. List of Nymphalidae taxa (*sensu* Harvey, 1991) used in cladistic analysis and the main study sites for each species. Localities presented as "country, state: municipalities." (Continued)

Subfamily	Tribe	Species	Localities
	Dynaminini	*Dynamine mylitta* (Cramer, 1782)	Brazil, São Paulo: Campinas, Jundiaí
	Eunicini	*Cybdelis phaesyla* (Hübner, 1827)	Brazil, São Paulo: Campinas, Jundiaí
		Eunica bechina (Hewitson, 1852)	Brazil, São Paulo: Itirapina
		Sallya natalensis (Boisduval, 1847)	Van Son, 1963
	Catonephelini	*Myscelia orsis* (Drury, 1782)	Brazil, São Paulo: Campinas, Jundiaí; Espírito Santo: Aracruz
		Catonephele numilia penthia (Hewitson, 1852)	Brazil, São Paulo: Campinas, Jundiaí, São Vicente
	Ageroniini	*Ectima thecla* (Fabricius, 1769)	Brazil, São Paulo: Campinas, Jundiaí, São Vicente
		Hamadryas epinome (Felder and Felder, 1867)	Brazil, São Paulo: Campinas
	Epiphilini	*Pyrrhogyra ophni* Butler, 1870	Brazil, São Paulo: Cubatão, São Vicente
		Temenis laothoe (Cramer, 1777)	Brazil, São Paulo: Campinas, Jundiaí, São Vicente
		Nica flavilla (Hübner, 1826)	Brazil, São Paulo: Castilho; Acre: Marechal Thaumaturgo
		Epiphile orea (Hübner, 1823)	Brazil, São Paulo: Cotia, Jundiaí
	Callicorini	*Diaethria clymena* (Cramer, 1775)	Brazil, São Paulo: Campinas, Jundiaí, São Vicente; Acre: Marechal Thaumaturgo
		Callicore hydaspes (Drury, 1782)	Brazil, São Paulo: Campinas
		Callicore sorana (Godart, 1823)	Brazil, São Paulo: Itirapina
Nymphalinae	Coeini	*Historis odius* (Fabricius, 1775)	Brazil, São Paulo: Campinas; Costa Rica, Guanacaste: Santa Rosa
		Smyrna blomfildia (Fabricius, 1781)	Brazil, São Paulo: Águas da Prata, Campinas, Cubatão
		Colobura dirce (Linnaeus, 1758)	Brazil, São Paulo: Campinas, Cubatão, Jundiaí, São Vicente
	Nymphalini	*Vanessa myrinna* (Doubleday, 1849)	Brazil, São Paulo: Campos do Jordão, Jundiaí
		Hypanartia lethe (Fabricius, 1793)	Brazil, São Paulo: Campinas, Jundiaí, São Vicente
	Kallimini	*Anartia amathea roeselia* (Eschscholtz, 1821)	Brazil, São Paulo: Campinas
		Siproeta stelenes meridionalis (Fruhstorfer, 1909)	Brazil, São Paulo: Campinas, São Vicente
		Junonia evarete (Cramer, 1779)	Brazil, São Paulo: Campinas, Cubatão, Jaguariúna
	Melitaeini	*Chlosyne lacinia saundersi* (Doubleday, 1847)	Brazil, São Paulo: Campinas
		Tegosa claudina (Eschscholtz, 1821)	Brazil, São Paulo: Cubatão, São Vicente
		Eresia lansdorfi (Godart, 1819)	Brazil, São Paulo: Campinas, São Sebastião, São Vicente
Heliconiinae	Pardopsini	*Pardopsis punctatissima* (Boisduval, 1833)	Van Son, 1963
	Acraeini	*Bematistes aganice* (Hewitson, 1852)	Van Son, 1963
		Acraea encedon (Linnaeus, 1758)	Van Son, 1963
		Actinote pellenea Hübner, 1821	Brazil, São Paulo: Cubatão, São Vicente
	Cethosiini	*Cethosia hypsea* Doubleday, 1847	Material sent by Stephen Hall from a butterfly house
	Heliconiini	*Phalanta phalanta* (Drury, 1773)	Van Son, 1979; Lee and Wang, 1995 (pp. 145–147).
		Argynnis paphia (Linnaeus, 1758)	Material from the Allyn Museum, Sarasota, FL, USA
		Euptoieta hegesia (Cramer, 1779)	Brazil, São Paulo: Campinas
		Vindula erota (Fabricius, 1793)	Material sent by Stephen Hall from a butterfly house
		Dione juno juno (Cramer, 1779)	Brazil, São Paulo: Campinas, Cubatão, São Vicente
		Dryadula phaetusa (Linnaeus, 1758)	Brazil, São Paulo: Campinas
		Dryas iulia alcionea (Cramer, 1779)	Brazil, São Paulo: Campinas, São Vicente
		Agraulis vanillae maculosa (Stichel, 1907)	Brazil, São Paulo: Campinas, Jundiaí, São Vicente
		Philaethria wernickei (Röber, 1906)	Brazil, São Paulo: Cubatão, São Vicente
		Eueides isabella dianasa (Hübner, 1806)	Brazil, São Paulo: Campinas, São Vicente
		Heliconius erato phyllis (Fabricius, 1775)	Brazil, São Paulo: Campinas, Cubatão, Jundiaí, São Vicente

they might be useful in future morphological studies in defining some lineages.

Separate analyses were carried out for three data partitions: (1) characters of immatures (eggs, larvae, and pupae) only; (2) characters of adults only; and (3) characters combined. Analyses were conducted using PAUP* 4.0b10 (Swofford, 1998), under the heuristic option with 1000 random-taxon-addition replicates. Tree searches were also conducted using the parsimony ratchet (Nixon, 1999) as implemented in PAUPRat (Sikes and Lewis, 2001). Successive approximations weighting analyses were carried out under the heuristic search option with 500 random-taxon-addition replicates. Both strict consensus and majority-rule consensus trees were calculated for the sets of most-parsimonious trees discovered by these search procedures. Nonparametric bootstrap analyses (Felsenstein, 1985) were conducted using NONA 1.8 (Goloboff, 1993), with 1000 pseudoreplicates and 10 random additions per pseudoreplicate. Bremer support and partitioned Bremer support (values (to obtain the contribution of each data set to the Bremer support values of the combined analysis) (Bremer, 1988; Baker and DeSalle, 1997; Baker et al., 1998) were calculated using TreeRot (Sorensen, 1999). The analysis was conducted with 25 random taxon addition replicates, TBR branch swapping, and 200 trees held in each replicate. The trees were drawn and printed using Tree Gardener 2.2.1 (Ramos, 1997).

Possible incongruence between the immature and adult data sets was explored using the incongruence length difference (ILD) test (Farris et al., 1994) as implemented in the program Winclada (Nixon, 2002). One thousand ILD replicates were conducted, each consisting of 10 random-taxon-addition replicates and employing TBR branch-swapping. Recent studies have shown many flaws in the ILD test (see Barker and Lutzoni, 2002), and some authors call attention to the sensitivity of the test to unequal sample sizes in the two data sets (Dowton and Austin, 2002). In the present study, however, the two data sets have nearly the same size (134 and 100 characters), and the ILD was used as a measure of heterogeneity between the two data sets (as originally proposed by Farris et al., 1994), and not as a way to validate or invalidate the combined analysis.

RESULTS

List of Characters

In all, 234 characters were obtained for the 95 species used in the analysis (Appendix 1). Of these, 134 were from immatures and 100 from adults (Table 2). Several additional characters were evaluated in previous phases of the work and were discarded as they proved to be ambiguous, not informative, with much intraspecific variation, or of difficult definition.

Phylogenetic Analysis

Combined data.—The ratchet search found 16632 equally parsimonious trees, and the random taxon addition search found 16926 trees (294 trees more than ratchet, including all found by the ratchet) with the same length (1240 steps), with CI of 21 and RI of 71. The strict consensus tree is presented in Figure 1. In the successive weighting analysis, the six subgroups were the same, but the positions of some taxa within these main groups were somewhat different (Fig. 2). The data on all subgroups and their supporting characters are in Tables 3 and 4.

TABLE 2. Number of characters used in the cladistic analysis, arranged according to the source of information.

Source of information	Number of characters		
Immature stages		134	
Eggs	15		
First instar larvae	21		
Last instar larvae	79		
General morphology			27
Scoli positions			26
Filiform setae			7
Head capsule			13
Larval behavior			6
Pupae	19		
Adults		100	
Hindwing	12		
Forewing	15		
Thorax	5		
Behavioral and chemical	7		
Head	4		
Legs	5		
Abdomen	14		
Male genitalia	25		
Female genitalia	13		
Total		234	

TABLE 3. Main subgroups of Nymphalidae based on the results of the equally weighted and successive weighting analyses.

Group	Subgroups used by Harvey (1991)
G1	Libytheinae
G2	Danainae, Tellervinae, Ithomiinae
G3	Heliconiinae, Nymphalinae (plus Coeini [= Coloburini] in the equally weighted analysis)
G4	Limenitidini and Cyrestini (plus Coeini in the successive weighting analysis)
G5	Apaturinae, Charaxinae, Morphinae, Brassolinae, Satyrinae, Calinaginae, and Biini
G6	Biblidini

Partitioned data.—In the analysis of only immatures, the ratchet search found 30355 trees with 612 steps, CI of 25 and RI of 76, the random taxon addition search found 29254 trees with same length, including 4 trees not found by ratchet. The strict consensus tree based on the total 30359 trees is showed in Figure 3. In the analysis with adults only, both ratchet and random taxon addition search found 3214 trees with 555 steps, CI of 20 and RI of 68, but each search found 2 trees not obtained by the other (3212 trees were common to both). The strict consensus tree based on the total 3216 trees is shown in Figure 4. There was significant incongruence between the two data sets (ILD test; $P = 0.002$), a fact clearly seen when the separate trees were compared (Figs. 3, 4).

If we accept the results of the combined analysis as the best estimate of nymphalid phylogeny, then a comparison of the trees in Figures 1, 3, and 4 suggests that the data from adults are the main source of conflict. The adult data set is in conflict with 27 of the 75 nodes of the combined analysis tree, whereas the data set from immatures shows only 19 nodes in conflict; 29 nodes are nonconflicting. Considering only the 26 nodes above the main tribal and subfamilial ranking (presented in Table 4), the adult data set is in conflict with the combined analysis tree in 12 nodes, against only 6 conflicting nodes of the data set of immatures; 8 nodes are nonconflicting. A recent point of view (DeBry, 2001) points out the limitations of Decay Index values used in Bremer and PBS analyses, for comparing support in a parsimony analysis; they need to be interpreted in the light of branch lengths.

Natural Groups and Subfamilies of Nymphalidae

The tree in Figure 5 was derived from the majority rule consensus, and summarizes the main results, showing the six major groups and all recognized subgroups within Nymphalidae (Table 3). Of the 37 major clades, only 4 appeared in less than 98% of the 16632 most-parsimonious trees found in the combined data analysis. The names of the three principal clades discussed below (danaoid, nymphaloid, and satyroid) are based on Freitas (1999).

