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Phylogeny of the genus *Morpho* Fabricius, 1807, revisited (Lepidoptera, Nymphalidae)

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Summary. – Although the genus *Morpho* Fabricius, 1807, is an important component of the international butterfly trade, it is still poorly understood phylogenetically. The first phylogenetic analysis of the genus, based on morphological characters, was published in 2002, and its results contested the monophyly of three of the nine recognized subgenera, and suggested abandoning the subgeneric classification altogether. The present study re-evaluates the characters used previously, and adds new data derived from the microstructure of the wing scales. In contrast to previous studies, eight of the nine subgenera were recovered as monophyletic. *M. absoloni* was confirmed to be closely related to *M. aurora* within subgenus *Balachowskyna*. Subgenus *Cytheritis* was found to comprise two widely separated monophyletic groups centered on *M. portis* and *M. marcus*. The latter is described as a new subgenus. However, the evolutionary relationships among the subgenera remain poorly supported, except for two clades each composed of two subgenera: (*Morpho, Pessonia*) and (*Iphimedeia, Laurschwartzia*). We then use this new phylogeny to gain further understanding of the evolution of the famous blue wing coloration of certain *Morpho* species. In particular, we demonstrate that not all blue *Morpho* are blue in the same way.

Résumé. – Quoique les espèces du genre *Morpho* Fabricius, 1807, soient très recherchées par les collectionneurs et activement commercialisées, ce genre reste très mal connu sur le plan phylogénétique. La première analyse phylogénétique basée sur des caractères morphologiques a été publiée en 2002. Ses résultats contestent la monophylie de trois des neuf sous-genres reconnus et suggérent d'abandonner l'ensemble de la classification subgénérique. La présente étude reprend l'analyse des caractères utilisés et en aborde de nouveaux concernant la microstructure des écailles alaires. Contrairement à la précédente analyse, ce nouveau travail montre que huit des neuf sous-genres sont monophylétiques. *Morpho absoloni* est confirmé comme étant proche parent de *M. aurora* dans le sous-genre *Balachowskyna*. Le sous-genre *Cytheritis* est révélé comme formé de deux groupes bien séparés centrés sur *Morpho portis* and *M. marcus.* Ce dernier est décrit comme un nouveau sous-genre. Cependant les relations évolutives entre les différents sous-genres sont peu soutenues, excepté pour deux clades, chacun composé de deux sous-genres : (*Morpho, Pessonia*) et (*Iphimedeia, Laurschwartzia*). Nous avons donc utilisé cette nouvelle phylogénie pour obtenir une meilleure compréhension de l'évolution de la fameuse coloration bleue des ailes de certaines espèces de *Morpho*. En particulier, nous démontrons que tous les *Morpho* bleus ne le sont pas de la même façon.

Keywords. – Lepidoptera, Nymphalidae, *Morpho,* phylogeny, subgenus *Balachowskyna, Deyrollia.*

For many people the name *Morpho* is immediately evocative of large blue butterflies encountered in Amazonian forests. But the genus *Morpho* Fabricius, 1807, also includes some very different species, such as the giant orange-brown *M. hecuba* (L., 1771) from the Guianas, the small *M. sulkowskyi* Kollar, 1850, with its brilliant transparent mother-of-pearl wings, which flies in the Andean cloud forests, the large white *M. polyphemus* Westwood, [1850], common in Mexican forests, or the black and blue *M. achilles* (L., 1758), the type species of the genus. Evidently, the genus *Morpho* is heterogeneous in colour pattern.

The first comprehensive study of the genus was published by FRUHSTORFER (1912-1913), who listed 30 species, and divided the genus into two species groups: *Iphimedeia* Fruhstorfer and *Morpho*. Fifty years later, LE MOULT & RÉAL (1962, 1963) distinguished 80 species. Using wing venation, male genitalia and wing color pattern, they divided the genus into

eight subgenera: *Iphimedeia* Fruhstorfer (10 species); *Iphixibia* Le Moult & Réal (1 sp.); *Cytheritis* Le Moult & Réal (16 sp.); *Balachowskyna* Le Moult & Réal (2 sp.: in Tome II, Le Moult & Réal indicated that *M. absoloni* is a distinct species belonging to *Balachowskyna*); *Cypritis* Le Moult & Réal, a junior synonym of *Megamede* Hübner, [1819] (6 sp.); *Pessonia* Le Moult & Réal (5 sp.); *Grasseia* Le Moult & Réal (9 sp.) and *Morpho s. str.* (31 sp.). LE MOULT & RÉAL (1962) considered that subgenus *Cytheritis* was probably the group from which the other subgenera had emerged and they suggested it should be divided into new groups after further study. This represented the first attempt to suggest evolutionary relationships among members of *Morpho*.

Revisiting the systematics of *Morpho*, BLANDIN (1988) followed Le Moult & Réal's subgeneric classification, but created subgenus *Schwartzia*, later named *Laurschwartzia* (BLANDIN, 2007b), for two species previously included in subgenus *Iphimedeia*. BILOTTA (1992, 1994a, b) elevated all the subgenera to genus level, based on the marked morphological variation he observed among seven Brazilian *Morpho* species representing six subgenera.

No modern phylogenetic study of *Morpho* was published until that of PENZ & DEVRIES (2002). The aim of that work was to test explicitly the monophyly of the nine subgenera. They studied a sample of 27 *Morpho* species, used three Antirrheina species as outgroups, and defined 118 morphological and 2 ecological and behavioral characters. Although the monophyly of the genus *Morpho* was not challenged, they obtained a consensus tree in which subgenera *Iphimedeia*, *Schwartzia*, *Cypritis* and *Pessonia* were monophyletic, but *Cytheritis*, *Grasseia* and *Morpho* were paraphyletic.

Based on these results, PENZ & DEVRIES (*l. c.*) rejected the subgeneric classification of *Morpho* butterflies, making all subgenera synonyms of *Morpho*. However, that work involved a relatively small sample of taxa. In this study, we revisit the phylogenetic relationships of genus *Morpho*, studying a more complete sample of species and including additional characters, especially from the genitalia and the microstructure of the scales on the upperside of wings, to bring new arguments to bear on the validity of the subgenera. We also aim to infer the evolution of wing color and certain biological traits within the genus.

MATERIAL AND METHODS

Increasing the support for and/or resolution of a phylogenetic hypothesis usually consists of increasing the number of characters analyzed, the number of taxa included, or both. We therefore added 43 ingroup taxa to the sample studied by PENZ & DEVRIES (2002) and an additional outgroup (Table I). Thus, all *Morpho* species, as recognized by BLANDIN (2007a, c), are included, several represented by more than one subspecies, to gain a better representation of their geographical range. The outgroups comprised three species of Antirrheina, the sister-subtribe of Morphina (DEVRIES *et al.*, 1985) within the tribe Morphini, and one species of Brassolini, the sister-tribe of Morphini within the subfamily Morphinae, according to the most recent higher classification (PEÑA *et al.*, 2006).

