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Molecular phylogenetics of the neotropical butterfly subtribe Oleriina (Nymphalidae: Danainae: Ithomiini)

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ABSTRACT

The Oleriina is one of the most speciose subtribes of the neotropical nymphalid butterfly tribe Ithomiini. They are widely distributed across the Andes and Amazonian lowlands and like other ithomiines they are involved in complex mimicry rings. This subtribe is of particular interest because it contains the most diverse ithomiine genus, *Oleria*, as well as two genera, *Megoleria* and *Hyposcada*, that feed on hostplants not utilized elsewhere in the tribe. Here we present the first comprehensive species-level phylogeny for the Oleriina, representing 83% of recognised species in the group, and based on 6698 bp from eight mitochondrial (mt) and nuclear (nc) genes. Topologies are largely congruent for ncDNA and the concatenated dataset and the genera *Oleria*, *Hyposcada* and *Megoleria* are recovered and well-supported, although strongly discordant genealogy between mtDNA and ncDNA suggest possible introgression among *Hyposcada* and *Megoleria*. A fourth clade containing the type species of *Ollantaya* is consistently recovered, and this recently synonymized name is resurrected. Clear subdivisions within *Oleria* separate the genus into four species groups, *onega*, *amalda*, *makrena* and *aegle*, which also correspond to differing biogeographic and elevation range characteristics.

Unlike other ithomiine genera, the Oleriina show homogeneity in mimetic wing pattern, in sharp contrast to the emerging paradigm that mimetic shifts have enhanced diversification in the tribe. Our results show a potentially more important role for geographic isolation in the diversification of the Oleriina compared to other Ithomiini studied to date and provide a framework for more detailed biogeographical studies, in addition to a rare opportunity for comparative analyses with other neotropical groups.

1. Introduction

The nymphalid butterfly tribe Ithomiini form a diverse and widespread neotropical group of approximately 370 species and over 1500 geographical races (Lamas, 2004; Willmott and Freitas, 2006). They are dominant members of complex mimicry rings that involve ithomiine, heliconiine, nymphaline and riodinid butterflies, notodontid day-flying moths and other insects (Beccaloni, 1997a). Adults of all Ithomiini use dehydropyrrolizidine alkaloids as defensive compounds, in the synthesis of pheromones to attract mates (Brown, 1987) and in the formation of aggregations of butterflies in ithomiine 'pockets' (Haber, 1978; Pinheiro et al., 2008). Most Ithomiini larvae feed on Solanaceae and the use of this family as a hostplant is seen as a key to the diversification of the butterfly group (Brown, 1987; Willmott and Freitas, 2006).

Knowledge of their systematics, biology and distribution is relatively advanced and the tribe has provided excellent models in studies on mimicry (Beccaloni, 1997a,b; Joron et al., 2001; Willmott and Mallet, 2004), biogeography (Elias et al., 2009), evolution (Whinnett et al., 2005a,b; Jiggins et al., 2006; Elias et al., 2007, 2008) and chemical ecology (Brown, 1987; Schultz et al., 2004). However, species-level molecular phylogenies have yet to be elucidated and currently only two out of 50 genera (Mallarino et al., 2005; Elias et al., 2009) have been completed.

Ten Ithomiini subtribes (one unnamed) are currently recognised based on morphological characteristics (Lamas, 2004; Willmott and Freitas, 2006) and molecular data (Brower et al., 2006). The subtribe Oleriina contains 63 species and is of particular interest because one of its three constituent genera, *Oleria*, is the most speciose ithomiine genus (52 species) (Lamas, 2004). In contrast, the other two genera are relatively depauperate, with nine species of *Hyposcada* (Willmott and Lamas, unpublished data) and two species of *Megoleria* (Willmott and Lamas, 2008) currently recognised. The biogeography of this group is also of interest. *Oleria* and *Hyposcada* are both widely distributed, occurring from Mexico to Brazil at varying altitudes from sea level to 3000 m, with the former genus diverse in both lowland and montane habitats. Con-

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versely, *Megoleria* is restricted to the high Andes of Colombia, Ecuador and Peru at altitudes ranging from 1200 to 2700 m.

Elucidating the systematics of the Ithomiini has proved particularly problematic because of their involvement in complex mimicry rings and geographical polymorphism. Additionally, association of the sexes in *Oleria* is sometimes complicated by sexual dimorphism in mimicry pattern (Willmott and Mallet, 2004).

Fox (1956) first proposed the subtribe Oleriina (then considered a tribe called Oleriini) including *Hyposcada*, *Oleria*, *Aeria* and an undescribed genus, but Harvey (1991) revised the constituent genera to include *Hyposcada*, *Oleria* and two new undescribed genera later named *Ollantaya* (Brown and Freitas, 1994) and *Megoleria* (Constantino, 1999). The genus *Ollantaya* was synonymized with the *Oleria* (Lamas, 2004), although recent morphological work suggests *Ollantaya* should be resurrected to include *O. canilla*, *O. aegineta*, *H. olerioides* and a fourth undescribed species from the Peruvian Andes (Willmott and Freitas, 2006).

Recent higher-level systematics of the Ithomiini using morphological characters confirmed Oleriina as monophyletic and sister to the Napeogenina and Ithomiina (Brown and Freitas, 1994; Willmott and Freitas, 2006). The monophyly of this group is corroborated from molecular data (Brower et al., 2006), based on 2335 bp of the mitochondrial (mtDNA) *cytochrome oxidase subunits I* and *II* (COI–COII) and the nuclear (ncDNA) genes, *wingless* and *elongation factor 1-alpha* (EF-1α). These data provide conflicting signal regarding the relationships of *Megoleria* to other oleriines, with morphological characters suggesting that *Megoleria* is sister to *Hyposcada* (Willmott and Freitas, 2006), while molecular data place *Megoleria* as sister to all other Oleriina (Brower et al., 2006). With the exception of *Megoleria*, there are few clear morphological synapomorphies supporting the remaining genera, and molecular data thus offer a promising solution to resolve relationships within this group.

The first molecular phylogenetic study of the Oleriina included 41 species (103 samples) based on 1619 bp of the mtDNA COI–COII and the ncDNA genes, *wingless* and EF-1α (Whinnett, 2005), and recovered the four genera, *Oleria*, *Hyposcada*, *Megoleria* and *Ollantaya*. Analyses using neighbour-joining (NJ) and maximum parsimony (MP) recovered *Hyposcada* as sister to all other Oleriina (Whinnett, 2005), whereas Bayesian inference (BI) of the concatenated data identified *Megoleria* as sister to all other Oleriina as in Brower et al. (2006).

Here we present one of the first comprehensive molecular phylogenetic analyses for any diverse butterfly tribe. Our sampling includes 52 of the 63 known Oleriina species, based on six gene regions comprising three mtDNA and five ncDNA genes for multiple individuals from the whole of the Oleriina subtribe. Our phylogenetic hypotheses allow revision of the generic classification of the tribe as a basis for generic revisions currently in preparation. In addition, this study forms part of a collaborative effort to generate species-level molecular phylogenetic hypotheses for the whole of the Ithomiini (Mallarino et al., 2005; Elias et al., 2009). As a result, we are also able to further assess the general importance of biogeographic processes identified as critical in the evolution of the few other tropical Andean butterfly genera studied to date (e.g., Willmott et al., 2001; Hall, 2005; Jiggins et al., 2006; Elias et al., 2009).

2. Materials and methods

2.1. Taxon sampling

A total of 52 Oleriina species (Lamas, 2004; Willmott and Lamas, 2008), represented by 228 specimens were included in our phylogenetic analyses. This includes 43 of the 52 known species of *Oleria*, seven of nine species of *Hyposcada* and both species of *Megoleria*. At least three samples of each species were sequenced where available. In order to maximize geographical coverage of each species and to test species validity as many subspecies as possible were included: 87 of the 262 known Oleriina subspecies including 64 of 188 subspecies of *Oleria*, 21 of 68 subspecies of *Hyposcada* and two of six subspecies of *Megoleria*. We were unable to obtain samples of 11 rare and/or geographically restricted species, including four undescribed species of *Oleria*, *O. flora*, *O. similigena*, *O. synnova*, *O. thiemei*, *O. zea*, *H. attilodes* and *H. dujardini*.