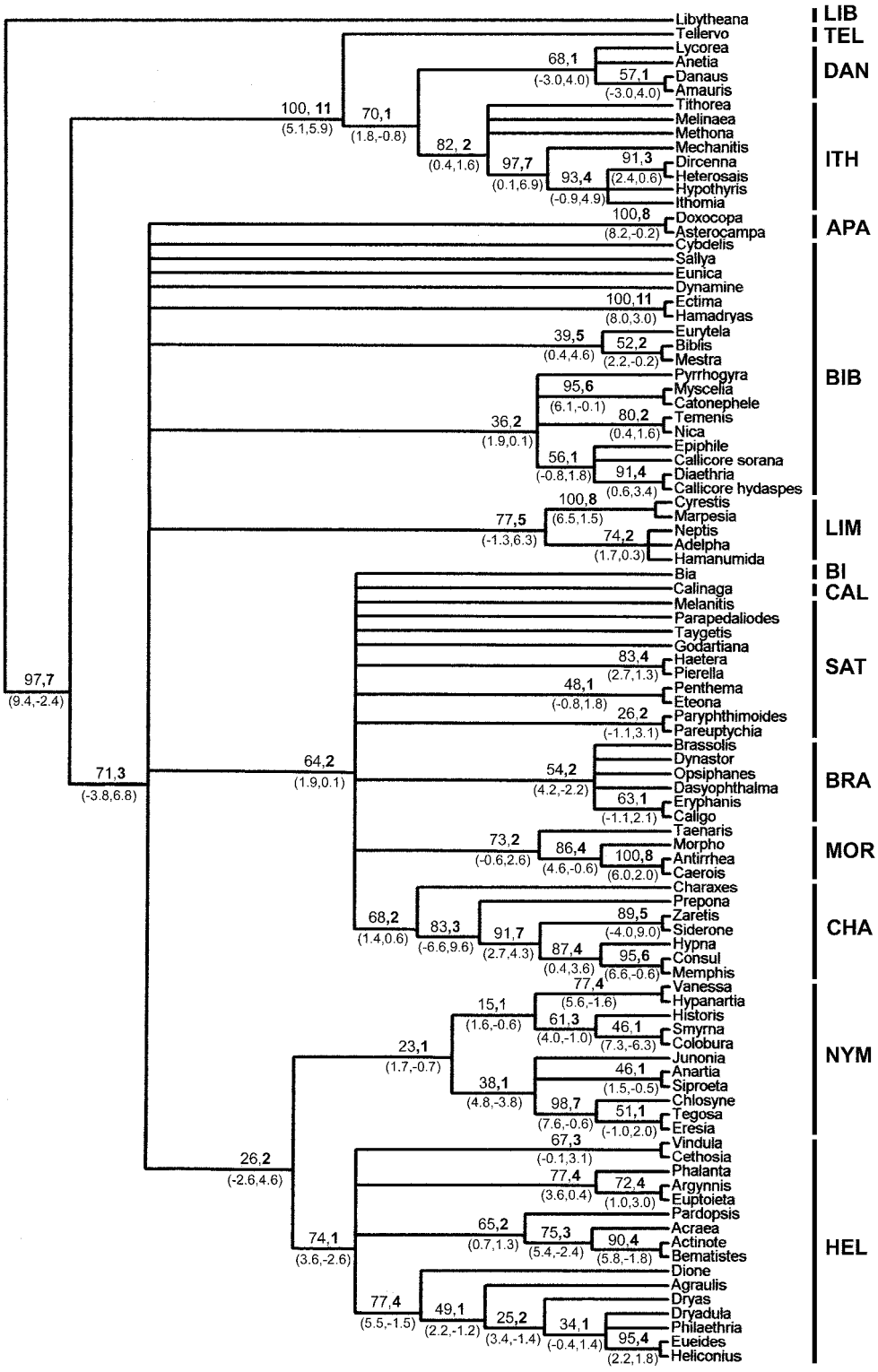

FIGURE 1. Strict consensus of 16632 equally parsimonious cladograms for the data matrix with equal weights. The numbers above the branches represent bootstrap values (regular font) and Bremer support (bold), respectively, for the node to the right of the numbers. Numbers in parentheses below the branches are the contributions of the partitions of immatures and adult characters, respectively, to the Bremer support value of the combined analysis. The subfamily codes at the right of the tree are: LIB = Libytheinae; TEL = Tellervinae; DAN = Danainae; ITH = Ithomiinae; APA = Apaturinae; BIB = Biblidinae; LIM = Limenitidinae; BI = Biinae; CAL = Calinaginae; SAT = Satyrinae; BRA = Brassolinae; MOR = Morphinae; CHA = Charaxinae; NYM = Nymphalinae; HEL = Heliconiinae.

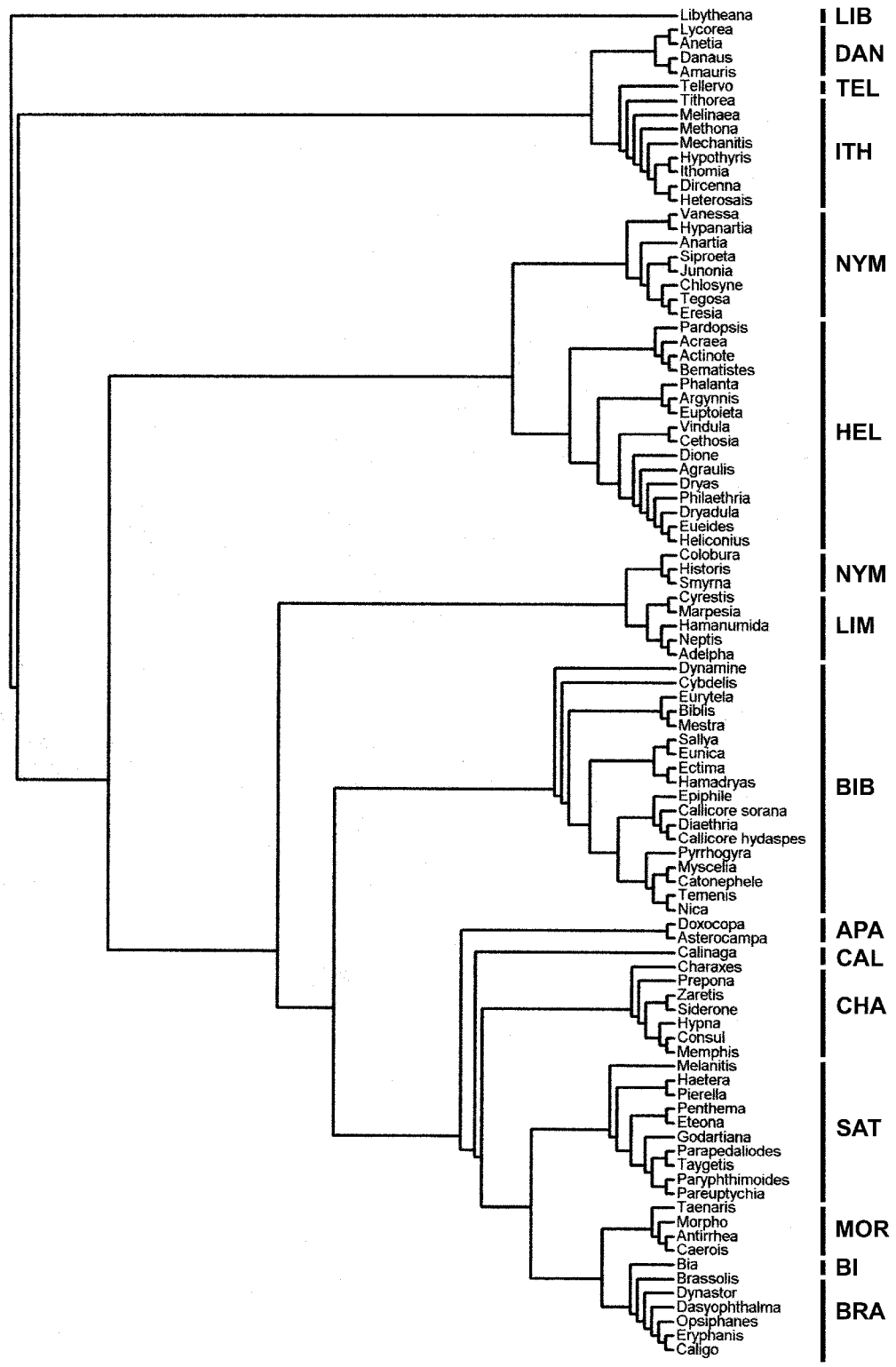

FIGURE 2. Tree obtained from successive weighting of the data matrix of Nymphalidae. Codes to the subfamilies follow Figure 1.

Group 1.—This group included only *Libytheana*, which appeared always isolated, as the basal branch of the family Nymphalidae.

Group 2.—This group (the danaoid clade) appeared as the outgroup of the remaining Nymphalidae. The three subfamilies belonging to this group, Tellervinae, Danainae, and Ithomiinae, emerged as monophyletic in all trees. Danainae + Ithomiinae were sister groups, and Tellervinae appeared as the basal group of this clade in the equal weighted analysis, but basal to the Ithomiinae

TABLE 4. Character support for the different branches of Nymphalidae (equal weighted analysis, majority consensus). The number of the character is followed by the state in the branch (in parentheses). Groups follow Table 3.

