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Phylogeny and diversification of the cloud forest *Morpho sulkowskyi* group (Lepidoptera, Nymphalidae) in the evolving Andes

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The monophyletic *Morpho sulkowskyi* butterfly group, endemic of Andean cloud forests, was studied to test the respective contributions of Mio-Pliocene intense uplift period and Pleistocene glacial cycles on Andean biodiversity. We sampled nine taxa covering the whole geographical range of the group. Two mitochondrial and two nuclear genes were analysed using a Bayesian method. We established a dated phylogeny of the group using a relaxed clock method and a wide-outgroup approach. To discriminate between two hypotheses, we used a biogeographical probabilistic method. Results suggest that the ancestor of the *M. sulkowskyi* group originated during the Middle–Late Miocene uplift of the Eastern Cordillera in northern Peru. Biogeographical inference suggests that the *M. sulkowskyi* and *Morpho lympharis* clades diverged in the northern Peruvian Andes. The subsequent divergences, from the Late Miocene to the Late Pliocene, should have resulted from a dispersal towards the Northern Andes (*M. sulkowskyi* clade), after the closure of the West Andean Portal separating the Central and Northern Andes, and a southwards dispersal along the Peruvian and Bolivian Eastern Cordilleras (*M. lympharis* clade). Only a few divergences occurred at the very end of the Pliocene or during the Pleistocene, a period when the more recent uplifts interfered with Pleistocene glacial cycles.

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Introduction

The tropical Andes, the richest and most diverse region on Earth (Mittermeier *et al.* 2004), are a good example of interactions between mountain building and diversification processes that decisively affected present biodiversity patterns (Hoorn *et al.* 2013). To explain the diversification of floras and faunas throughout the vast and complex Andean mountains, different processes were considered, including dispersal and subsequent diversification driven by the rise of mountain ranges that create connections, barriers and new ecological belts, as well as more recent cycles of areas fragmentation and expansion driven by Pleistocene climatic oscillations (e.g. Descimon 1986; Pennington & Dick 2004; Wahlberg & Freitas 2007; Antonelli *et al.* 2009; Couvreur *et al.* 2011; Condamine *et al.* 2012; Blandin & Purser 2013). That recent processes (Pleistocene glacial cycles) played a major role, or not, in the diversification of Andean lineages is a matter of debate (Rull 2008, 2014; Garzón- Orduña *et al.* 2014, 2015). Various studies, based on robust time-calibrated phylogenies, demonstrated that several plant and animal lineages diversified during the Mio-Pliocene intense uplift period (e.g. Chaves *et al.* 2011; Fiedler & Strutzenberger 2013; Castroviejo-Fisher *et al.* 2014; Luebert & Muller 2015; Sanín *et al.* 2016). However, the role of Pleistocene geoclimatic dynamism has been suggested by other studies (e.g. García-Moreno *et al.* 1999; Chesser 2000; Casner & Pyrcz 2010). As suggested by meta-analyses, the Mio-Pliocene and Pleistocene hypotheses are complementary (Turchetto- Zolet *et al.* 2013; Rull 2014).

Several works on diversification in the Andes focussed on butterflies or moths (e.g. Adams 1985; Descimon 1986; Willmott *et al.* 2001; Hall 2005; Elias *et al.* 2009; Casner & Pyrcz 2010; Strutzenberger & Fiedler 2011; Matos-Maraví *et al.* 2013; De-Silva *et al.* 2016). The diversification of the Neotropics-endemic genus *Morpho* Fabricius, 1807 (Nymphalidae), that includes the emblematic, metallic blue butterflies, was studied by Penz *et al.* (2012), and its relation with the geodynamics of the Andes was discussed by Blandin & Purser (2013). This monophyletic genus (Cassildé *et al.* 2010, 2012; Penz *et al.* 2012) originated at ca. 32 Ma (Wahlberg *et al.* 2009) in the east side of the proto-Andes (Penz *et al.* (2012) and Blandin & Purser (2013)). The genus diversified into species living in lowland forests, low mountain forests and cloud forests, during a period of major and complex orogenic processes, high elevations being attained at different times in Central Andes and Northern Andes, as well as in western and eastern cordilleras (Garzione *et al.* 2008; Sempere *et al.* 2008; Eude *et al.* 2015). In order to discriminate between the Mio-Pliocene and Pleistocene diversification hypotheses, Andean-endemic cloud forest butterflies should provide ideal models, as they diversified within a single ecological belt that could develop only when cordilleras attained suitable elevations. We have selected a group of common butterflies, the *Morpho sulkowskyi* group, that includes closely related taxa distributed from Colombia to Bolivia at 1.8–3.5 km altitudes (Krüger 1924; Schultze 1928; Salazar 1998; Prieto *et al.* 2005; personal observations in Bolivia and Peru), where they occur in stands of *Chusquea* bamboos (Poaceae, Chusqueinae), known as their host plants (Heredia & Alvarez 2007).

Here, we established a phylogeny to clarify the systematics of the group, and we used a dated phylogeny with distribution data to localize the origin of the group in time and space, and then to discriminate two biogeographical scenarios: (i) a recent vicariant diversification of a widespread species, as a result of the Pleistocene glacial cycles; (ii) an older diversification, resulting from dispersal throughout the Andes driven by geodynamics processes, with two opposite predictions: (i) origin in the Northern Andes (Colombia and Ecuador), with subsequent dispersal towards the Central Andes (Peru and Bolivia); or (ii) origin in the Central Andes and dispersal towards the Northern Andes.

Material and methods

Taxonomy

Morpho sulkowskyi Kollar, 1849 was described from Colombia. Several morphologically very similar taxa were subsequently described, which have been considered as either species or subspecies. Le Moult & Réal (1962) identified one polytypic and five monotypic species, but Lamas (2004) recognized only two polytypic species and Blandin (1993, 2007) only one (Appendix S1). In the present state of knowledge, we recognize 11 taxa of the species group: *sulkowskyi* Kollar, 1849 (Colombian cordilleras); *lympharis* Butler, 1873 (from southern Peru (Cuzco Department) to Bolivia (La Paz Department)); *eros* Staudinger, 1892 (Bolivia, Cochabamba Department); *sirene* Niepelt, 1911 (eastern Ecuador); *selenaris* Le Moult & Réal 1962 (northern Peru, upper Huallaga valley, Huánuco Department); *stoffeli* Le Moult & Réal 1962 (central Peru, Pasco and Junín Departments); *descimokoenigi* Blandin 1993 (southern Peru, Cuzco Department); *nieva* Lamas & Blandin [2007] (northern Peru, Amazonas and San Martín Departments); *calderoni* Blandin & Lamas [2007] (Amazonas Department); *achiras* Fisher, 2009 (northern Peru, Abiseo National Park, San Martín Department); *zachi* Schäffler & Frankenbach, 2009 (northern Peru, San Martín Department). Diagnostic characters are indicated and taxa are figured, with their geographical distribution, in Appendix S1, which also includes a historical survey of the taxonomy of the group.

Field studies

Three recently described taxa from northern Peru (*calderoni* Blandin & Lamas [2007], *nieva* Lamas & Blandin [2007] and *zachi* Schäffler & Frankenbach, 2009) were discovered on different slopes of the Eastern Cordillera in its northern part (Blandin & Lachaume 2014). We performed field surveys to check for the allopatry or, alternatively, the

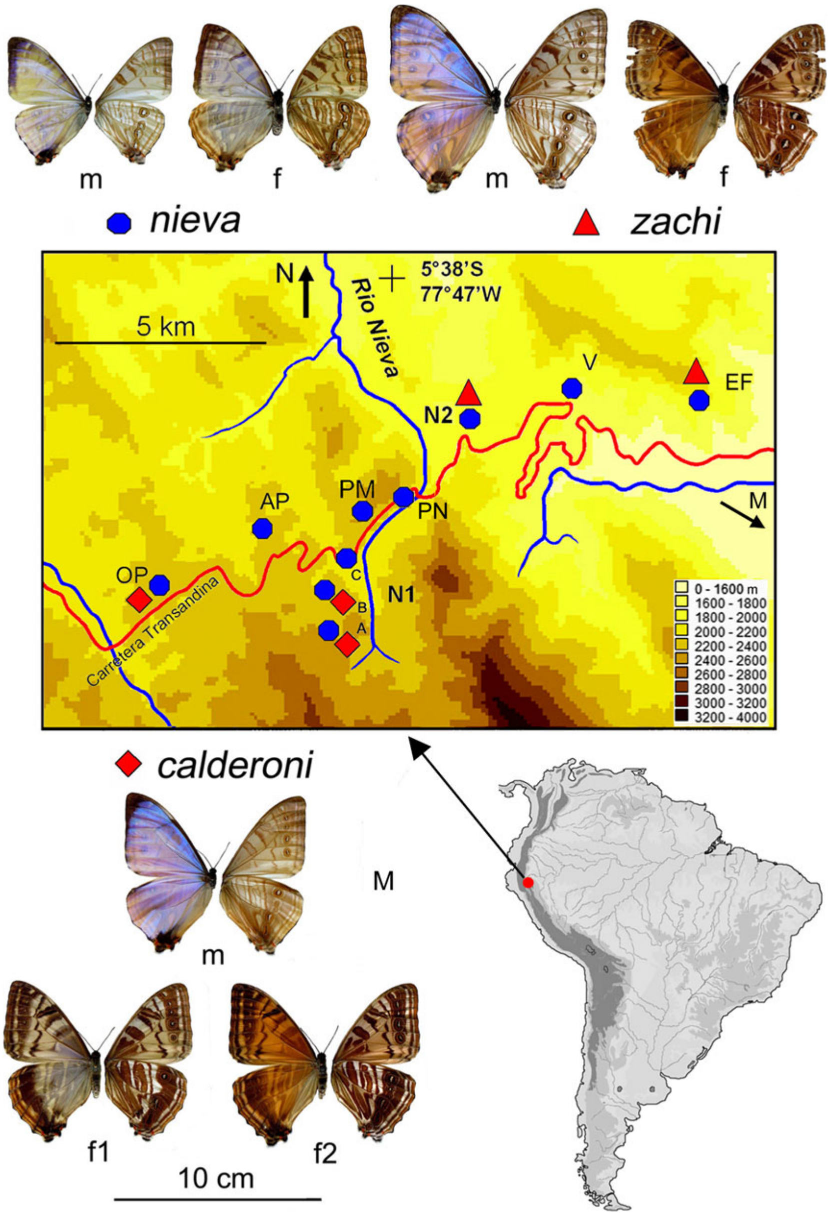

Fig. 1 Map of the region where field studies were carried out, with habitus of the taxa *calderoni*, *zachi* and *nieva* (m: male; f: female; f1 and f2: female morphs within the *calderoni* population). N1 and N2: sampling areas along the upper Río Nieva. Other localities where specimens were collected: AP: Abra Patricia; EF: El Afluente; OP: Oso Perdido; PM: Abra Pardo Miguel; V: Venceremos. Two specimens of *nieva* were also collected at Santa Cruz del Mirador (M), at ca. 20 km ESE from El Afluente.

sympatry of these taxa. After a first survey which allowed us to locate a key area close to the Carretera Transandina near the border between the Amazonas and San Martín Departments, specimens were collected along two parts of the upper Río Nieva that are separated by a mountain range that this river cut across through a narrow gorge (Fig. 1, N1 & N2). Specimens were also collected in neighbouring localities, notably in Oso Perdido (OP; Amazonas) and El Afluente (San Martín) (Fig. 1, OP; EF). The collected specimens have been deposited either in the Museo de Historia Natural, Universidad Nacional Mayor de San Marcos (MUSM, Lima) or the Muséum National d'Histoire Naturelle (MNHN, Paris).

Molecular sampling

Field sampling along Río Nieva (Fig. 1, N1 & N2) provided specimens of *calderoni*, *nieva* and *zachi* (deposited in the MNHN). Specimens as recent as possible of *sulkowskyi* Kollar, 1849 (Colombia: Central Cordillera, Medellín Area, Antioquia Department), *sirene* Niepelt, 1911 (Ecuador: Baños Area, Tungurahua Province), *selenaris* Le Moult & Réal 1962 (Peru: Carpish Area, Huánuco Department), *lympharis* Butler, 1873 (Peru: Pillahuata Area, Cuzco Department), *descimokoenigi* Blandin 1993 (Peru: Cordillera Urubamba, Cuzco Department) and *eros* Staudinger, 1892 (Bolivia: Incachaca Area, Chapare, Cochabamba Department) were selected from MNHN collections. There were no available specimens of *stoffeli* Le Moult & Réal 1962 (Junín Department, Peru). The taxon *achiras* Fisher, 2009, from Abiseo National Park (San Martín Department, Peru), could not be studied, as it is not represented in either MUSM or MNHN. Thus, we studied nine taxa and 91 specimens (Appendix S2a and Fig. 2), covering the whole geographical range of the group.