To increase objectivity in our search for phylogenetically informative characters, we initially coded characters without reference to PENZ & DEVRIES (2002). Wing characters were observed on males and we use a terminology based on BLANDIN (1988, 2007a; Appendix 1, plates 1 and 2). Scales were observed in discal cell of forewing upperside using a stereo magnifier (up to $\times 64$) for characters related to scale shape and organization, whereas their microstructure was examined with a scanning electronic microscope following gold metallization by cathodic plasma deposition (Appendix 1, plate 3). Presence or absence of pigments, and their density in scales, was determined by transmission photon microscopy. *Morpho* scale terminology and optics were described, richly explained and illustrated by BERTHIER (2007).

Table I. – Taxa sampled.

All specimens are deposited in the general collection and the Laurent Schwartz and Patrick Blandin collections at the Muséum national d'Histoire naturelle, Paris. Ingroup taxa marked with * were also studied by PENZ & DEVRIES (2002).

Outgroups

Brassolini, Brassolina
Caligo ilioneus (Cramer, 1775)

Morphini, Antirrheina
Caerois chorinaeus (Fabricius, 1775)
Antirrhea pterocopha Salvin & Godman, 1868
Antirrhea tomasia (L., 1758)

Ingroup: Morphini, Morphina

Subgenus ***Iphimedeia*** Fruhstorfer, 1912
M. hercules hercules (Dalman, 1823)*
M. theseus theseus Deyrolle, 1860*
M. theseus aquarius Butler, 1872
M. theseus juturna Butler, 1870
M. theseus oaxacensis Le Moult & Réal, 1962
M. niepelti Röber, 1927
M. telemachus telemachus (L., 1758)*
M. telemachus lillianae Le Moult, 1927
M. telemachus martini Niepelt, 1933
M. telemachus exsusarion Le Moult & Réal, 1962
M. amphitryon amphitryon Staudinger, 1887*

Subgenus ***Laurschwartzia*** Blandin, 2007
M. hecuba hecuba (L. 1771)*
M. hecuba obidonus Fruhstorfer, 1905
M. cisseis cisseis Felder & Felder, 1860*
M. cisseis phanodemus Hewitson, 1869
M. cisseis cisseistricta Le Moult & Réal, 1962

Subgenus ***Cytheritis*** Le Moult & Réal, 1962
M. marcus marcus (Schaller, 1785)*
M. eugenia eugenia Deyrolle, 1860*
M. eugenia uraneis H. W. Bates, 1865
M. sulkowskyi sulkowskyi Kollar, 1850*
M. sulkowskyi eros Staudinger, 1892
M. sulkowskyi lympharis Butler, 1873
M. sulkowskyi calderoni Blandin & Lamas, 2007
M. zephyritis Butler, 1873
M. portis portis (Hübner, [1821])*
M. portis thamyris Felder & Felder, 1867
M. aega aega (Hübner, [1822])*
M. rhodopteron rhodopteron Godman & Salvin, 1880

Subgenus ***Balachowskyna*** Le Moult & Réal, 1962
M. aurora aurora Westwood, 1851*
M. aurora aureola Fruhstorfer, 1913
M. absoloni May, 1924

Subgenus ***Cypritis*** Le Moult & Réal, 1962
M. cypris cypris Westwood, 1851*
M. rhetenor rhetenor (Cramer, 1775)*
M. rhetenor cacica Staudinger, 1876
M. rhetenor helena Staudinger, 1890
M. rhetenor augustinae Le Cerf, 1925

Subgenus ***Pessonia*** Le Moult & Réal, 1962
M. polyphemus polyphemus Westwood, [1850]*
M. polyphemus luna Butler, 1869
M. epistrophus epistrophus (Fabricius, 1796)*
M. epistrophus catenaria (Perry, 1811)*
M. epistrophus nikolajevna Weber, 1951
M. iphitus iphitus Felder & Felder, 1867
M. iphitus titei Le Moult & Réal, 1962

Subgenus ***Iphixibia*** Le Moult & Réal, 1962
M. anaxibia (Esper, [1801])*

Subgenus ***Grasseia*** Le Moult & Réal, 1962
M. menelaus menelaus (L., 1758)*
M. menelaus coeruleus (Perry, 1810)
M. menelaus eberti Fischer, 1962
M. menelaus occidentalis Felder & Felder, 1862
M. amathonte Deyrolle, 1860*
M. godartii godartii Guérin-Méneville, [1844]*
M. godartii didius Hopffer, 1874*
M. godartii julanthiscus Fruhstorfer, 1907

Subgenus ***Morpho*** Fabricius, 1807
M. achilles achilles (L., 1758)*
M. achilles phokylides Fruhstorfer, 1912
M. achilles vitrea Butler, 1866
M. helenor helenor (Cramer, 1776)
M. helenor achillaena (Hübner, [1823])*
M. helenor peleides Kollar, 1850*
M. helenor peleus Röber, 1903
M. helenor theodorus Fruhstorfer, 1907
M. helenor coelestis Buttler, 1866
M. helenor anakreon Fruhstorfer, 1910
M. helenor maculata Röber, 1903
M. helenor macrophtalmus Fruhstorfer, 1913
M. helenor montezuma Guenée, 1859
M. deidamia deidamia (Hübner, [1819])*
M. deidamia jacki Nield, 2008
M. deidamia electra Röber, 1903
M. granadensis granadensis Felder & Felder, 1867*
M. granadensis lycanor Fruhstorfer, 1913

The abdomens of *Morpho* specimens are often removed just after the capture, to avoid "greasing" of the wings and unfortunately, they are often discarded or lost. Most specimens included in this analysis were intact but for some species, such specimens were not available. In those cases, we used specimens only when an unambiguous label was pinned with the abdomen, certifying its origin. We dissected at least one male (Appendix 1, plate 4) and one female (App. 1, plate 5) for each taxon. Only one female was available for *Antirrhea tomasia* and *Caerois chorinaeus*. Both of these specimens were old, very fragile and were badly damaged during dissection. Nevertheless, we could still record most of the characters included in the analysis. We followed KRISTENSEN'S (2003) nomenclature for genitalia.

After our initial character search, we compared our morphological data matrix with that of PENZ & DEVRIES (2002). We evaluated how they treated and defined their characters, and how they were coded for all taxa. We then rechecked our characters and added several more. Among the characters coded by Penz & DeVries, we rejected most of those related to degree of sclerotization because we observed that it can be strongly affected by the length of time the preparations were heated in the aqueous potassium hydroxide solution used for maceration of soft tissues. Instead, we focused on the shapes of the same structures, which are never affected by such treatment. We omitted a further 23 characters that concerned structures we could not identify or recognize (Appendix 1). Our final matrix thus included 140 morphological characters (App. 1), of which 49 were new to this study. Of the remaining 91, which were also used by PENZ & DEVRIES (2002), 62 characters were either coded differently for some taxa (disagreement in observations) or treated differently: in case of conflicting observations, we favored our own character states as they were confirmed by many specimens; some characters were changed from binary to multistate characters and vice versa, depending on the number of states we could effectively observe on the structure (sometimes less, sometimes more than those indicated by Penz & DeVries). Moreover, we took care to code characters in such a way as to avoid redundancy and hence hidden weighting in the matrix. To avoid subjective assessments of color and differences caused by different viewing angles, color of wing upperside was coded only from the microstructure and not as it appears to the naked eye.