We used sequences of *Ithomia salapia* and *Napeogenes pharo*, and the more distantly related *Mechanitis mazeus* and *Pseudoscada timna*, as outgroups (Brower et al., 2006). All outgroup sequences for the ncDNA genes, *Ef1α* and *Wg* and the mtDNA sequence for *Mechanitis mazeus* were taken from Genbank. Samples are listed in Table S1 (Supplementary material).

2.2. Molecular methods

Samples were typically stored in 20% dimethylsulphoxide, 0.25 M EDTA and saturated NaCl solution. Wings were removed and preserved as vouchers and are held in the Mallet Lab Collections at University College London. Donated samples were dried or stored in alcohol. DNA was extracted from one third of the thorax or from legs of donated samples using Qiagen's DNeasy Blood & Tissue Kit or Qiagen's QIAamp DNA Micro Kit for small samples. The manufacturer's protocol was followed with a minimum 3-h incubation period at 56 °C and a final elution volume of 200 μl or 50 μl to concentrate DNA extracted from museum specimens.

Genes selected for sequencing are widely used across phylogenetic studies of Lepidoptera and were selected to allow comparison and use in further studies. As such, we amplified and sequenced eight gene regions: the mtDNA genes *COI*, *leucine tRNA* and *COII* and the autosomal genes *Wingless* (*Wg*), *Elongation factor 1-alpha* (*Ef1α*), *Tektin*, *Ribosomal Protein L5* (*RpL5*) and the sex-linked gene, *Triose phosphate isomerase* (*Tpi*) (Table 1). We used published primers and modified these where a significant number of specimens failed to amplify (Table S2). PCR primers used for gene amplification were also used for sequencing. PCR amplification conditions are given in Table S2. Cycle sequencing products were purified and sequenced on an Applied Biosystems 3730xl DNA Analyser using the manufacturer's instructions.

The resulting chromatograms were edited using ChromasPro v1.33 (Technelysium Pty Ltd.) and aligned in the program BioEdit (Hall, 1999) using ClustalW (Thompson et al., 1994). Heterozygous base calls were coded using IUPAC ambiguity codes. Variable length indels were found in *RpL5* and *Tpi*. These regions were aligned by eye and inferred gaps were coded as missing data.

2.3. Phylogenetic analyses

Data were analysed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI) for the individual mtDNA and ncDNA datasets as well as for the entire, concatenated dataset of 6698 bp totalling 232 taxa including four outgroups.

MP analyses were performed using TNT (Goloboff, 1999) using the New Technology search algorithm, implementing tree-fusing, tree-drifting, ratchet and sectorial searches. Bootstrap support (BS) was evaluated with 1000 replicates and 100 random taxon additions. Traditional MP searches were also performed using a heuristic search with multiple random addition sequences and tree bisection reconnection branch swapping. All characters were equally weighted. Multiple equally most parsimonious trees were summarised by a strict consensus tree in the program WinClada (Nixon, 1999). WinClada was also used to calculate the consistency index (CI) and retention index (RI).

Table 1
Summary statistics for the genes used in this study.

Gene region	*COI–COII*	*Wg*	*Ef-1α*	*Tektin*	*RpL5*	*Tpi*	Concatenated
Taxa amplified	218	196	194	156	180	78	228
Base pairs	2291	463	1072	760	808	1304	6698
Parsimony informative	821	113	180	229	285	552	2204
Estimates of sequence evolution							
Model	GTR + *I* + *G*			GTR + *I* + *G*			GTR + *I* + *G*
		SYM + *I* + Γ	GTR + *I* + *G*	GTR + *I* + *G*	K81uf + *I* + Γ	HKY + *I* + Γ	
Invariable sites (*I*)	0.5343	0.3808	0.5513	0.3055	0.3493	0.2658	0.4497
Gamma distribution (α)	0.6215	0.6311	0.9570	1.0454	1.4773	3.8701	0.7753
Base composition							
a	0.4656	0.2716	0.2685	0.3756	0.3538	0.3725	0.3264
c	0.0621	0.2206	0.2466	0.1547	0.1657	0.1424	0.1661
g	0.0986	0.2518	0.2303	0.2161	0.1429	0.1328	0.1552
t	0.3737	0.2560	0.2546	0.2536	0.3377	0.3524	0.3523
Substitution rates							
Tr							
a–g	15.4313	2.2997	3.6693	3.7042	2.3637	–	6.1185
c–t	13.2194	4.6897	7.2800	6.5490	2.3637	–	9.7127
Tv							
a–c	0.3303	0.7044	1.1509	1.7872	1	–	1.5531
a–t	0.6098	1.1116	1.9141	1.2769	1.1914	–	1.9642
c–g	0.5150	0.2119	0.8449	2.4520	1.1914	–	1.9559
g–t	1	1	1	1	1	–	1
Tr/Tv	–	–	–	–	–	0.8817	–

The best fit model of DNA sequence evolution was determined by Modeltest v3.7 (Posada and Crandall, 1998) using the Akaike Information Criterion (AIC). The model GTR + *I* + *Γ* was selected for the mtDNA, ncDNA and concatenated datasets, whereas partitioning the mtDNA and ncDNA genes to assess individual behaviour returned alternative models for some genes (Table 1). We remove parameter *I* in all subsequent analyses as it has been shown that gamma shape parameter *Γ* and parameter *I* are highly correlated and should not be estimated together (Ren et al., 2005; Wahlberg and Freitas, 2007).

ML analyses were performed using GARLI (Genetic Algorithm for Rapid Likelihood Inference) v.0.96 (Zwickl, 2006) for each dataset applying the model GTR + *Γ*. BS was estimated with 100 replicates due to the large size of the data matrix.

BI was implemented using MrBayes v3.1 (Huelsenbeck and Ronquist, 2001) with four simultaneous Markov Chain Monte Carlo (MCMC) chains run for 2 million generations starting from random trees and sampling every 100 generations. Chain convergence was corroborated by the program TRACER v1.4 (Rambaut and Drummond, 2007). A burn-in of 5000 generations was applied once log-likelihood values had stabilised. Branch support was evaluated with Bayesian posterior probabilities (BPP).

BI was performed on each dataset partitioning by gene to account for potential variation in behaviour of each region. The mtDNA dataset was partitioned into its three constituent gene regions, *COI–tRNA–COII,* as was ncDNA using individual models of DNA sequence evolution for each gene region (Table 1).

Plots of transitions against transversions suggested that codon positions in the mtDNA dataset had not become saturated (data not shown) but we additionally partitioned the mtDNA Bayesian analyses by codon position, separating the first and second position from the third, to corroborate this result.

To assess confidence in tree selection alternative tree topologies under ML and BI for mtDNA, ncDNA and the combined data were evaluated using the KH test (Kishino and Hasegawa, 1989; Shimodaira, 2002) implemented in PAUP (Swofford, 2002).

3. Results

3.1. Sequence data

The concatenated dataset consists of 6698 bp. Of 2291 bp from mtDNA, 929 sites were found to be variable of which 821 were parsimony informative. The combined ncDNA dataset of 4407 bp contained 2155 variable sites of which 1350 were parsimony informative. mtDNA showed strong A:T nucleotide bias (47.0:6.2:9.8:37.0% A:C:G:T) also found in other butterfly and *Drosophila* genomes (Wahlberg et al., 2003 and references therein). The ncDNA genes showed almost equal nucleotide base composition (30.2:22.5:21.7:25.6% A:C:G:T).

