

Phylogenetic relationships of butterflies of the tribe Acraeini (Lepidoptera, Nymphalidae, Heliconiinae) and the evolution of host plant use

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Received 19 April 2007; revised 14 November 2007; accepted 28 November 2007

Available online 10 January 2008

Abstract

The tribe Acraeini (Nymphalidae, Heliconiinae) is believed to comprise between one and seven genera, with the greatest diversity in Africa. The genera *Abananote*, *Altinote*, and *Actinote* (*s. str.*) are distributed in the Neotropics, while the genera *Acraea*, *Bematistes*, *Miyana*, and *Pardopsis* have a Palaeotropical distribution. The monotypic *Pardopsis* use herbaceous plants of the family Violaceae, *Acraea* and *Bematistes* feed selectively on plants with cyanoglycosides belonging to many plant families, but preferentially to Passifloraceae, and all Neotropical species with a known life cycle feed on Asteraceae only. Here, a molecular phylogeny is proposed for the butterflies of the tribe Acraeini based on sequences of COI, EF-1 α and wgl. Both Maximum Parsimony and Bayesian analyses showed that the tribe is monophyletic, once the genus *Pardopsis* is excluded, since it appears to be related to Argynnini. The existing genus *Acraea* is a paraphyletic group with regard to the South American genera, and the species of *Acraea* belonging to the group of “Old World *Actinote*” is the sister group of the Neotropical genera. The monophyly of South American clade is strongly supported, suggesting a single colonization event of South America. The New World *Actinote* (*s. str.*) is monophyletic, and sister to *Abananote* + *Altinote* (polyphyletic). Based on the present results it was possible to propose a scenario for the evolution in host plant use within Acraeini, mainly concerning the use of Asteraceae by the South American genera.

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Keywords: *Actinote*; *Acraea*; Asteraceae; Biogeography; Host plant shifts; Molecular phylogeny; *Pardopsis*; Passifloraceae; Urticaceae

1. Introduction

The classification of the Nymphalidae butterflies of the tribe Acraeini (Heliconiinae), as employed by the most

recent revisions (Harvey, 1991; Lamas, 2004), has been a puzzle. Members of the tribe Acraeini are characterized by several distinct morphological characters in larvae and pupae, and also by adult wing veins and scales, shape of prothoracic legs, and male and female genitalia and abdomen (Penz and Peggie, 2003; Freitas and Brown, 2004). At present, the tribe is believed to comprise between one and seven genera distributed in the Neotropics and Old World,

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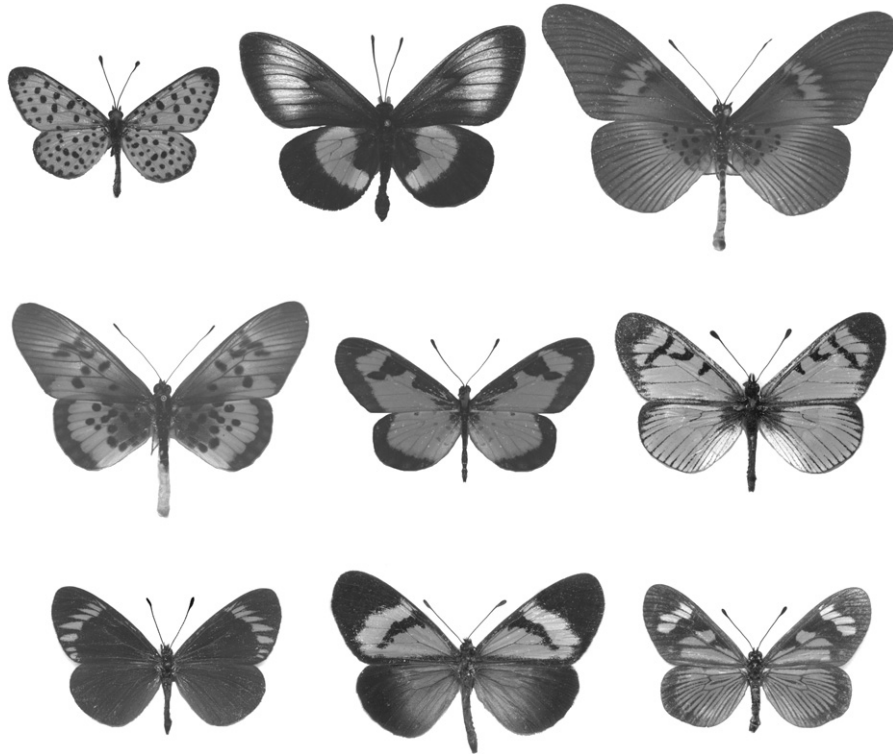