Total DNA was extracted from the legs or the abdomens of the specimens, using a Qiagen DNeasy Tissue kit (Courtaboeuf, France). Two mitochondrial (cytochrome oxidase 1 and cytochrome b) and two nuclear (EF1a – translation elongation factor 1 alpha and RPS5 – ribosomal protein S5) DNA fragments were amplified by polymerase

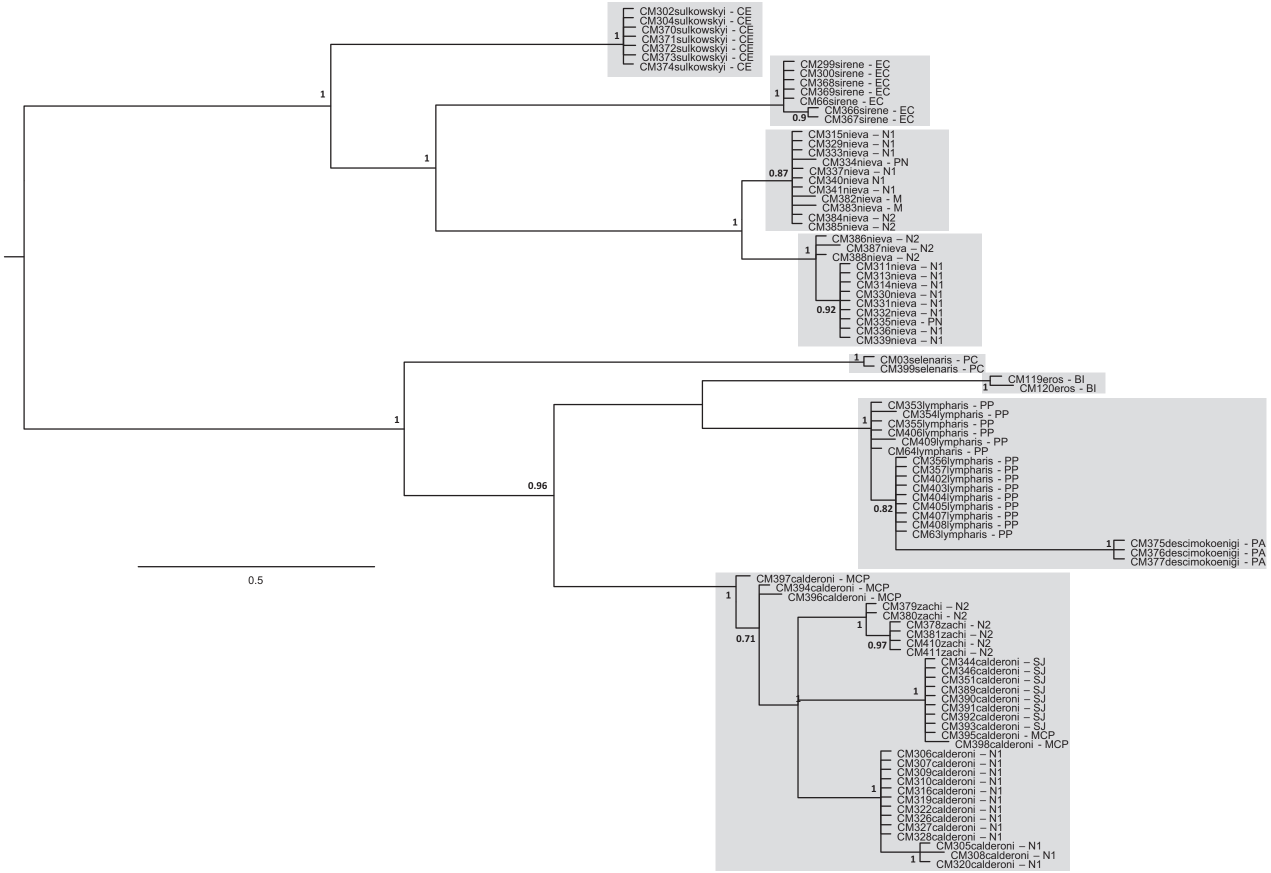

Fig. 2 Topology obtained by Bayesian inference with the concatenation of the two mitochondrial genes (CO1 and Cytb). Numbers above each node represent the posterior probabilities. The 8 clusters recognized after analyses conducted for Cytb on the poisson tree process web server are highlighted in grey.

chain reaction. For additional information on sequencing protocols, see Appendix S3.

Molecular phylogenetic analysis

The sequences of the two mitochondrial genes were concatenated for each individual, since both are from mitochondrial DNA. To conduct Bayesian analyses, the substitution model of evolution was estimated using JMODELTEST v 2.1.4 (Darriba *et al.* 2012), and the Akaike information criterion corrected for small samples (AICc) (Akaike 1973, 1974) was used to select the GTR + G model. Analyses were performed with MrBayes 3.2.2 (Ronquist & Huelsenbeck 2003). Four Markov chains were run simultaneously for 50 million generations, sampling every 1000 generations to ensure independence of samples. The first 10 000 trees generated were discarded as burn-in and determined empirically from the log-likelihood values using TRACER V1.6 (Rambaut & Drummond 2007). The remaining trees were used to construct 50% majority-rule consensus trees. Two independent runs were performed to check whether convergence was reached on the same posterior distribution and whether the final trees converged on the same topology. The statistical confidence of each node was evaluated by posterior probabilities. *Morpho anaxibia* (Esper [1801]) (subgenus *Iphixibia* Le Moult & Réal 1962) was chosen as outgroup, as this species belongs to one of the two major *Morpho* clades (see Cassildé *et al.* 2012; Penz *et al.* 2012), while the *M. sulkowskyi* group belongs to the other.

Dating analyses

To minimize the effect of increased mutation rates at the intraspecific level in dating methods (Ho *et al.* 2005; Ho 2007), we used the Poisson tree process (PTP) model to infer putative species boundaries (Zhang *et al.* 2013). This method models speciation or branching events in terms of the number of substitutions and can use the phylogenetic tree directly without needing the difficult and error prone procedures of time calibration required by other methods such as generalized mixed Yule-coalescent (Zhang *et al.* 2013). Analyses were conducted for CO1 gene using the PTP web server (http://species.h-its.org/ptp/) to select one individual per cluster for dating analyses.

Because no suitable fossils for direct calibrations exist within our study group, and in order to be fully independent from regional geological knowledge, we attempted to use a balanced approach to calibrate our analyses using a wide-outgroup approach, which is considered to be a reasonable alternative (Hedges & Kumar 2004; Strijk *et al.* 2012). We extended a published dated genus-level data set (Wahlberg *et al.* 2009) with our new sequences and sequences available in GenBank for several other *Morpho* species. The sampling for the dating analysis contained 31 Nymphalidae butterflies (including 17 *Morpho* species) and three DNA fragments (CO1, EF1a and RPS5; Appendix S2b).

To improve the estimates of node ages, we used multiple calibration points (Lee 1999; Wang *et al.* 1999; Sauquet *et al.* 2012) on basal and apical nodes. We also checked whether all calibration points were compatible with the reference topology. The dating analysis was conducted using five secondary calibration points (Wahlberg *et al.* 2009) with the entire data set (Fig. 3A). The split between Calinaginae (*Calinaga davidis* Oberthür, 1881) and all other Nymphalidae was set at 75.7 Myr (standard deviation: 7.9 Myr, node A): the split between *Euxanthe* Hübner [1819] and *Charaxes* Ochsenheimer, 1816 at 18.1 Myr (4.6 Myr, node B); the diversification of Satyrini at 52.1 Myr (6.4 Myr, node C); the split between *Neope* Moore, 1866 and *Lethe* Hübner [1819] at 36.6 Myr (5.0 Myr, node D); and the diversification of Morphini + Brassolini at 48.5 Myr (5.0 Myr, node E). The calibration of the node D is consistent with fossil data; two fossil *Lethe* species are known, the age of the oldest being estimated at ca. 32 Myr (Miller *et al.* 2012).

We used a normal distribution for the tree prior to node calibration, which is particularly suitable for modelling secondary calibration points (Ho 2007), with the same standard deviation as the highest posterior density (HPD) of Wahlberg *et al.* (2009). *Calinaga davidis* was set as a sister group of all the other species and calibration nodes were constrained on the topology, while all other relationships were left free to vary, so that topological uncertainty was incorporated into posterior estimates of divergence dates. We used the Bayesian relaxed phylogenetic approach, performed in BEAST 1.7.5 (Drummond *et al.* 2012), to estimate the relative age of divergence of the lineages studied. We partitioned the data by gene, with unlinked partitions according to the GTR + G substitution model for each and by codon for the CO1. We used the uncorrelated lognormal relaxed clock model and a birth–death process of speciation. We confirmed the results by using two independent analyses over 50 million generations and sampled every 2500th generation. The two analyses converged on similar posterior estimates. We then used TRACER 1.6 (Rambaut & Drummond 2007) to assess convergence, measure the effective sample size of each parameter and calculate the mean and 95% HPD interval for divergence times. We assessed whether a sample size >200 was achieved for all parameters after the analyses. Results of the two runs were combined with LOGCOMBINER 1.7.5 (Drummond *et al.* 2012), and the maximum clade credibility tree was compiled with TREEANNOTATOR 1.7.5 (Drummond *et al.* 2012).

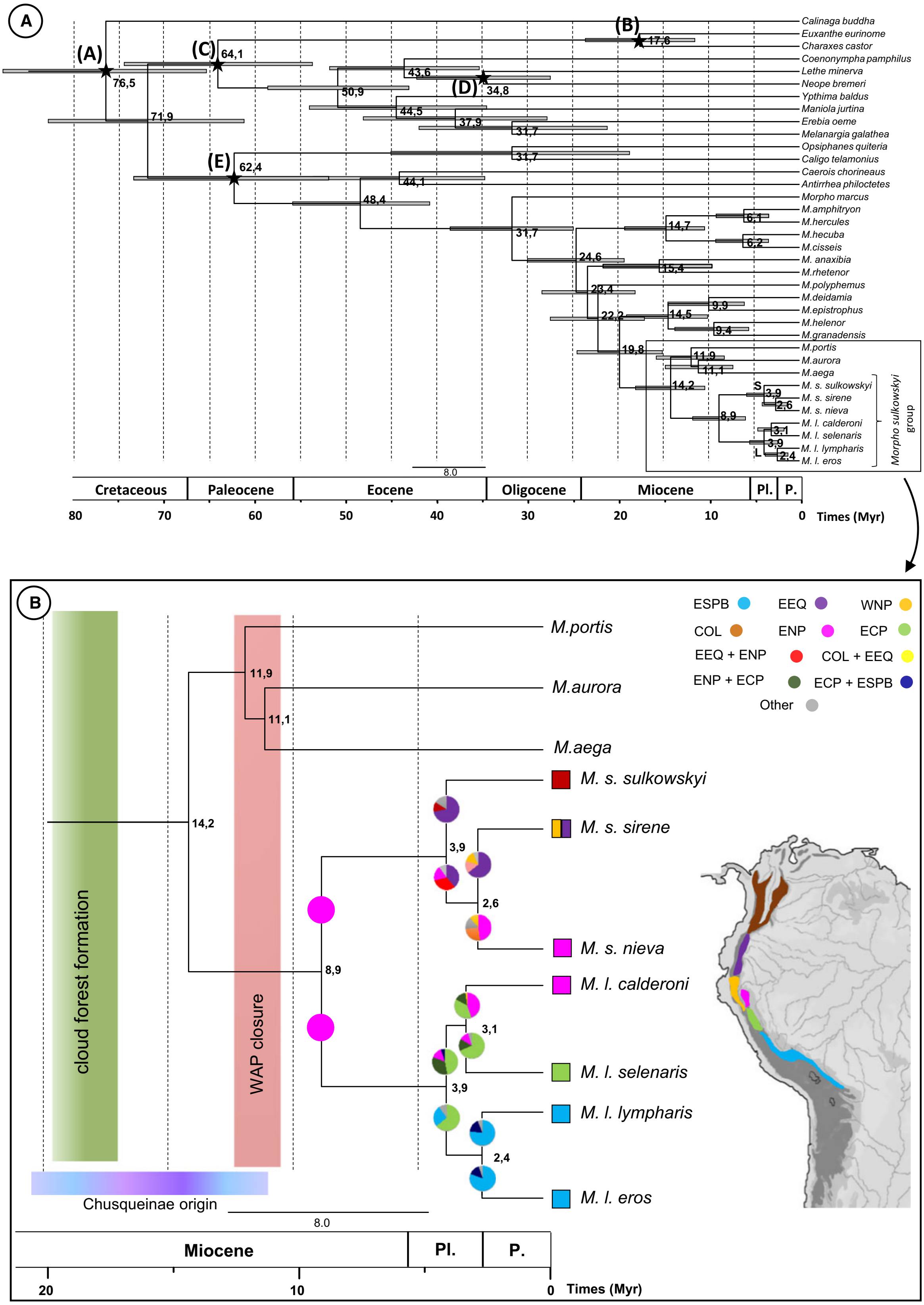
A
(A)
76,5
71,9
(C)
64,1
50,9
(B)
17,6
43,6
(D)
34,8
44,5
37,9
31,7
31,7
(E)
62,4
44,1
48,4
31,7
14,7
6,1
6,2
24,6
15,4
23,4
22,2
9,9
14,5
9,4
19,8
11,9
11,1
14,2
S
3,9
2,6
8,9
3,1
3,9
L
2,4
Calinaga buddha
Euxanthe eurinome
Charaxes castor
Coenonympha pamphilus
Lethe minerva
Neope bremeri
Ypthima baldus
Maniola jurtina
Erebia oeme
Melanargia galathea
Opsiphanes quiteria
Caligo telamonius
Caerois chorineaus
Antirrhea philoctetes
Morpho marcus
M.amphitryon
M.hercules
M.hecuba
M.cisseis
M. anaxibia
M.rhetenor
M.polyphemus
M.deidamia
M.epistrophus
M.helenor
M.granadensis
M.portis
M.aurora
M.aega
M. s. sulkowskyi
M. s. sirene
M. s. nieva
M. l. calderoni
M. l. selenaris
M. l. lympharis
M. l. eros
Morpho sulkowskyi group
8.0
Cretaceous
Paleocene
Eocene
Oligocene
Miocene
Pl.
P.
80
70
60
50
40
30
20
10
0
Times (Myr)
B
ESPB
EEQ
WNP
COL
ENP
ECP
EEQ + ENP
COL + EEQ
ENP + ECP
ECP + ESPB
Other
cloud forest formation
WAP closure
M.portis
11,9
M.aurora
11,1
M.aega
14,2
M. s. sulkowskyi
M. s. sirene
3,9
2,6
M. s. nieva
8,9
M. l. calderoni
3,1
M. l. selenaris
3,9
M. l. lympharis
2,4
M. l. eros
Chusqueinae origin
8.0
Miocene
Pl.
P.
20
10
0
Times (Myr)

Fig. 3 —A. Chronogram representing the divergence times of the principal lineages of nymphalid butterflies including the *Morpho sulkowskyi* group. —B. Chronogram representing the divergence times of the *M. sulkowskyi* group. Node positions indicate mean estimated divergence times, and pie charts represent relative probabilities of ancestral ranges.

