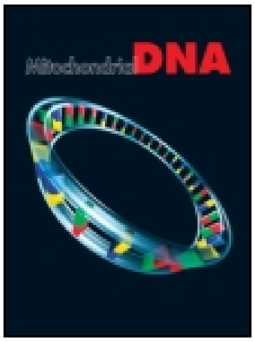

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Tropical montane nymphalids in Mexico: DNA barcodes reveal greater diversity

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Abstract

Materials and methods: DNA sequences obtained for the Barcode of Life library in the All Lepidoptera Campaign project Nymphalidae of Central Mexico were analyzed as a test of species limits and to explore possible phylogenetic groupings in the Preponini tribe. Using specimens in the National Insect Collection of the Instituto de Biología of the Universidad Nacional Autónoma de México, 78 specimens were assayed for cytochrome oxidase *c* subunit 1.

Results: Disregarding the missing data, there were 458 conserved sites, 200 variable sites and 187 parsimony-informative sites. The neighbor-joining and maximum likelihood analyses indicate that none of the three genera of Preponini as currently circumscribed are reciprocally monophyletic. As per species limits, high levels of barcode variation in the *Prepona deiphile* complex suggest the existence of at least two new endemic species to Mexico. The divergent taxa were *escalantiana* from the Tuxtlas region in Veracruz, and *ibarra* from Sierra Madre del Sur in the Pacific states of southern Mexico. The genetic distance in the CO1 fragment between them and the other *deiphile* populations ranged from 2.7 to 8.0%.

Conclusion: We recommend that morphological data need to be re-examined and that additional molecular data for species ought to be gathered before a particular biogeographic model can be proposed for the group in Mesoamerica.

Keywords: *Preponini, phylogeography, neotropics, cytochrome oxidase* c *subunit 1*

Introduction

The International Barcode of Life project is an initiative of great appeal to wildlife and resource managers. One of the more active campaigns in this project is the Lepidoptera Barcode of Life (http://www.lepbarcoding.org) because there are considerable number of specimens in collections that could be barcoded, and because adequate protocols are now in place. Additionally, butterflies are quite amenable and prized by people as collectable items, and government agencies should have an efficient method to verify species identification and countries of origin. These goals are achievable through an international initiative in a relatively short time. Because of these considerations, we undertook DNA barcoding of the most attractive component of the lepidopteran fauna of central Mexico, the Nymphalidae. Here, we present the very first results and their interpretation for the tribe Preponini (subfamily Charaxini).

Preponini are large, canopy-dwelling, fruit-feeding nymphalids of the neotropics, most of them with cryptic wing patterns on their underside, and striking iridescent patterns on their dorsal surface. There is as yet very few molecular data at the species level for this group. The Lepidoptera of Mexico have been studied at great geographical detail (i.e. Luis-Martínez et al. 2003), but a revision using a new suite of characters such as mitochondrial DNA sequences has not yet been produced. In our paper, we present a preliminary phylogenetic analysis of closely related species in the subfamily Charaxinae, tribe Preponini Rydon 1971, mostly from the west of the Isthmus of Tehuantepec, using cytochrome oxidase *c* subunit 1 (*cox1*) sequences.

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The tribe Preponini is currently composed of 21 species in four genera, distributed mainly in the Neotropical Region. In Mexico, there are representatives of 11 species in three genera. The current taxonomy (Savela 2010) of this tribe acknowledges these: *Prepona* (seven species, of which four are in Mexico), *Archaeoprepona* (eight species, of which five are in Mexico), *Agrias* (five species, of which two are in Mexico) and *Noreppa* (one species, none in Mexico). *Anaeomorpha*, from South America, is sometimes recognized as a monotypic genus in this tribe, too.

As updated by Wahlberg and Brower (2009), the sister group of Preponini are members of the Anaeini tribe. This is also in accordance with a recent cladistic analysis of morphological characters for the subfamily Charaxinae (Marconato 2008), in which, except for *Anaeomorpha splendida*, Preponini is monophyletic. At the generic level, *Archaeoprepona* and *Prepona* were polyphyletic, since *Archaeoprepona* should include the monotypic genus *Noreppa*, and *Prepona* should include *Agrias*, to be monophyletic. With these findings, we chose a member of *Anaeia* as the sister group for our analysis, and as additional outgroups we included seven other Nymphalids from the Barcode of Life Data Systems (BOLD) public database.