We also added the two ecological and behavioral characters used by PENZ & DEVRIES (2002) and three more following a field study of flight behavior using a watchtower in the río Shilcayo valley, near Tarapoto, department San Martín, Peru. Larval host plants are known with certainty for only a small number of *Morpho* butterflies and what is mostly reported is just whether the caterpillars feed on monocotyledonous or dicotyledonous plants. Five larval characters (Appendix 1) and as much data as possible about ecology and behavior were extracted from the literature (FRUHSTORFER, 1912-1913; OTERO, 1966; DEVRIES, 1987; ACKERY *et al.*, 1998; OTERO & MARIGO, 1990; TÁKÁCS & TELLO, 1993, 1994; DEVRIES & MARTINEZ; 1994; CONSTANTINO, 1997; BRÉVIGNON, 2003; MILLER *et al.*, 2007; NEILD, 2008; GUERRA-SERRUDO & LEDEZMA-ARIAS, 2008).

Most parsimonious cladograms (MPCs) and bootstraps values (FELSEINSTEIN, 1985) were found using TNT (GOLOBOFF *et al.*, 2003) by heuristic searching with tree bisection-reconnection (10 addition sequence replications and 10 rounds of branch swapping). As some replicates could overflow because of buffer capacity, many independent analyses were run, as recommended by GOLOBOFF *et al.* (2008), each with a different starting seed, until we found the most parsimonious score ten times. Strict consensus trees were generated from the results of these ten analyses. Two datasets were analyzed. The first comprised only the morphological characters, the second also included the ecological and behavioral characters. In each case, all characters were equally weighted and multistate characters were unordered. Values of Bremer's Decay Index (BREMER, 1994) were calculated under *TreeRot.v2* (SORENSON, 1999).

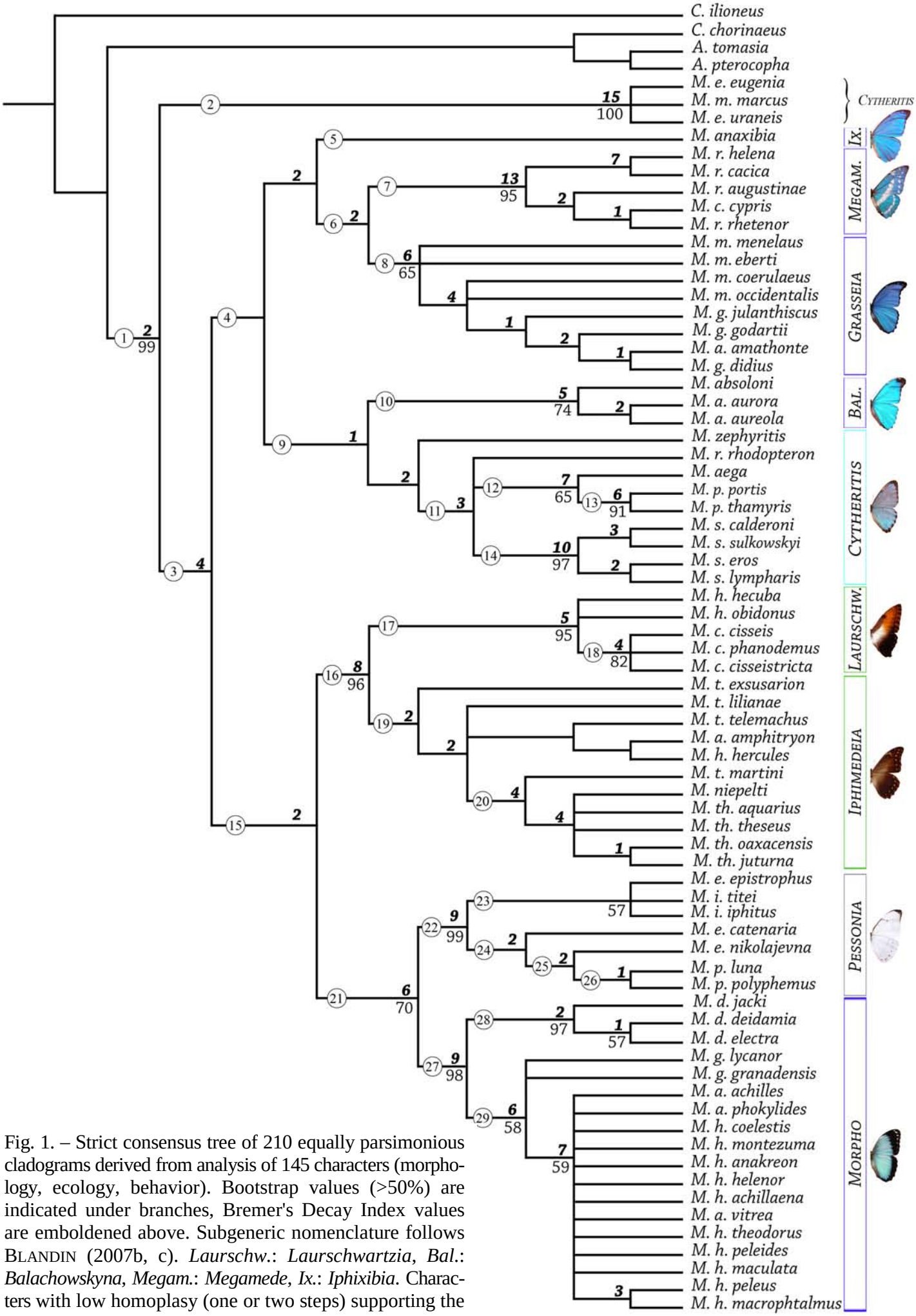


Fig. 1. – Strict consensus tree of 210 equally parsimonious cladograms derived from analysis of 145 characters (morphology, ecology, behavior). Bootstrap values (>50%) are indicated under branches, Bremer's Decay Index values are emboldened above. Subgeneric nomenclature follows BLANDIN (2007b, c). *Laurschw.*: *Laurschwartzia*, *Bal.*: *Balachowskyna*, *Megam.*: *Megamede*, *Ix.*: *Iphixibia*. Characters with low homoplasy (one or two steps) supporting the numbered clades are listed in Table II.

Table II. – List of synapomorphies with one to three steps. Clades are as numbered on fig. 1. Autapomorphies of the clades are emboldened. FW: forewing, HW: hindwing, SC: scales, ♂ and ♀: male and female genitalia.