Biogeographical reconstructions

To test the dispersal and subsequent speciation hypotheses, we used a probabilistic method implemented in LAGRANGE (Ree & Smith 2007) using biogeographical speciation scenarios and parameters for dispersal and extinction (Ree *et al.* 2005). Terminal taxa were assigned to one or several of the following geographical areas: Colombian Cordilleras (COL), Ecuadorian eastern cordilleras (EEQ), western cordillera in northern Peru (WNP), eastern cordillera in northern Peru (ENP), eastern cordillera in central Peru (ECP), eastern cordilleras in southern Peru and Bolivia (ESPB). We confined possible dispersals to adjacent areas only, specifically between the following combinations: COL–EEQ, EEQ–WNP, EEQ–ENP, ECP–WNP, ECP–ENP, ECP–ESPB. The BEAST chronogram was used for LAGRANGE calculation and the root of the tree was calibrated at 8.9 Ma, as estimated age of the most recent ancestor of all lineages of the *M. sulkowskyi* group. Separate analyses were also conducted to determine the ancestral root area, using local optimizations conditional on the root state (considering either single or multiple area ranges, but with only adjacent areas, following Condamine *et al.* (2013)). Three LAGRANGE models were run: an unconstrained model, where dispersals among all areas and any combination of ancestral areas are allowed (M0), and two stepping-stone models where dispersals are prohibited between non-adjacent areas and only combinations of adjacent ancestral areas are allowed: the first with dispersal allowed only from southern to northern adjacent areas (M1), while this constraint does not exist in the second (M2).

Results

Field studies

Sympatry was discovered between *nieva* and *calderoni* along the upper valley of the Nieva River (transect N1) and westwards at OP, while sympatry between *nieva* and *zachi* was observed downstream the Nieva (transect N2), and eastwards near El Afluente (EF) (Fig. 1). Specimens of *zachi* were never collected or observed with *calderoni* specimens and reciprocally. Therefore, it is likely that *zachi* and *calderoni* populations are parapatric.

Phylogeny and divergence time estimates

The *M. sulkowskyi* group forms two clades, one including the taxa *sulkowskyi*, *sirene* and *nieva* (clade *sulkowskyi*) and the other *selenaris*, *lympharis*, *eros*, *descimokoenigi*, *calderoni* and *zachi* (clade *lympharis*) (Fig. 2). In total, eight clusters were recognized under PTP, corresponding to *sulkowskyi*, *sirene*, *nieva* (two clusters), *calderoni* + *zachi*, *selenaris*, *lympharis* + *descimokoenigi* and *eros* (Fig. 2). We selected one individual per cluster for dating analyses, except for *nieva* specimens, which were considered as belonging to a unique cluster. The two combined BEAST runs yielded high effective sample sizes (>200) for all relevant parameters, indicating adequate sampling of the posterior distribution.

The *M. sulkowskyi* group diverged from the (*Morpho portis* (Hübner [1821]) + *Morpho aega* (Hübner [1822]) + *Morpho aurora* Westwood, 1851) clade at 14.2 Ma (95% HPD: 18.1 – 10.4) (Fig. 3A). The subsequent divergence into the *sulkowskyi* clade (S, Fig. 3.A) and the *lympharis* clade (L, Fig. 3A) occurred at 8.9 Ma (95% HPD: 11.8–5.9 Ma). Within the *sulkowskyi* clade, two subclades originated at 3.9 Ma (95% HPD: 5.8–2.1 Ma), one corresponding to the Colombian *sulkowskyi* and the other to the Ecuadorian *sirene* and the Peruvian *nieva*. The divergence of *nieva* ancestors is estimated at 2.6 Ma (95% HPD: 4.1–1.2 Ma). Within the *lympharis* clade, two subclades originated at 3.9 Ma (95% HPD: 5.5–2.4 Ma): a northern one, corresponding to *selenaris* and *calderoni*, and a southern one, including *lympharis* and *eros*. It should be noted a topological incongruence concerning the divergence of *selenaris*, which possibly results from differences in molecular sampling (see Figs 2 and 3); the dating analysis being based on mitochondrial and nuclear markers, its result will be used. The divergence of *selenaris* and *calderoni* ancestors is estimated at 3.1 Ma (95% HPD: 4.6–1.7 Ma). Within the Peruvian–Bolivian subclade, *eros* ancestors diverged from *lympharis* ancestors at 2.4 Ma (95% HPD: 3.8–1.3 Ma).

Biogeographical inference

Adjacent area optimizations recovered the ENP as the most likely root state (logL = −22.11). The M2 stepping-stone model had a higher log-likelihood (lnL) than the other models (lnL = −18.04 vs. lnL = −19.75 for M0 and lnL = −22.77 for M1), and only results from this best-fitting model are reported (Fig. 3B). LAGRANGE inference suggests that the *M. sulkowskyi* and *M. lympharis* clades diverged in the northern Peruvian Andes. Later, ancestors of the *M. sulkowskyi* clade dispersed from northern Peru towards the Ecuadorian Andes and subsequently towards the Colombian cordilleras (divergence between *sirene* and *sulkowskyi* subclades). The more recent divergence of *nieva* ancestors from the *sirene* lineage possibly resulted from a dispersal towards the northern end of the Peruvian Eastern

Cordillera. Within the *M. lympharis* clade, the major event was the separation of a northern branch and a southern branch that occurred within the Peruvian Eastern Cordillera. Within the northern branch, *calderoni* ancestors diverged from *selenaris* ancestors in the northern end of the Eastern Cordillera, while *eros* ancestors diverged from the *lympharis* lineage in Bolivia (Fig. 3B).

Discussion

Taxonomy: towards a clarification

Our results confirm the monophyly of the *M. sulkowskyi* group (Cassildé *et al.* 2012) and clarify relationships between taxa. Their partition into a *sulkowskyi* clade and a *lympharis* clade is congruent with the intuition of Le Moult & Réal (1962), who divided the *Morpho sulkowskyi* group into a *sulkowskyi* subgroup and a *lympharis* subgroup. Moreover, our field data show that the *sulkowskyi* and *lympharis* clades are represented by sympatric populations in the north of the Peruvian eastern Cordillera (Fig. 1). Therefore, these two clades should be considered as distinct species, according to Lamas (2004) taxonomic choice. However, the analysis from PTP resulted in eight clusters, which might be considered as different species (Fig. 2). Nevertheless, it should be emphasized that the morphologically very similar taxa *sulkowskyi* and *sirene* correspond to distinct clusters, while the morphologically different taxa *zachi* and *calderoni* are included in a single cluster, as well as are the taxa *lympharis* and *descimokoenigi*. On another hand, *nieva* specimens are distributed between two clusters independently of their spatial origin: in each cluster, there are specimens from N1, N2 and PN collecting areas (Figs 1 and 2). In this context, a final taxonomic decision needs to be supported by several analyses with different genes, combined with morphological data and geographical distribution in an integrative taxonomy approach (Dayrat 2005; Schlick-Steiner *et al.* 2010). At the moment, we adopt Lamas's viewpoint, and the taxonomic changes resulting from our results are synthesized in Table 1.

The origin of the group: when and where?

The *M. sulkowskyi* group originated between 18 and 10 Ma, according to our estimation, or even later, between 10 and 5 Ma, according to Penz *et al.* (2012). This incongruence between age estimations may be explained by different methodological choices. Recently, Garzón- Orduña *et al.* (2015) emphasized the difficulties of choosing between alternative scenarios based on divergence times estimated by different secondary calibrations that may produce incompatible ages. Moreover, van Tuinen & Torres (2015) showed that many methodological factors influence differently the estimation of age for deep nodes and shallow nodes. Therefore, differences in species samples and calibration points may explain why we obtained an older age for the Morphini – ca. 48 Ma vs. ca. 32 Ma – than Wahlberg *et al.* (2009). Possibly, we also overestimated other ages, including that of the *M. sulkowskyi* group and of subsequent divergences. However, we used a wide-outgroup approach and five secondary calibration points selected in Wahlberg *et al.* (2009), one of them being related to the age of a precisely dated fossil, while Penz *et al.* (2012) calibrated only the root of their tree, using the divergence time of the clade *Morpho* provided by Wahlberg *et al.* (2009). This method strongly underestimates divergence times, as demonstrated by Sauquet *et al.* (2012). Therefore, it is possible that the M. *sulkowskyi* group diverged earlier than Penz *et al.* (2012) supposed.

From a S-DIVA ancestral area reconstruction, Penz *et al.* (2012) concluded that *M. sulkowskyi* and its relatives *M. aurora*, *M. aega* and *M. portis* originated in what they called the Eastern Andean region, an immense area extending from Colombia to southern Bolivia. Blandin & Purser (2013), taking into account the other *M. sulkowskyi* and *M. lympharis* relatives (*Morpho absoloni* May, 1924, that lives in Peruvian southern and central lowlands and Andean piedmonts, *Morpho zephyritis*, Butler, 1873, that lives along the Andes in southern Peru and Bolivia, from lowlands to low Andean slopes, and *Morpho rhodopteron*, Godman & Salvin, 1880, that occurs in the Sierra Nevada de Santa Marta, northern Colombia), also concluded that the

Table 1 Taxonomic changes within the *Morpho sulkowskyi* group, resulting of the present study

Taxa				
Original names	Origin	Names in Lamas (2004)	Names in Blandin (2007)	Present study
Morpho sulkowskyi Kollar, 1849	Colombia	*M. s. sulkowskyi*	*M. s. sulkowskyi*	*M. sulkowskyi sulkowskyi*
Morpho sulkowskyi sirene Niepelt, 1911	Ecuador	*M. s. sirene*	*M. s. sulkowskyi*	*M. sulkowskyi sirene*
Morpho sulkowskyi nieva Lamas & Blandin [2007]	Peru	–	*M. s. nieva*	*M. sulkowskyi nieva*
Morpho lympharis Butler, 1873	Peru	*M. l. lympharis*	*M. s. lympharis*	*M. lympharis lympharis*
Morpho sulkowskyi calderoni Blandin & Lamas [2007]	Peru	–	*M. s. calderoni*	*M. lympharis calderoni*
Morpho sulkowskyi zachi Schäffler & Frankenbach, 2009	Peru	–	–	*M. lympharis zachi*
Morpho sulkowskyi selenaris Le Moult & Réal 1962	Peru	*M. s. selenaris*	*M. s. selenaris*	*M. lympharis selenaris*
Morpho sulkowskyi descimokoenigi Blandin 1993	Peru	*M. l. descimokoenigi*	*M. s. descimokoenigi*	*M. lympharis descimokoenigi*
Morpho eros Staudinger, 1892	Bolivia	*M. l. eros*	*M. s. eros*	*M. lympharis eros*

ancestors of all these species originated somewhere along the eastern side of the Andes. All these species occur at elevations lower than 2.0 km, except *M. rhodopteron* that is found from 0.6 to 2.4 km, and *M. sulkowskyi* and *M. lympharis* that live from ca. 1.8 to 3.5 km (Blandin 2007; Blandin & Purser 2013). Therefore, we hypothesize that the divergence of the *M. sulkowskyi* group ancestors resulted from an altitudinal shift and adaptation to the cloud forest belt, in areas where altitudes suitable for the development of cloud forests were attained.

In the southern Central Andes (Bolivia – southern Peru), at least 2.0 km elevations were attained by ca. 20–16 Ma (Sébrier *et al.* 1988; Leier *et al.* 2013; Saylor & Horton 2014), and it has been suggested that cloud forests originated in this area around 20–18 Ma (Sempere *et al.* 2005). During the Early Miocene, in northern Peru, the western cordillera was lower that southern ranges (Picard *et al.* 2008), but it attained locally 3 km elevations around 15 Ma (Margirier *et al.* 2015). Thus, the divergence of *M. sulkowskyi* group was possible in either the southern Central Andes or the northern western cordillera. However, our biogeographical analysis indicates that the *M. sulkowskyi* group originated probably in the Eastern Cordillera, in northern Peru. Here, from ca. 24 to 17 Ma, a foredeep depozone existed in the place of the Eastern Cordillera, the uplift of which resulted from an intense horizontal shortening between 17 Ma and 8 Ma (Eude *et al.* 2015). Our estimation for the divergence time of the *M. sulkowskyi* group (ca. 18–10 Ma) is consistent with these data. However, as suitable elevations were attained towards the end of the shortening period, Penz *et al.* (2012) estimation (ca. 10–5 Ma) is also compatible. The main point is that the group should have originated in the northern part of the Peruvian Eastern Cordilleran from low or mid-altitude ancestors, when this mountain range attained cloud forest elevations, around 10 Ma, consistent with the divergence between ca 14.5 and 6.5 Myr of the *Chusquea* clade that includes *Calinaga scandens*, the host plant of *M. sulkowskyi* in Colombia (Heredia & Alvarez 2007; Fisher 2011).

Mio-Pliocene or Pleistocene diversification?

The first divergence within the *M. sulkowskyi* group occurred during the Late Miocene, at ca. 8.9 Ma, probably a very few millions of years after the group originated in the Peruvian Eastern Cordillera. The ancestors of the *sulkowskyi* clade possibly dispersed towards the western cordillera, where cloud forests today exist in large areas near the Ecuadorian border, and southwards as restricted, isolated patches surrounded by cultivated areas (Young & León, 2001). This hypothesis is supported by the discovery in one of these relictual cloud forests (Cutervo Area, Naranja, 2300 m; Cajamarca Department), in 1998, of four males similar to Colombian and Ecuadorian specimens and consequently identified as *sulkowskyi* by Blandin (2007), who considered *sirene* as a junior synonym of *sulkowskyi* (these specimens are housed in MUSM; see Appendix 1, Fig. S1–2e). Such a distribution, covering the north of the Peruvian western cordillera and the eastern Ecuadorian cordilleras, is not surprising: it is known in other cloud forest groups, for example in the hummingbird genus *Metallura* Gould, 1847, which has an Andean distribution almost similar to the distribution of the *M. sulkowskyi* group (Benham *et al.* 2015).