Synapomorphies →	Exclusive		Homoplasious	
Group	Early stages	Adults	Early stages	Adults
Libytheinae (G1)	absent	absent	29(1), 31(1), 44(1), 97(1), 116(1)	138(1), 149(1), 197(1), 198(1), 232(1), 233(1)
Danainae	absent	184(1)	absent	174(1), 178(1), 197(1), 200(1), 201(1), 217(1)
Tellervinae	absent	absent	17(1), 37(1), 116(1)	173(1), 218(1)
Ithomiinae	absent	147(1)	118(1)	175(2), 206(1)
Danainae + Ithomiinae	absent	absent	absent	143(1), 152(1), 181(1)
Heliconiinae	77(1), 79(1), 90(1)	absent	1(2), 64(0), 67(0)	171(1), 183(1)
Cethosini	absent	absent	42(1), 101(3), 113(1), 127(1)	absent
Argynnini	absent	absent	4(1), 14(1), 43(0), 101(0)	159(1), 222(0)
Acraeini	absent	185(1)	absent	135(1), 154(1)
Heliconiini	absent	195(1)	absent	135(1), 143(1), 149(1), 153(1)
Nymphalinae	78(1)	absent	6(1), 14(1), 66(1)	138(1), 149(1), 197(1), 228(1)
Kallimini + Melitaeini	88(1), 91(1)	absent	85(1), 86(1)	207(1), 220(1)
Melitaeini	absent	absent	1(2), 6(0), 13(1), 14(0), 69(1), 80(1), 92(1)	181(1), 183(1), 200(1), 201(1), 214(1), 217(1)
Nymphalini	absent	absent	92(1), 101(0), 113(1)	absent
Coeini	absent	absent	52(1), 54(0), 57(1), 115(1)	168(2), 169(0), 170(1), 175(0)
Coeini + Nymphalini	absent	absent	absent	208(1), 211(2)
Limenitidini	9(1)	absent	11(1), 64(0), 67(0)	208(1), 230(1)
Cyrestini	50(1), 64(2)	absent	1(1), 6(1), 125(1)	173(1), 181(1), 197(1), 217(1)
Apaturinae	absent	absent	6(1), 121(1)	135(1), 138(1), 149(1), 175(0), 197(1), 200(1), 224(1), 225(1), 231(1)
Charaxinae	12(1)	163(1)	10(1), 120(1)	164(0)
Morphinae	59(1)	absent	5(1), 10(1), 23(1), 28(1), 120(1)	absent
Brassolinae	absent	151(1)	44(0)	172 (1), 207(0)
Satyrinae	96(1)	absent	5(1), 10(1), 32(1)	absent
Calinaginae	absent	absent	3(1), 25(0), 35(0)	135(1), 154(1), 159(1), 209(0), 211(0), 213(1)
Biinae	absent	absent	7(1), 14(3), 33(1), 44(0), 101(3), 102(1), 108(1), 117(1), 122(1), 134(1)	136(1), 137(1), 141(1), 160(1), 172(1), 200(1)
G2	21(1), 22(1), 94(1), 114(1), 123(1)	167(1), 177(1)	25(1), 27(1), 42(1), 120(1), 133(1)	183(1), 208(1)
G3	absent	absent	1(1), 23(1)	141(1)
G4	74(2)	absent	14(1), 24(1), 68(0), 81(1), 83(1), 115(1)	213(1), 216(1)
G5	16(1), 60(1)	absent	51(0), 126(0), 133(1)	absent
(Biblidinae) G6	absent	187(1)	4(1), 23(1), 127(1)	absent
G2, G3, G4, G5, G6	26(1), 39(1)	162(1), 165(1), 182(1)	119(1)	156(1), 175(1), 222(1)
G3, G4, G5, G6	51(1)	144(1), 166(1), 169(1)	28(1) 101(1)	absent
G4, G5, G6	absent	168(1)	17(1)	159(1)
G5,G6	absent	absent	104(1), 119(0)	146(1), 207(1)
Total	23	16	96	88

in the weighted analysis. The bootstrap value for this group was always very high.

Group 3.—This group (the nymphaloid clade) was formed by the subfamilies Nymphalinae and Heliconiinae (*sensu* Harvey, 1991), and is the sister group of the next three groups. Both Nymphalinae and Heliconiinae emerged as monophyletic in all trees. Within the Nymphalinae, only the tribe Kallimini was not monophyletic. The Coeini emerged as a tribe of Nymphalinae, and the relationships among the tribes show that Melitaeini + Kallimini form the sister group of Nymphalini + Coeini. In the successive weighting, the Coeini appeared together with group 4. The bootstrap support for this group was low, but the bootstrap value for Heliconiinae was moderately high.

Group 4.—This group represents the Limenitidinae minus the Biblidini (*sensu* Harvey, 1991). It is formed by two monophyletic tribes, Limenitidini and Cyrestini. The Coeini could be the sister group of these, as shown by the successive weighting analysis. The bootstrap values for this group were moderately high.

Group 5.—Seven subfamilies (the satyroid clade minus Biblidinae and Limenitidinae) belong to this group in

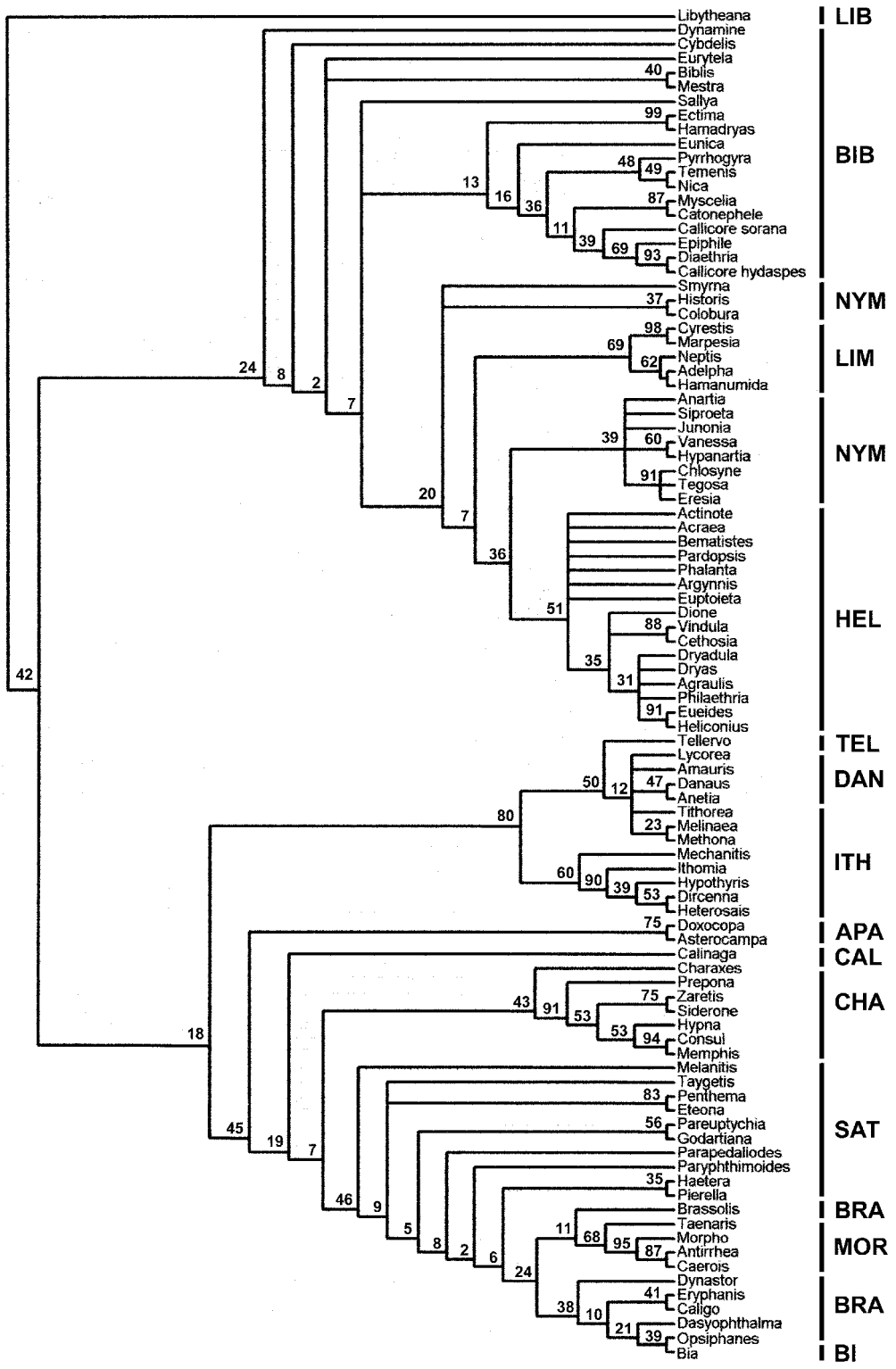

FIGURE 3. Strict consensus of 30355 equally parsimonious cladograms for the data matrix of immatures only. The numbers above the branches represent bootstrap values. Codes to the subfamilies follow Figure 1.

the majority rule consensus. Even though all subfamilies are shown as monophyletic, some of the relationships are not resolved. The Apaturinae form the basal group of this clade, in the majority rule, but not included in this group in the strict consensus. The subfamily Charaxinae always appeared in this group, as basal after Apaturinae in the majority rule and without clear relationships in the strict consensus. In the remaining subfamilies,

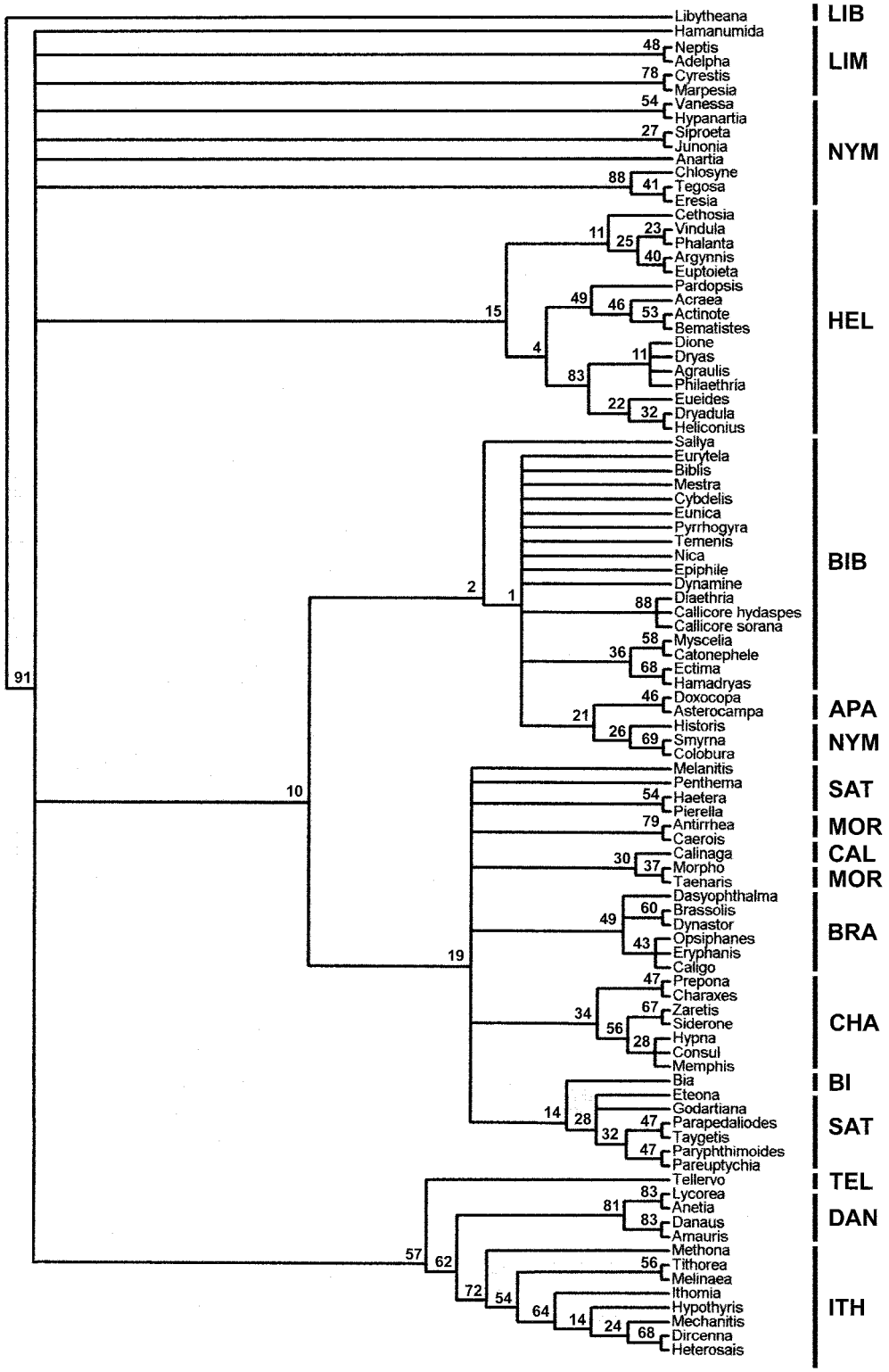

FIGURE 4. Strict consensus of 1669 equally parsimonious cladograms for the data matrix of adults only. The numbers above the branches represent bootstrap values. Codes to the subfamilies follow Figure 1.