Clade 1: Genus *MORPHO*

8:1 Labial palpus white
10:1 Tuft of white scales on patagium present
11:1 Tegula with a white spot at base
14:1 ♂: Midleg, four rows of ventral spines on tarsomere 5
17:1 ♀: Foreleg, pulvillus not fused medially
24:1 HW: Discal cell open
42:1 HW: Discal area absent ♂
65:1 SC: Discal cell cover scales not pigmented
70:0 SC: Cover scales not pigmented near crossveins m_{1-2}
82:1 ♂: Scale tufts attached to vinculum
105:0 ♂: Base of valva rounded
118:1 ♀: *Papilla anales* semicircular
139:1 ♀: Ductus bursae short
143:1 Diurnal flight

Clade 2: *marcus* species group

15:1 ♂: Male midleg, ventral pulvillar process blunt
48:1 HW: No eyespot in cell 3
71:2 SC: Cover scale folded like an accordion
72:1 SC: Cover scales forming a uniform multi-layered coat
77:1 SC: Each ridge a single *lamella* developed lengthwise
92:2 ♂: Apex of uncus truncated
97:2 ♂: Gnathos stick-shaped
110:1 ♂: Presence of one strong spine at inner side of valva
112:1 ♂: Valva conspicuously convex
117:1 ♂: Juxta developing a strong backward process
132:1 ♀: Flattened processes on *lamella postvaginalis*

Clade 3

89:2 ♂: Dorsal fissure ending near the apex of uncus
142:1 Larval host plant: dicots

Clade 4

66:1 SC: Apex of cover scales nearly smooth and straight
78:1 SC: Discal cell ground scales: high ridge density

Clade 5: Subgenus *IPHIXIBIA*

12:1 Strongly iridescent blue scales on thorax
71:3 SC: Semicircular cover scales
97:0 ♂: Gnathos slightly constricted

Clade 6: (*MEGAMEDE, GRASSEIA*)

8:3 Red scales on labial palpus

Clade 7: Subgenus *MEGAMEDE*

9:1 Labial palpus red: a white line on the internal edge
54:1 HW: Eyespot in cell 1b present
69:1 SC: Atrophied cover scales
83:1 ♂: Segment IX setae white
89:1 ♂: Dorsal fissure short

M. rhetenor

140:1 ♀: *Signa* short

M. cypris

62:1 HW: Eyespot pupils white and blue

Clade 8: Subgenus *GRASSEIA*

6:1 Hairy eyes
40:2 HW: Areas over the cellular and basal zones greenish
43: 1 HW: Discal area thin
44:1 HW: Discal area disrupted by veins
100:1 ♂: Small spines on gnathos present

M. didius

10:0 No white scales on patagium

Clade 9: (*BALACHOWSKYNA, CYTHERITIS*)

142:0 Larva feeds on monocots

Clade 10: Subgenus *BALACHOWSKYNA*

43:1 HW: Discal area thin
66:2 SC: Apex of discal cell cover scales concave
83:1 ♂: Tegumen scales white
105:1 ♂: Base of valva making an angle
108:0 ♂: Valva inner side: spiny bulge present

M. zephyritis

66:1 SC: Apex of discal cell cover scales nearly smooth and straight
113:1 ♂: *Carina penis* present
125:1 ♀: *Lamella postvaginalis* developed forward

Clade 11

7:1 Labial palpus unkempt
8:2 Labial palpus orange
66:0 SC: Apex of cover scales deeply indented
89:1 ♂: Dorsal fissure short
121:0 ♀: *Papilla anales* setae inserted in long tubercles

Clade 12: (*M. aega, M. portis* ssp.)

91:1 ♂: Uncus flattened lateral processes present
112:1 ♂: Valva conspicuously convex
130:1 ♂: Posterior area entirely sclerotized

M. aega

7:0 Labial palpus smooth
8:0 ♀: Foreleg, pulvillus fused medially
79:1 SC: Upper *lamella* of ridges ending curved outward
98:0 ♂: Gnathos pointed and sharp
111:1 ♂: Costa of valva projected at base

Clade 13: *M. portis* ssp.

92:1 ♂: Apex of uncus bifid
96:1 ♂: Gnathos atrophied

Clade 14: *M. sulkowskyi* ssp.

53:1 HW: Eyespots very distorted or oblate
67:1 SC: Basal scales not pigmented in discal cell
73:1 SC: Basal scales not pigmented around m_{1-2}, m_{2-3}
121:0 ♀: *Papilla anales* setae inserted in long tubercles
128:1 ♀: Integument strongly wrinkled on posteriorarea

M. eros

98:0 ♂: Gnathos pointed and sharp

Clade 15

8:2 Labial palpus orange
70:1 SC: Cover scales pigmented around m_{1-2}, m_{2-3}
81:0 Color dimorphism weak
113:1 ♂: *Carina penis* present

Clade 16: (*LAURSCHWARTZIA, IPHIMEDEIA*)

32:1 FW: Marginal and/or postmarginal spots orange

65:0 SC: Discal cell cover scales pigmented
97:0 ♂: Gnathos slightly constricted
100:1 ♂: Gnathos spinose
144:1 Gliding flight

Clade 17: Subgenus *LAURSCHWARTZIA*

40:1 HW: Cellular and basal areas: deep orange

Clade 18: *M. cisseis* ssp.

32:2 FW: Marginal and/or postmarginal spots blue
53:1 HW: Eyespots very distorted or oblate

Clade 19: Subgenus *IPHIMEDEIA*

25:0 HW: Tail appendix on vein M3
113:0 ♂: *Carina penis* absent
129:1 ♀: Supernumerary depression dorsal to the *lamella postvaginalis* present

M. hercules

10:0 No white scales on patagium
140:1 ♀: Signa short

Clade 20: (*M. niepelti, M. theseus* ssp.)

29:1 HW: Small tail-like appendices pointed

Clade 21: (*MORPHO, PESSONIA*)

9:1 Labial palpus with a white line on the internal edge
75:1 SC: Thin scales present at discal cell base
83:1 ♂: Segment IX setae white
101:1 ♂: Gnathos basal process present
102:1 ♂: Gnathos subterminal basal process present
125:1 ♀: *Lamella postvaginalis* developed forward
134:1 ♀: *Lamella antevaginalis* developed backward

Clade 22: Subgenus *PESSONIA*

11:2 Tegula nearly entirely white with brown edges
67:1 SC: Basal scales not pigmented in discal cell
114:1 ♂: *Carina penis* near apex of the *rostellum*

Clade 23

53:1 HW: Eyespots very distorted or oblate
76:2 SC: Upperside *lamina*: present with large perforations

Clade 24

81:1 Color dimorphism strong

Clade 25

134:0 *Lamella antevaginalis* not developed backward

Clade 26: *M. polyphemus* ssp.

76:1 SC: Upperside *lamina*: present with small perforations
109:1 ♂: Strong spine halfway along valva posterior edge

Clade 27: Subgenus *MORPHO*

17:0 Female foreleg, pulvillus fused medially
40:2 HW: Areas over the cellular and basal zones greenish
43:1 HW: Discal area thin
60:1 HW: Claret shadow over the eyespot disk present
66:1 SC: Apex of discal cell cover scales nearly smooth and straight
69:2 SC: – At apex of discal cell, cover scales over developed
70:0 SC: – *Idem*, not pigmented
71:1 SC: – *Idem*, cover scale distal part enlarged
72:1 SC: Cover scales forming a uniform multi-layered coat

Clade 28: *M. deidamia* ssp.