The divergence of *sulkowskyi* ancestors from *sirene* ancestors occurred during the Pliocene, at ca. 3.9 Myr. Their common ancestors should have dispersed from the Peruvian western cordillera towards the Ecuadorian eastern cordilleras. Until the Middle Miocene, a lowland corridor situated approximately between 3°S and 5°S, the 'Western Andean Portal' (WAP) separated the Ecuadorian Andes from the Peruvian Andes (Antonelli *et al.* 2009; Hoorn & Wesselingh 2010). The WAP landscapes included wetlands, and temporary marine incursions from the Pacific Ocean might have occurred (Hungerbühler *et al.* 2002). Thus, the WAP constituted a long-lasting barrier for mountain organisms (Antonelli *et al.* 2009). Starting from 13 to 11 Ma, the uplift of mountain ranges closed the WAP (Coltorti & Ollier 2000; Hungerbühler *et al.* 2002). Migration of cloud forest species towards Ecuadorian and Colombian Andes became possible when suitable elevations were attained. Luebert & Weigend (2014) quote several examples of plants that dispersed from the Central to the Northern Andes, and vice versa, during the Late Miocene and Early Pliocene (10–3.8 Ma), following the closure of the WAP. For example, the ancestors of species of the genus *Puya* (Bromeliaceae) that diversified in the high Northern Andes originated in the Central Andes and crossed the WAP around 6–4 Ma (Givnish *et al.* 2011, 2014; Jabaily & Sytsma 2013). We hypothesize that the ancestors of *sirene* and *sulkowskyi* lineages also crossed the WAP during this period, corresponding approximately to the Early Pliocene.

In Colombia, the western and central cordilleras already had rather high elevations at the Oligocene–Miocene boundary (24–21 Ma) (Duque-Caro 1979; Gómez *et al.* 2005; Roddaz *et al.* 2010). A major phase of uplift followed, and the rise of the Eastern Cordillera began at that time (Restrepo- Moreno *et al.* 2009). This cordillera reached 2.0–2.5 km elevations by 3.7 Ma at the latitude of Bogotá (Van der Hammen & Hooghiemstra 2001). As we estimated the divergence of *sulkowskyi* ancestors from *sirene* ancestors at ca. 3.9 Myr, a synchronous dispersal from the eastern Ecuadorian slopes towards the three cordilleras is plausible during the Late Pliocene.

The diversification of the *lympharis* clade occurred during a period encompassing the Pliocene and Pleistocene, so it is difficult to differentiate the roles, on the one hand, of latitudinal range expansion across existing topographical barriers and subsequent allopatric divergence, and, on the other hand, the fragmentation of already established areas provoked by glacial cycles. Our results suggest a north-to-south dispersal of the *lympharis* lineage after its divergence, in the Peruvian Eastern Cordillera, at ca. 3.9 Ma, followed around 2.4 Ma by the divergence of the southernmost *eros* lineage, perhaps as a consequence of Late Pliocene or early Pleistocene climatic changes. In our present state of knowledge, we suppose that *descimokoenigi* populations, which are geographically separated from *lympharis* populations (Appendix S1), diverged during the Pleistocene.

Within the northern subclade, the divergence of *calderoni* ancestors also occurred during the Late Pliocene (ca. 3.1 Ma). It possibly resulted from a dispersal towards the north-eastern extremity of the Eastern Cordillera, but the role of climatic changes at the very end of the Pliocene or the beginning of the Pleistocene cannot be excluded. Our field studies provided evidence that *zachi* and *calderoni* populations are parapatric in the north-eastern extremity of the Eastern Cordillera. We hypothesize that their divergence resulted from Pleistocene fragmentation. However, it should be emphasized the fact that the easternmost mountain ranges raised during the very last few millions of years and that they probably attained cloud forest altitudes during the Pleistocene; therefore, dispersal of cloud forest species from older ranges may have been controlled by climate-driven altitudinal shifts of ecosystems.

The divergence of *nieva* ancestors from the *sirene* lineage occurred approximately at the Pliocene–Pleistocene transition (ca. 2.6 Ma), logically in the north of the Peruvian western cordillera. As *nieva* is known only from the northernmost part of the Eastern Cordillera, which is separated from the western cordillera by the dry and deep Marañón Valley, we hypothesize that changes in altitudinal distribution of ecological belts associated with Pleistocene climatic variations after 2.6 Ma may have favoured dispersion between the western and the eastern cordilleras.

On the whole, the expansion and diversification of the *M. sulkowskyi* group essentially occurred before the Quaternary. However, climate-driven cycles of fragmentation and expansion possibly played a role in the ultimate diversification of the group. An almost similar pattern was observed in the butterfly genus *Lymanopoda* Westwood, 1851 (Nymphalidae, Satyrinae), a species-rich montane group distributed along the Andes. It includes a majority of cloud forest species, the larvae of which eat *Chusquea* bamboos (Casner & Pyrcz 2010). The genus first diversified between 15 and 10 Ma, the formation of major clades occurred by 10–8 Ma, and most of the species-level diversification occurred since the end of the Miocene, ca. 6 Ma, more than half of the studied species being of Pleistocene or even post-Pleistocene origin. The diversification of another high elevation (1.9–4.8 km) Andean species, the hummingbird genus *Metallura* followed an almost similar pattern, with Mio-Pliocene first divergences, but with the majority (81%) of divergence events occurring during the mid–late Pleistocene (Benham *et al.* 2015). Thus, such examples suggest that the precise timing of diversification could differ between Andean groups, Pleistocene climatic cycles having a more or less important influence.

Conclusions

Simple relationships between Andean uplift and the diversification of various plant and animal groups, implying pre-Pleistocene driving processes, have been supposed by various authors. Doan (2003), for example, proposed the south-to-north speciation hypothesis, where the process of speciation should be related to the south-to-north progression of uplift throughout the Andes. Other authors emphasized the possible role of a rapid uplift that occurred during the Late Miocene and Early Pliocene, but often without establishing clear links between dated divergences and local geologic events (e.g. Casner & Pyrcz 2010; Mulch *et al.* 2010; Matos-Maraví *et al.* 2013; Lagomarsino *et al.* 2016). From a geological point of view, the concept of a progressive, general south-to-north uplift is an oversimplified view of a much more complex reality (Sempere *et al.* 2008). In the Central Andes, palaeo-elevation histories differ not only between the south and the north, but also between the western and the eastern cordilleras, notably in northern Peru (Picard *et al.* 2008; Eude *et al.* 2015; Margirier *et al.* 2015). The idea that the Northern Andes, as a whole, uplifted later than the Central Andes, as suggested by Doan (2003), and often admitted by other authors, is not supported by geological studies that also demonstrate that the timing of palaeo-elevation differed between the three Colombian Cordilleras (Restrepo- Moreno *et al.* 2009). Consistent with many other examples, notably the clearwing Oleriina butterflies (De-Silva *et al.* 2016), the *M. sulkowskyi* group illustrates the diversity of diversification histories throughout the Andes. It also demonstrates that Mio-Pliocene orogenic and Pleistocene climatic diversification drivers should not be opposed.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Appendix S1. The *Morpho sulkowskyi* group: geographical distribution and taxonomy.

Appendix S2. List of all taxa included in the analyses and their Genbank accession numbers: (a) phylogenetic analysis and species delimitation; (b) dating analysis. In GenBank, geographical origins of many specimens are not available (n/a).

Appendix S3. Additional information on sequencing protocols.

Phylogeny and diversification of the cloud forest *Morpho sulkowskyi* group (Lepidoptera, Nymphalidae) in the evolving Andes

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Appendix S1

The *Morpho sulkowskyi* group: geographical distribution and taxonomy

This supplementary information is not a taxonomic revision. It aims to provide a clarification of the nomenclature that changed several times in the past, and a survey of the geographical distribution of taxa, that are illustrated and briefly described.

Historical background

The name *Morpho sulkowskyi* Kollar (lectotype ♂ from Pacho, Cundinamarca, Colombia, Eastern Cordillera) was not published in 1850, as currently quoted (Blandin, 1993, 2007, Lamas, 2004) but in a preprint issued by Kollar (1849) (Lamas, 2016). According to Lamas (2004), *Morpho ganymede* Westwood, 1851 (syntype ♀ from "Bogotá", Colombia) is a junior synonym of *sulkowskyi*.

When Fruhstorfer (1912-1913) revised the genus *Morpho*, the *Morpho sulkowskyi* group was poorly known: *M. lympharis* Butler, 1873 had been described from Peru (male and female syntypes from Cuzco, "Huasampilla" (= Huaisampillo, Huaisampilla, Huaysampillo, Huaysampilla), 9000 feet, on the upper Cosñipata valley), *M. eros* Staudinger, 1892, from Bolivia (lectotype ♂ from Locotal, a locality in Cochabamba department), and *M. sulkowskyi sirene* Niepelt. 1911 from Ecuador (four male syntypes without precise locality [Ecuador, "Oriente"]).

Subsequently, several taxa were described and different classifications were proposed (tab. S1-1). Le Moult & Réal (1962-1963) performed a comprehensive study of specimens housed in major European collections. They divided the group into two sub-groups, taking into account the shape of hindwings and the shape of subunci (male genitalia): i) - the *M. sulkowskyi* sub-group, including a single polytypic species (*M. sulkowskyi* Kollar, 1849); ii) - the *M. lympharis* sub-group, including five monotypic species (*M. lympharis* Butler, 1873, *M. eros* Staudinger, 1892, *M. ockendeni* Rothschild, 1916, *M. nymphalis* Le Moult & Réal, 1962, and *M. stoffeli* Le Moult & Réal, 1962).

Blandin (1993) noted several confusing points in Le Moult & Réal (1962-1963), and emphasized the fact that known populations were geographically separated (sometimes by hundreds of kilometres); consequently, he preferred to consider the whole *M. sulkowskyi* group as a single, polytypic species. Lamas (2004) distinguished two species, *M. sulkowskyi* and *M. lympharis*, based on male genitalia differences (unpublished observations), and corresponding to the sub-groups of Le Moult & Réal (1962-1963), but Blandin (2007) maintained his viewpoint, waiting for field studies that might, perhaps, allow the finding of sympatric populations. In the meanwhile, Blandin & Lamas ([2007]) described two new subspecies from northern Peru that they decided to refer to *M. sulkowskyi*: *M. sulkowskyi nieva* Lamas & Blandin, [2007], and *M. sulkowskyi calderoni* Blandin & Lamas, [2007]. Later, also from northern Peru, two other subspecies were described and referred to *M. sulkowskyi*: *M. sulkowskyi achiras* Fisher, 2009, and *M. sulkowskyi zachi* Schäffler & Frankenbach, 2009.

Table S1-1. Successive classifications of specific and/or subspecific taxa included in the *Morpho sulkowskyi* group.

Taxa	Origin	Le Moult & Réal (1962)	Blandin (1993)	Lamas (2004)	Blandin (2007)
sulkowskyi Kollar, 1849	Colombia	*M. s. sulkowskyi*	*M. s. sulkowskyi*	*M. s. sulkowskyi*	*M. s. sulkowskyi*
ganymede Westwood, 1851	Colombia	*M. s. sulkowskyi*	ignored	*M. sulkowskyi sulkowskyi*	*M. sulkowskyi sulkowskyi*
lympharis Butler, 1873	Peru	*M. lympharis*	*M. s. lympharis*	*M. l. lympharis*	*M. s. lympharis*
eros Staudinger, 1892	Bolivia	*M. eros*	*M. s. eros*	*M. l. eros*	*M. s. eros*
psyche Staudinger, 1892	Bolivia	= *eros*	ignored	*M. l. eros*	ignored
sirene Niepelt, 1911	Ecuador	*M. s. sirene*	*M. s. sirene*	*M. s. sirene*	*M. s. sulkowskyi*
ockendeni Rothschild, 1916	Peru	*M. ockendeni*	*M. s. lympharis*	*M. l. lympharis*	*M. s. lympharis*
hoppiana Niepelt, 1923	Colombia	*M. s. hoppiana*	*M. s. sulkowskyi*	*M. s. hoppiana*	*M. s. hoppiana*
orientalis E. Krüger, 1925	Colombia	ignored	ignored	*M. s. sulkowskyi*	*M. s. sulkowskyi*
howarthi Le Moult & Réal, 1962	« Costa Rica »	*M. s. howarthi*	doubtful taxon	*M. s. sulkowskyi*	*M. s. sulkowskyi*
selenaris Le Moult & Réal, 1962	Peru	*M. s. selenaris*	*M. s. selenaris*	*M. s. selenaris*	*M. s. selenaris*
nymphalis Le Moult & Réal, 1962	Bolivia	*M. nymphalis*	*M. s. eros*	*M. l. eros*	= *M. s. eros*
stoffeli Le Moult & Réal, 1962	Peru	*M. stoffeli*	*M. s. stoffeli*	*M. l. stoffeli*	*M. s. stoffeli*
descimokoenigi Blandin, 1993	Peru	-	*M. s. descimokoenigi*	*M. l. descimokoenigi*	*M. s. descimokoenigi*
nieva Lamas & Blandin, [2007]	Peru	-	-	-	*M. s. nieva*
calderoni Blandin & Lamas, [2007]	Peru	-	-	-	*M. s. calderoni*

Geographical distribution and nomenclatural problems

The figure S1-1 illustrates the distribution of taxa.

Morpho sulkowskyi exists in the three Colombian Cordilleras. It was discovered several decades ago in the Venezuelan Cordillera de Mérida (Blandin & Deroche, 2011). The ♂ holotype of *howarthii* Le Moult & Réal, 1962, supposedly collected in Costa Rica (where representatives of the *M. sulkowskyi* group do not exist) is similar to the holotype of *sulkowskyi*. The nominate *sulkowskyi* was described from the Colombian Cordillera Oriental. One of the two known ♂ syntypes of the form *hoppiana* Niepelt, has a rather precise locality, "Frontina [sic] bei Ipiales", located in Nariño department, between the Central and the Western Cordilleras. The variety *orientalis* E. Krüger, 1925 was described from the Eastern Cordillera, without any more precision (based on an unstated number of male and female specimens, whereabouts of original specimens unknown).

Lamas (2004) and Blandin (2007), considered *hoppiana* as a distinct taxon, but it is similar to specimens from the Western and Central Cordilleras, and from different localities along the Eastern Cordillera (Huila department to the south, and Santander department to the north). The lectotype of the nominate *sulkowskyi* was collected in a geographically intermediate area (Cundinamarca department). It differs from the *hoppiana* syntypes by the presence of a black, elongated mark along the hindwing costa. The two males known from Venezuela also have this black mark (Blandin & Deroche, 2011). Thus, the relationships between the nominate taxon and *hoppiana* are unclear, and further studies are needed to disentangle the problem. For the purpose of the present work, we use the name *sulkowskyi* to designate all Colombian specimens (NB: specimens analyzed in this study are uniquely from the area of Medellín, in the Central Cordillera).