Many species of Preponini have a number of described subspecies. One such example is the *Prepona deiphile* complex reviewed by Llorente-Bousquets et al. (1992), in which differentiating isolated populations occurring in various mountainous ranges are given subspecies status according to a particular morphological interpretation and a previously chosen biogeographical hypothesis, which is understood as a "major vicariant pattern in southern Mexico" with one component in the east and southeast part of the country, and the second component in the south and west (Llorente-Bousquets et al. 1993; Vargas-Fernández et al. 2006). DNA barcodes can help by providing data to test this biogeographic hypothesis.

Materials and methods

Samples

Tissue samples were collected from specimens deposited in the National Insect Collection at the Instituto de Biología, Universidad Nacional Autónoma de México (CNIN-LEP, IBUNAM). Legs were removed and sent for genetic analysis to the Biodiversity Institute of Ontario at the University of Guelph (Guelph, Ontario, Canada). The specimens were photographed and entered into the database of the Unidad de Informática de la Biodiversidad (Instituto de Biología, UNAM; http://www.unibio.ibiologia.unam.mx), and subsequently into BOLD (http://www.boldsystems.org). Most of the subspecies described for this tribe in Mexico were represented in this data set.

DNA extraction, PCR amplification and sequencing

The samples were subjected to the regular laboratory procedures used in the All-Lepidoptera Campaign for lysis and DNA extraction, but a "minibarcode" amplification was undertaken to account for the specimens' age (collected between the 1960s and 1990s). Rather than a PCR to amplify a 650 bp fragment in a single reaction for each sample, two smaller fragments were amplified in separate PCR reactions. Only 10 samples failed to amplify for both fragments, while only one of the two fragments was successfully amplified for about 24 of the 84 specimens. Here, we only analyze the specimens belonging to the Tribe Preponini (*Archaeoprepona*, *Prepona*, and *Agrias* spp.) with a minimum sequence length of 307 bp (68 samples, of which 36 were 658 bp in length, 22 were 407–602 bp and 10 were 307 bp). All species/populations were represented by at least two full barcodes. The sequences obtained were deposited in BOLD and GenBank (Table I).

Alignment and phylogenetic reconstruction

The 68 DNA sequences were aligned in ClustalX (Thompson et al. 1997) and then we used the neighbor-joining (NJ) tree tool with the Kimura two-parameter substitution model (Kimura 1980) in BOLD with node support estimated through 100 bootstrap replicates. We translated our nucleotide sequences into amino acids in DnaSP 5.10.01 (Librado and Rozas 2009) and no stop codons were encountered. For outgroups, we chose a sequence from Costa Rica of *Anaea aidea*, already available in BOLD and seven other outgroups (see Table I, for full data and GenBank accession numbers), following Peña and Wahlberg (2008).

Disregarding missing data, there were 458 conserved sites, 200 variable sites and 187 parsimony-informative sites. Nucleotide composition was 39.2% T, 16.5% C, 30% A and 14.3% G; that is, within the expected range for a coding gene (Saccone et al. 1999; Junqueira et al. 2004).

We selected the best-fit substitution model for our alignment using jModelTest 0.1.1 (Posada 2008), and then used those parameters for a maximum likelihood (ML) phylogenetic analysis in PAUP* 4b10 (Swofford 2003) using the NJ tree as the start tree for a heuristic search. The settings from the best-fit model (TIM2 + I + G) selected by the Bayesian information criterion used in PAUP* were: Lset base = (0.3001 0.1425 0.1382 0.4192) nst = 6 rmat = (10.2850 24.5568 10.2850 1.0000 84.4402 1.0000) rates = gamma shape = 1.1510 ncat = 4 pinvar = 0.5880. Four trees were obtained with the same likelihood score and were summarized through a majority-rule consensus tree. For visualization, rooting the tree and labeling the tips, we used FigTree (Rambaut 2007). For constructing the map in Figure 1 using a niche

Table I. Specimen data used in the present study.