8:1 Labial palpus white

Clade 29

6:1 Hairy eyes
8:3 Red scales on labial palpus
126:1 ♀: Lamella postvaginalis: laterally overdeveloped
133:1 ♀: Integument anterolateral area: strongly wrinkled

Table III. – Character consistency (ci) values of morphological, ecological and behavioral characters. Characters with ci values between 0.5 and 1 undergo at the most three state changes. The number of characters having a ci value equal to 1 is given in parentheses.

	Number of characters			
Character categories	per category	with ci < 0.5	with ci ≥ 0.5 (ci = 1)	novel characters[a] (with ci = 1)
Caterpillars	5	-	5 (5)	5 (5)
General morphology	12	1	11 (6)	1[b]
Wing structure	12	7	5 (4)	6 (3)
Wing color pattern	33	21	12 (10)	12[b] (3)
Scales	17	2	15 (10)	7[c] (5)
Sexual dimorphism	2	2	-	1
Genitalia ♂	36	18	18 (11)	10[d] (3)
Genitalia ♀	23	7	16 (9)	6 (2)
Ecology and behavior	5	3	2 (2)	3 (2)
Total for morphology only	140	58	82 (55)	48 (21)
Total for MEE	145	61	84 (57)	51 (23)

a: characters not previously used for phylogenetic studies of the genus; **b**: including one character with ci ≥ 0.5; **c**: including two characters with ci ≥ 0.5; **d**: including three characters with ci ≥ 0.5

RESULTS

Analyses with and without the ecological and behavioral characters produced respectively 210 MPCs (L = 457, CI = 0.383, RI = 0.845) and 140 trees (L = 443, CI = 0.381, RI = 0.845). Howewer, the two strict consensus trees have the same topology (fig. 1). Forty-five characters are autapomorphies of subclades within the genus *Morpho* (*i.e.* characters having ci = 1; emboldened in Table II) and slightly more than half the characters have a character consistency index, ci, ≥ 0.5. The morphological structures that generated characters with lower homoplasy were the scales of the forewing upperside and the female genitalia. A few other characters with high ci values came from general morphology and larvae (Table III).

Monophyly of the genus *Morpho* is confirmed, but contrary to the results of PENZ & DEVRIES (2002), only subgenus *Cytheritis* appears polyphyletic. Moreover, all the subgenera are quite well supported except *Iphimedeia*, and there are strongly supported sister-group relationships between *Laurschwartzia* and *Iphimedeia*, and *Morpho* and *Pessonia*. The deepest relationships between larger clades are not supported.

DISCUSSION

At the subgeneric level, the new phylogeny is better supported than that previously published (PENZ & DEVRIES, 2002), as at least one autapomorphy was found for eight of the nine subgenera. However, with regard to the deeper nodes, none receives improved support (with and without ecological and behavioral characters), and the most homoplastic characters in Penz & DeVries study remained homoplastic in the present study.

Phylogenetic relationships within the genus Morpho

Contrary to the results of the previous work of Penz & DeVries, subgenus *Morpho* is recovered as monophyletic, as it now includes *M. deidamia*. The topologies contradict Lamas' opinion (2004) that *M. granadensis* is a subspecies of *M. deidamia* but support a basal position of the latter. A sister-group relationship between subgenera *Morpho* and *Pessonia* was found. This pattern suggests interesting biogeographical questions, given that *Pessonia* is split into Mata Atlantica species-pair (*M. epistrophus*, *M. iphitus*) and a Mesoamerican species (*M. polyphemus*), and subgenus *Morpho* includes two strictly Amazonian species (*M. deidamia*, *M. achilles*), a trans-Andean species (*M. granadensis*) and the *Morpho* species with the largest range (from South-East Brazil to Mexico), *M. helenor*.

Also in contrast to the results of PENZ & DEVRIES (2002), subgenus *Grasseia* was also found to be monophyletic. Penz & DeVries did not study *M. absoloni*, which was considered to be a subspecies of *M.* (*Grasseia*) *amathonte* by LE MOULT & RÉAL (1962), but later placed by them in *Balachowskyna* (LE MOULT & RÉAL, 1963). This latter decision is strongly supported by our results, as the clade (*M. absoloni*, *M. aurora*) has good bootstrap and Bremer's Decay Index values.

Morpho hecuba and *M. cisseis* were originally included within subgenus *Iphimedeia* by FRUHSTORFER (1912), but when revisiting the subgenus taxonomy, BLANDIN (1988) placed these two species in a different subgenus (now called *Laurschwartzia* Blandin, 2007b). Unsurprisingly and as already demonstrated by PENZ & DEVRIES (2002), these two subgenera were distinct from each other in our phylogenetic analysis, yet they remained closely related.

LE MOULT & RÉAL (1962) included in subgenus *Cytheritis* those species they considered to be « *among the most primitive* » of genus *Morpho*. However, because these species have rather similar external appearance but markedly divergent genitalia, they believed that subgenus *Cytheritis* constituted a non-homogeneous group, a view supported by PENZ & DEVRIES (2002). On our cladogram, subgenus *Cytheritis* appeared as two widely separated groups. The first, which we term the *marcus* species group (*M. marcus* and *M. eugenia*), forms the most basal

Morpho clade. We refer to the second clade as *Cytheritis s. str*. It includes the type-species, *M. portis*, and this is placed as the sister clade of *Balachowskyna*. The distance between the *marcus* group and the *Cytheritis* group derives mainly from the very divergent male and female genital structures and wing upperside scales of the *marcus* species group. In all, we found seven striking autapomorphic traits for this group (Table II, clade 2), making it very different from *Cytheritis s. str*. but also very different from all other *Morpho* species. Therefore, we decided to erect a new subgenus, *Deyrollia*, n. subgen., which is described in Appendix 2 (p. 249).

Blues in blue: a phylogenetic test of the origin of blue coloration

Many *Morpho* butterflies are instantly recognizable by the dazzling blue color of males, which is due to optical phenomena produced by the upper lamina of the wing upperside scales. Consequently, we used scales organization characteristics, microscopic observations and the optical characteristics of *Morpho* wing upperside scales to test a hypothesis of color evolution within the genus. As the outgroup taxa are either not iridescent or iridescence is restricted to very small areas of hindwing, one would expect non-iridescent *Morpho* butterflies to constitute the more basal clades within the genus.

In *Morpho*, with the exception of the new subgenus *Deyrollia*, the iridescent blue coloration is produced by the Christmas tree-like structure of the ground scales (BERTHIER *et al*, 2006). This characteristic structure is generated by a number of piled chitinous lamellae that form high ridges on the upper membrane of the scale. The ground scales also contain pigments and vary in size and shape. In general, the larger the ridge density, the number of lamellae per ridge and degree of melanization, the more dazzling is the resulting color. Although they do not produce color, the cover scales act to diffuse the light and thus reduce the spectral purity of the blue color (BERTHIER, 2007). They are therefore also important to our understanding of the structural colors. For example, *Morpho (Megamede) cypris* and *Morpho (Morpho) helenor* have ground scales that are similar in shape, size and pigment concentration. However, in *M. cypris* the structural color is intense and relatively pure because the cover scales are atrophied and cannot interact with the light reflected by the ground scales. In contrast, the spectral purity of the blue coloration on the wings of *M. helenor* is quite low because the enlarged and overlapping cover scales diffuse the light reflected by the ground scales in a large range of directions across the plane of the wing membrane.