Brassolinae + Morphinae appeared as sister groups in all trees, and Satyrinae was a monophyletic group (but appeared as a paraphyletic group in the strict consensus tree). The positions of two taxa, *Calinaga* and *Bia*, remained unresolved in the current analysis. The genus *Bia* appeared off the Satyrinae, and is considered as a subfamily (Biinae). The position of this taxon is ambiguous, as it appeared in three different positions in the trees: as

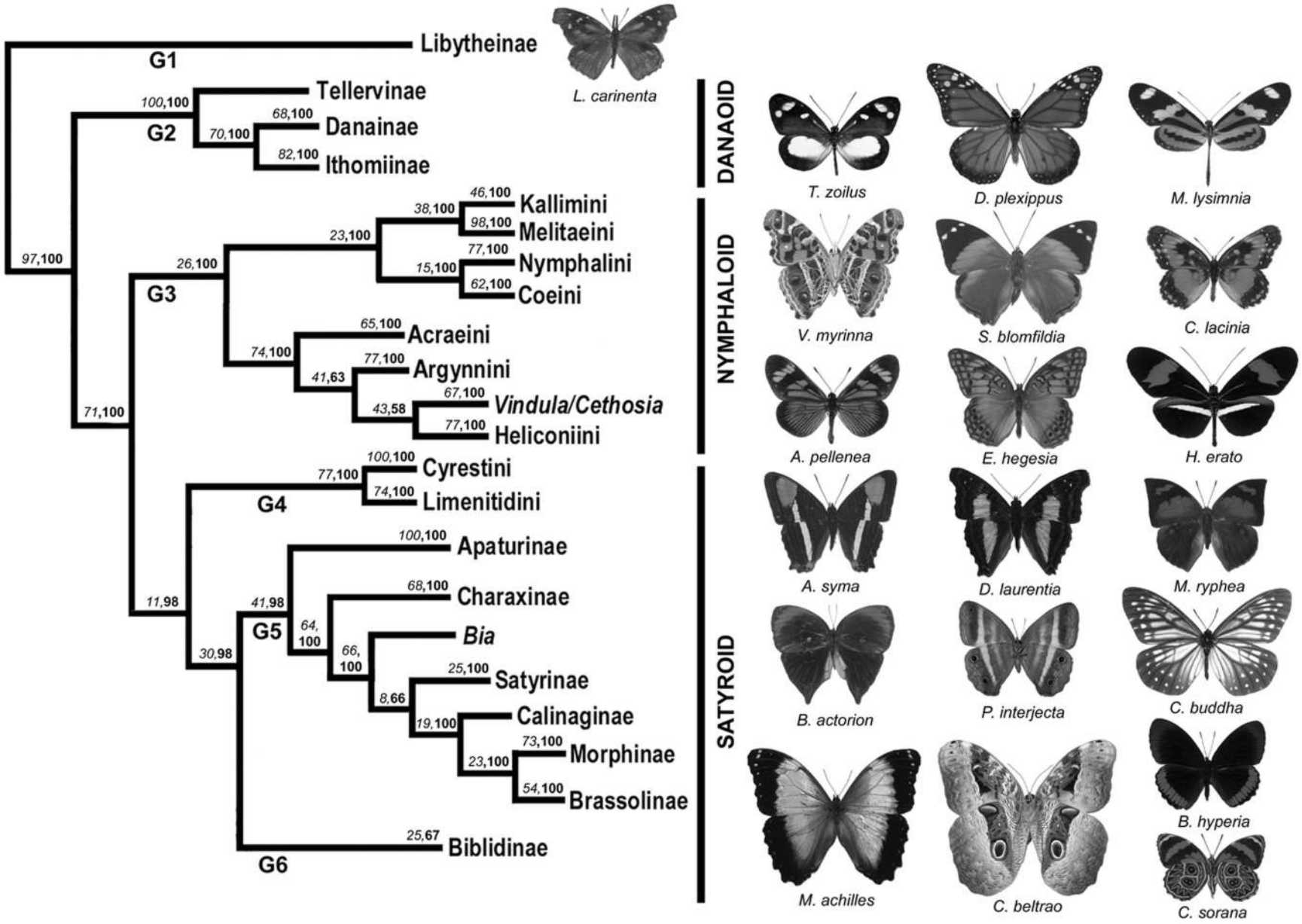

FIGURE 5. Summary tree based on the majority rule consensus of the combined data, showing the relationships among the subfamilies (as in Figure 1). The numbers above each branch represent its bootstrap value (in italics), and the percentage of the 16632 most parsimonious trees in which this branch is found (bold). The groups discussed in Table 4 are shown in the major branches (G1 to G6), and the three main clades are indicated at right. Values are not given for single taxon branches (Libytheinae, Tellervinae, *Bia*, and Calinaginae).

the basal group of the clade formed Satyrinae + Morphinae + Brassolinae + Calinaginae; as the sister group of Morphinae + Brassolinae + Calinaginae; and as basal to the Satyrinae. However, in the successive weighting analysis, *Bia* appeared together with the Brassolinae, as the basal taxon (see additional information in Freitas, Murray, and Brown, 2002). The genus *Calinaga* also is considered as belonging to its own subfamily, the Calinaginae. This taxon appeared together with the group Brassolinae + Morphinae in most trees, as the outgroup of this clade or as the basal taxon of Morphinae. After successive weighting, *Calinaga* became a basal taxon in group 5, just after Apaturinae, as the outgroup of Charaxinae + Satyrinae + Brassolinae + Morphinae. The bootstrap value for this group (without Apaturinae) is moderately high.

Group 6.—The single subfamily Biblidinae (= Eurytelinae auctt.) forms this group. It corresponds to the tribe Biblidini of Harvey (1991), which is here removed from the Limenitidinae to gain the status of a subfamily. This group it is the sister of group 5. In the strict consensus tree, Biblidinae appeared as paraphyletic.

DISCUSSION

The Combined Analyses and Relationships Within Nymphalidae

All 13 Nymphalidae subfamilies recognized by Harvey (1991) and many additional subgroups were covered in this study. All major recognized groups appeared as monophyletic in the majority consensus, and the relations within them were stable. In strict consensus, however, Biblidinae and Satyrinae appear as paraphyletic, reflecting the need for additional characters defining these two groups. The remaining subgroups were stable even with the great number of trees, and most of the remaining variation affects only the positions of some terminal taxa.

All recognized monophyletic subfamilies and groups of Nymphalidae were supported by one or more characters. The results obtained give support to Harvey's (1991) classification, probably in part due to the use of data from immature stages in both studies. Characters from immature stages were important in giving support for many branches (see Table 4), especially within the Nymphalinae clade.

The subfamily Libytheinae (represented by *Libytheana* in the present study) appeared as basal to the remaining Nymphalidae; its consistent appearance as an isolated branch confirms its distance from the other taxa, and agrees with most previous hypotheses based on adult morphological studies (Ehrlich, 1958; Scott, 1985; De Jong et al., 1996). Modern studies using molecular data (Weller et al., 1996; Brower, 2000) have also emphasized the isolation of the Libytheinae, which, due to several similarities in the immatures with Pieridae (Freitas, 1999) and hierarchic reasons (Vane-Wright, 2003), could be evidence for supporting familial rank (even if in Brower, 2000, Libytheinae did not appear as the basal taxon of Nymphalidae). Evidence from host plant use (Freitas, 1999), morphology (Häuser, 1993; De Jong et al., 1996) and geographic distribution (Ackery, 1984) together continue to suggest that this group is the outgroup of the remaining Nymphalidae (Vane-Wright, 2003), as a basal subfamily. The position of this taxon depends to a considerable extent on the accuracy of our character-state polarizations, used to code the hypothetical ancestor that served as an outgroup. Additional data will be needed to define the basal position of Libytheinae.

The position of Tellervinae + Danainae + Ithomiinae as basal to the remaining Nymphalidae agrees with previous morphology-based studies (Ehrlich, 1958; Ehrlich and Ehrlich, 1967; Scott, 1985; De Jong et al., 1996), but not with analyses of molecular data (Martin and Pashley, 1992; Weller et al., 1996; Brower, 2000). This conflict could result from the limited sampling of tropical taxa in molecular studies, or from the choice of a derived species to represent Danainae (usually a species of *Danaus*) in the early studies. This problem could be solved with the inclusion of additional species of Ithomiinae in the analysis (as proposed by Martin and Pashley, 1992), or through a search for additional molecular data for the analysis (Weller et al., 1996; Brower and Egan, 1997). In fact, in the study of Wahlberg et al. (2003) with one mitochondrial and two nuclear genes, the Danainae + Ithomiinae clade appeared as basal to the whole Nymphalidae (except Libytheinae).

The position of Calinaginae as basal to the Morphinae + Brassolinae is new. Even though the larva was recognized as bearing caudae (Ehrlich, 1959), this position within the satyroid clade (near the Apaturinae or within the Satyrinae) was previously recognized by very few authors (e.g., Moore *in* Horsfield and Moore, 1858; Felder, 1861:27; Butler, 1885:309). Recently, Wahlberg et al. (2003) placed *Calinaga* as outgroup of the Charaxinae, in the satyroid clade, but combination of these molecular data with the present morphological set showed *Calinaga* as basal to the entire satyroid clade (Wahlberg and Freitas, in preparation).

The Coeini have been recently suggested as part of the Nymphalinae (Freitas, 1999; Brower, 2000; Wahlberg et al., 2003), and Brown (1992) recognized that the coeine genus *Smyrna* could be near to *Hypanartia* (tribe Nymphalini), in the Nymphalinae. It is interesting to note that many temperate species of Nymphalini such as *Polygonia* and *Nymphalis* are known to feed on fruits (Scott, 1986, and personal observations), giving support to the appearance of a primarily fruit feeding group deriving from Nymphalini.

The definition of Biblidinae as a monophyletic group separate from the Limenitidinae *sensu latu* was not recognized by early authors, perhaps because few taxa were included in the analysis. Harvey (1991) pointed out the homogeneity of the Biblidini, but placed it as a tribe of Limenitidinae. Recent molecular studies have found Biblidinae independent of Limenitidinae (Brower, 2000; Wahlberg et al., 2003). This group is well supported by the presence of an hypandrium in the adult males (Jenkins, 1990), and recent information confirms its monophyly and position separate from

Limenitidinae (Freitas, 1999 and Vane-Wright, personal communication). The present results could end the discussion about the unity of the Limenitidinae sensu latu, separating this paraphyletic group into at least two (or three if Coeini be considered) monophyletic clades that are not obligatory sister groups.