The mtDNA dataset ($N = 218$ sequences) was largely complete with the exception of the following ten taxa (02K *Hyposcada zarepha zarepha*, M27 *Megoleria susiana susiana,* KW09 *Oleria amalda faunula*, 7-502 *Oleria antaxis antaxis*, 5-735 *Oleria estella estella*, MC3 *Oleria phenomoe phenomoe*, KW12 *Oleria santineza* ssp. nov. 4-30 *Oleria tigilla* ssp. nov., 5-752 *Oleria tigilla* ssp. nov. and 8498 *Oleria vicina*) from which no mtDNA sequence data were obtained. This is mostly likely a result of poorly preserved or degraded sample material. Only partial sequences were obtained for some other samples (Table S1).

We had variable success sequencing the ncDNA coding genes, obtaining 196 sequences for *Wg*, 194 sequences for *EF1α*, 156 sequences for *Tektin*, 180 sequences for *RpL5* and 78 sequences for the *Tpi* gene. We thus combined the ncDNA gene data into a single dataset and analysed all sequences together. All sequences that were missing or incomplete were coded as missing data in the phylogenetic analyses.

One sample *Oleria tigilla* ssp. nov. PE18-24, was amplified from only a single mtDNA marker while others samples from this species were amplified by complementary gene regions (Table S1). To ensure correct taxonomic assignment of samples with complementary missing data we first reduced our dataset to individual taxa ensuring all had gene regions in common and analysed our

data using BI (data not shown). We compared mtDNA and ncDNA analyses using all samples to the reduced dataset ensuring that samples clustered according to the reduced species tree. Additionally we ensured that *Oleria tigilla* formed a species clade with additional samples in alternative mtDNA and ncDNA trees (Figs. S1–S4, S6 and S7) before including all *Oleria tigilla* samples in the combined dataset and analysing them together.

3.2. Phylogenetic analyses

The monophyly of the Oleriina is recovered with maximum support across all analyses (Fig. 1 and Figs. S1–S8). Constituent genera, *Oleria*, *Megoleria* and *Hyposcada* and a clade containing the *Ollantaya* species are recovered under BI and ML analyses by ncDNA (Figs. S3 and S4) and the concatenated dataset (Fig. 1 and Fig. S5). Support for these clades is strong under ncDNA (>94 BPP, BS) but, with the exception of *Ollantaya* (100 BPP, 87 BS), relatively weak under the concatenated data. Resulting phylogenetic hypotheses were found to be largely congruent from BI and ML analyses. Partitioning of the mtDNA third codon position under BI had very little effect on tree topology. MP did not recover the same level of resolution as BI and ML methods of phylogenetic inference (Figs. S6–S8). MP analyses of the concatenated data found 24 most parsimonious trees supporting the genera *Ollantaya* (93 BS), *Hyposcada* (84 BS) and Megoleria (100 BS) but not *Oleria*; hence rendering the backbone of the resulting consensus largely unresolved (Fig. S8). As such, we mainly discuss results from BI and ML analyses.

While phylogenetic hypotheses generated for the concatenated, mtDNA and ncDNA datasets are for the most part congruent (Fig. 1 and Figs. S1–S5), there are areas of conflict between the mtDNA and ncDNA data. The ncDNA and concatenated datasets both recover similar results with each genus recovered as monophyletic. The concatenated and ncDNA datasets recover *Hyposcada* as sister to a clade consisting of *Megoleria*, *Ollantaya* and *Oleria*. Support for these relationships is good for the ncDNA data (>94 BPP and BS), but less so for the concatenated data. However, analyses of mtDNA render *Hyposcada* and *Ollantaya* paraphyletic, although support for this hypothesis is weaker than that obtained by ncDNA. Further incongruence is found between ncDNA and mtDNA data as the phylogenetic hypothesis from the mtDNA alternatively embeds *Megoleria* within a clade containing *Hyposcada taliata*. Results for each genus are discussed further in the following sections.

Comparison of alternative ML and BI trees for the mtDNA, ncDNA and concatenated data using the KH test recovered the mtDNA and ncDNA trees as a significantly worse fit (Table 2). As such we represent the phylogenetic hypothesis of the BI consensus based on the concatenated dataset (Fig. 1).

3.3. Oleria

A monophyletic *Oleria* is recovered with high support for BI of mtDNA (Fig. S1) and BI and ML of ncDNA (Figs. S3 and S4), whereas support is considerably weaker for ML of mtDNA (Fig. S2) and the concatenated data (Fig. 1 and Fig. S5). Within this genus four species groups, *makrena*, *amalda*, *onega* and *aegle*, are recovered from the concatenated and ncDNA data under BI and ML analyses (Fig. 1 and Figs. S3–S5), but mtDNA renders the *amalda* group non-monophyletic (Figs. S1 and S2). Support for these relationships is good for all datasets. The *amalda* group aside, support is generally stronger from the different partitions (BI > 72 BPP), than the concatenated data.

Relationships between the species groups are generally not well-supported under ML and the concatenated data (Figs. S2, S4 and S5 and Fig. 1), although the clade *onega* and the clade uniting *makrena* and *amalda* is consistently recovered across all datasets and analyses (Figs. S1–S5). The position of *aegle* is unstable as this monotypic taxon is alternatively recovered as sister to *onega* in ncDNA (Figs. S3 and S4) and concatenated analyses, but is sister to the clade (*makrena*, *amalda*) from mtDNA data (Figs. S1 and S2).

3.3.1. aegle species group

BI of mtDNA recovers the *aegle* group as sister to the *amalda* and *makrena* species groups (81 BPP) (Fig. S1), whereas its position in the ML and MP analyses is unresolved relative to the rest of the *Oleria* (Figs. S2 and S6). Conversely, ncDNA recovers *O. aegle* as sister to the *onega* group (94 BPP, ML 77 BS) as does the combined ncDNA and mtDNA data but with less support (Figs. S3–S5 and Fig. 1).

3.3.2. onega species group

The *onega* species group is recovered with high support when datasets are analysed separately (BI 100 BPP; ML > 87 BS; MP > 82 BS) (Figs. S1–S4, S6 and S7), and while consistently recovered with the combined data, support is weaker (Fig. 1 and Figs. S5 and S8). All internal relationships within the *onega* group are also highly congruent and well-supported across mtDNA analyses, with the exception of the clade containing *O. ilerdina*, *O. didymaea* and *O. onega* subspecies, which is weakly supported under BI (Fig. S1) and unresolved under ML and MP (Figs. S2 and S6). This is also the case under ML of ncDNA where relationships between the aforementioned species and *O. astrea*, *O. quintina* and *O. alexina* are unresolved (Fig. S4).

3.3.3. amalda species group

The *amalda* group is recovered as sister to the high-elevation Andean *makrena* clade with good support under ncDNA and combined analyses (Fig. 1 and Figs. S3–S5). The resulting hypotheses split the *amalda* group into two constituent clades, the first composed of *O. zelica*, *O. rubescens* and *O. amalda* and the second composed of *O. estella*, *O. gunilla* and *O.* sp. nov. 1. However, mtDNA does not support a monophyletic *amalda*, with the latter constituent clade sister to the former (100 BPP) (Fig. S1). Relationships between these clades are unclear from ML and MP analyses (Figs. S2 and S6).

3.3.4. makrena species group

This species group is recovered in all analyses and is relatively well-supported. Interestingly, while there is good support for a number of clades within the *makrena* group, in which topologies are reasonably well resolved, branches deeper in this clade are very short and unsurprisingly those deeper nodes are largely unresolved. The positions of *O. deronda valida* and *O. derondina* ssp. nov. are largely unresolved under all analyses, presumably because these taxa are represented by single museum specimens, from which only COI could be sequenced.

BI of the combined dataset recovers the clade (*O. bioculata*, (*O. attalia*, *O. cyrene*)) as sister to ((*O. tremona* (*O.tremona*, *O.makrena*)) (*O. makrena*, *O. padilla*)) (100% BPP) (Fig. 1). mtDNA strongly supports this clade but *O. bioculata* is recovered as sister to the remaining clades (100 BPP) (Fig. S1). BI of ncDNA recovers these relationships with the exception of *O. attalia* whose subspecies are separated and presented as sisters (72 BPP) (Fig. S3).