In Ecuador, the *M. sulkowskyi* group is unknown along the Pacific side of the Andes. Blandin (1993) noted that Ecuadorian specimens closely resemble Colombian ones. Lamas (2004) considered *sirene* as a distinct subspecies, but Blandin (2007) established the synonymy: *M. sulkowskyi sirene* Niepelt, 1911 = *M. sulkowskyi sulkowskyi* Kollar, 1849. Here we follow Lamas (2004) and use the name *sirene* to designate the sampled Ecuadorian population, a decision supported by our phylogenetic analysis.

Several taxa exist in northern Peru. We provisionally refer to *sirene* a population discovered in the Peruvian western cordillera (Cajamarca department). The taxon *nieva* Lamas & Blandin, [2007] is distributed in the northern end of the eastern cordillera, in Amazonas and San Martín departments. It is partly sympatric with *calderoni* Blandin & Lamas, [2007], which extends to the south along the eastern cordillera. The taxon *zachi* was based on commercial specimens with an imprecise, but not erroneous locality (« Peru, Dep. San Martin, Alto Mayo, westlich Nueva Cajamarca, 1750 m », in Schäffler & Frankenbach (2009)). Actually, it is known only in a limited area, at about 70 km in straight line to the WNW of the town of Nueva Cajamarca, near the limit between the San Martín and Amazonas departments. It is sympatric with *nieva*, and parapatric with *calderoni*.

Thanks to our field studies, the taxon *zachi* is well characterized. On the contrary, the taxon *achiras* is intriguing: according to illustrations in Fisher (2009), males are similar to *zachi* males, while females definitely differ from *zachi* ochre-yellow females, and resemble *calderoni* bluish females. The area where the *achiras* type series was supposedly collected is situated in the Abiseo National Park, at more than 200 km, in straight line, to the south of the area of *zachi*, and more than 100 km to the south of the area of *calderoni*. The name *achiras* was published a few monthes before *zachi*; if they were to be regarded as subjective synonyms, *achiras* would have priority.

The taxon *selenaris* Le Moult & Réal, 1962 was described as a subspecies of *M. sulkowskyi* after a single male labelled from Pozuzo, a locality situated in central Peru (Pasco department), along the Eastern Cordillera. Blandin (1993) identified as *selenaris* very similar specimens from Carpish (Huánuco department), north of Pozuzo. Le Moult & Réal (1962-1963) described as a distinct species the taxon *stoffeli*, after a single male from Oxapampa, less than 60 km south of Pozuzo, in the same river basin. Populations from the Chanchamayo area (Junín department), to the south of Oxapampa (in a different river basin), have been identified as *stoffeli* (Blandin, 1993, 2007). Two males collected in the area of Jano (12°46'S-74°00'W; Ayacucho department) are also identified as *stoffeli* (Museo de Historia Natural, Universidad Nacional Mayor de San Marcos, Lima). In the present work, we only analyzed two specimens from Carpish, which we identified as *selenaris*.

In southern Peru, two taxa have been identified. The taxon *descimokoenigi* Blandin, 1993 was described from males collected in the Cordillera Urubamba, (Cuzco department), and it occurs at Machu Picchu (Lamas, 2002). The syntypes of *lympharis* Butler, 1873 were collected in Cuzco, on the upper Cosñipata valley; specimens from Puno department (Peru), and from La Paz department (Bolivia) are similar (Blandin, 1993). At the extreme south of the distribution of the *M. sulkowskyi* group, in Bolivia, the taxon *eros* Staudinger, 1892 is known only in Cochabamba department.

Characterization of taxa

***sulkowskyi* Kollar, 1849** (Figures S1-2a, b; S1-3a, b)

Male. Wingspan about 85-90 mm. Contour of the hindwing regular, with a short cubital lobe, and very reduced undulations at the ends of the veins. Apex of the forewings black, a colour which extends a little backwards, forming a marginal band rapidly contracting into a fine edging. On the hindwing underside, there is generally no ocellus in space M1-M2.

Female. Wingspan about 85-100 mm. Wing shape more rounded than in the male; distal edge of the forewings often convex. Contour of the hindwing with undulations slightly more pronounced than in males. A light-blue metallic colouring extends on a pale ivory-yellow background; marginal decoration dark brown on the forewings, ochre on the hindwings. On the hindwing underside, there is generally no ocellus in space M1-M2.

***sirene* Niepelt, 1911** (Figures S1-2c, d, e f; S1-3c, d)

There is no obvious difference with *sulkowskyi*.

***nieva* Lamas & Blandin [2007]** (Figures S1-2g, h; S1-3e, f)

Males and females are smaller than in *sulkowskyi* and *sirene* (wingspan about 70-80 mm). On the hindwing underside, the ocellus in space M1-M2 is more often missing. Forewing of the male with a 4-5 mm wide dark margin, covered by deep violet-blue reflections. The pale metallic colouring of the female is less brilliant than in *sulkowskyi* and *sirene*.

***zachi* Schäffler & Frankenbach, 2009** (Figures S1-4a, b; S1-5a, b)

Males and females are larger than in *sulkowskyi* and *sirene*: the wingspan of many males and females attains or even exceeds 100 mm. The contour of the hindwing is very regular in males, while it is slightly undulated in females. In both sexes, the ocellus in space M1-M2 (hindwing) is generally present. Forewing of males with a narrow black margin, hindwing with black marks covered by blue reflections at the ends of the veins. Ventral surface of males with a general whitish background, and silvery reflections. All females have a yellow-ochre colouring on the dorsal surface, with brownish drawings.

***calderoni* Blandin & Lamas [2007]** (Figures S1-4c, d; S1-5c, d, e, f)

Males and females are smaller than in *zachi*: the wingspan varies between 75 and 85 mm in males, 85 and 95 mm in females. The contour of the hindwing is regular in males, while it is slightly undulated in females, with a small projection at the end of vein Cu1. In both sexes, the apex of the hindwing is more elongated along the veins R and M1, than in *sulkowskyi*, *sirene*, and *nieva*: consequently, the hindwing has a more triangular shape. In both sexes, the ocellus in space M1-M2 (hindwing) is generally present. The black margin of the forewing, in males, narrows rapidly; the hindwing has a fine, black marginal line, but no dark marks at the ends of the veins; near the apex, along the costa, there is an elongated black mark. Ventral surface of males with a pale yellow-ochre background colour. In some populations, there are two female morphs, which are equally common: the morph 1 (Figure S1-5c, d) has a yellow-ochre colouring on the dorsal surface, as in *zachi* females, but brown drawings are darker; the morph 2 (Figure S1-5e, f) has a pale ivory colouring, with blue reflections, and dark-brown drawings. In other populations, there is only one morph, almost similar to morph 2, but often with a more yellow colouring.

***achiras* Fisher, 2009** (not illustrated)

Male similar to *zachi* males. Female similar to the morph 2 of *calderoni* females.

***selenaris* Le Moult & Réal, 1962** (Figures S1-4e, f; S1-6a, b, c, d)

Male and female wingspan between 80 and 90 mm. The contour of the hindwing is regular in males, while it is undulated in females, with projections at the end of veins, notably Cu1. In both sexes, the apex of the hindwing is elongated along the veins R and M1. In both sexes, the ocellus in space M1-M2 (hindwing) is generally present. The black margin of the forewing, in males, narrows rapidly; the hindwing has a fine, black marginal line, but no dark marks at the ends of the veins; in most cases, there is no black mark near the apex. There are two female morphs (Figure S1-6a, b, c, d), but the ochre-yellow morph is much less frequent than in *calderoni*.

***stoffeli* Le Moult & Réal, 1962** (Figures S1-4g, h; S1-6e, f)

Male and female wingspan between 80 and 90 mm. The contour of the hindwing is regular in some males, but with small projections at the ends of veins in others; it is undulated in females, with sharp projections at the end of veins, notably Cu1. In both sexes, the apex of the hindwing is often less elongated than in *selenaris*, the shape of the hindwing thus being less triangular; consequently, the line of ocelli (underside) is more or less parallel to the edge of the wing. In both sexes, the ocellus in space M1-M2 (hindwing) is generally present. There is no dimorphism in females.

***descimokoenigi* Blandin, 1993** (Figures S1-7a, b; S1-8a, b, c, d)

Male and female wingspan between 75 and 85 mm. Forewings and hindwings very elongated distally. The contour of the hindwing is regular in males, however with a small Cu1 projection; it is undulated in females, with sharp projections at the end of veins, notably Cu1. In both sexes, the apex of the hindwing is strongly elongated along the veins R and M1, and the line of ocelli strongly diverges from the edge of the wing. In both sexes, the ocellus in space M1-M2 (hindwing) is often reduced, or even missing.

The ventral surface, in males, can be coloured with a strong ochre tint, as the specimen illustrated (Figure S1-7b), or it can be much more pale. Similarly, there are females differing by the color of the ventral surface, but also dorsally (Figure, S1-8a, c). Intermediate specimens do exist.

***lympharis* Butker, 1873** (Figures S1-7c, d; S1-8e, f)

Male and female wingspan between 75 and 85 mm. Contour of the hindwing with at least a marked projection at the end of Cu1 in males, and several in females. In both sexes, the ocellus in space M1-M2 (hindwing) is missing in about 75% of specimens. Variations of colour, as in *descimokoenigi*, are unknown. Otherwise, differences between the two taxa are weak.

***eros* Staudinger, 1892** (Figures S1-7e, f; S1-8g, h)

Male and female wingspan between 85 and 95 mm. Contour of the hindwing with marked projections in males and females. In both sexes, the ocellus in space M1-M2 (hindwing) is missing in almost all specimens. Variations of colour, as in *descimokoenigi*, are unknown.

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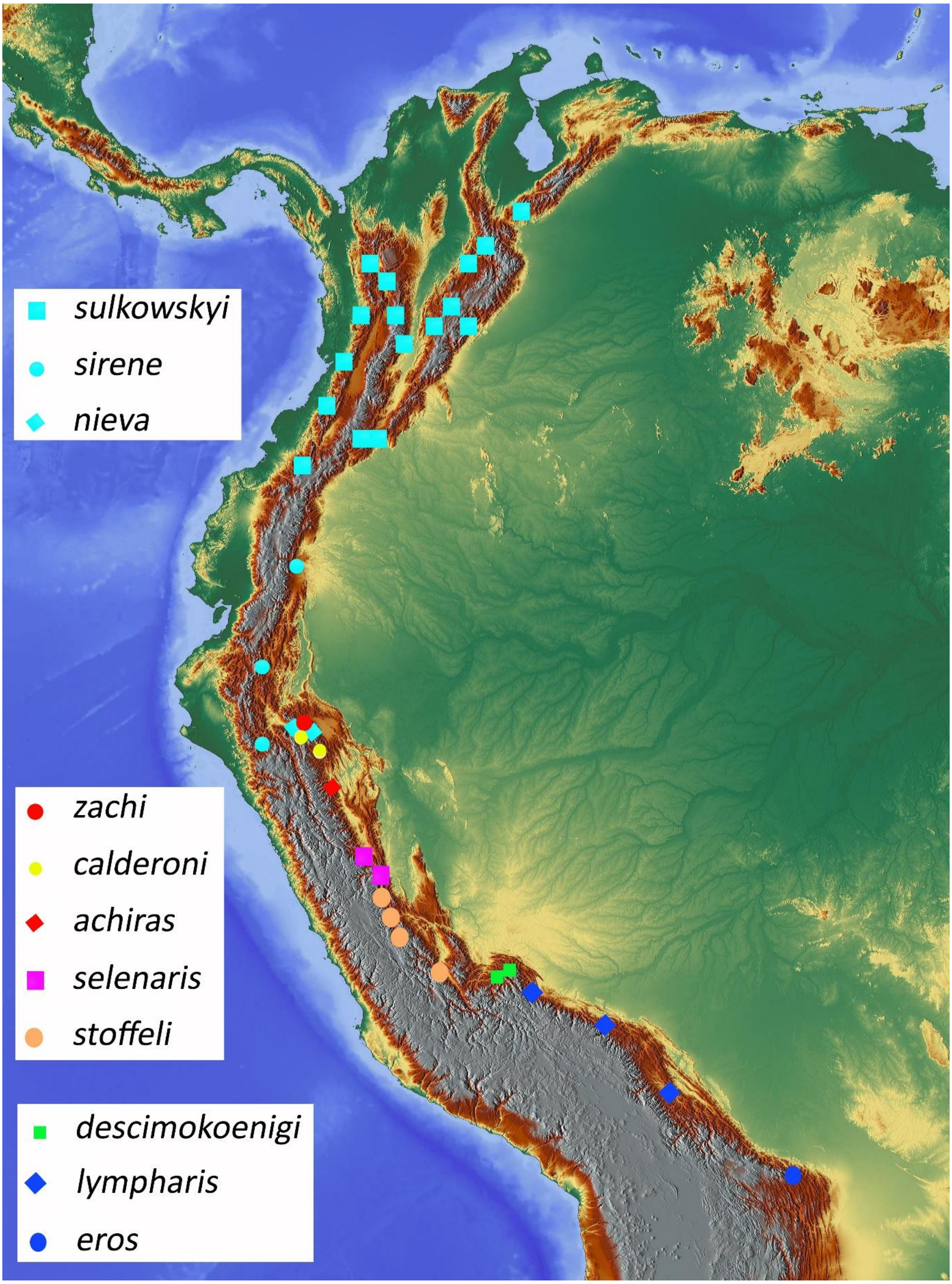

Figure S1-1. Geographical distribution of the *Morpho sulkowskyi* group.