Three main groups can be recognized within *Morpho* based on their color. The first is formed of the iridescent blue species of subgenera *Cytheritis* and *Deyrollia, Iphixibia, Grasseia, Megamede, Balachowskyna* and *Morpho*. The second includes the white species of subgenus *Pessonia*. The last group comprises subgenera *Iphimedeia* and *Laurschwartzia*. This last group is a peculiar case in that it includes *M. cisseis*, which shows slightly iridescent blue areas, and species having dull colored areas that can be blue (some forms of *M. telemachus, M. niepelti, M. theseus schweizeri*), greenish and grayish-blue (*M. hercules, M. amphitryon*), white (*M. theseus juturna*), ocher-bronze or even yellow-orange (some forms of *M. telemachus*; *M. hecuba hecuba*).

The iridescent blue *Morpho* species do not form a monophyletic unit (fig. 2). With the naked eye, we could differentiate the deep blue species from the pale blue ones. In the first group are the subgenera *Iphixibia, Grasseia, Megamede, Balachowskyna* and *Morpho*. Their ground scales show a high ridge density, with 5 to 12 piled lamella, and strong melanin density (although not in some populations of *M. aurora*). Their cover scales are not pigmented and, depending on their size, they can modulate the spectral purity of the blue, as noted above. In the pale blue group, we could separate the new subgenus *Deyrollia* from *Cytheritis str*. The color difference between them is quite subtle when viewed with the naked eye but striking when the scale shape, organization and microstructure are considered. In the subgenus *Deyrollia*, the

Christmas tree-like structure does not exist. Each ridge is made from a single lamella, developed and not disrupted lengthwise, and having a circular cross-section. The piling of the lamellae, essential to produce iridescence, is replaced by a piling of the scales to form a multiple layer coat. Nonetheless, this piling is not sufficient to produce a very dazzling color. In contrast, *Cytheritis* str. species show the characteristic lamella piling of iridescent *Morpho*. The color difference between *Cytheritis* str. and the deep blue species does not come from differences in fundamental microstructure but from low melanin density or even, in the case of *M. sulkowskyi*, the complete absence of pigments in the scales of the wing upperside.

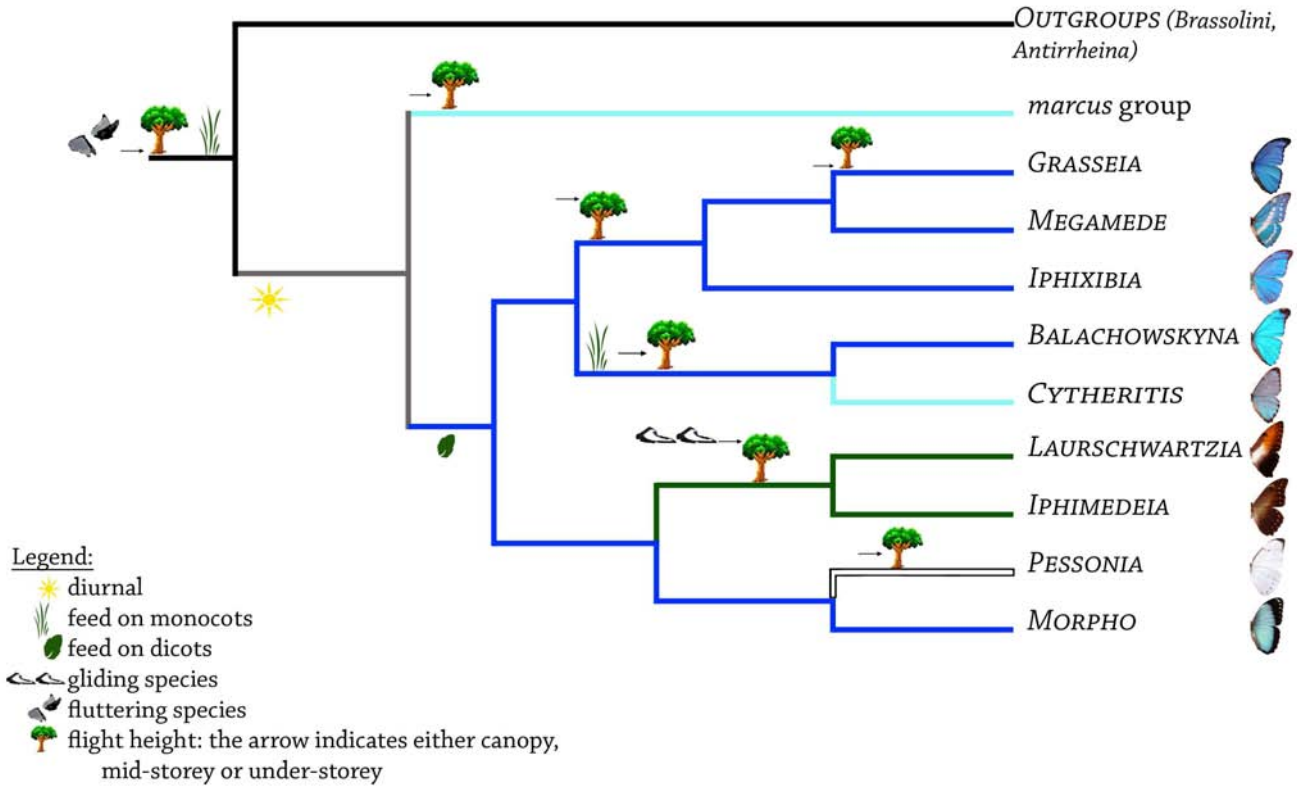


Fig. 2. – Evolution of the ecological and behavioral characters. Gregariousness data are really to scares to be included in character matrices.

Whiteness is the most notable characteristic of subgenus *Pessonia* and supports the monophyly of the subgenus. This color results from absence of pigments, except for small black areas, and ability of the scales to reflect all wavelengths of visible light. At the microscopic level, we observed that they are the only *Morpho* butterflies having the upperside lamina developed between the ridges, but fenestrated at different degrees. Moreover, the ridges are only one lamella high, the lamella themselves being short compared with that observed in iridescent scales.

Our results also support the grouping of all the non-white/iridescent blue *Morpho*. In this clade (*Iphimedeia*, *Laurschwartzia*), scale microstructure cannot generate iridescence as each ridge is only one lamella high and both cover and ground scales are deeply pigmented. Greenish and grayish-blue could have different origins. Some of this type of coloration could come from optical phenomena that modify the wavelength reflected by underlying pigment grains. Alternatively, diverse tones of green, purple and blue can derive from biliary pigments, such as pterobiline, as in *Graphium weiskei* (Papilionidae). However a generally weak and localized iridescence in diverse colors, generated by both cover and ground scales, can be observed in few subspecies but it is a relatively unusual phenomenon and its microstructural origins remain unknown. Other candidates for iridescence include *M. theseus schweizeri* and some specimens of *M. hercules* but the occurrence and underlying physics of structural color in these taxa needs to be investigated. The only confirmed exception is *M.* (*Laurschwartzia*) *cisseis*, which is iridescent even though its ground scale ridges are only two lamellae high.