The positions of *O. boyeri* and *O. quadrata quadrata* are unstable. BI of mtDNA places *O. boyeri* as sister to *O. victorine* (BPP > 74) (Fig. S1) and as sister to the rest of the *makrena* group (BPP 72) with ncDNA (Fig. S3). *O. quadrata quadrata* is recovered as sister to an internal clade containing *O. bioculata*, *O. cyrene*, *O. attalia*, *O. tremona*, *O. makrena* and *O. padilla* in BI of mtDNA and ncDNA (Figs. S1 and S3), but the combined data place it as sister to *O. vicina* (Fig. 1). The positions of both taxa are unresolved under ML and MP (Figs. S2, S4, S6 and S7).

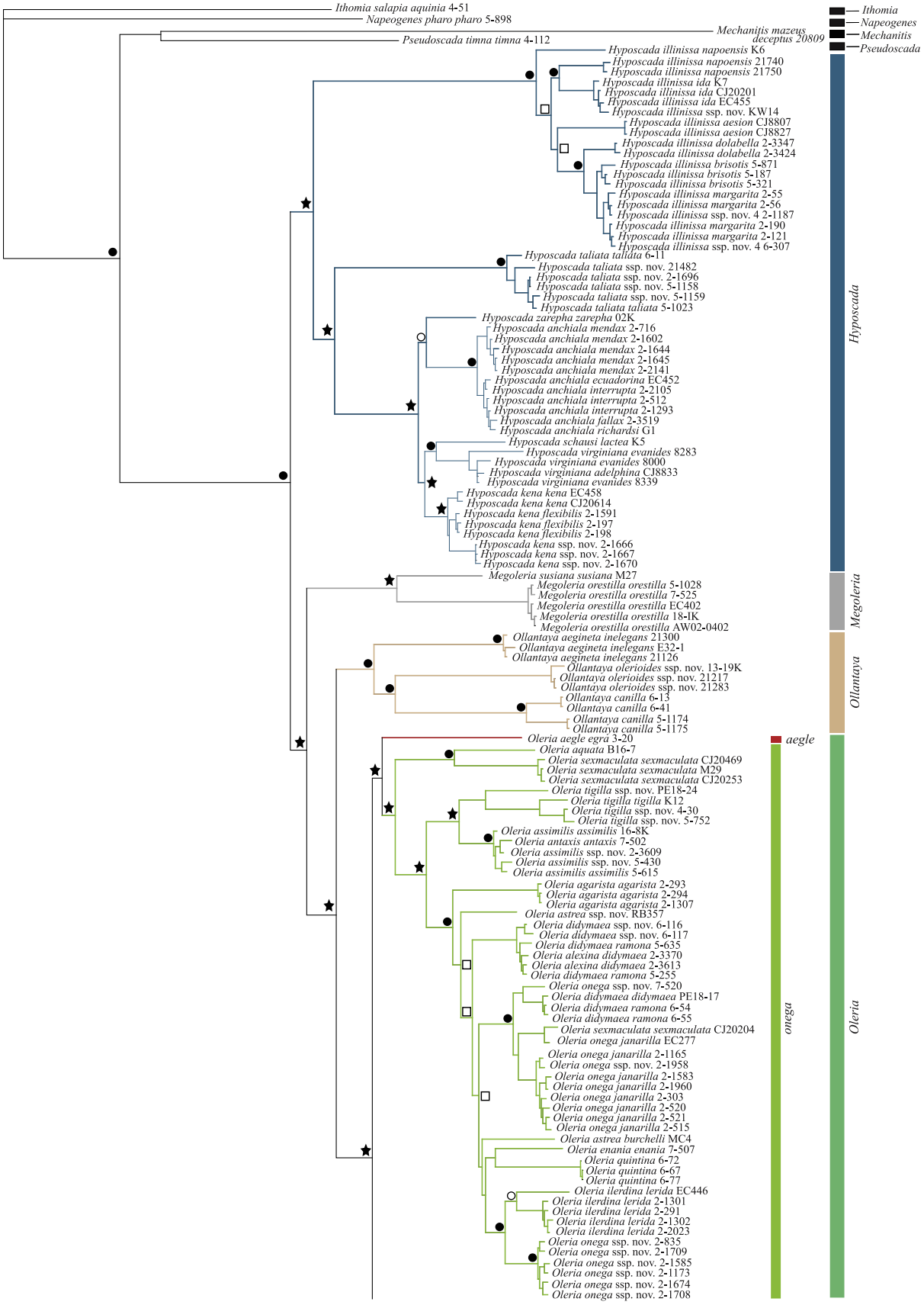

Fig. 1. Bayesian consensus tree of concatenated data with branch lengths proportional to the number of changes. The combined dataset consists of 228 taxa and 6698 nucleotides. ● indicates nodes with 100% BPP; ○ indicates nodes with >95% BPP; □ indicates nodes with >75% BPP; ★ indicates nodes with >55% BPP.

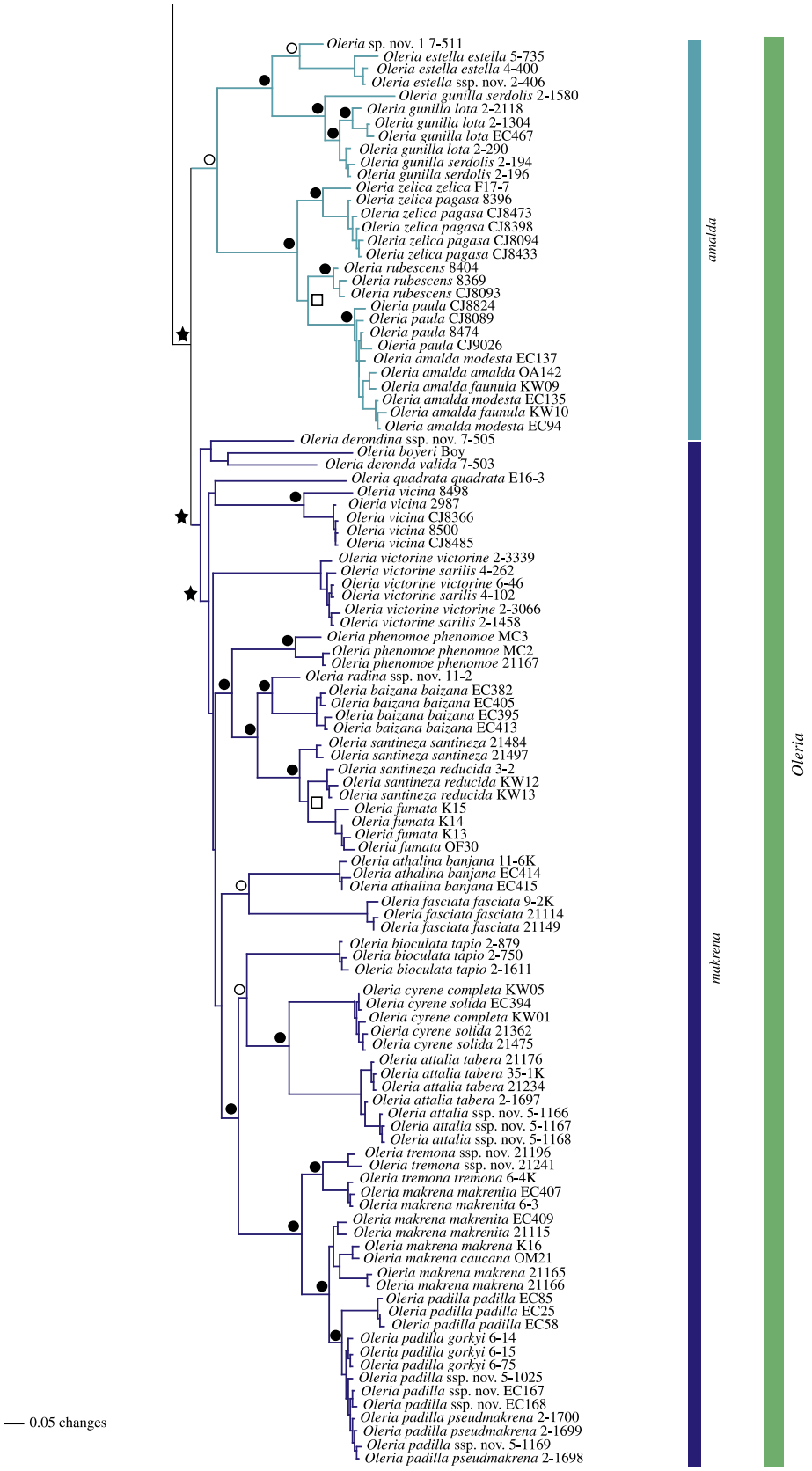

Fig. 1 (*continued*)