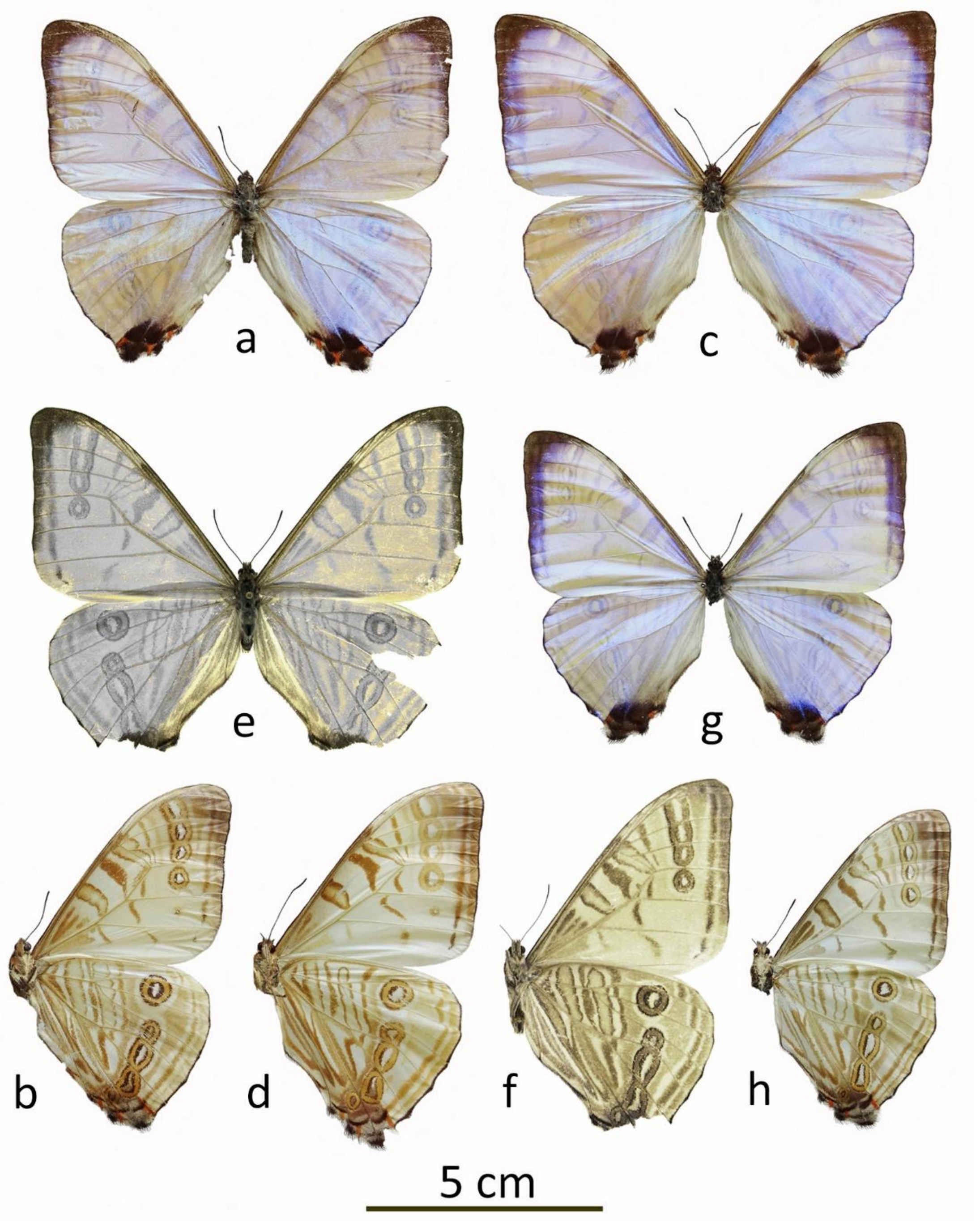

Figure S1-2 (D: upperside. V: underside). **a**: *sulkowskyi* ♂ (Guarne, 2000 m, Antioquia, Colombie; MNHN), D. - **b**: idem, V. - **c**: *sirene* ♂ (río Blanco, 2000 m, Baños, Tungurahua, Equateur; MNHN), D. - **d**: *idem*, V. - **e**: *sirene* ♂ (Naranja, 2300 m, 06°16'S 78°51'W, Cajamarca, Peru; MUSM), D. - **f**: idem, V. - **g**: *nieva* ♂ (Alto Nieva, 1900-2000 m, 5°39-40'S 77°46-47'W, Amazonas, Pérou; MNHN), D. - **h**: *idem*, V.

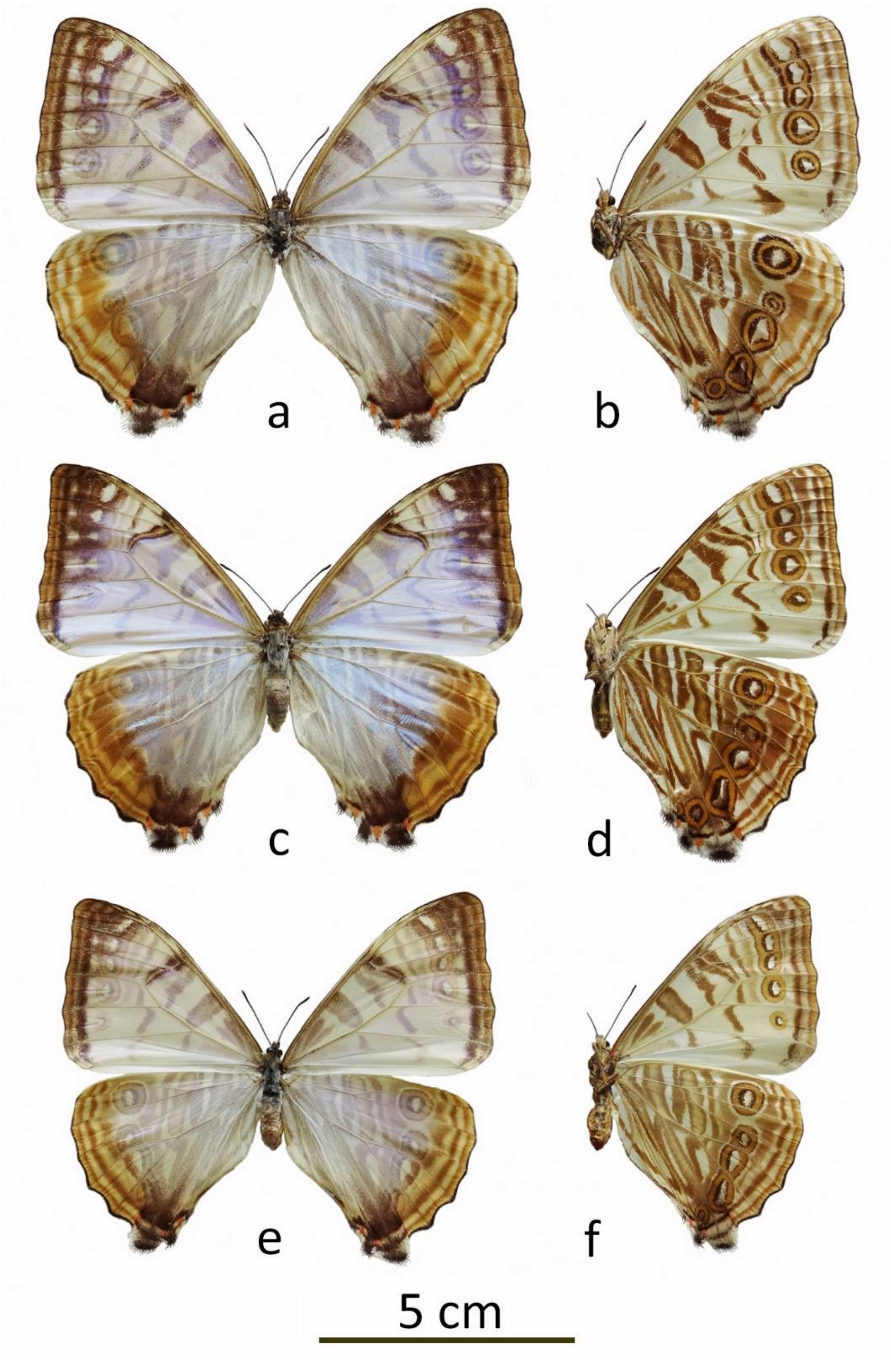

Figure S1-3 (D: upperside. V: underside). **a**: *sulkowskyi* ♀ (Huila, Colombie; MNHN), D. - **b**: *idem*, V. - **c**: *sirene* ♀ (río Blanco, Santana, 1600 m, Equateur; MNHN), D. - **d**: *idem*, V. - **e**: *nieva* ♀ (Alto Nieva, 1900-2000 m, 5°39-40'S 77°46-47'W, Amazonas, Pérou; MNHN), D. - **f**: *idem*, V.

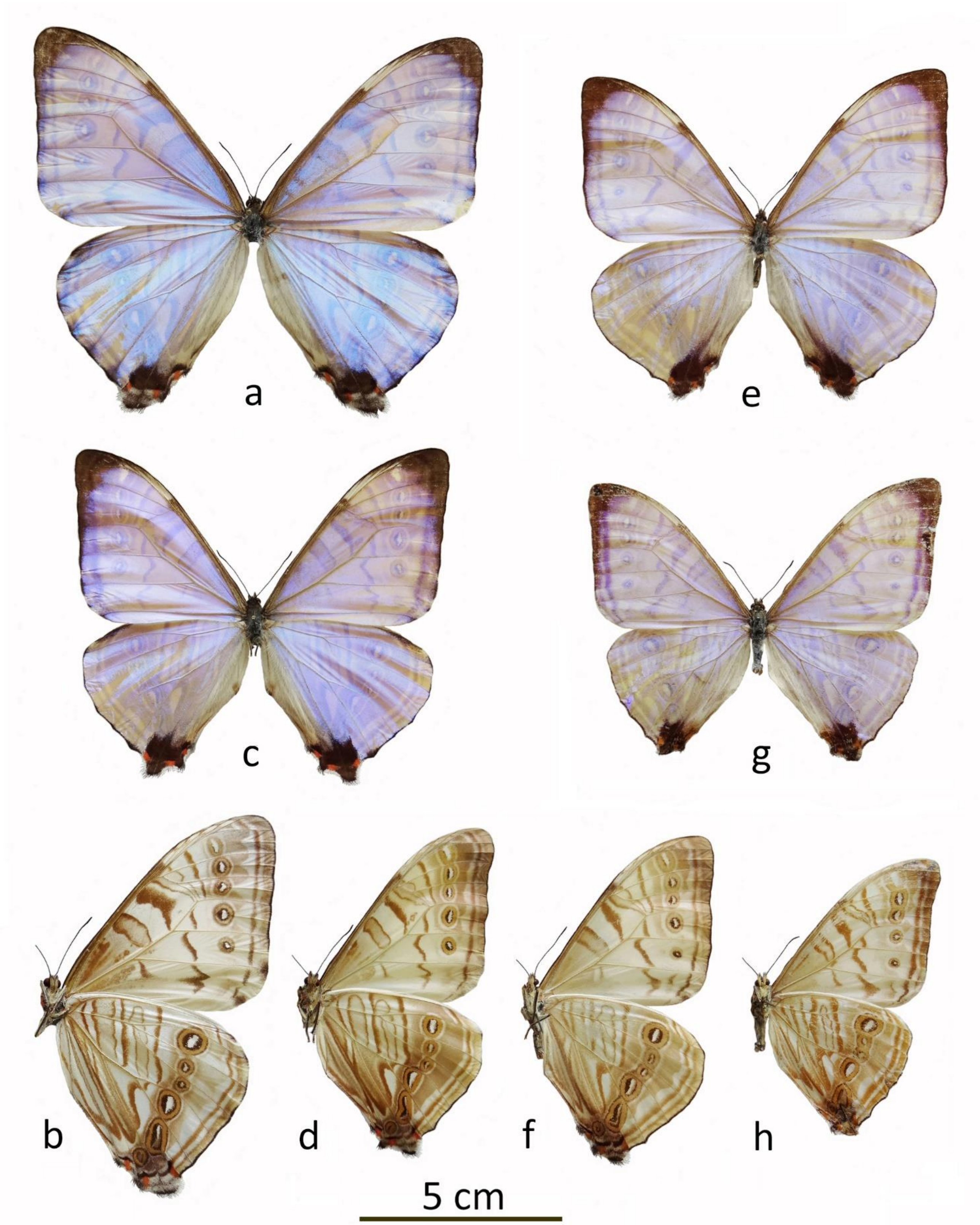

Figure S1-4 (D: upperside. V: underside). **a**: *zachi* ♂ (Alto Nieva, 1900-2000 m, 5°39-40’S 77°46-47’W, Amazonas, Pérou; MNHN), D. - **b**: *idem*, V. - **c**: *calderoni* ♂ (alto Río Nieva, 2250 m, 5°43’S 77°47’W, Amazonas, Pérou; MNHN), D. - **d**: *idem*, V. - **e**: *selenaris* ♂ (Carpish, 2500 m, Huánuco, Pérou; MNHN), D. - **f**: *idem*, V. - **g**: *stoffeli* ♂ (Holotype, Oxapampa, Pérou; MNHN), D. - **h**: *idem*, V.