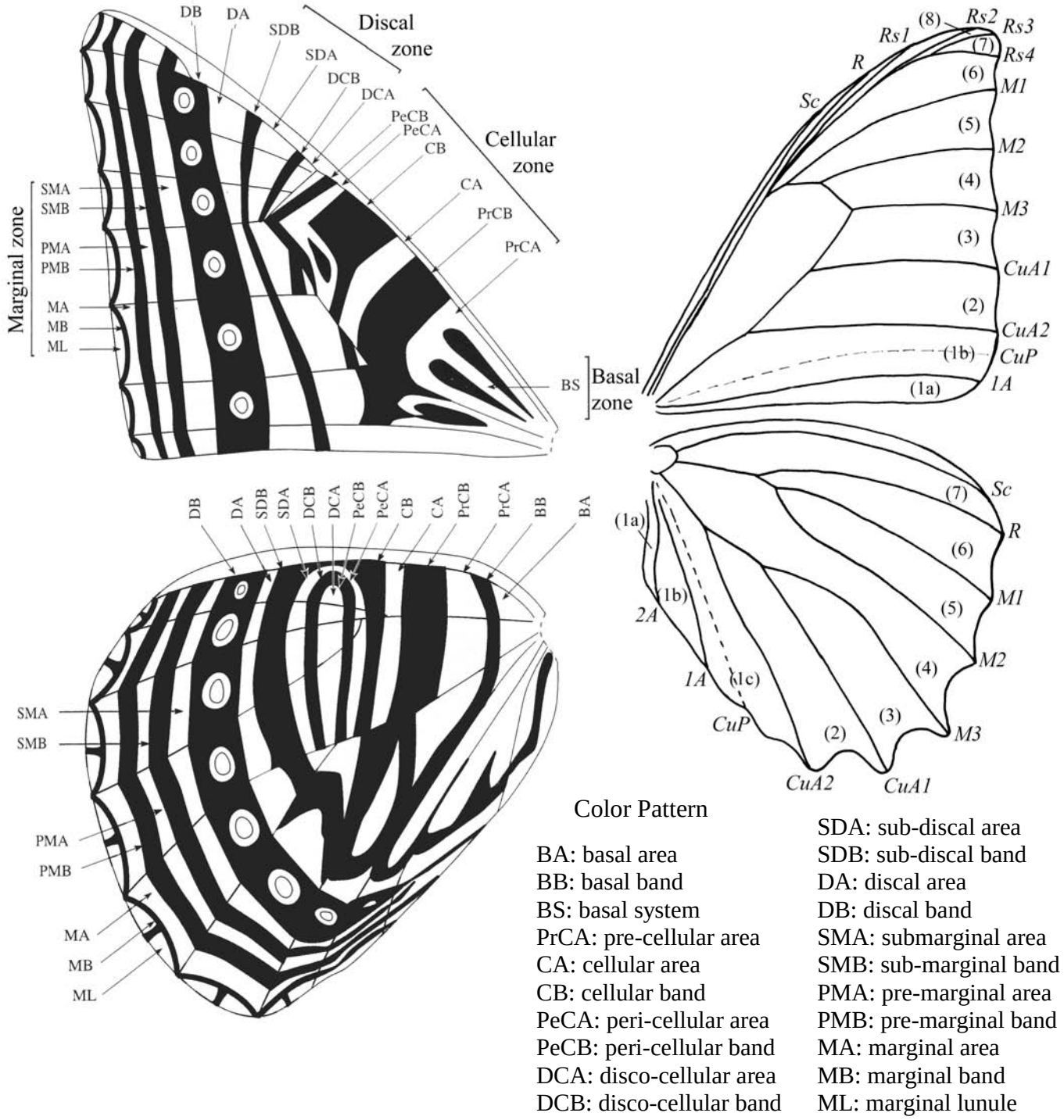
In conclusion, iridescence appears to be created by two different mechanisms – the subgenus *Deyrollia* and the *Morpho* type. Among the deep blue butterflies, optical and colorimetrical differences occur (BERTHIER, 2007), which are the result of variations in ridge height and density, scale shape and organization. Considering this, all blue *Morpho* are differently blue. With the exception of the peculiar case of the subgenus *Deyrollia*, three major events could have generated much of the observed variation within the genus. In the clade (*Iphimedeia*, *Laurschwartzia*), species became drab and lost their blue coloration as the ridge density strongly decreased and ridge height reduced to one lamella. *Pessonia* species also lost their blue coloration and became white by losing the black pigment in ground scales. Finally, the blue color became pale within *Cytheritis* str. as the melanin concentration decreased.

Flight behavior of **Morpho** *butterflies, host plants and larval behavior*

Flight characteristics, derived from literature information and numerous oral communications, are much better known for males than for females. Inclusion of the male flight height character, coded following PENZ & DEVRIES (2002) and completed using personal observations in Peru, only brought further homoplasy to the results of our analysis (fig. 2). We observed that *Morpho* flying in the understorey never fly at canopy height but that the reverse is not true. For example, we observed *M. cisseis* flying quite high (i.e. 15 meters) in the canopy, but also at only a meter above a sandy river bank (see also NEILD, 2008). This behavior was also reported for *M. polyphemus* (D. Janzen, M. Balcazár-Lara, pers. comm.) and it certainly occurs in other taxa. Moreover, indications of species flying in "midstorey" are not precise as individuals can fly just under the lower branches of canopy, quite low over shrubs or throughout the entire space between – a space that can be very important in sites where trees are very tall. Furthermore, we noted some occasional, slight or marked variations in flight height between cloudy and sunny days, depending on the species group. Linking flight height to environmental parameters such as host plant stratification or weather / microclimatic data should be investigated to determine the different parameters that influence this trait and then assess the part of it that is inherited. Now, it is more a locally useful diagnostic trait for recognizing species in the field than a phylogenetically significant character. Flight style – fluttering versus gliding – only distinguishes the clade (*Iphimedeia*, *Laurschwartzia*) (fig. 2). As we noticed in the field, perhaps more detailed and standardized observations would provide further significant characters.

Data related to larval host plants are scarce and exist only for about 20 *Morpho* taxa (data from various sources in CONSTANTINO, 1997; TÁKÁCS & TELLO, 1993; 1994; DEVRIES & MARTINEZ, 1994; BRÉVIGNON, 2003; MILLER *et al*., 2007; NEILD, 2008; GUERRA-SERRUDO & LEDEZMA-ARIAS, 2008). According to our most parsimonious interpretation, evolution of larval host-plant type (monocots versus dicots, character 142) involves only two steps (Table II), a change from monocots to dicots in clade 3 followed by a reversal to monocot feeding again in clade 9. These data are very coarse-grained but they do suggest that the first host plant shift, to dicots, was associated with the diversification of the genus (fig. 2).