Fig. 1. Acraeini butterflies. From the top left as follow: *Pardopsis punctatissima*, *Miyana meyeri*, *Bematistes alcinoe*, *Acraea egina*, *Acraea acerata*, *Altinote eresa*, *Abananote radiata*, *Altinote dicaeus*, and *Actinote pelenea*.

with the greatest diversity in Africa (Fig. 1), and with generic-level taxonomy not satisfactorily reconciled between these major regions. In the Neotropics, the genera *Abananote* Potts 1943 and *Altinote* Potts 1943 are distributed exclusively in northwestern South America, on the Andean slopes from Bolivia to Colombia. *Actinote* Hübner 1819 occurs through northern South America and Central America to southern Brazil, where the genus is more diversified. However, *Actinote* is also currently used by most authorities as a subgenus of the genus *Acraea* Fabricius 1807 to classify a Palaeotropical group of Acraeini related

with the Neotropical species (e.g. Pierre, 1987, followed by Ackery et al., 1999 and Larsen, 2005) (Fig. 2). In the Neotropics, the genus *Actinote* (*s. str.*) has 31 described species, followed by *Altinote*, with 15, and *Abananote*, with only five (Francini et al., 2004; Lamas, 2004; Paluch, 2006; Paluch et al., 2006). Twenty-three species of *Actinote* (*s. str.*) (21 formally described) are known just for southern Brazil, thus becoming the second largest generic-level radiation of Nymphalidae in this region, after *Adelpha* Hübner [1819] (Francini, 1992; Willmott, 2003b). These butterflies are gregarious in all stages and, as a rule, the developing

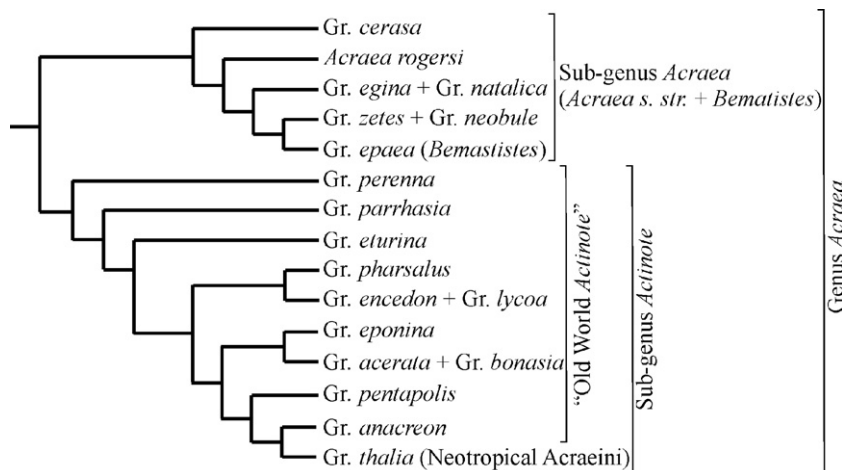


Fig. 2. Phylogenetic hypothesis for Acraeini proposed by Pierre (1987) and based on morphological data (modified).

time of larvae (up to eight months) is much longer than the adult life span (about three days on the average) (Francini, 1989; Francini et al., 2005; Paluch et al., 2005).

In the Palaeotropics, the genus *Acraea* has approximately 220 species distributed over African and Asian forests and savannas (Larsen, 2005), which include, as mentioned above, many species currently arranged in *Actinote*—thereafter “Old World *Actinote*”—although there has been great disagreement about the generic and subgeneric arrangement of African forms (Pennington et al., 1997; Larsen, 2005). *Miyana* Fruhstorfer [1914] has a single species (*M. moluccana* Felder, 1860) and could thus easily be accommodated elsewhere, unless found to be sister to a valid genus. The other two genera are *Bematistes* Hemming 1935, with approximately 20 species restricted to African forests (Owen, 1971) [currently classified as *Acraea* by some authorities—e.g. Larsen (2005)], and *Pardopsis* Trimen 1887, with a single species; the small orange black-spotted butterfly *Pardopsis punctatissima* (Boisduval, 1833) is found only in the East African and Malagasy savannas (Van Son, 1963; Francini, 1992; Penz and Francini, 1996; Penz and Peggie, 2003; Francini et al., 2004).

Acraeini species are supposed to be unpalatable and unpleasant to possible predators, especially vertebrates (Francini, 1989; Raubenheimer, 1989; Brown and Francini, 1990; Brown, 1992; DeVries, 2002; Nishida, 2002). This unpalatability is suggested to be due to the presence of cyanoglycosides secreted by a thoracic gland, although the chemical basis is not completely understood (Ackery, 1988; Kassarov, 2001). These butterflies have aposematic coloration and slow flight, and are mimetic models for a variety of other Nymphalidae, Pieridae, Papilionidae, Lycaenidae, and some moths, especially Castniidae (Owen, 1971; Francini, 1989; Brown, 1992; Owen et al., 1994; Larsen, 2005).