Table 2
KH test comparison of alternative phylogenetic hypotheses inferred from different datasets and phylogenetic methods.

Tree	−1n L	Δ−1n L	*P*-value
mtDNA			
BI (three-partition)	60055.63263	547.00102	0.000*
BI (codon)	60067.99340	559.36179	0.000*
ML	60192.86573	684.23412	0.000*
ncDNA			
BI	61569.10819	2060.47658	0.000*
ML	61600.54295	2091.91134	0.000*
Concatenated			
BI	59593.82673	85.19512	0.077
ML	59508.63161	–	–

* $P < 0.05$.

3.4. Hyposcada & Megoleria

BI and MP analyses of mtDNA strongly support *H. taliata* as the sister taxon to *Megoleria* (93 BPP, 71 BS), rendering *Hyposcada* paraphyletic (Fig. 2). This relationship is unresolved under ML (Fig. S2). Alternatively, ncDNA supports *Megoleria* as sister to *Ollantaya* and *Oleria* (95 BPP, 98 BS), whilst *Hyposcada* is monophyletic and sister to the rest of the Oleriina (Figs. S3 and S4). The concatenated data support the finding of ncDNA data, albeit with lower support, revealing that ncDNA has a stronger signal.

All basal branches in the mtDNA for *Hyposcada* are well-supported under BI (>76 BPP) (Fig. S1). ML and MP both fail to resolve relationships at deeper nodes (Figs. S2 and S6). Under BI and ML, *H. kena* is recovered as sister to *H. anchiala* across all analyses to the exclusion of *H. schausi* and *H. virginiana*. MP places *H. schausi* as sister to *H. virginiana*, *H. kena* and *H. anchiala*.

All ncDNA topologies for *Hyposcada* are congruent and all species cluster together and are well-supported (Figs. S3, S4 and S7). In contrast, with mtDNA all *H. kena* are recovered as sister to *H. virginiana* and *H. schausi* and *H. anchiala* is revealed as sister to these. As with mtDNA, *H. illinissa* forms a well-supported clade under ncDNA. Topologies for the concatenated dataset are congruent with ncDNA across BI and ML. *H. zarepha zarepha* is recovered as sister to *H. anchiala* across all BI and ML analyses, however under MP, *H. zarepha zarepha* is recovered as sister to all *H. kena* subspecies.

mtDNA relationships within *Megoleria* are congruent across all topologies (Figs. S1, S3 and S6). BI and ML of ncDNA and the concatenated data similarly recover *M. susiana* as sister to the remaining *Megoleria* (Fig. 1 and Figs. S3–S5) while relationships under MP of ncDNA support a single specimen of *M. orestilla orestilla* 5-1028 as sister to *M. susiana* and other samples of *M. orestilla orestilla* (Fig. S7). Relationships under MP of the concatenated data are unresolved (Fig. S8).

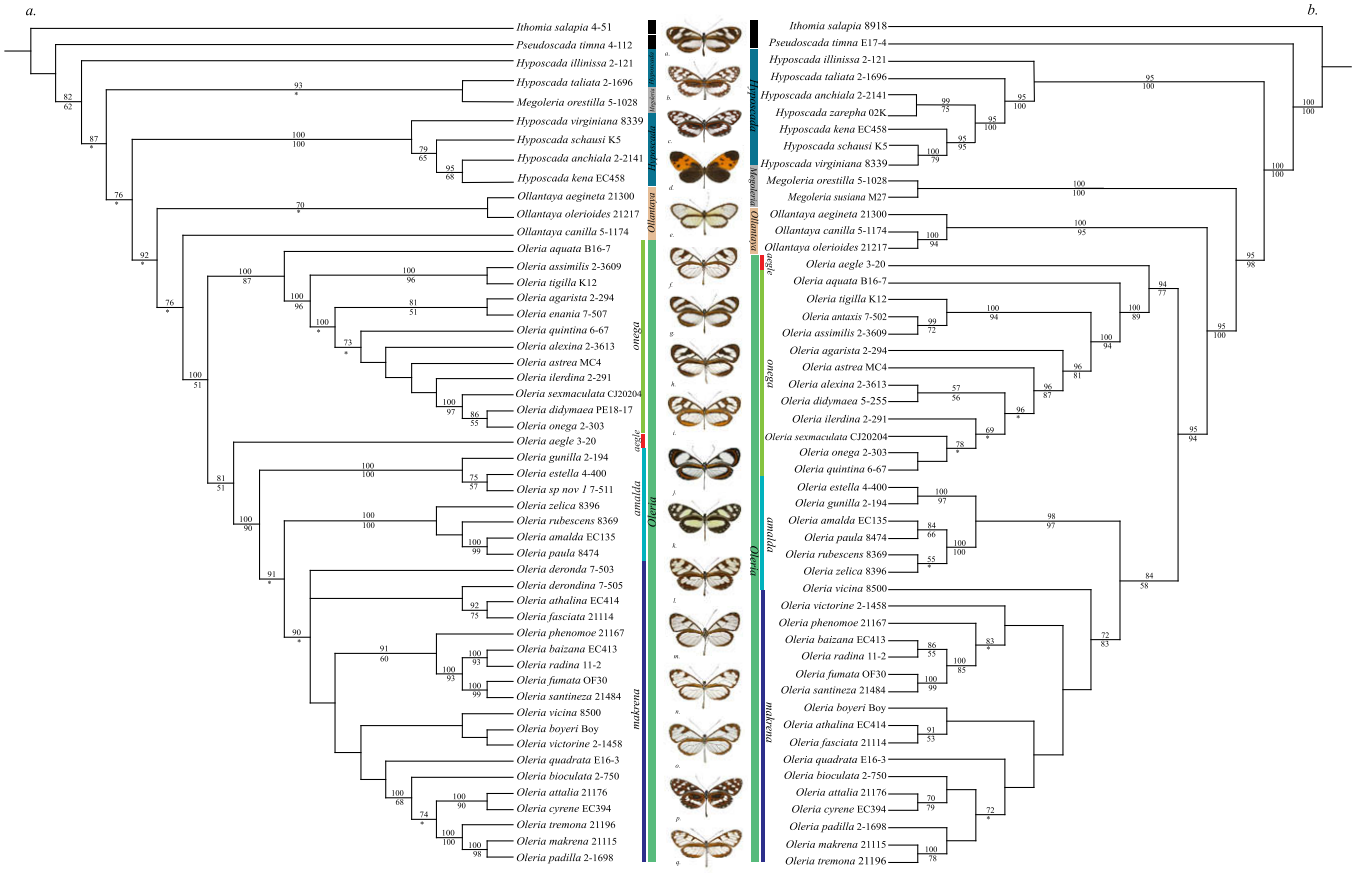

Fig. 2. 50% Majority-rule consensus tree of (a) mtDNA (BI three-partition analyses, BI partitioned by codon position, ML of 100 bootstraps) and (b) ncDNA (BI and ML) trees for individual species of *Hyposcada*, *Megoleria*, *Ollantaya* and *Oleria*. Numbers show BPP above the line and maximum likelihood BS below the line. * Indicates unresolved relationship. Photographs from top: (a) *H. illnissa brisotis*, (b) *H. taliata taliata*, (c) *M. orestilla orestilla*, (d) *H. anchiala mendax*, (e) *Ollantaya canilla*, (f) *O. aquata*, (g) *O. quintina*, (h) *O. astrea*, (i) *O. aegle egra*, (j) *O. estella estella*, (k) *O. rubescens*, (l) *O. deronda*, (m) *O. phenomoe phenomoe*, (n) *O. fumata*, (o) *O. victorine sarilis*, (p) *O. cyrene completa* and (q) *O. tremona tremona*.