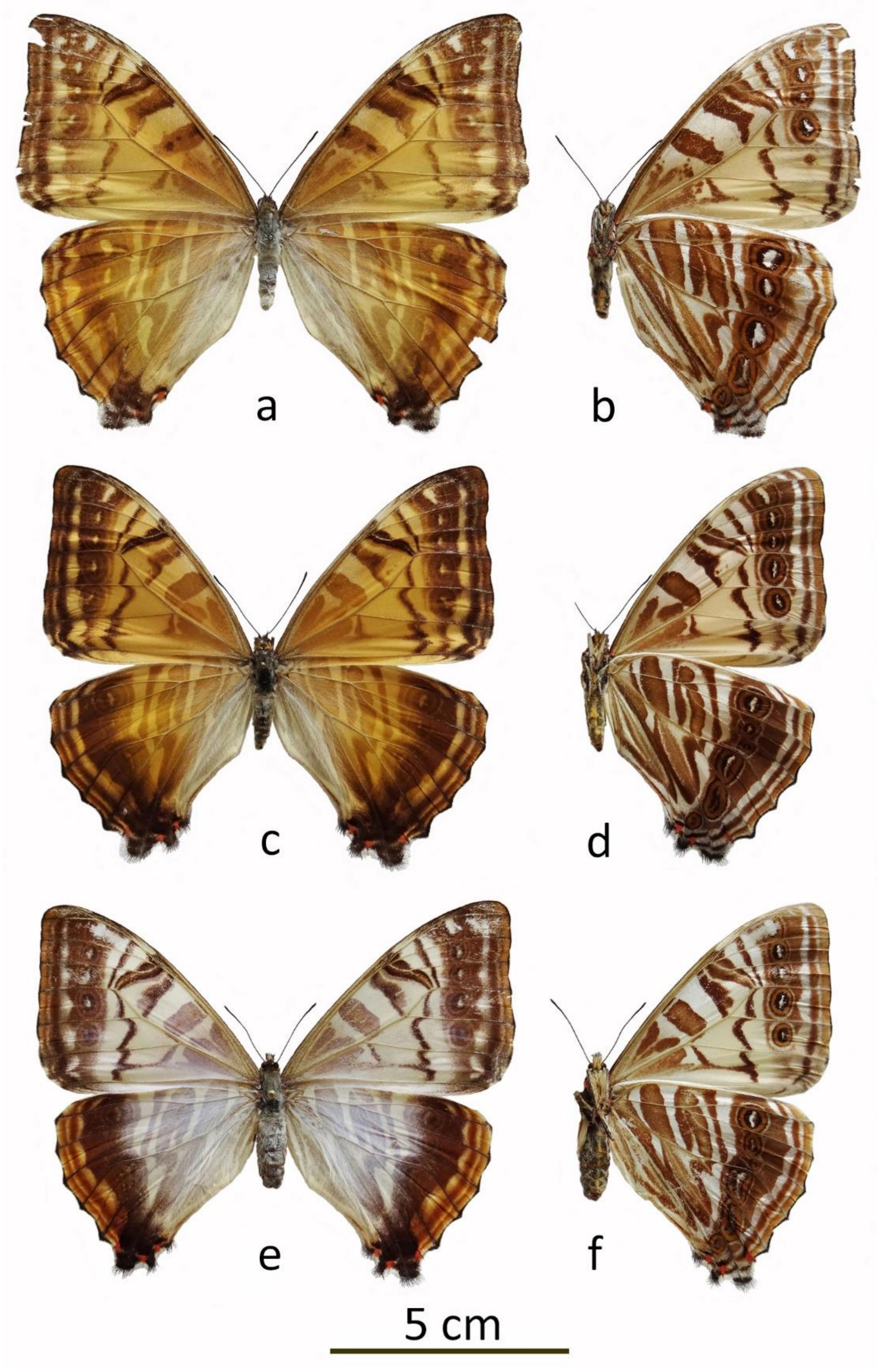

Figure S1-5 (D: upperside. V: underside). **a**: *zachi* ♀ (Nieva, 1900-2000 m, 5°39-40’S 77°46-47’W, Amazonas, Pérou; MNHN), D. - **b**: *idem*, V. - **c**: *calderoni* ♀ morph 1 (alto Río Nieva, 2250 m, 5°43’S 77°47’W, Amazonas, Pérou; MNHN), D. - **d**: *idem*, V. - **e**: ♀ morph 2 (alto Río Nieva, 2200 m, 5°43’S 77°47’W, Amazonas, Pérou; MNHN), D. - **f**: *idem*, V.

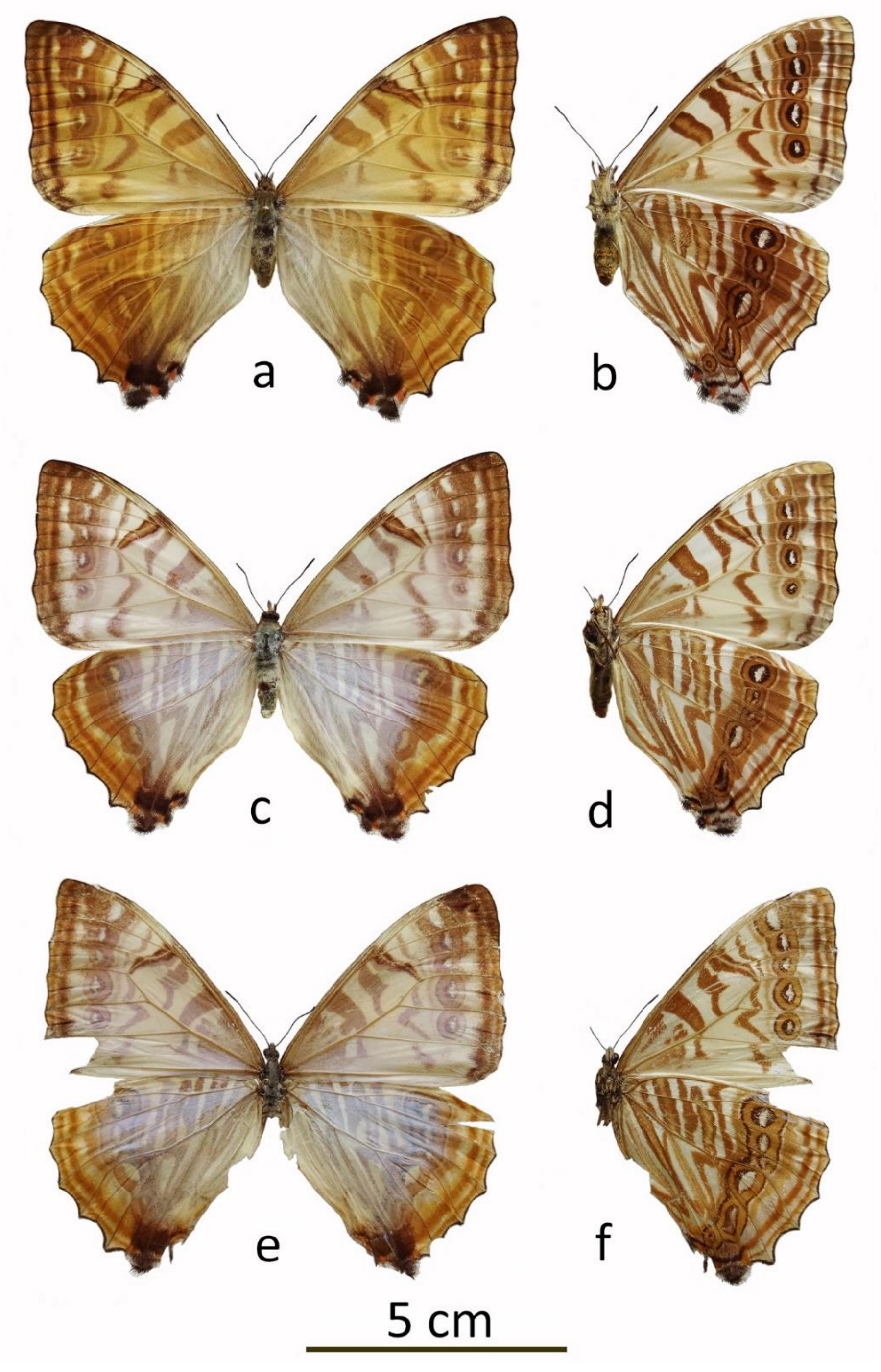

Figure S1-6 (D: upperside. V: underside). **a**: *selenaris* ♀ morph 1 (Carpish, 2500 m, Huánuco, Pérou; MNHN), D. - **b**: *idem*, V. - **c**: *selenaris* ♀ morph 2 (Carpish, 2500 m, Huánuco, Pérou; MNHN), D. - **d**: *idem*, V. - **e**: *stoffeli* ♀ (San Francisco, Chanchamayo, Junín, Pérou; MNHN), D. - **f**: *idem*, V.

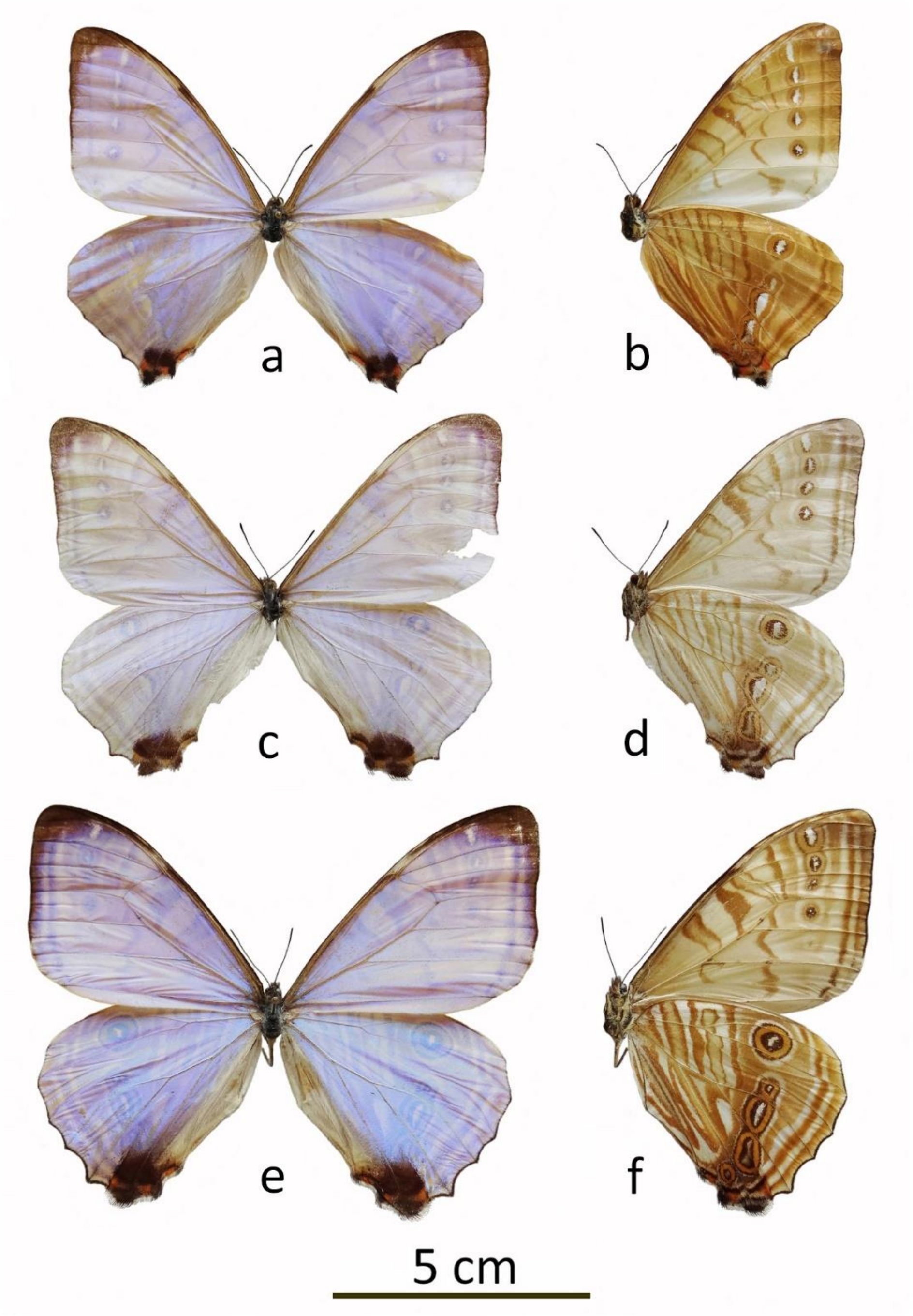

Figure S1-7 (D: upperside. V: underside). **a**: *descimokoenigi* ♂ (environs d’Alfamayo, Cuzco, Pérou ; MNHN), D. - **b**: *idem*, V. - **c**: *lympharis* ♂ (Coroico – Uchumachi, 2600 m, La Paz, Bolivie ; MNHN), D. - **d**: *idem*, V. - **e**: *eros* ♂ (El Limbo, 2200 m, Provincia Chapare, Cochabamba, Bolivie ; MNHN), D. - **f**: *idem*, V.

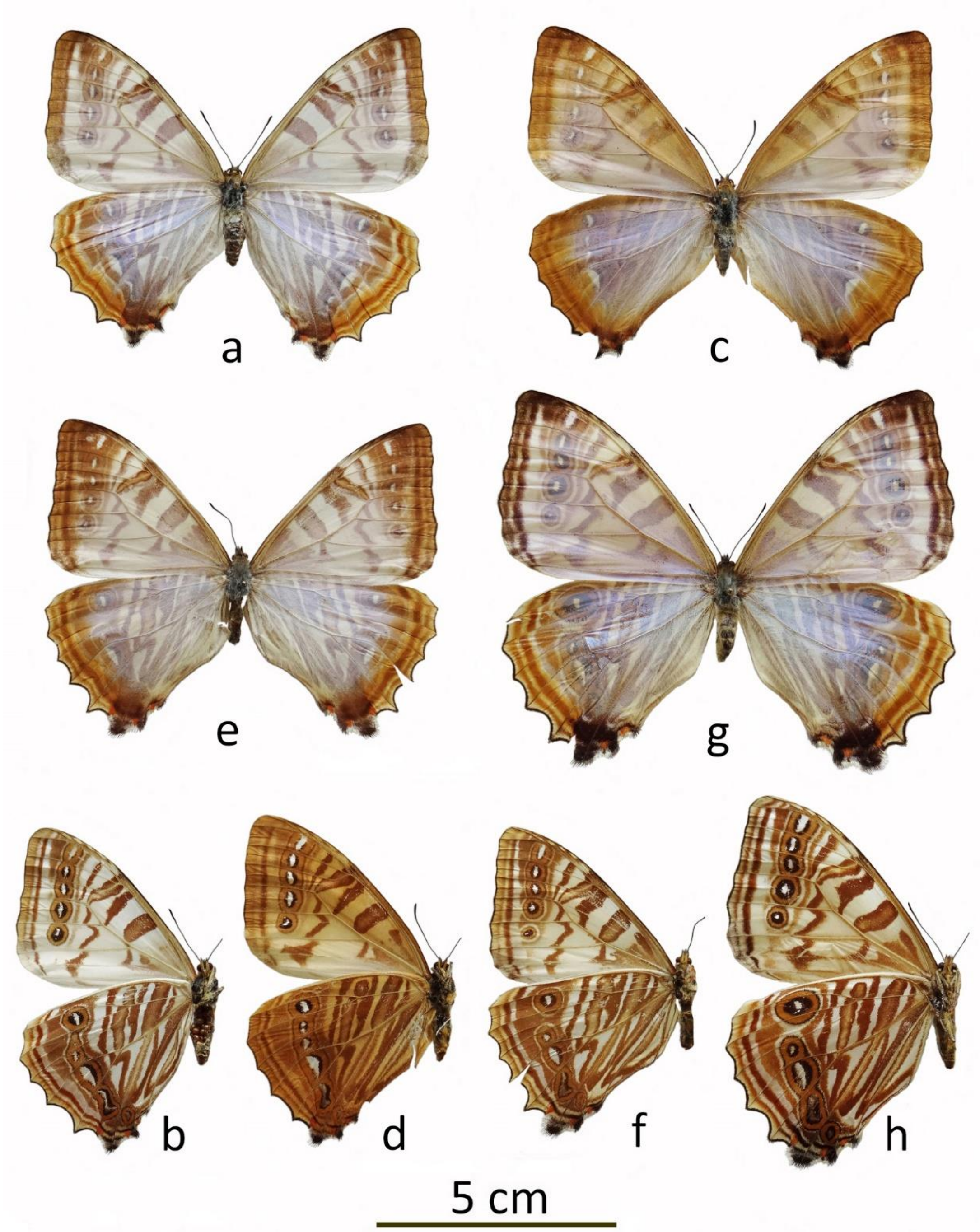

Figure S1-8 (D: upperside. V: underside). **a**: *descimokoenigi* ♀ form 1 (environs d'Alfamayo, Cuzco, Pérou; MNHN), D. - **b**: *idem*, V. - **c**: *descimokoenigi* ♀ form 2 (environs d'Alfamayo, Cuzco, Pérou; MNHN), D. - **d**: *idem*, V. - **e**: *lympharis* ♀ (Cuzco, 2200 m, Pérou; MNHN), D. - **f**: *idem*, V. **g**: *eros* ♀ (El Limbo, 2200 m, Provincia Chapare, Cochabamba, Bolivie; MNHN), D. - **h**: *idem*, V.