Sample ID	GeneBank accession number	Museum voucher ID	Scientific name	Locality	Latitude	Longitude	Collection date
AIV 001	HM888242	CNIN-LEP 37688	*Archaeoprepona amphimachus amphiktion*	Puebla, Jopala, Patla	20.2480	−97.8530	4 May 1980
AIV 002	HM888243	CNIN-LEP 37687	*Archaeoprepona amphimachus amphiktion*	Puebla, Zihuateutla, Barranca de Patla	20.2350	−97.8880	8 Oct 1977
AIV 003	HM888244	CNIN-LEP 37686	*Archaeoprepona amphimachus amphiktion*	Puebla, Zihuateutla, Barranca de Patla	20.2350	−97.8880	8 Oct 1977
AIV 004	HM888245	CNIN-LEP 37683	*Archaeoprepona amphimachus amphiktion*	Puebla, Zihuateutla, Barranca de Patla	20.2350	−97.8880	1 Jul 1992
AIV 005	HM888246	CNIN-LEP 37722	*Archaeoprepona amphimachus baroni*	Oaxaca, San Pedro Pochutla, Chacalapilla, Candelaria Loxicha	15.8940	−96.4760	31 Oct 1977
AIV 006	HQ025031	CNIN-LEP 37721	*Archaeoprepona amphimachus baroni*	Oaxaca, Candelaria Loxicha, Candelaria Loxicha	15.9260	−96.4920	9 Feb 1982
AIV 007	HM888247	CNIN-LEP 37720	*Archaeoprepona amphimachus baroni*	Guerrero, La Union, El Faisanal, Paraiso	17.9250	−101.6240	12 Dec 1980
AIV 008	HM888248	CNIN-LEP 37771	*Archaeoprepona demophon centralis*	Puebla, Zihuateutla, Barranca de Patla	20.2350	−97.8880	25 Jul 1978
AIV 009	HM888249	CNIN-LEP 37769	*Archaeoprepona demophon centralis*	Veracruz, San Andres Tuxtla, Estacion de Biologia, Los Tuxtlas	18.5900	−95.0690	11 Dec 1984
AIV 011	HQ025032	CNIN-LEP 37753	*Archaeoprepona demophon centralis*	Chiapas, Arriaga, Arriaga	16.2330	−93.9000	
AIV 012	HQ025033	CNIN-LEP 37806	*Archaeoprepona demophon occidentalis*	Morelos, Xochitepec, Alpuyeca	18.7440	−99.2580	1 Dec 1981
AIV 013	HM888250	CNIN-LEP 37805	*Archaeoprepona demophon occidentalis*	Guerrero, Acapulco de Juarez, Playon	17.1550	−99.6480	1 Sep 1991
AIV 014	HM888251	CNIN-LEP 37785	*Archaeoprepona demophon occidentalis*	Oaxaca, Pluma Hidalgo, Finca el Pacifico	15.8630	−96.5080	20 Oct 1990
AIV 015	HM888252	CNIN-LEP 37784	*Archaeoprepona demophon occidentalis*	Guerrero, Acapulco de Juarez, Playon	17.1550	−99.6480	1 Sep 1991
AIV 017	HM888253	CNIN-LEP 37808	*Archaeoprepona demophoon gulina*	Veracruz, San Andres Tuxtla, Volcan San Martin	18.5550	−95.2000	1 Aug 1987
AIV 018	HQ025034	CNIN-LEP 37814	*Archaeoprepona demophoon gulina*	Puebla, Zihuateutla, Barranca de Patla	20.2350	−97.8880	16 Mar 1980
AIV 019	HM888254	CNIN-LEP 37823	*Archaeoprepona demophoon gulina*	Oaxaca, San Jose Chiltepec, Chiltepec	17.9470	−96.1710	1 Aug 1978
AIV 020	HM888255	CNIN-LEP 37842	*Archaeoprepona demophoon mexicana*	Guerrero, Chilpancingo de los Bravo, Acahuizotla	17.3600	−99.4620	20 Jun 1977
AIV 021	HM888256	CNIN-LEP 37844	*Archaeoprepona demophoon mexicana*	Colima, Manzanillo, Manzanillo	19.0520	−104.3160	22 Dec 1952
AIV 023	HM888257	CNIN-LEP 37846	*Archaeoprepona demophoon mexicana*	Guerrero, Chilpancingo de los Bravo, Acahuizotla	17.3600	−99.4620	15 Jul 1978
AIV 024	HQ025035	CNIN-LEP 37850	*Archaeoprepona meander phoebus*	Chiapas, Ocosingo, Bonampak	16.7020	−91.0640	1 Sep 1964
AIV 025	HM888258	CNIN-LEP 37851	*Archaeoprepona phaedra aelia*	Puebla, Zihuateutla, Barranca de Patla	20.2350	−97.8880	18 Jul 1981
AIV 026	HM888259	CNIN-LEP 37854	*Archaeoprepona phaedra aelia*	Puebla, Zihuateutla, Barranca de Patla	20.2350	−97.8880	1 Jul 1992
AIV 027	HM888260	CNIN-LEP 37863	*Archaeoprepona phaedra aelia*	Puebla, Zihuateutla, Barranca de Patla	20.2350	−97.8880	31 Jul 1981
AIV 028	HM888261	CNIN-LEP 37861	*Archaeoprepona phaedra aelia*	Oaxaca, San Jose Chiltepec, Chiltepec	17.9470	−96.1710	1 Jun 1967
AIV 029	HM888262	CNIN-LEP 37864	*Archaeoprepona phaedra* ssp.	Oaxaca, Candelaria Loxicha, Portillo del Rayo	15.9760	−96.4910	26 Aug 1977
AIV 030	HQ025037	CNIN-LEP 37867	*Archaeoprepona phaedra* ssp.	Chiapas, La Independencia, San Antonio Buena Vista (Sta. Rosa)	16.1510	−91.6510	Ago 1970
AIV 031	HQ025036	CNIN-LEP 37868	*Archaeoprepona phaedra* ssp.	Chiapas, La Independencia, San Antonio Buena Vista (Sta. Rosa)	16.1510	−91.6510	1 Aug 1961
AIV 033	HM888263	CNIN-LEP 37874	*Archaeoprepona phaedra* ssp.	Chiapas, La Independencia, San Antonio Buena Vista (Sta. Rosa)	16.1510	−91.6510	Ago 1976