4. Discussion

4.1. Oleriina phylogeny and evolutionary history

Phylogenetic hypotheses for the Oleriina are largely concordant with morphology and previous molecular analyses with the exception of the relationships of *Hyposcada* and *Megoleria*. The former is recovered as sister to all other Oleriina confirming the findings of Whinnett (2005) but challenging those of Brower et al. (2006) and Willmott and Freitas (2006). Morphological analyses place *Megoleria* as sister to *Hyposcada* and these as sister to all other Oleriina (Brown and Freitas, 1994; Willmott and Freitas, 2006), while higher-level molecular analyses found *Megoleria* to be sister to all other Oleriina (Brower et al., 2006). The strongest character supporting a sister relationship for *Hyposcada* and *Megoleria* is the shared use of the host plant family Gesneriaceae (Drummond and Brown, 1987; Willmott and Freitas, 2006), unique among the Ithomiini, while *Oleria* feed on Solanaceae similar to most other Ithomiini. Our hypotheses suggest, instead, that *Megoleria* and *Hyposcada* may independently have switched from Solanaceae to Gesneriaceae, or that the ancestor of *Oleria* plus *Ollantaya* reverted from feeding on Gesneriaceae to Solanaceae. However, none of these datasets provide very strong support for relationships among *Hyposcada*, *Megoleria* and *Oleria*/*Ollantaya*, and it is unsurprising that there are alternative topologies.

The phylogenetic hypothesis based on mtDNA results in the paraphyly of *Hyposcada*; with *H. taliata* placed as sister to *Megoleria* (Fig. 2), whereas ncDNA, the combined data and morphology recover each genus as a well-supported clade (Figs. 1 and 2) (Willmott and Freitas, 2006). *Megoleria* and *H. taliata* also share biogeographic and ecological similarities. Both groups are Andean in distribution and occur sympatrically at elevations above 1200 m (pers. obs.). They share near indistinguishable mimetic wing patterning distinct from other *Hyposcada* (Fig. 2) and potentially share the same larval host plant family (that of *H. taliata* is unknown but is assumed to be Gesneriaceae, as in other *Hyposcada* and *Megoleria*) (Drummond and Brown, 1987). One possible explanation for the phylogenetic discordance between mtDNA and ncDNA and morphology is that this shared mtDNA is the result of ancient introgression together with the selective, perhaps adaptive, introgression of loci for colour pattern and host plant use (Mallet, 2009). Introgression has been shown to affect some parts of butterfly genomes, while other regions affected by divergent selection remain largely isolated (Bull et al., 2006; Gompert et al., 2006; Kronforst et al., 2006; Mavárez et al., 2006; Mallet, 2007). On the other hand, introgression seems rather unlikely among such distantly related taxa. The other alternative is that the shared ancestry of these taxa may have resulted in the retention of an unusual ancient polymorphism. Further widespread sampling and sequencing of additional markers together with coalescence-based methods can be used to determine which of these scenarios is more likely.

4.2. Oleriina systematics

The ncDNA and concatenated dataset corroborate previous morphological studies (Willmott and Freitas, 2006) in recovering all currently recognised Oleriina genera as monophyletic. Within *Oleria* there is a basal split between a clade containing *O. canilla*, *O. aegineta* and *O. olerioides*, and a clade containing the remaining *Oleria* species. Brown and Freitas (1994) described the genus *Ollantaya* with type species *O. canilla* based on several putative morphological synapomorphies, and also included *O. aegineta* and *O. baizana* within the genus. While *O. baizana* appears to be unrelated to the other two species, Willmott and Freitas (2006) confirmed that *O. canilla* and *O. aegineta* are morphologically distinct from other *Oleria*, forming a clade sister to all other *Oleria*, along with *O. olerioides* and an additional undescribed species from Peru. Given that molecular divergence between these two clades is high in comparison with that among other *Oleria* clades, that both groups can be diagnosed by morphological synapomorphies (Willmott and Freitas, 2006), and that the larva of *O. aegineta* is rather different from other known *Oleria* (Willmott and Elias, unpublished data), we resurrect the name *Ollantaya* for *O. canilla*, *O. aegineta* (revised status) and *O. olerioides*.

The phylogeny of *Oleria* is highly congruent across all analyses, with the consistent recovery of four main clades. *O. aegle* is recovered as sister to the *amalda* and *makrena* groups by mtDNA and it is recovered as sister to the *onega* species group by ncDNA and by the much stronger signal of the latter partition in the combined dataset. Although the relationship of *O. aegle* with other species groups is inconclusive, it clearly clusters with remaining *Oleria*, confirming its inclusion in the genus.

At the species-level, our study suggests the need for a revision of species limits in *Hyposcada* and the *Oleria onega* group, at least. *H. zarepha zarepha* failed to cluster with any other *Hyposcada* taxa, and, in concert with morphological characters (Willmott and Lamas, unpublished data). This result suggests that Guianan *H. zarepha* should be regarded as a species distinct from west Amazonian *Hyposcada kena* (revised status). Our data also support the recognition of *H. kena* as a species distinct from its west Andean sister, *H. schausi* (Lamas, 2004). Finally, marked molecular divergence among races of *H. illinissa* suggest that an intensive phylogeographic study of this highly polymorphic species would be desirable to test the current classification. Within *Oleria*, both *O. astrea* and *O. onega* are polyphyletic, with different geographic races failing to cluster together, suggesting that at least some of these might represent distinct species. mtDNA differentiation between *Oleria onega* subspecies is around 6.5% (Whinnett et al., 2005b), which is notably high given that divergences within butterfly species are typically less than 2% (Brower, 1994).

4.3. Biogeography

One of the most remarkable features of the Oleriina is the relative mimetic homogeneity of the group (Fig. 2). Most other Ithomiini genera are diverse in wing pattern, with mimetic shifts often occurring between sister species (e.g., Jiggins et al., 2006). In contrast, within single communities of *Oleria*, most species mimic one another, with few co-mimics from other genera. The Oleriina are thus in sharp contrast to the emerging paradigm that mimetic shifts have enhanced diversification in the tribe (Jiggins et al., 2006), being mimetically similar but highly diverse. We might therefore expect a more important role for geography and biological factors other than mimicry in Oleriina speciation.

The four species groups identified in *Oleria* are similar to those identified in a preliminary morphological survey (Willmott, unpublished data), which suggests the monophyly of the *amalda* and *makrena* groups and the phylogenetic isolation of *O. aegle*. Furthermore, the four species groups are characterised by different preferred elevation ranges (Table 3). The *amalda* group species are characteristic of montane foothill forests from 0 to 1550 m. The *aegle* group is represented by a single species found below 500 m also in the Guianas and lower Amazon basin. The *onega* group species are found between 0 and 2100 m, though the great majority are characteristic of lowland Amazonian forests. In contrast, the *makrena* group species are mostly high-elevation Andean cloud forest species found between 1000 and 2850 m (Table 3).