The next steps to understanding remaining problems in the subgroups of the family Nymphalidae could be:

1. Study of the subgroups of Nymphalidae (like the subfamilies Heliconiinae, and Biblidinae and the danaoid and satyroid lineages), with refined data adapted to the subgroup. Within these subgroups, characters that are homoplastic in the Nymphalidae could be stable and consistent, revealing different internal relationships among the tribes and genera. Some intermediate character states, which make little sense in the family analysis (for example the different kinds of hairpencils in Ithomiinae), become useful in a more restricted analysis.
2. Examine in more detail the positions of the tribe Coeini and the subfamilies Tellervinae, Biinae and Calinaginae.
3. Look carefully for evidence supporting positions of Pseudergolini and other exclusively Old World taxa.
4. Search the "total known evidence," using the largest and most complete data set possible, by combining all known characters used in previous works with those of the present study and with molecular data available in the literature.

Separate Analyses

Separate analyses showed that different sources of characters can result in very different hypotheses of internal relationships among the major groups of Nymphalidae. Some groups and relationships were constant in both juveniles and adults and also in combined analyses, including the establishment of monophyletic groups such as Tellervinae + Danainae + Ithomiinae, and the subfamilies Heliconiinae, Charaxinae, and Limenitidinae. Characters from immatures were important in defining the Morphinae and the subfamily Nymphalinae (*sensu* Harvey, 1991; a polyphyletic and poorly defined group when based on characters from adults). Characters from adults defined the Brassolinae and the satyroid lineage, but all internal relationships were lost, resulting in a tree very similar to those proposed in previous works based mostly on adult characters (Ackery, 1984, 1988). As shown by all analyses, data from immatures contributed much to the topology of the trees obtained from the combined data, including the fact that characters from immatures were most useful in defining the main lineages if compared with characters from adults (both exclusive and homoplasious, Table 4).

The present results show that, based on morphological characters, we should assume that:

1. Only combined analyses gives a good resolution for the phylogeny of Nymphalidae.
2. Data from immatures can be extremely important in defining the topology of the combined trees.

Previous results without resolution in the internal branches of Nymphalidae and the nonrecognition of the several different subfamilies hidden within this group could be ascribed to lack of knowledge of immature characters (many of these groups such as Nymphalinae *sensu strictu*, emerged only in the analysis of the immature data set, and later in the combined analysis), because many groups were well defined by these (such as the Nymphalinae).

This scenario, especially when compared with previous studies (DeVries et al., 1985; Kitching, 1985; Motta, 1989, 1998, 2003; Brown and Freitas, 1994; Freitas et al., 1997; Penz, 1999), suggests that characters from immatures are crucial to understanding the evolution of Lepidoptera; in a broader view, this could be usefully applied to all holometabolous insects.

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APPENDIX 1

LIST OF CHARACTERS

Characters used in the cladistic analysis. All characters (including multistate) are ordered. The figures show some of the characters as number and (in parentheses) state. Most apomorphies are shown, but plesiomorphic states are also illustrated in some cases. Additional information on immatures can be found in Freitas (1991, 1993, 1996, 1999), Freitas and Oliveira (1992), Brown and Freitas (1994), and Freitas et al. (1997).

Eggs

Most characters for eggs are shown in Figure A1. Some data on egg color and shape can be found also in Brown and Freitas (1984) and Freitas et al. (1997).

1. Color: white (0), green (1), yellow (2) [unordered]
2. Surface: smooth (0), hairy (1)
3. Ratio length/diameter: more than 1.0 (0), between 0.99 and 0.61 (1), equal to or less than 0.6 (hemispheric egg) (2). This character was based on Motta (1989) who studied Ithomiinae only, and adapted to the Nymphalidae. The intervals were based on general shapes of the eggs, separating elongated from spherical eggs. Based on preliminary analyses excluding this character, the hemispheric egg (restricted to the Morphinae) was considered the following step of this transformation series.
4. General shape: rounded (oval, slender or hemispheric) (0), trapezoid/truncate (1)
5. Longitudinal ridges: present (0), absent (1)
6. Longitudinal ridges strongly marked: absent (0), present (1)
7. Average number of longitudinal ridges: less than 30 (0), 31 or more (1). The number of longitudinal ridges in eggs of the outgroup taxa

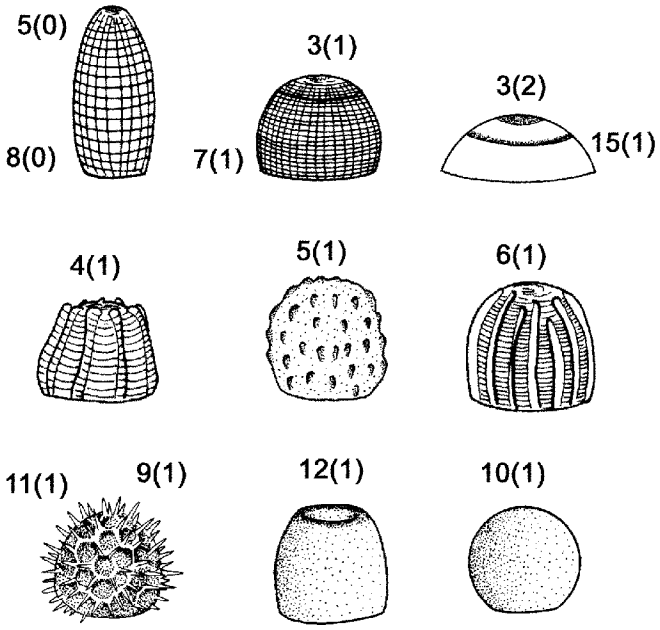

FIGURE A1. Eggs of Nymphalidae (not to scale). Left to right, top to bottom: *Mechanitis*, *Opsiphanes*, *Morpho*, *Myscelia*, *Ectima*, *Doxocopa*, *Adelpha*, *Memphis*, and *Pareuptychia*.

and in most Nymphalidae ranges from 8 to 25. Eggs with 31 to 60 longitudinal ridges were found only in some Brassolinae. The results were not changed with the exclusion of this character.

8. Longitudinal ridges: most reach the egg apex (0), several ending/anastomosing near half the height (1)
9. Cell shape: quadrangular (0), hexagonal (1), irregular (2) [unordered]
10. Transverse ridges: present (0), absent (1)
11. Spiny projections at the intersection of the ridges: absent (0), present (1)
12. Apex: convex (0), concave (1)
13. Oviposition pattern: isolated eggs (0), grouped eggs (1)
14. Position in nature: under leaf (0), upper leaf surface (1) tendrils (2), other places (3) [unordered]
15. Fertile egg with concentric circles appearing after laying: absent (0), present (1)

First Instar

A general scheme of setal distribution is presented in Figure A2, and specific characters are shown in Figure A3.

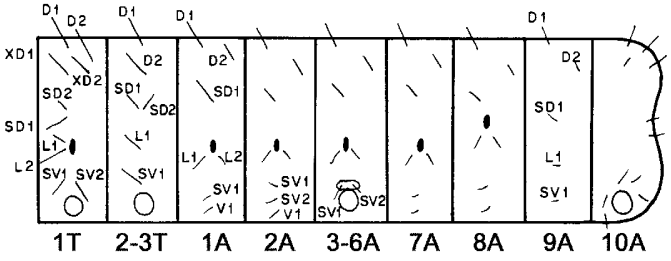

FIGURE A2. Scheme of a first instar of Nymphalidae illustrating the setae (names following Nakanishi, 1988).

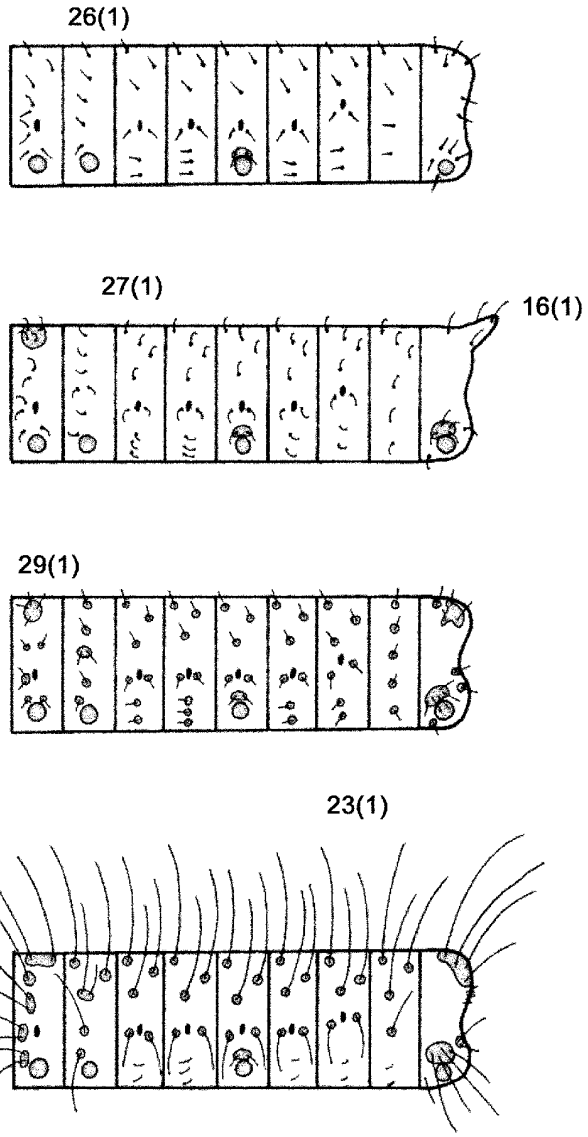

FIGURE A3. Schemes of first instar of Nymphalidae showing the main setal types and chaetotaxy. Top to bottom: *Libytheana*, *Archaeoprepona*, *Myscelia*, and *Euptoieta*.

16. Caudae: absent (0), present (1)
17. Thoracic leg colour: dark (0), light (1)
18. Prolegs dark laterally: present (0), absent (1)
19. Crochets on prolegs: up to 25 (0), more than 25 (1). The number of crochets on prolegs of the outgroup taxa and most Nymphalidae vary from 15 to 20. Prolegs with more than 25 crochets were considered as apomorphic. In fact, most of the species included in the latter category present a number much higher than 25 crochets (40 or more).
20. Crochets on prolegs: eight or more (0), fewer than eight (1). Only two of the studied taxa presented less than 8 crochets, defining the apomorphic state in this case.
21. Thoracic tubercles: absent (0), present (1)
22. Body rings: absent (0), present (1)
23. Relative length of the primary setae: less than half the segment height (0), equal to or longer than the segment height (1)
24. Colour of the setae: dark (0), light (1)
25. Pinacula: sclerotised (0), not sclerotised (1)

26. Number of SD setae on 2T and 3T: one (0), two to five (1)
27. Additional SD setae on the abdominal segments: absent (0), present (1)
28. Additional XD seta on prothorax: absent (0), present (1)
29. Prothoracic plate: present (0), absent (1)
30. Head capsule: dark (black, dark brown, red, orange) (0), light (green, yellow, transparent) (1)
31. In dark head capsules, light areas: absent (0), present (1)
32. A pair of short scoli on head capsule: absent (0), present (1)
33. Head capsule: few setae (0), pilose (1)
34. In pilose head capsules, setae: rounded (0), flat (1)
35. Head capsule "neck": normal (0), projecting as a tube section (1)
36. Relative size of the third stemmata: same as the others (0), larger than the others (1)

Last Instar (Except 102)

The last instar (essentially always the fifth, but can vary from four to seven, see Scott, 1986:21; DeVries, 1987:6) was always used because it bears fully developed structures, many times difficult to see in earlier instars (except for characters of the first instar only). A scheme combining most of the possible body structures of a last instar of Nymphalidae is shown in Figure A4 (adapted from Scott, 1986:11); the general distribution of scoli is presented in Figure A5. Additional information on some structures can be found in Freitas and Oliveira (1992), Brown and Freitas (1994), Freitas et al. (1997), and Freitas (1999).