Appendix S2. List of all taxa included in the analyses and their Genbank accession numbers: a - phylogenetic analysis and species delimitation; b – dating analysis. In GenBank, geographical origins of many specimens are not available (n/a).

Appendix S2a.

Tribe	**Species**	**Geographical area**	**Voucher specimen**	**GenBank accession number**	
				CO1	**Cytb**
Morphini	*Morpho lympharis eros* Staudinger, 1892	Bolivia: Incachaca (Cochabamba) - BI	CM119eros	KC578546	KC578566
			CM120eros	KC578546	KC578567
	Morpho lympharis lympharis Butler, 1873	Peru: Pilcopata area (Cuzco) - PP	CM353lympharis	KC578540	KC578560
			CM354lympharis	KC578540	KC578561
			CM355lympharis	KC578540	KC578560
			CM356lympharis	KC578541	KC578560
			CM357lympharis	KC578541	KC578560
			CM63lympharis	KC578541	KC578560
			CM64lympharis	KC578540	KC578560
			CM402lympharis	KC578541	KC578560
			CM403lympharis	KC578541	KC578560
			CM404lympharis	KC578541	KC578560
			CM405lympharis	KC578541	KC578560
			CM406lympharis	KC578540	KC578560
			CM407lympharis	KC578541	KC578560
			CM408lympharis	KC578541	KC578560
			CM409lympharis	KC578540	KC578565
	Morpho lympharis descimokoenigi Blandin, 1993	Peru: Alfamayo (Cuzco) - PA	CM375descimokoenigi	KC578542	KC578562
			CM376descimokoenigi	KC578542	KC578562
			CM377descimokoenigi	KC578542	KC578562
	Morpho lympharis selenaris Le Moult & Réal, 1962	Peru: Carpish (Huánuco) - PC	CM03selenaris	KC578545	KC578564
			CM399selenaris	KC578545	KC578564
	Morpho lympharis calderoni Blandin & Lamas, 2007	Peru: Mendoza area, quebrada Cedro Pirruro (Amazonas) - MCP	CM394calderoni	KC578539	KC578556
			CM395calderoni	KC578544	KC578555
			CM396calderoni	KC578539	KC578557

			CM397calderoni	KC578539	KC578558
			CM398calderoni	KC578544	KC578559
		Peru: along the upper río Nieva, upstream from the Puente Nieva (Amazonas) - N1	CM305calderoni	KC578535	KC578554
			CM306calderoni	KC578536	KC578554
			CM307calderoni	KC578536	KC578554
			CM308calderoni	KC578537	KC578554
			CM309calderoni	KC578536	KC578554
			CM310calderoni	KC578536	KC578554
			CM316calderoni	KC578536	KC578554
			CM319calderoni	KC578536	KC578554
			CM320calderoni	KC578535	KC578554
			CM322calderoni	KC578536	KC578554
			CM326calderoni	KC578536	KC578554
			CM327calderoni	KC578536	KC578554
			CM328calderoni	KC578536	KC578554
		Peru: San José de Molinopampa area (Amazonas) - SJ	CM344calderoni	KC578544	KC578555
			CM346calderoni	KC578544	KC578555
			CM351calderoni	KC578544	KC578555
			CM389calderoni	KC578544	KC578555
			CM390calderoni	KC578544	KC578555
			CM391calderoni	KC578544	KC578555
			CM392calderoni	KC578544	KC578555
			CM393calderoni	KC578544	KC578555
	Morpho sulkowskyi nieva Lamas & Blandin, 2007	Peru: along the upper río Nieva, upstream from the Puente Nieva (Amazonas) - N1	CM311nieva	KC578531	KC578549
			CM313nieva	KC578531	KC578549
			CM314nieva	KC578531	KC578549
			CM315nieva	KC578532	KC578550
			CM329nieva	KC578532	KC578550
			CM330nieva	KC578531	KC578549
			CM331nieva	KC578531	KC578549
			CM332nieva	KC578531	KC578549

		CM333nieva	KC578532	KC578550
		CM336nieva	KC578531	KC578549
		CM337nieva	KC578532	KC578550
		CM339nieva	KC578531	KC578549
		CM340nieva	KC578532	KC578550
		CM341nieva	KC578532	KC578550
	Peru: : near the Puente Nieva (Amazonas) - PN	CM334nieva	KC578532	KC578551
		CM335nieva	KC578531	KC578549
	Peru: Mirador de Santa Cruz (San Martín) - M	CM382nieva	KC578533	KC578550
		CM383nieva	KC578532	KC578552
	Peru: along the upper río Nieva, downstream from the Puente Nieva (Amazonas) - N2	CM384nieva	KC578532	KC578550
		CM385nieva	KC578532	KC578550
		CM386nieva	KC578531	KC578553
		CM387nieva	KC578534	KC578553
		CM388nieva	KC578531	KC578553
Morpho lympharis zachi S chaffler & Frankenbach, 2009	Peru: along the upper río Nieva, downstream from the Puente Nieva (Amazonas) - N2	CM378zachi	KC578543	KC578563
		CM379zachi	KC578544	KC578563
		CM380zachi	KC578544	KC578563
		CM381zachi	KC578543	KC578563
		CM410zachi	KC578543	KC578563
		CM411zachi	KC578543	KC578563
Morpho sulkowskyi sirene Niepelt, 1911	Ecuador: Baños area (Tungurahua) - EC	CM66sirene	KC578528	KC578548
		CM299sirene	KC578529	KC578548
		CM300sirene	KC578529	KC578548
		CM366sirene	KC578530	KC578548
		CM367sirene	KC578530	KC578548
		CM368sirene	KC578529	KC578548
		CM369sirene	KC578529	KC578548
Morpho sulkowskyi sulkowskyi Kollar, 1850	Colombia: Envigado area (Antioquia) - CE	CM302sulkowskyi	KC578527	KC578547
		CM304sulkowskyi	KC578527	KC578547
		CM370sulkowskyi	KC578527	KC578547

			CM371sulkowskyi	KC578527	KC578547
			CM372sulkowskyi	KC578527	KC578547
			CM373sulkowskyi	KC578527	KC578547
			CM374sulkowskyi	KC578527	KC578547
	Morpho anaxibia (Esper, 1801)	Brazil	CM166anaxibia	JN625398	JN625499

Appendix S2b.

Tribe	**Species**	**Geographical area**	**GenBank accession number**		
			CO1	**EF1a**	**RPS5**
Morphini	*Morpho lympharis eros* Staudinger, 1892	Bolivia: Incachaca (Cochabamba) - BI	KC578546	GBK***	GBK***
	Morpho lympharis lympharis Butler, 1873	Peru: Pilcopata area (Cuzco) - PP	KC578541	GBK***	GBK***
	Morpho lympharis selenaris Le Moult & Réal, 1962	Peru: Carpish (Huánuco) - PC	KC578545	GBK***	GBK***
	Morpho lympharis calderoni Blandin & Lamas, 2007	Peru: Mendoza area, quebrada Cedro Pirruro (Amazonas) - MCP	KC578539	GBK***	GBK***
	Morpho sulkowskyi nieva Lamas & Blandin, 2007	Peru: : near the Puente Nieva (Amazonas) - PN	KC578532	GBK***	GBK***
	Morpho sulkowskyi sirene Niepelt, 1911	Ecuador: Baños area (Tungurahua) - EC	KC578530	GBK***	GBK***
	Morpho sulkowskyi sulkowskyi Kollar, 1850	Colombia: Envigado area (Antioquia) - CE	KC578527	GBK***	GBK***
	Morpho aega (Hübner, [1822])	BRAZIL: SP, Santa Virginia	JN696160	JN696188	JN696272
	Morpho anaxibia (Esper, 1801)	Brazil	JN625398	GBK***	GBK***
	Morpho aurora Westwood, 1851	PERU: JU, Quebrada Siete Jeringas	JN696164	JN696191	JN696275
	Morpho cisseis C. Felder & R. Felder, 1860	BRAZIL: Mato Grosso	JN696165	JN696192	JN696276
	Morpho deidamia (Hübner, [1819])	n/a	JN696167	JN696193	JN696277
	Morpho epistrophus (Fabricius, 1796)	BRAZIL: SP, Santa Virginia	JN696168	JN696194	JN696278
	Morpho granadensis C. Felder & R. Felder, 1867	COSTA RICA: Area de Conservacion Guanacaste	JN696171	JN696195	JN696279
	Morpho helenor (Cramer, 1776)	n/a	JN696174	JN696197	EU141407
	Morpho hecuba (Linnaeus, 1771)	BRAZIL: Pará	JN696172	JN696196	JN696280
	Morpho hercules (Dalman, 1823)	BRAZIL: SP, Atibaia	JN696175	JN696198	JN696283
	Morpho marcus (Schaller, 1785)	Amazonas	JN696176	JN696199	JN696284
	Morpho polyphemus Westwood, [1850]	MEXICO: Morales, Road Yautopec-Jojutla	JN696180	JN696202	JN696285
	Morpho portis (Hübner, [1821])	n/a	JN696181	JN696203	JN696286
	Morpho rhetenor (Cramer, 1775)	n/a	JN696182	JN696204	JN696287
	Morpho amphitryon Staudinger, 1887	PERU: Oxapampa	JN696186	JN696207	JN696290
	Caerois chorinaeus (Fabricius, 1775)	PERU: Madre de Dios, Los Amigos Biological Station	EU528315	EU528292	EU528426
	Antirrhea philoctetes (Linnaeus, 1758)	n/a	DQ338577	DQ338885	EU528424
Euxanthini	*Euxanthe eurinome* (Cramer, [1775])	n/a	EU141357	EU136664	EU141390
Charaxini	*Charaxes castor* (Cramer, [1775])	n/a	AY090219	AY090185	EU141422
Elymniini	*Lethe minerva* Fabricius 1775	n/a	DQ338768	DQ338909	EU141387
	Neope bremeri Felder, 1862	n/a	DQ338770	DQ338911	EU528448

Satyrini	*Coenonympha pamphilus* (Linnaeus, 1758)	n/a	DQ338777	DQ338920	EU528428
	Erebia oeme (Hübner, 1804)	n/a	DQ338780	DQ338923	EU141375
	Maniola jurtina (Linnaeus, 1758)	n/a	AY090214	AY090180	EU141376
	Melanargia galathea (Linnaeus, 1758)	n/a	DQ338843	DQ338993	EU528444
	Ypthima baldus Fabricius, 1775	n/a	DQ338875	DQ339033	EU528469
Brassolini	*Caligo telamonius* (C. Felder & R. Felder, 1862)	n/a	AY090209	AY090175	EU141414
	Opsiphanes quiteria (Stoll, 1780)	n/a	DQ018957	DQ018924	EU528451
Calinagini	*Calinaga buddha* Moore, 1857	n/a	AY090208	AY090174	EU141406

Appendix S3. Additional information on sequencing protocols.

Specific primers were defined: K698D (5’-AAATCATAAAGATATAGGAMCWYT-3’) and NANCYB (5’TTGCTCCAGCTAAHACWGG-3’) for *CO1;* MORCYF2 (5’-TTTAYTTTCAYATTGGNCGAA3’) and MORCYR2 (5’-TTGATTTAADGGRTAAAATTGDA-3’) for *Cyt b*. Nuclear markers (EF1a and RPS5) were selected following Wahlberg & Wheat (2008). Each amplification contained 1 µl of DNA, 1 unit of reaction buffer, 3mM MgCl2, 0,4mM dNTP, 0,4 µM of each primer and 1 unit of GoTaq Flexi DNA Polymerase (Promega). The PCR reactions were performed with an initial denaturation step at 94°C for 5 minutes, followed by 40 cycles of the three following steps: denaturation at 94°C for 1minute, annealing at 50°C (CO1, Cytb and EF1a) or 55°C (RpS5) for 1 min 30 s, and extension at 72°C for 1 minute. A final extension was then made at 72°C for 5 minutes. Most samples were then processed by the French sequencing centre Genoscope using a BigDye v3.1 sequencing kit and Applied Biosystems 3730xl sequencers. For the remaining samples, PCR products were purified with the Wizard® SV Gel and PCR Clean-Up System (Promega). Fragments were then sequenced with the ABI Prism 3130 automatic sequencer using a Big-Dye Terminator v3.1 Sequencing Kit. Both strands were sequenced for all specimens to minimize PCR artefacts and ambiguities. All sequences were deposited in GenBank. Alignment of sequences was straightforward as no gaps were detected. We also used Mesquite 2.75 (available at: www.mesquiteproject.org) to check the coding frame for possible errors or stop codons.

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