Although this pattern of broad elevational sympatry within clades and regions is suggestive of diversification *in situ* driven by ecological adaptation, on closer inspection there are many

Table 3
Oleria species groups based on morphological and molecular analyses with known elevation range.

Oleria species groups							
aegle		*amalda*		*makrena*		*onega*	
O. aegle	0–500 m	*O. amalda*	0–1500 m	*O. athalina*	1200–2700 m	*O.* sp. nov. 4	0–500 m
		O. estella	600–1470 m	*O. attalia*	1200–2400 m	*O. agarista*	120–600 m
		O. gunilla	100–850 m	*O. baizana*	2000–2450 m	*O. alexina*	120–1300 m
		O. paula	30–1750 m	*O.* sp. nov. 2	1310–2000 m	*O. antaxis*	100–450 m
		O. rubescens	30–1540 m	*O. bioculata*	1250–1600 m	*O. aquata*	0–1100 m
		O. sp. nov. 1	500–1000 m	*O. boyeri*	900–1475 m	*O. assimilis*	120–900 m
		O. zelica	0–1550 m	*O.* sp. nov. 3	1600–2400 m	*O. astrea*	25–1000 m
				O. cyrene	1600–2600 m	*O. didymaea*	100–1200 m
				O. deronda	1400–2200 m	*O. enania*	130–650 m
				O. derondina	1800–2850 m	*O. flora*	100–820 m
				O. fasciata	1300–2200 m	*O. ilerdina*	10–1400 m
				O. fumata	1000–2500 m	*O. onega*	100–1550 m
				O. makrena	950–2500 m	*O. quintina*	500–2100 m
				O. padilla	500–2500 m	*O. sexmaculata*	120–600 m
				O. phenomoe	480–1835 m	*O. similigena*	400–820 m
				O. quadrata	900–1550 m	*O. synnova*	50–120 m
				O. radina	1700–2400 m	*O. thiemei*	400–500 m
				O. santineza	1200–2400 m	*O. tigilla*	12–1200 m
				O. tremona	1300–2400 m		
				O. vicina	1200–2000 m		
				O. victorine	25–1650 m		
				O. zea	1200–2000 m		

examples of geographically allopatric, or, in some cases, elevationally parapatric sister species. The *amalda* group has two allopatric sub-clades, the first comprising the transandean *O. rubescens*, *O. zelica*, *O. paula* and *O. amalda* and the second the Amazonian *O. estella*, *O. gunilla* and *O.* sp. nov. 1. Within these sub-clades, sister species *O. amalda* and *O. paula* are geographically allopatric, while *O. gunilla* and *O. estella*/*O.* sp. nov. 1 are elevationally allopatric. Among the highland *O. makrena* group, allopatric or only partially sympatric sister species include *O. makrena*/*O. padilla*, *O. vicina*/*O. victorine*, *O. radina*/*O. baizana*, *O. santineza*/*O. fumata* and *O. cyrene*/*O. attalia*. Among the lowland *onega* group, there are a notable number of species restricted to the Guianas, lower Amazon or southeastern Brazil, including *O. antaxis*, *O. aquata*, *O. astrea*, *O. flora*, *O. similigena* and one undescribed species. By contrast, for example, only a single *Ithomia* and no *Napeogenes* species are restricted to these regions (Jiggins et al., 2006; Elias et al., 2009). Both *O. aquata* and *O. astrea* are sister to west Amazonian species or clades with which they are largely or completely allopatric.

Little or no support is shown for the relationships of the high elevation *makrena* group although internal clades uniting species are well-resolved suggesting rapid radiation of this group rather than insufficient data. Recent evidence suggests that young species may be more prevalent in montane areas (Hall, 2005; Weir, 2006) and many taxa have been found to diversify into highland habitats after uplift of the Andes (Bates and Zink, 1994; Bleiweiss, 1998).

To conclude, our results hint at a potentially more important role for local geographic isolation in the diversification of Oleriina than in other Ithomiini groups studied to date (e.g., Jiggins et al., 2006; Elias et al., 2009) and provide a framework for future more detailed biogeographical studies. In addition, comparative studies with other butterfly taxa in addition to groups such as reptiles (Torres-Carvajal, 2007), amphibians (Santos et al., 2009) and birds (Brumfield and Edwards, 2007), could improve our understanding of the general processes involved in colonisation and diversification within the planet's most biodiverse region.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2010.01.010.

References

Bates, J.M., Zink, R.M., 1994. Evolution into the Andes: molecular evidence for species relationships in the genus *Leptopogon*. The Auk 111 (3), 507–515.
Beccaloni, G.W., 1997a. Ecology, natural history and behaviour of ithomiine butterflies and their mimics in Ecuador (Lepidoptera: Nymphalidae: Ithomiinae). Trop. Lepid. 8, 103–124.
Beccaloni, G.W., 1997b. Vertical stratification of ithomiine butterfly (Nymphalidae: Ithomiinae) mimicry complexes: the relationship between adult flight height and larval host plant height. Biol. J. Linn. Soc. 62, 313–341.
Bleiweiss, R., 1998. Origins of hummingbird faunas. Biol. J. Linn. Soc. 65, 77–97.
Brower, A.V.Z., 1994. Phylogeny of *Heliconius* butterflies inferred from mitochondrial DNA sequences (Lepidoptera: Nymphalidae). Mol. Phylogenet. Evol. 3, 159–174.
Brower, A.V.Z., Freitas, A.V.L., Lee, M.-M., Silva-Brandão, K.L., Whinnett, A., Willmott, K.R., 2006. Phylogenetic relationships among the Ithomiini (Lepidoptera: Nymphalidae) inferred from one mitochondrial and two nuclear gene regions. Syst. Entomol. 31, 288–301.
Brown, K.S., 1987. Chemistry at the solanaceae/ithomiinae interface. Ann. Missouri Bot. Gard. 74, 359–397.
Brown, K.S., Freitas, A.V.L., 1994. Juvenile stages of Ithomiinae: overview and systematics (Lepidoptera: Nymphalidae). Trop. Lepid. 5 (1), 9–20.

Brumfield, R.T., Edwards, S.V., 2007. Evolution into and out of the Andes: a Bayesian analysis of historical diversification in *Thamnophilus* antshrikes. Evolution 61 (2), 346–367.

Bull, V., Beltrán, M., Jiggins, C.D., McMillan, W.O., Bermingham, E., Mallet, J., 2006. Polyphyly and gene flow between non-sibling *Heliconius* species. BMC Biol. 4, 11.

Constantino, L.M., 1999. Nuevas especies, subespecies y un nuevo género de ropalóceros del occidente de Colombia (Lepidoptera: Papilionidae, Nymphalidae, Charaxinae, Ithomiinae, Heliconiinae). Boletín Cientifico. Museo de Historia Natural. Universidad de Caldas 3, 57–68.

Drummond, B.A., Brown, K.S., 1987. Ithomiinae (Lepidotera: Nymphalidae): summary of known larval food plants. Ann. Missouri Bot. Gard. 74, 341–358.

Elias, M., Gompert, Z., Jiggins, C., Willmott, K., 2008. Mutualistic interactions drive ecological niche convergence in a diverse butterfly community. PLoS Biol. 6, e300.

Elias, M., Hill, R.I., Willmott, K., Dasmahapatra, K., Brower, A.V.Z., Mallet, J., Jiggins, C.D., 2007. Limited performance of DNA barcoding in a diverse community of tropical butterflies. Proc. R. Soc. Lond. B 274, 2881–2889.

Elias, M., Joron, M., Willmott, K., Silva-Brandão, K.L., Kaiser, V., Arias, C.F., Gomez Piñeres, L.M., Uribe, S., Brower, A.V.Z., Freitas, A.V.L., Jiggins, C., 2009. Out of the Andes: patterns of diversification in clearwing butterflies. Mol. Ecol. 18, 1716–1729.

Fox, R.M., 1956. A monograph of the Ithomiidae (Lepidoptera). Part I. Bull. Am. Mus. Nat. Hist. 111, 1–76.