37. Color of the legs: dark (black, brown, or red) (0), light (green, yellow) (1)
38. Proleg shield: dark (0), light (1)
39. Lateral crochets: present (0), absent (1)
40. Lateral stripe: present (0), absent (1)
41. Additional longitudinal stripes: absent (0), present (1)
42. Body rings: absent (0), present, continuous (1), broken (2). A good explanation of polarization of this character in Ithomiinae is present in Brown and Freitas (1994), showing that broken body rings are a second step of modification of continuous rings. Body rings are absent in most of the outgroup taxa, and also in *Libytheana*. Preliminary analyses excluding this character showed that the broken rings should be a state derived from the continuous rings of basal Ithomiinae.
43. Predominant ventral color: light (green, white, or yellow) (0), dark (black, brown, or red) (1)
44. Color of the anal plate (or cauda if present): dark (black, brown, red) (0), light (green, white, or yellow) (1)
45. Segment 1A: same size as remaining (0), enlarged (1), enlarged bilobed (2)
46. Subdorsal slender flexible scoli: absent (0), present (1)
47. Subdorsal tubercles on mesothorax: absent (0), present (1)
48. Subdorsal tubercles on metathorax: absent (0), present (1)
49. Subdorsal tubercles on 8A: absent (0), present (1)
50. Dorsal slender rigid scoli: absent (0), present (1)
51. Rigid scoli: absent (0), present (1), present and strongly reduced (2)
52. Color of the branched scoli: dark (0), light (1)
53. Median and subspiracular scoli in larvae with subdorsal scoli developed: present (0), reduced (1), absent (2)

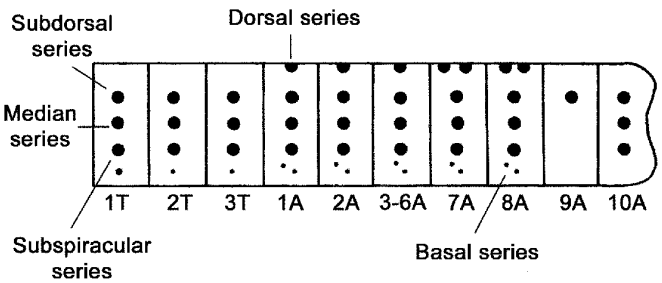

FIGURE A4. Scheme of a last instar of Nymphalidae illustrating the main types of morphological structures in the body.

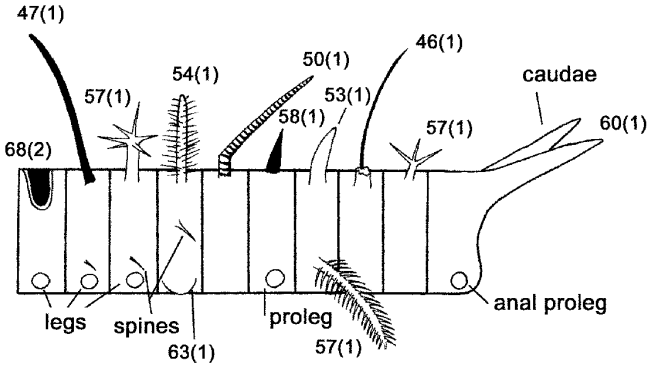

FIGURE A5. Scheme of a last instar of Nymphalidae showing all possible series of body scoli.

54. Feathered rigid scoli: absent (0), present (1)
55. Feathered rigid scoli: longer than half segment height (0), shorter than half segment height (1)
56. Prothoracic feathered rigid scoli longer than the others: absent (0), present (1)
57. Branched rigid scoli: absent (0), present (1)
58. Dorsal cuneiform projections: absent (0), present (1)
59. Body covered by abundant soft hairs: absent (0), present (1)
60. Caudae: absent (0), present (1)
61. Relative size of caudae: shorter than segment A10 (0), up to four times segment A10 (1), more than four times segment A10 (2)
62. Caudae with both arms free (0) or fused (1)
63. Sublateral expansions semicircle-shaped: absent (0), present (1)

Scoli

Dorsal series

64. Scoli from 1A to 7A: absent (0), present (1), present on few segments (2)
65. Additional distal scolus on 7A: absent (0), present (1)
66. Additional proximal scolus on 8A: absent (0), present (1)
67. Additional distal scolus on 8A: absent (0), present (1)

Subdorsal series

68. Scolus on 1T: absent (0), present, spine-shaped (1), modified as a plate (2)
69. If scolus on 1T present: single (0), several short (1)
70. Scoli on 2T and 3T: equal in size to scoli on 3A to 6A (0), larger than scoli on 3A to 6A (1)
71. Scolus on 10A: absent (0), present (1)

Median series

72. Scolus on 1T: absent (0), present (1)
73. Scoli on 2T and 3T: present (0), absent (1)
74. Median series present on 1A to 8A and 10A (0), absent from 1A to 8A and present on 10A (1), absent on 10A (2)
75. Median series present on 1A to 8A and 10A (0), absent on 1A (1)
76. Median series present on 1A to 8A and 10A (0), absent on 2A (1)
77. Scoli on 2T and 3T: aligned with remaining scoli (0), shifted to anterior portion of the segment (1)
78. Additional scolus on 9A posteroventral to the filiform setae: absent (0), present (1)
79. Additional scolus on 9A dorsal to the filiform setae: absent (0), present (1)

Subspiracular series

80. Scoli on 2T and 3T: absent (0), present (1)
81. Subspiracular series from 1A to 7A: present (0), absent (1)

82. Subspiracular series from 1A to 7A: single (0), a lateral pair of short scoli (1)
83. Additional scolus on 8A: present (0), absent (1)
84. Additional scolus on 10A: absent (0), present (1)

Basal series

85. Scolus on 1T: absent (0), present (1)
86. Bas al series from 2T to 8A: absent (0), present (1)
87. Basal series from 2T to 8A: one or two present (0), three present (1)
88. Basal series from 2T to 8A: present and complete (0), absent on 8A (1)
89. Additional scolus on 9A: absent (0), present (1)

Filiform setae

These characters were based entirely on Harvey (1991, and personal communication), who used for the first time the positions of filiform setae in the classification of the Nymphalidae.

90. Insertion of filiform setae from 3A to 8A: on the body (0), on the base of the scolus or above it (1)
91. Filiform seta on 9A: arising from the body (0), arising from the base of the scolus (1)
92. Filiform setae on 1A and 2A: absent (0), present (1)
93. Anterior filiform seta on 1T: absent (0), present (1)
94. Filiform setae on 2T and 3T: present (0), absent (1)
95. Filiform seta on 8A: present (0), absent (1)
96. Filiform setae on 3A and 8A: present (0), absent (1)

Head capsule

97. General colour: dark (black, brown, red, or orange) (0), light (green, yellow, or transparent) (1)
98. In dark head capsules, light areas: absent (0), present (1)
99. Furry head capsule: absent (0), present (1)
100. General shape: rounded (0), triangular (1)
101. Scoli on head vertex: absent (0), up to 1/3 head height (1), up to 3 times head height (2), more than 3 times head height (3)
102. Scoli on head vertex in second instar: short (half head height) (0), long (up to twice head height) (1)
103. Scoli on head vertex: dark (0), light (1)
104. Scoli on head vertex: "naked", without ornaments (0), with ornaments (1)
105. Scoli on head vertex: without apical crown (0), with apical crown (1)
106. Scoli on head vertex: simple (0), forked at the apex (1)
107. Scoli on head vertex curved posteriorly: absent (0), present (1)
108. Three additional pairs of scoli: absent (0), present (1)
109. Frontal pair of short tubercles (cones): absent (0), present (1)

Larval behavior

110. Takes refuge in rolled leaf: absent (0), present (1)
111. Takes refuge on island-like leaf segments: absent (0), present (1)
112. Inflation of the thorax when disturbed: absent (0), present (1)
113. Construct shelters of leaf pieces and silk: absent (0), present (1)
114. Rests in "J" position: absent (0), present (1)
115. Constructs frass chains: absent (0), present (1)

Pupae

The principal kinds of Nymphalidae pupae are illustrated in Figure A6. Additional information is found in Brown and Freitas (1994) and Freitas (1999).

116. General profile: elongate (0), short (1)
117. Reflective areas: absent (0), present (1)
118. Reflective areas present: restricted (0), widespread (1), widespread but weak (2). This character was also coded using data from the Ithomiinae (see Brown and Freitas, 1994). Previous analysis excluding this character showed that when reflective areas are present, the restricted distribution is the first step, followed by a widespread distribution of the reflection. The following step was present only in species with widespread reflective areas, and was considered as an additional step following the latter.
119. General color: green (0), yellow (1), brownish tones (2)
120. Abdominal segments: mobile (0), immobile (1)
121. Pupal position: suspended (0), on top of leaf (1)
122. Suspended pupae: perpendicular to the substrate (0), parallel to substrate (1)
123. Dorsal shelf on the third abdominal segment: absent (0), present, well developed (1), reduced (2) – This character is relevant only to the Ithomiinae, and an explanation of the polarity is in Brown and Freitas (1994). The placement of a reduced dorsal shelf as an additional step of a well developed dorsal shelf was obtained after previous analyses excluding this character.
124. Dorsal shelf on the fourth abdominal segment: absent (0), present (1)
125. Ocular caps: very short bumps or absent (0), up to 1/4 pupal length (1), more than 1/4 pupal length (2)
126. Cremaster in ventral view: slender (0), wide (1)
127. Mesothorax: not projecting (0), a conspicuous bump present (1), strongly projecting as an appendix (2)
128. Segment 2A: not projecting (0), projecting (1)
129. Spines on abdomen: absent (0), present, short (1), present, long (2)
130. Dorsal abdomen: profile rounded (0), flat, with lateral crests (1)
131. Dorso-lateral crests on abdomen: simple (0), one pair of short projections between 2A and 3A (1), projections long with spines (2)
132. Pupal angle (between thorax and abdomen): more than 120° (straight pupa) (0), 120° to 90° (bent pupae) (1)
133. Lateral thoracic bumps: four (0), two or absent (1)
134. Alar caps: not projecting (0), projecting laterally (1)

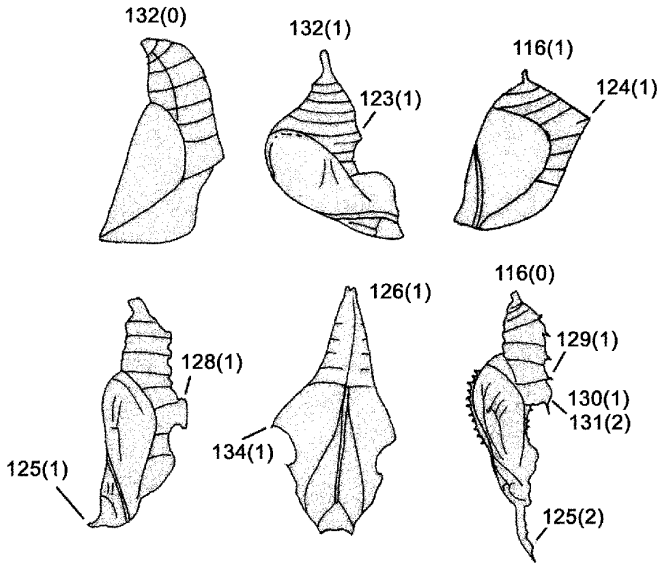

FIGURE A6. Pupae of Nymphalidae (not to scale). Left to right, first row: *Libytheana*, *Tithorea*, and *Memphis*; second row: *Adelpha*, *Callicore*, and *Heliconius*.