Table I – *continued*

Sample ID	GeneBank accession number	Museum voucher ID	Scientific name	Locality	Latitude	Longitude	Collection date
AIV 034	HM888264	CNIN-LEP 37901	*Prepona deiphile brooksiana*	Puebla, Zihuateutla, Barranca de Patla	20.2350	−97.8880	1 Jul 1994
AIV 035	HM888265	CNIN-LEP 37882	*Prepona deiphile brooksiana*	Veracruz, Coatepec, Coatepec	19.4570	−96.9580	14 Aug 1977
AIV 036	HM888266	CNIN-LEP 37898	*Prepona deiphile brooksiana*	Puebla, Zihuateutla, Barranca de Patla	20.2350	−97.8880	1 Jul 1994
AIV 037	HM888267	CNIN-LEP 37822	*Prepona deiphile brooksiana*	Oaxaca, San Jose Chiltepec, Chiltepec	17.9470	−96.1710	1 Sep 1964
AIV 038	HM888268	CNIN-LEP 37910	*Prepona deiphile brooksiana*	Puebla, Zihuateutla, Barranca de Patla	20.2350	−97.8880	1 Aug 1991
AIV 039	HM888269	CNIN-LEP 37884	*Prepona deiphile brooksiana*	Puebla, Xicotepec, Tequezquitla	20.2760	−97.8880	1 Jul 1991
AIV 040	HM888270	CNIN-LEP 37921	*Prepona deiphile* ssp.	Chiapas, Pijijiapan, Pijijiapan	15.6870	−93.2090	20 Dec 1966
AIV 042	HM888271	CNIN-LEP 38614	*Prepona deiphile* ssp.	Chiapas, Ocozocoautla de Espinosa, Laguna Bélgica	16.0000	−93.0000	3 Ago 1980
AIV 043	HM888272	CNIN-LEP 38024	*Prepona deiphile* ssp.	Chiapas, Ocozocoautla de Espinosa, Ocozocoautla	16.7480	−93.3710	12 Aug 1980
AIV 044	HM888273	CNIN-LEP 37953	*Prepona deiphile escalantiana*	Veracruz, Catemaco, La Perla de San Martin, Los Tuxtlas	18.5400	−95.1220	1 Jul 1991
AIV 045	HM888274	CNIN-LEP 37986	*Prepona deiphile escalantiana*	Veracruz, Catemaco, Santa Martha	18.3990	−95.0120	1 Jul 1992
AIV 046	HM888275	CNIN-LEP 37978	*Prepona deiphile escalantiana*	Veracruz, Santiago Tuxtla, Cerro Blanco	18.4930	−95.3460	1 Aug 1982
AIV 047	HM888276	CNIN-LEP 37977	*Prepona deiphile escalantiana*	Veracruz, Santiago Tuxtla, Cerro Blanco	18.4930	−95.3460	1 Aug 1984
AIV 048	HM888277	CNIN-LEP 38006	*Prepona deiphile diaziana*	Chiapas, La Independencia, San Antonio Buena Vista (Sta. Rosa)	16.1510	−91.6510	1 Jun 1991
AIV 049	HM888278	CNIN-LEP 38005	*Prepona deiphile diaziana*	Chiapas, La Independencia, San Antonio Buena Vista (Sta. Rosa)	16.1510	−91.6510	1 Aug 1975
AIV 050	HM888279	CNIN-LEP 38025	*Prepona deiphile diaziana*	Chiapas, La Independencia, San Antonio Buena Vista (Sta. Rosa)	16.1510	−91.6510	1 Sep 1992
AIV 051	HM888280	CNIN-LEP 38022	*Prepona deiphile diaziana*	Chiapas, La Independencia, San Antonio Buena Vista (Sta. Rosa)	16.1510	−91.6510	1 Sep 1992
AIV 052	HM888281	CNIN-LEP 38028	*Prepona deiphile ibarra*	Michoacan, Uruapan, Uruapan	19.4260	−102.0610	23 Aug 1993
AIV 053	HQ025038	CNIN-LEP 38027	*Prepona deiphile ibarra*	Michoacan, Uruapan, Uruapan	19.4260	−102.0610	28 Aug 1992