Goloboff, P.A., 1999. Analyzing large data sets in reasonable times: solutions for composite optima. Cladistics 15, 415–428.

Gompert, Z., Fordyce, J.A., Forister, M., Shapiro, A.M., Nice, C.C., 2006. Homoploid hybrid speciation in an extreme habitat. Science 314, 1923–1925.

Haber, W.A., 1978. Evolutionary Ecology of Tropical Mimetic Butterflies (Lepidoptera: Ithomiinae). Ph.D. Thesis, University of Minnesota, Minnesota.

Hall, J.P.W., 2005. Montane speciation patterns in *Ithomiola* butterflies (Lepidotera: Riodinidae): are they consistently moving up in the world? Proc. R. Soc. Lond. B 272, 2457–2466.

Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp. Ser. 41, 95–98.

Harvey, D.J., 1991. Higher classification of the Nymphalidae. In: Nijhout, H.F. (Ed.), The Development and Evolution of Butterfly Wing Patterns. Smithsonian Institution, Washington, pp. 255–273.

Huelsenbeck, J.P., Ronquist, F., 2001. MrBayes: Bayesian inference of phylogenetic trees. Bioinformatics 17, 754–755.

Jiggins, C.D., Mallarino, R., Willmott, K.R., Bermingham, E., 2006. The phylogenetic pattern of speciation and wing pattern change in neotropical *Ithomia* butterflies (Lepidoptera; Nymphalidae). Evolution 60 (7), 1454–1466.

Joron, M., Wynne, I.R., Lamas, G., Mallet, J., 2001. Variable selection and the coexistence of multiple mimetic forms in *Heliconius numata*. Evol. Ecol. 13 (7/8), 721–754.

Kishino, H., Hasegawa, M., 1989. Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in the Hominoidea. J. Mol. Evol. 29, 170–179.

Kronforst, M.R., Young, L.G., Blume, L.M., Gilbert, L.E., 2006. Multilocus analysis of admixture and introgression among hybridizing *Heliconius* butterflies. Evolution 60, 1254–1268.

Lamas, G., 2004. Ithomiinae. In: Heppner, J.B. (Ed.), Atlas of Neotropical Lepidoptera. Checklist: Part 4A Hesperioidea – Papilionoidea. Association of Tropical Lepidoptera. Scientific Publishers, Gainesville, FL.

Mallarino, R., Bermingham, E., Willmott, K.R., Whinnett, A., Jiggins, C.D., 2005. Molecular systematics of the butterfly genus *Ithomia* (Lepiodoptera: Ithomiinae): a composite phylogenetic hypothesis based on seven genes. Mol. Phylogenet. Evol. 34, 625–644.

Mallet, J., 2007. Hybrid speciation. Nature 446, 279–283.

Mallet, J., 2009. Rapid speciation, hybridization and adaptive radiation in the *Heliconius melpomene* group. In: Butlin, R., Bridle, J., Schluter, D. (Eds.), Speciation and Patterns of Diversity. Cambridge University Press, pp. 177–194.

Mavárez, J., Salazar, C., Bermingham, E., Salcedo, C., Jiggins, C.D., Linares, M., 2006. Speciation by hybridization in *Heliconius* butterflies. Nature 441, 868–871.

Nixon, K.C., 1999. The parsimony ratchet, a new method for rapid parsimony analysis. Cladistics 15, 407–414.

Pinheiro, C.E.G., Medri, Í.M., Salcedo, A.K.M., 2008. Why do the ithomiines (Lepidoptera, Nymphalidae) aggregate? Notes on a butterfly pocket in central Brazil. Revista Brasileira de Entomologia 52 (4), 610–614.

Posada, D., Crandall, K., 1998. MODELTEST: testing the model of DNA substitution. Bioinformatics 14, 817–818.

Rambaut, A., Drummond, A.J., 2007. Tracer v1.4. <http://www.beast.bio.ed.ac.uk/Tracer>.

Ren, F.R., Tanaka, H., Yang, Z.H., 2005. An empirical examination of the utility of codon-substitution models in phylogeny reconstruction. Syst. Biol. 54, 808–818.

Santos, J.C., Coloma, L.A., Summers, K., Caldwell, J.P., Ree, R., Cannatella, D.C., 2009. Amazonian amphibian diversity is primarily derived from late Miocene Andean lineages. PLoS Biol 7 (3), E56.

Schultz, S., Beccaloni, G., Brown Jr., K.S., Boppré, M., Freitas, A.V.L., Ockenfels, P., Trigo, J.R., 2004. Semiochemicals derived from pyrrolizidine alkaloids in male ithomiine butterflies (Lepidoptera: Nymphalidae: Ithomiinae). Biochem. Syst. Ecol. 32, 699–713.

Shimodaira, H., 2002. An approximately unbiased test of phylogenetic tree selection. Syst. Biol. 53 (3), 492–508.

Swofford, D.L., 2002. PAUP*. Phylogenetic Analysis Using Parsimony (* and Other Methods), Version 4.0b10. Sinauer Associates, Sunderland, MA.

Thompson, J.D., Higgins, D.G., Gibson, T.J., 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22, 4673–4680.

Torres-Carvajal, O., 2007. Phylogeny and biogeography of a large radiation of Andean lizards (Iguania, *Stenocercus*). Zool. Scripta 36, 311–326.

Wahlberg, N., Freitas, A.V.L., 2007. Colonization of and radiation in South America by butterflies in the subtribe Phyciodina (Lepidoptera: Nymphalidae). Mol. Phylogenet. Evol. 44, 1257–1272.

Wahlberg, N., Weingartner, E., Nylin, S., 2003. Towards a better understanding of the higher systematics of Nymphalidae (Lepidoptera: Papilionoidea). Mol. Phylogenet. Evol. 28, 473–484.

Weir, J., 2006. Divergent timing and patterns of species accumulation in lowland and highland neotropical birds. Evolution 60 (4), 842–855.

Whinnett, A., 2005. The Phylogeography and Molecular Evolution of Ithomiine Butterflies. Unpublished Thesis, University College London, UK.

Whinnett, A., Willmott, K.R., Brower, A.V.Z., Simpson, F., Lamas, G., Mallet, J., 2005a. Mitochondrial DNA provides an insight into the mechanisms driving diversification in the ithomiine butterfly *Hyposcada anchiala* (Lepidoptera: Nymphalidae, Ithomiinae). Eur. J. Entomol. 102, 633–639.

Whinnett, A., Zimmermann, M., Willmott, K.R., Herrera, N., Mallarino, R., Simpson, F., Joron, M., Lamas, G., Mallet, J., 2005b. Strikingly variable divergence times inferred across an Amazonian butterfly 'suture zone'. Proc. R. Soc. Lond. B 272, 2525–2533.

Willmott, K.R., Freitas, A.V.L., 2006. Higher-level phylogeny of the Ithomiinae (Lepidoptera: Nymphalidae): classification, patterns of larval hostplant colonization and diversification. Cladistics 22, 297–368.

Willmott, K.R., Hall, J.P.W., Lamas, G., 2001. Systematics of *Hypanartia* (Lepidoptera: Nymphalidae: Nymphalinae), with a test for geographical speciation mechanisms in the Andes. Syst. Entomol. 26, 369–399.

Willmott, K.R., Lamas, G., 2008. A revision of the genus *Megoleria* (Lepidoptera: Nymphalidae; Ithomiinae). Trop. Lepid. Res. 18 (1), 46–59.

Willmott, K.R., Mallet, J., 2004. Correlations between adult mimicry and larval host plants in ithomiine butterflies. Proc. R. Soc. Lond. B: Biol. Lett. 271 (S5), S266–S269.

Zwickl, D.J., 2006. Genetic Algorithm Approaches for the Phylogenetic Analysis of Large Biological Sequence Datasets under the Maximum Likelihood Criterion. Ph.D. Dissertation, The University of Texas at Austin.