Adults

Data for adult Nymphalidae are available in many major papers, especially data for wing venation and morphology of legs (see Ehrlich, 1958; Ehrlich and Ehrlich 1967; DeVries et al., 1985; deJong et al., 1996; and Otero, 1990, for Vogel's organ morphology). Illustrations are provided only for morphological characters with little information available in the literature (thorax and genital apparatus of males and females). Character 177 for head adapted from Ehrlich (1958).

Forewing

135. Ratio between width and length: less than 1.9 (normal wing) (0), equal or more than 1.9 (elongate wing) (1). A general squared wing was considered plesiomorphic, because it is the state present in most outgroup taxa. Wings with the above ratio equal to or higher than 1.9 were considered as elongated.
136. Ratio between width and length: more than 1.5 (normal wing) (0), equal to or less than 1.5 (short wing) (1). Following character 135, wings were considered short when the above ratio was equal to or less than 1.5.
137. Inflated costal veins: absent (0), present (1)
138. Discal cell: closed (0), open (1)
139. Discal cell closed, position of recurrent vein: high (over M2) or absent (0), opposite to M2 (1), low (2)
140. Short vein at the base of the discal cell: absent (0), present (1)
141. Position of M2 in closed discal cell: arising proximal to m1–m2 (0), arising distal to m1–m2 (1)
142. Hair tuft on ventral hindwing: absent (0), present (1)
143. Ratio between forewing and hindwing length: equal to or more than 0.7 (0), less than 0.7 (1)
144. Vein 3A free at base: present (0), absent (1)
145. Transverse vein m2–m3: distal to M3 (0), proximal or on M3 (1)
146. Vogel's organ: absent (0), present (1)

Hindwing

147. Hairpencil on costal area: absent (0), present (1)
148. Hairpencil: simple (0), divided (1)
149. Discal cell: closed (0), open (1)
150. Discal cell closed: without recurrent vein (0), with recurrent vein (1)
151. Precostal cell: absent (0), present (1)
152. Humeral vein: single (0), forked (1)
153. Humeral vein single: distally curved (0), curved towards base (1)
154. Additional vestigial anal vein: absent (0), present (1)
155. Angle between 1D and R: open (0), acute (less than 100°) (1)
156. Angle between 1D and 2D: less than 90° (0), more than 90° (1)
157. Angle between 2D and 3D: open (0), acute (less than 100°) (1)
158. Angle between 3D and 4D: acute (0), open (1)
159. Vein 4D: present (0), M3 and CU2 arise together, 4D absent (1)
160. Dorsal hair tuft on the outer border of discal cell: absent (0), present (1)
161. Dorsal hair tuft in the inner part of discal cell: absent (0), present (1)

Thorax.—Characters adapted from Scott (1985). Figure A7 illustrates the main characters drawn from the thorax, showing the structures analyzed.

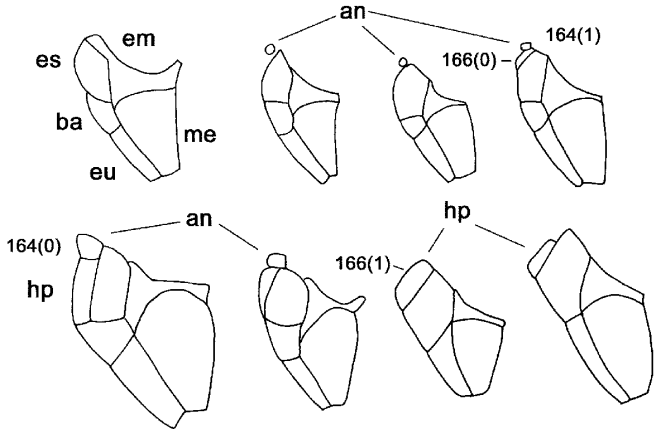

FIGURE A7. Thorax of Nymphalidae. Left to right, first row: *Libytheana*, *Tithorea*, *Bia*, and *Melanitis*; second row: *Morpho*, *Taenaris*, *Doxocopa*, and *Nica*. an = anepisternum; es = episternum; ba = basimerum; em = epimerum; hp = hipopteron; eu = eucoxa; me = meron.

162. Patagium: reduced (0), conspicuous (1)
163. Parapatagium: absent (0), present (1)
164. Anepisternum (mesothorax): conspicuous (0), reduced (1), absent (2)
165. Secondary externo-pleural groove: reduced (0), conspicuous (1)
166. Hypopterum (= pre-episternum): reduced (0), conspicuous (1)

Behaviour and chemistry of adults

167. Attracted to PA sources: absent (0), present (1)
168. Main food sources: flowers and enriched sand (0), flowers and fruits (1), fruits and enriched soil but not flowers (2)
169. Wing position when feeding: closed wings (0), open wings (1)
170. Perches with head down on tree trunks: absent (0), present (1)
171. Cyanogenic: absent (0), present (1)
172. Flight period: diurnal (0), crepuscular (1)
173. Roosting communally: absent (0), present (1)

Head

174. Scaled antennae: present (0), absent (1)
175. Ratio between antennae and body (abdomen excluded): less than 1.3 (0), between 1.3 and 1.9 (1), equal to or more than 2 (2). The limits of the clusters in this character and the following are mostly subjective. They show the relative size of the antennae, defining a route leading to long antennae in character 175, and a route to short antennae in 176. In both characters, the abdomen was excluded because its total length can show more alterations than the thorax.
176. Ratio between antennae and body (abdomen excluded): more than 1.0 (0), equal to or less than 1.0 (1)
177. Cranial extensor of proboscis: absent (0), present (1)

Legs

178. Prothoracic legs in females: with five tarsi (0), four tarsi or less (1)
179. Tarsi of prothoracic legs of females: elongate and developed (0), strongly reduced (1)
180. Prothoracic legs in males: tibia equal to or longer than half of femur (0), tibia shorter than femur (1)
181. Prothoracic legs in males: tarsi equal to or longer than half of femur (0), tarsi shorter than half of tibia (1), tarsi shorter than 1/4 of tibia (2)
182. Prolegs: strongly reduced in males, not in females (0), strongly reduced in both sexes (1)

Abdomen

Data on the hypandrium are presented in Figure A8. (Figure A15 shows a single character of female genitalia.) Additional data for characters in Jenkins (1990) and references therein, Emsley (1965) and Pierre (1986).

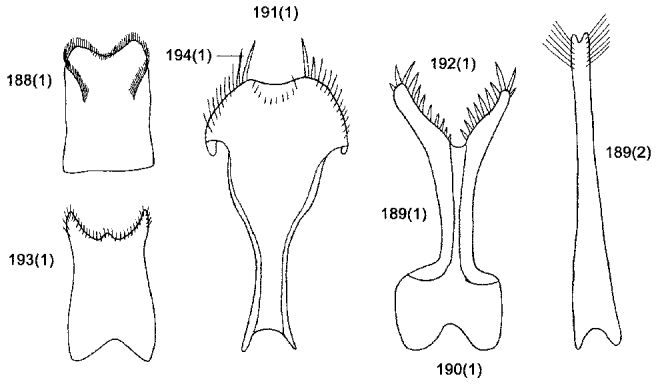

FIGURE A8. Hypandrium of Nymphalidae (Biblidinae). Top left *Dynamine*, lower left *Cybdelis*; next three (left to right): *Biblis*, *Catonephele*, and *Temenis*.

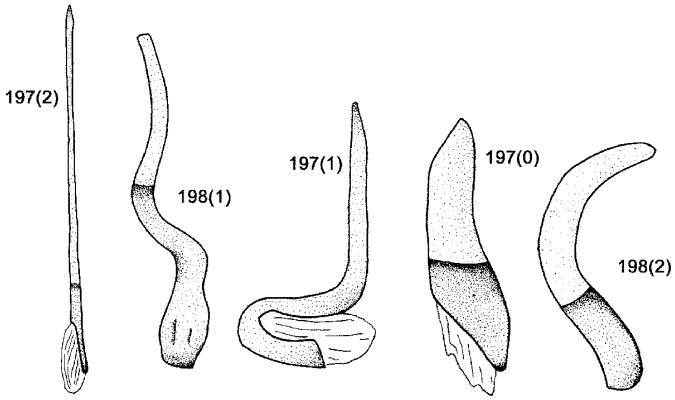

FIGURE A9. Penis of Nymphalidae. Left to right: *Hypoleria* (Ithomiinae, similar to *Heterosais*), *Colobura*, *Haetera*, *Dryadula* (Heliconiini), and *Cybdelis*.

183. Ratio between abdomen and total body length in males: less than 0.5 (0), 0.5 to 0.7 (1), more than 0.7 (2)
184. Abdominal hair tufts in males: absent (0), present (1), with a dorsal diverticulum (2)
185. "Gland sous-papillaire" in females: not sclerotized (0), strongly sclerotized (1)
186. Eversible dorsal glands in segments 7A and 8A in females: absent (0), present (1)
187. Hypandrium: absent (0), present (1)
188. Hypandrium: normal or elongate (0), short, ratio between length/width equal to or less than 1.5 (1)
189. Hypandrium: normal or short (0), elongate, length more than twice the width (1), very long, length more than four times the width (2)
190. Base of the hypandrium: same size as the terminal region (0), wide (1)
191. Terminal region of the hypandrium: same size as base (0), wide (1)
192. Distal portion of hypandrium: not cleft (0), strongly cleft (1)
193. Distal portion of hypandrium: lateral arms short or absent (0), lateral arms opening like a horseshoe (1)
194. Spines on hypandrium: present (0), absent (1)
195. Abdominal processes in females: absent (0), present (1)
196. Androconial lateral hair tufts on male genitalia: absent (0), present (1)

Male genitalia

Morphological characters of male genitalia are presented in Figures A9 (penis), A10, A11 (uncus, gnathos and tegumen), A12 (saccus), and A13 (valva).