AIV 054	HM888282	CNIN-LEP-Type144	*Prepona deiphile ibarra*	Guerrero, Chilpancingo – Acapulco Km.36	17.3000	−99.5000	30 Jul 1978
AIV 055	HM888283	CNIN-LEP-Type145	*Prepona dexamenes medinai*	Veracruz, Agua Dulce	18.1270	−94.1480	24 May 1977
AIV 056	HM888284	CNIN-LEP 38034	*Prepona dexamenes medinai*	Chiapas, Ocosingo, Chajul	16.1180	−90.9240	1 Apr 1981
AIV 057	HQ025039	CNIN-LEP 38037	*Prepona dexamenes medinai*	Chiapas, Ocosingo, Chajul	16.1180	−90.9240	1 Apr 1981
AIV 058	HM888285	CNIN-LEP 38038	*Prepona dexamenes medinai*	Chiapas, Ocosingo, Chajul	16.1180	−90.9240	1 Oct 1983
AIV 059	HQ025041	CNIN-LEP 38085	*Prepona leartes octavia*	Veracruz, Agua Dulce, Agua Dulce	18.1270	−94.1480	23 Jul 1977
AIV 060	HM888286	CNIN-LEP 38084	*Prepona leartes octavia*	Guerrero, Chilpancingo de los Bravo, Acahuizotla	17.3600	−99.4620	1 Jul 1991
AIV 061	HQ025040	CNIN-LEP 38073	*Prepona leartes octavia*	Veracruz, Veracruz, Veracruz	19.1740	−96.1320	1 Jan 1964
AIV 062	HM888287	CNIN-LEP 38113	*Prepona leartes octavia*	Guerrero, Acapulco de Juarez, Playon	17.1550	−99.6480	1 Sep 1991
AIV 063	HM888288	CNIN-LEP 38118	*Prepona pylene philetas*	Oaxaca, San Jose Chiltepec, Chiltepec	17.9470	−96.1710	1 Aug 1963
AIV 064	HQ025042	CNIN-LEP 38119	*Prepona pylene philetas*	Quintana Roo, Benito Juarez, Puerto Morelos	20.8590	−86.8730	14 Aug 1982
AIV 065	HM888289	CNIN-LEP 38124	*Prepona pylene philetas*	Quintana Roo, Cozumel, Playa del Carmen	20.6240	−87.0770	15 Mar 1982
AIV 066	HM888290	CNIN-LEP 40261	*Agrias aedon rodriguezi*	Chiapas, Ocosingo, Chajul	16.1180	−90.9240	1 Aug 1992
AIV 067	HQ025028	CNIN-LEP 40263	*Agrias aedon rodriguezi*	Chiapas, Ocosingo, Chajul	16.1180	−90.9240	1 Aug 1993
AIV 068	HM888291	CNIN-LEP 40264	*Agrias aedon rodriguezi*	Chiapas, Ocosingo, Chajul	16.1180	−90.9240	1 Aug 1993
AIV 069	HM888292	CNIN-LEP 40258	*Agrias amydon lacandona*	Chiapas, Ocosingo, Chajul	16.1180	−90.9240	1 Aug 1992
AIV 070	HQ025030	CNIN-LEP-Type AAL	*Agrias amydon lacandona*	Chiapas, Chajul, Río Lacantún	16.0830	−90.9330	20 Nov 1981

Table I – *continued*

Sample ID	GeneBank accession number	Museum voucher ID	Scientific name	Locality	Latitude	Longitude	Collection date
AIV 071	HQ025029	CNIN-LEP 40269	*Agrias amydon lacandona*	Chiapas, Chajul, Ocozingo	16.7020	−91.0640	Ago 1993
AIV 072	HM888293	CNIN-LEP 40251	*Agrias amydon oaxacata*	Oaxaca, San Juan Bautista Valle Nacional, Metates	17.6950	−96.3260	30 May 1993
AIV 074	HM888294	CNIN-LEP 40255	*Agrias amydon oaxacata*	Oaxaca, San Juan Bautista Valle Nacional, Metates	17.6950	−96.3260	26 Mar 1979

model, we used the program DIVA-GIS (Hijmans et al. 2005).