We did not include data about larval gregariousness in our analyses because it is too scarce, but this behavior should also be subjected to more intense scrutiny. For example, in subgenus *Iphimedeia*, *M. telemachus* and *M. hercules* larvae are gregarious throughout all instars, whereas *M. theseus* larvae are gregarious during the first four instars but solitary in the fifth (FRUHSTORFER, 1912-1913; OTERO, 1966; BRÉVIGNON, 2003; MILLER *et al*., 2007). The same situation occurs in subgenus *Pessonia*: *M. epistrophus* and *M. iphitus* larvae are gregarious, (FRUHSTORFER, 1912-1913; OTERO, 1966; OTERO & MARIGO, 1990) but *M. polyphemus* larvae are not (YOUNG & MUYSHONDT, 1972; MILLER *et al*., 2006). Within subgenus *Grasseia*, *M. amathonte* has solitary larvae (CONSTANTINO, 1997) but it has recently been observed that *M. godartii godartii* larvae in



Color Pattern

BA: basal area
BB: basal band
BS: basal system
PrCA: pre-cellular area
CA: cellular area
CB: cellular band
PeCA: peri-cellular area
PeCB: peri-cellular band
DCA: disco-cellular area
DCB: disco-cellular band
SDA: sub-discal area
SDB: sub-discal band
DA: discal area
DB: discal band
SMA: submarginal area
SMB: sub-marginal band
PMA: pre-marginal area
PMB: pre-marginal band
MA: marginal area
MB: marginal band
ML: marginal lunule

Plate 1. – *Morpho* butterfly wing color pattern of ventral surface (from BLANDIN, 2007a) and venation (*Sc*: subcostal; *R* : radius; *Rs*: radial sector; *M*: media ; *CuA*: anterior cubitus; *CuP*: posterior cubitus; *A*: anal vein).

Bolivia (GUERRA-SERRUDO & LEDEZMA-ARIAS, 2008) and *M. godartii tingomariensis* larvae in Northern Peru, are gregarious (Douglas Cotrina Sánchez, pers. comm.). In contrast, *M. menelaus occidentalis* larvae are solitary (César Ramirez & Stéphanie Gallusser, pers. comm.). In subgenus *Morpho*, *M. helenor* (*e.g.* CONSTANTINO & CORREDOR, 2004) and *M. deidamia* (TÁKÁCS & TELLO, 1993) both have solitary larvae. According to the limited information available, larvae are also solitary in subgenus *Megamede* (TÁKÁCS & TELLO, 1994; DEVRIES & MARTINEZ, 1994). Even given our poor present knowledge, it is important to emphasize that gregariousness exists in several species in several different subgenera. Most gregarious larvae (*M. theseus* is an exception) share a common red and yellow pattern, whereas solitary larvae show a different but rather similar pattern characterized by large rhomboidal yellow or green patches. Presently, we cannot draw any definitive conclusions regarding the apomorphic or plesiomorphic status of larval behavior and pattern characters.

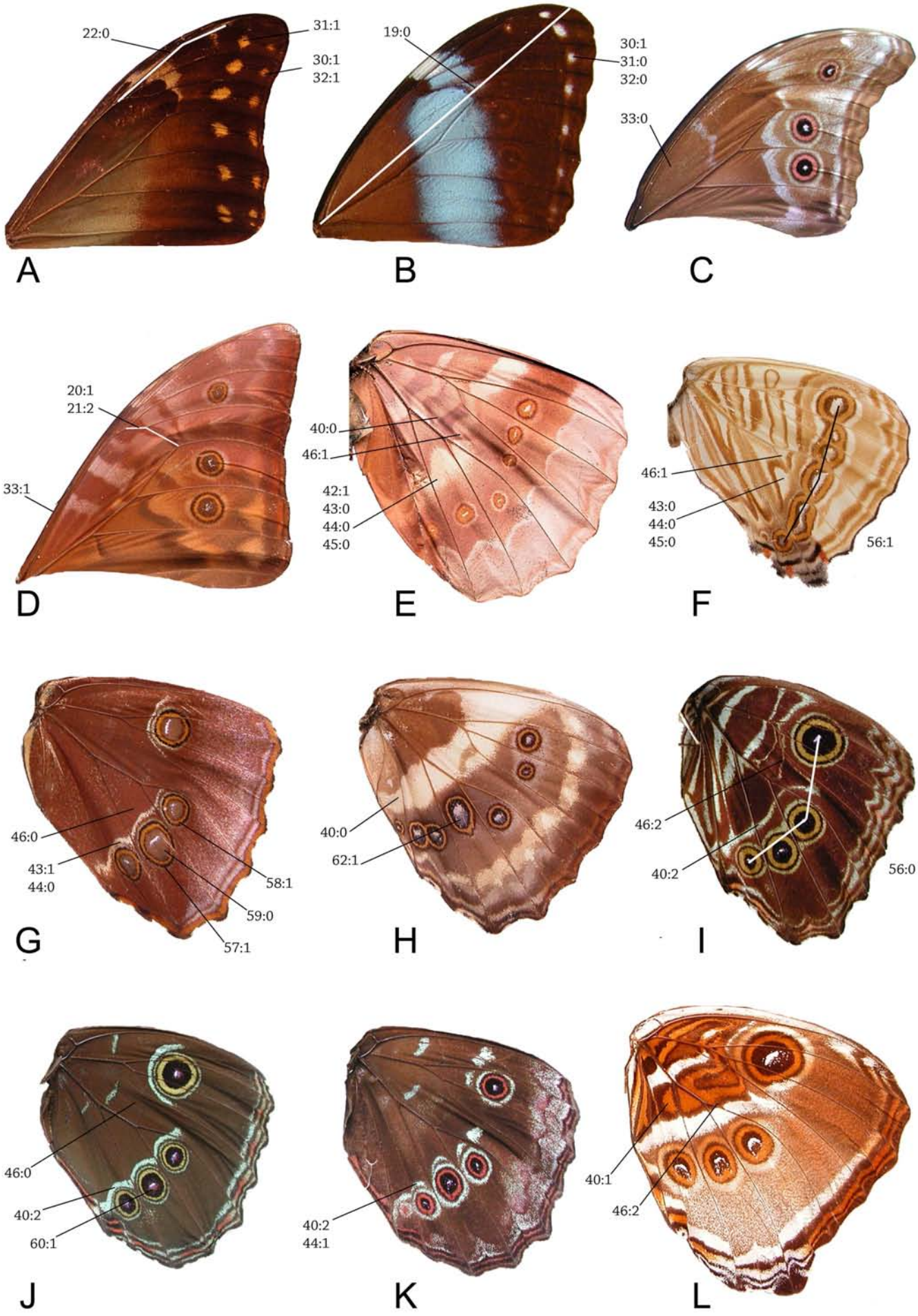


Plate 2. – Wing pattern. A-B, Forewing upperside – A: *M. hercules*; B: *M. helenor*. – C-D, Forewing underside – C: *M. godartii julanthiscus*; D: *M. theseus*. – E-L, Hindwing underside – E: *M. anaxibia*; F: *M. sulkowskyi*; G: *M. absoloni*; H: *M. cypris*; I: *M. deidamia*; J: *M. achilles phokylides*; K: *M. menelaus occidentalis*; L: *M. hecuba*.