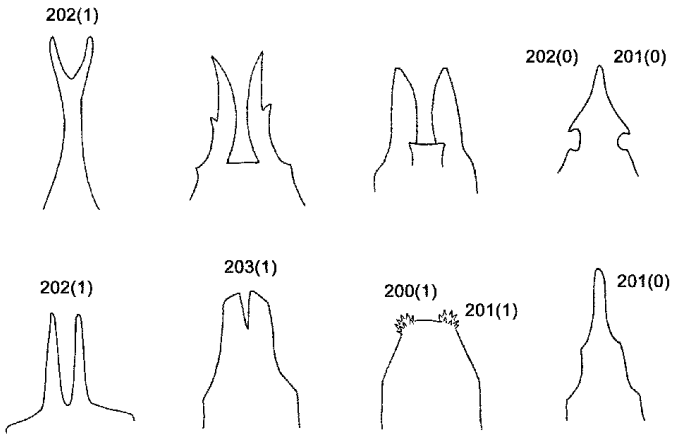

FIGURE A10. Dorsal view of the uncus of some Nymphalidae. Left to right, first row: *Caerois*, *Haetera*, *Pareuptychia*, and *Pseudoscada*; second row: *Anartia*, *Hypanartia*, *Tegosa*, and *Nica*.

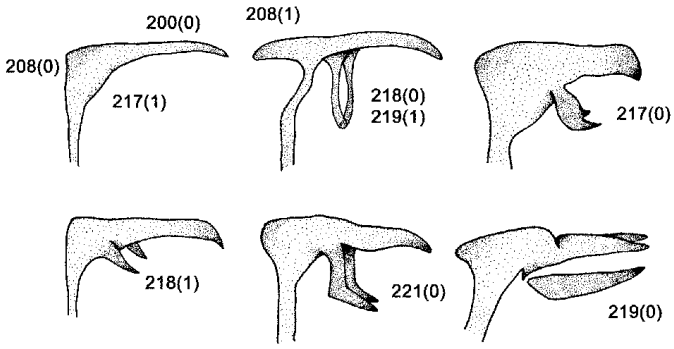

FIGURE A11. Lateral view of the uncus of some Nymphalidae (showing also the tegumen and gnathos). Left to right, first row: *Marpesia*, *Adelpha*, and *Dynastor*; second row: *Antirrhea*, *Dynamine*, and *Biblis*.

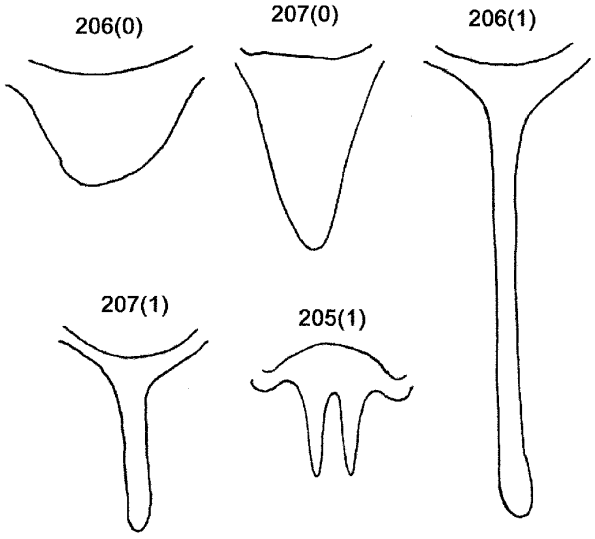

FIGURE A12. Ventral view of the saccus of some Nymphalidae. Left to right, first row: *Eueides*, *Cethosia*, and *Ectima* (the very long structure); second row: *Danaus* and *Tegosa*.

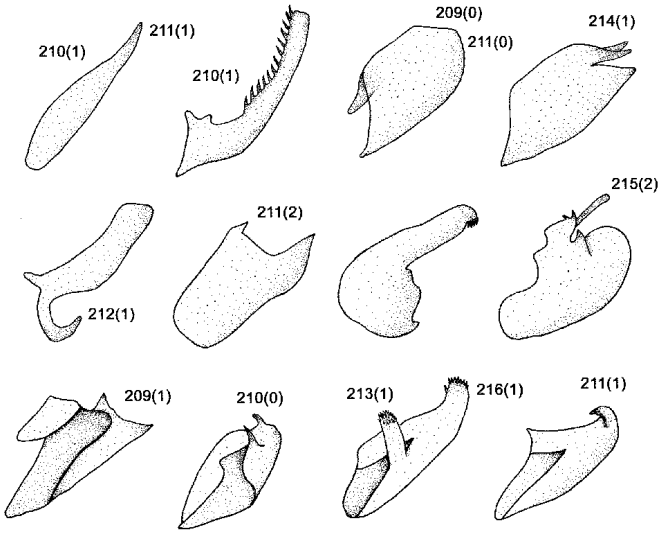

FIGURE A13. Lateral view of the valva of some Nymphalidae. Left to right, top row (external view): *Eteona*, *Eryphanis*, *Consul*, *Smyrna*; middle row (external view): *Diaethria*, *Hypanartia*, *Junonia*, *Euptoieta*; bottom row (internal view): *Haetera*, *Colobura*, *Adelpha*, *Caerois*.

197. Penis: short (length less than three times width) (0), medium (length up to five times width) (1), long (more than five times width) (2)
198. Penis: straight (0), slightly curved (1), strongly curved (2)
199. Uncus: present (0), absent, not sclerotized (1)
200. Uncus: elongate (0), short (1)
201. Uncus: pointed (0), not pointed (flat or rounded) (1)
202. Uncus: single point (0), forked (1)
203. Uncus: not cleft (0), cleft (1)
204. Uncus: straight (0), "broken," pointing down (1)
205. Saccus: single (0), forked (1)
206. Saccus: short (saccus equal to or shorter than total length of genital armature) (0), medium (saccus longer than total length of genital armature) (1)
207. Saccus: wide (length equal to or less than two times width) (0), narrow (length more than two times width) (1)
208. Tegumen: short, not projecting posteriorly (0), long, projecting posteriorly (1)
209. Valva: smooth contour (0), with appendages (1)
210. Valva: short (length equal to or less than width) (0), elongate (length more than twice the width) (1)
211. Distal point of valva: absent (0), present single (1), double (2)
212. Basal processes on valva: absent (0), present (1)
213. Sacculus of valva: simple (0), with appendages (1)
214. Superior internal border of valva: simple (0), with projections (1)
215. Superior lobe of valva: absent (0), present, projecting (1), with appendages (2)

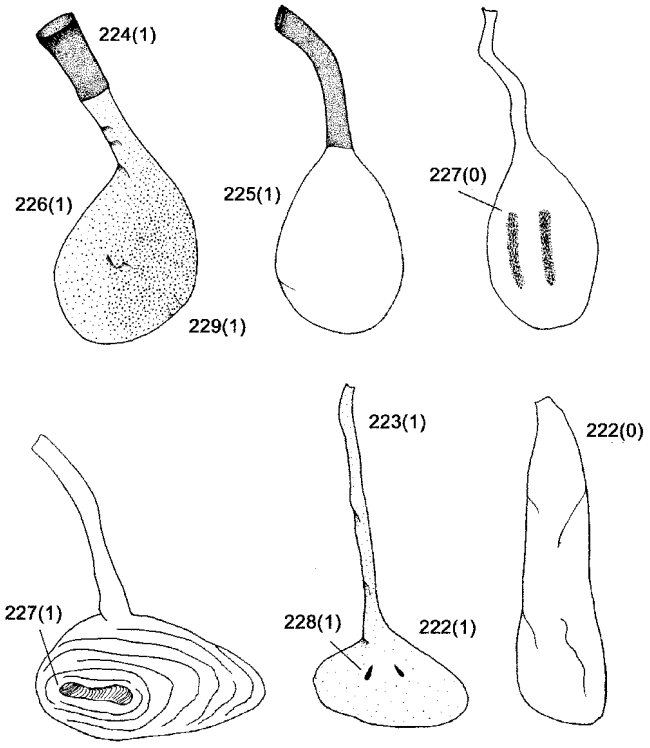

FIGURE A14. Types of bursa copulatrix found in Nymphalidae. Left to right, first row: *Lycorea*, *Asterocampa*, *Opsiphanes*; second row: *Cethosia*, *Ectima*, *Junonia*.

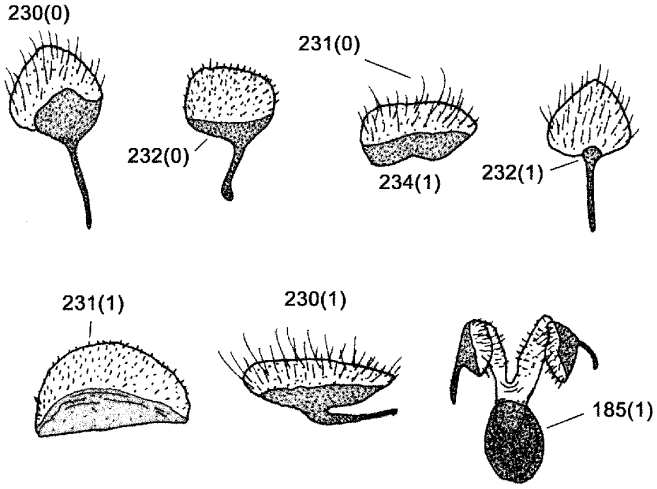

FIGURE A15. Lateral external view of the anal papillae of some Nymphalidae (first six) and ventral view of the "glande sous-papillaire" (second row, right). Left to right, first row: *Eryphanis*, *Anaea*, *Archaeoprepona*, *Pseudoscada*; second row: *Morpho*, *Adelpha*, *Actinote* (showing also the "glande sous-papillaire").

216. Small teeth on distal appendages and on the sacculus: absent (0), present (1)
217. Gnathos: present (0), absent (1)
218. Gnathos: continuous (0), discontinuous (two arms below the uncus) (1)
219. Gnathos: wide in dorsal view (0), narrow in dorsal view (1)
220. If gnathos wide in dorsal view: projecting (0), as a plate (1)
221. If gnathos projecting: projects ventrally (0), projects dorsally (1)

Female genitalia

Figure A14 shows some morphological characters of the bursa copulatrix (corpus bursae and ductus bursae) and signa, and Figure A15 the morphological characters of the anal papillae.

222. Corpus bursae: elongate (0), short, rounded (1)
223. Ductus bursae: short, length of ductus equal to or less than twice the length of the bursa (0), long, length of ductus more than twice the length of bursa (1)
224. Ductus bursae: membranous (0), sclerotized (1)
225. Signum bursae: present (0), absent (1)
226. Signum bursae present: localized (0), spread, diffuse (1)
227. If signum bursae localized: paired (0), single (1)
228. If signum bursae localized: elongate (0), short, small areas (1)
229. If signum bursae spread: weak, poorly marked (0), conspicuously marked (1)
230. Anal papillae: normal (0), elongate laterally (1)
231. Anal papillae: normal hairs (0), very short hairs (1)
232. Sclerotization on base of anal papillae: wide (0), small or not sclerotized (1)
233. Anterior apophyses: short, up to twice the length of the papilla (0), long, more than twice the length of the papilla (1)
234. Anterior apophyses: more than the length of the papilla (0), very short (less than the length of the papilla) or absent (1)