Results and discussion

Genetic distance analysis

For all the ingroup taxa, within-species distances ranged from 0 to 8.004. Within species level designations, distances above 2.87 and up to 8.0 were observed for *Archaeoprepona phaedra* (five out of 15 comparisons), *P. deiphile* (84 out of 171 comparisons) and *Prepona pylene philetas* (one out of one comparison). There were three peaks in the pairwise comparisons within species: one at 0.25, another at 3.12 and a third at 5.25. The NJ distance tree is shown in Figure 2.

Preliminary phylogenetic reconstruction

No previous genetic distance trees have been produced for Preponini at this level. The NJ tree indicated that *Archaeoprepona* is perhaps a polyphyletic genus, with *A. phaedra* more closely allied with the *Prepona–Agrias* group (Figure 2) than with the other three *Archaeoprepona* species. However, the ML tree (not shown) did not recover *Archaeoprepona* as monophyletic at any level. *Prepona* was found to be paraphyletic because it included *Agrias* in both the NJ and the ML trees, a result supported by a previous cladistic analysis (Marconato 2008). The *Prepona–Agrias* group did not show the same topology in the NJ and the ML analyses, but the same lower groups were recovered by both analyses. A comprehensive phylogenetic reconstruction of the Preponini will require greater taxon sampling and more informative characters.

Species limits

On the other hand, our results were consistent for distance and phylogenetic criteria regarding species-level groupings. The same groups can be recognized using either the $>2\%$ genetic distance criteria for species or the reciprocal monophyletic criterion in the likelihood analysis. Except for *Archaeoprepona meander*, all the other four *Archaeoprepona* species were recognized by both criteria, but *A. phaedra* deserves a more detailed geographical and phylogenetic analysis to better understand its taxonomic status.

Additional interesting results are emerging in the *Prepona* group. *Prepona laertes* and *Prepona dexamenes* were sister taxa. The *deiphile* group was polyphyletic; first, *deiphile ibarra* of the Sierra Madre del Sur was the sister group of *P. pylene*, although the two were separated by a considerable genetic distance. This relationship was suggested originally by Beutelspacher (1982). Another segregate outside the main *deiphile* subspecies was *deiphile escalantiana*, which occurs only

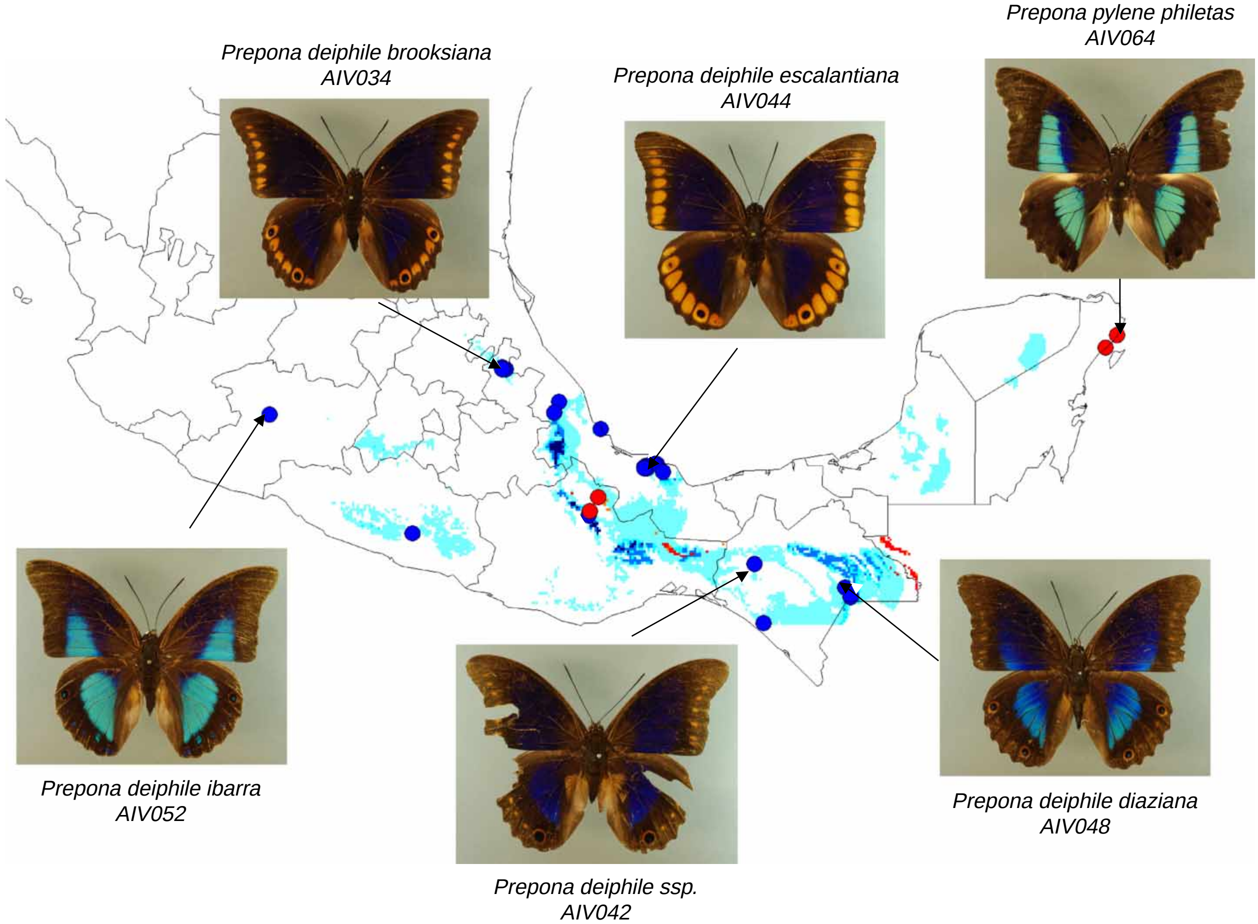

Figure 1. *Prepona deiphile* species group. Distributional ranges of the *P. deiphile* forms in Mexico as predicted with the DIVA-GIS program. Some representative specimens used in this study are depicted. Blue dots and shades represent localities and ranges for *deiphile* forms, while red dots and shades for *pylene*.

in Los Tuxtlas, Veracruz in the Gulf of Mexico coast, separated by a genetic distance of more that 2.97% from the main *deiphile* forms. The remaining populations from the cloud forests of the Sierra Madre Oriental and Chiapas form two closely related, perhaps incipiently differentiating forms.

Taxonomic recommendations

The *cox1* data collected thus far suggest that the current taxonomy of Preponini needs revision, and a closer scrutiny of morphological, genetic, and behavioral data will produce a better understanding and classification of Preponini. We would like to point out specific suggestions for lepidopterists to consider:

- (a) The possible merging of *A. meander* and *Archaeoprepona amphimachus*. The genetic distance between specimens from both species was negligible, and our observations of the available specimens indicate that there are very subtle and subjective morphological differences between these two species.
- (b) The merging of *Agrias* into *Prepona*. Because this was also suggested by the cladistic analysis of Marconato (2008), and recognized in the literature by previous authors, the mitochondrial DNA and new morphological interpretations could support this change.
- (c) The upgrade of *ibarra* (Beutelspacher 1982) to species status. By giving it only a subspecies status, some differentiation was recognized but this form is widely geographically separated, together with *lambertoana* from the rest of the *deiphile* forms, which seem to match perfectly with the mitochondrial separation. There also seem to have less sexual dimorphism than that in other forms of *deiphile*.
- (d) The elevation of *escalantiana* (Stoffel and Mast 1973) to the species level. This form is strikingly similar to *Prepona xenagoras* of Peru, especially in the presence of ocelli with orange rectangles on the external part of veins of the dorsal part of their wings. This character then shows a leapfrog geographic pattern in which perhaps *escalantiana*

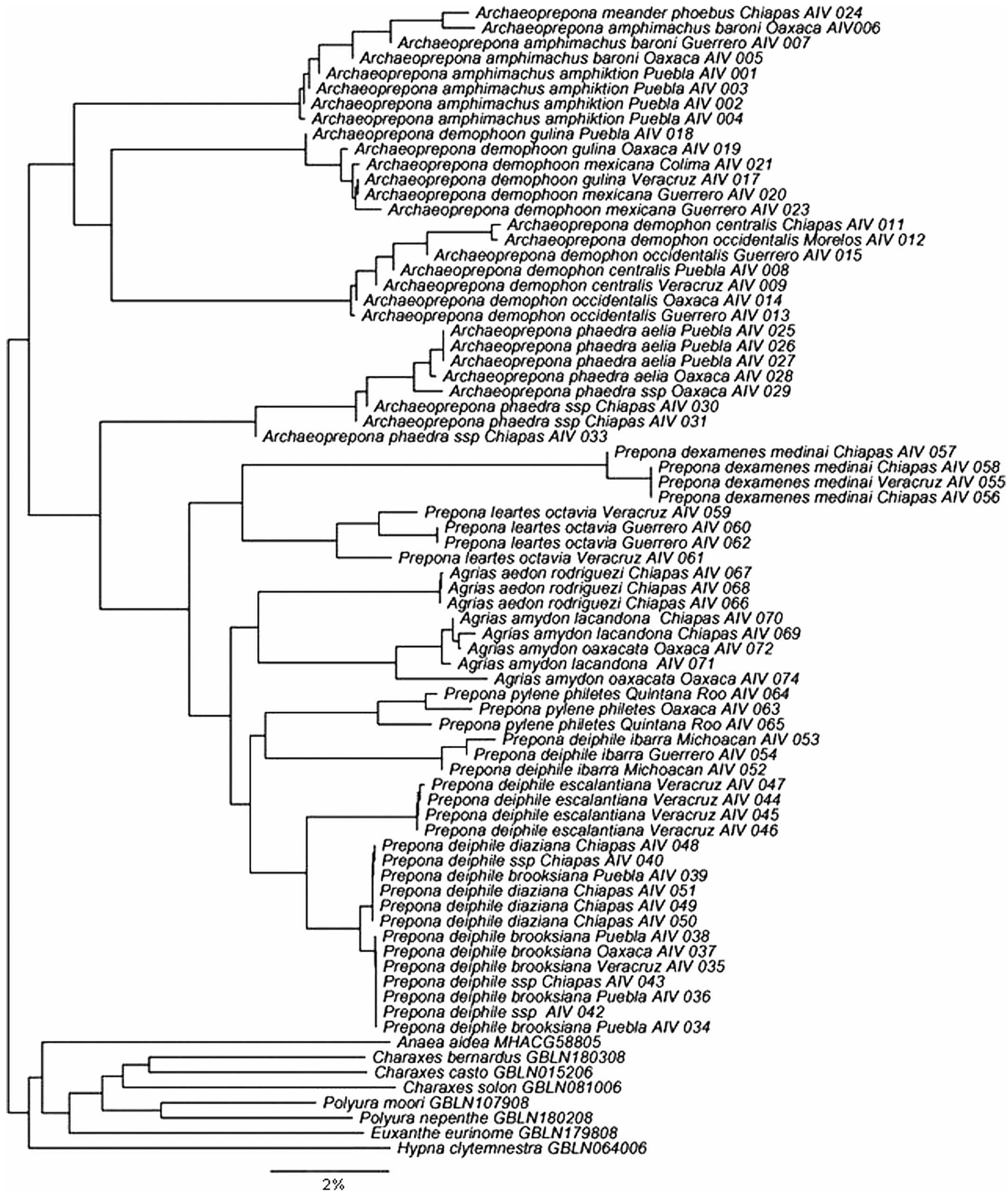

Figure 2. NJ distance tree of Preponini from Central Mexico. This tree was obtained with 68 *cox1* barcode sequences of Preponini and 8 putative outgroups taken from the BOLD database for a total of 658 bp per Operational Taxonomic Unit.

and *xenagoras* have retained a primitive character. Species *escalantiana* also has another important morphological characteristic that differs from the rest of the *deiphile* forms: very little sexual dimorphism in coloration exhibited by most forms of *deiphile*.

Biogeographic patterns

Although a phylogeographical analysis would be premature with the present data for the species groups represented in this study, it is clear that the vicariant model of eastern and western forms north of the

Isthmus of Tehuantepec proposed to explain differentiation in these series of species populations does not hold as a general pattern. None of the four *Archaeoprepona* species included in this study presented a clear significant differentiation between eastern and western forms that would give validity to the subspecies proposed as evidence of this pattern. The same could be said among the *Prepona* species represented in this study, and particularly in the *P. deiphile* group in which potentially new endemic species revealed by the *cox1* data could deserve recognition. These results suggest that the biogeographic history of Preponini in this region is more complicated and involves perhaps a series of dispersion and vicariant events at different ages and opportunities, with more than a single model applying for the species.

GenBank accession numbers for outgroups: *Anaeia aidea* MHACG58805, GU333743; *Charaxes bernardus* GBLN180308, EF534101; *Charaxes castor* GBLN 015206, AY090219; *Charaxes solon* GBLN081006, DQ810197; *Polyura moori* GBLN107908, EU528325; *Polyura nepenthe* GBLN180208, EF534102; *Euxanthe eurinome* GBLN179808, EU141357; *Hypna clytemnestra* GBLN064006, DQ338574.

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