1.1. Relationships between Acraeini and their host plants

African Acraeini feed selectively on plants with cyanoglycosides, like Passifloraceae, Turneraceae, and Flacourtiaceae; van Someren (1974) and Ackery (1988) give a list of host plants used by these Acraeini [see e.g. Larsen (2005) for further records]. Cyanogenesis was reported for some species of *Acraea* (Nahrstedt and Davis, 1981) and these butterflies are able to synthesize cyanoglycosides *de novo* (Raubenheimer, 1989).

The American Acraeini of the genera *Actinote* (*s. str.*), *Altinote*, and *Abananote* do not feed on cyanogenic plants; instead, they have been exclusively reported on plants of the family Asteraceae (mainly on the tribe Eupatorieae) (Francini, 1989, 1992), which are known to have several chemical compounds, such as pyrrolizidine alkaloids (PAs), sesquiterpene lactones, terpenoids, among others (Ferreira et al., 2004; Reimann et al., 2004). Although previous studies showed that PAs are not sequestered by these butterflies, cyanogenesis has been verified in all their stages, which is indicative of synthesis *de novo* of cyanogenic com-

pounds (Brown and Francini, 1990). The 20 species of *Actinote* (*s. str.*) with a well known life cycle feed on 47 species of Asteraceae in seven genera, of which the genus *Mikania* is the most used (Francini, 1989, 1992; Francini et al., 2004, 2005, and unpublished data). Many species of *Actinote* (*s. str.*) feed on a single species of host plant, while those more widely distributed usually feed on three or more host plants. The presence of an *Actinote* (*s. str.*) population is generally related to the occurrence of its host plant, and the abundance and the distribution of these plants can determine the distribution of the butterflies (Francini, 1989, 1992). Interestingly, the African *Acraea perenna* is also recorded to feed on *Mikania*, among other host plant families (Fontane, 1988; Larsen, 2005).

Thus, the general patterns of host plant use in the tribe Acraeini are: *Pardopsis* feeding on herbaceous plants of the family Violaceae, *Acraea* and *Bematistes* feeding preferentially on Passifloraceae, “Old World *Actinote*” feeding mainly on Urticaceae, and all Neotropical species with a known life cycle feeding on Asteraceae only (Van Son, 1963; Pierre, 1987; Francini, 1992).

1.2. Phylogenetic relationships in the tribe Acraeini

Pierre (1987) proposed the only phylogenetic hypothesis for the Acraeini published to date, based on morphological data of the sphragis, the sub-papillar gland and male and female genitalia (Pierre, 1985a,b, 1986) (Fig. 2). This phylogeny shows *Acraea* (containing *Bematistes*) as a paraphyletic genus that includes the “Old World *Actinote*” plus the Neotropical species forming a single clade. For Pierre, the genus *Pardopsis* is a plausible sister group of *Acraea*.

Francini (1992) showed a preliminary phylogenetic hypothesis for 17 species of *Actinote* (*s. str.*) from SE Brazil, based on 58 morphological characters of adults and larvae. This is the only proposal on the interspecific relationships for this group of butterflies from Brazil, and Francini’s results will be discussed in more detail in Section 4.

The main aim of this study was to propose a phylogenetic hypothesis for butterflies of the tribe Acraeini based on molecular data of three gene regions, cytochrome oxidase I (COI), elongation factor 1- α (EF-1 α) and wingless (*wgl*), with special attention to the species of *Actinote* (*s. str.*) found in SE Brazil. Based on this phylogenetic hypothesis, the evolution of host plant use in this tribe was investigated and a biogeographic scenario for colonization of South America was suggested.

2. Material and methods

2.1. Specimens

Individual butterflies of the tribe Acraeini were collected in the field (Table 1). One or two legs of each individual collected were separated from the body for later extraction of DNA. Vouchers were deposited in the Museu de Histó-

Table 1
Species of Acraeini sampled and outgroups used in the phylogenetic analyses

Tribe	Species	Voucher code	Source of specimen	Host plant family ^c	GenBank Accession No.		
					COI	EF-1a	Wingless
<i>Outgroup</i>							
Limnitiidini	<i>Adelpha bredowii</i> Geyer, 1837	NW107-16	Oregon, USA ^a	FAG	AY788591	AY788693	AY788457
Argynnini	<i>Argynnis paphia</i> (Linnaeus, 1758)	NW76-12	Stockholm, Sweden ^b	BRA, ROS, URT, VIO	AY090200	AY090166	AY090133
	<i>Boloria selene</i> ([Denis and Schiffermüller], 1775)	NW76-13	Stockholm, Sweden ^b	ERI, VIO	AY090201	AY090167	AY090134
Heliconiini	<i>Euptoieta hegesia</i> (Cramer, [1779])	NW127-22	Campinas, SP, Brazil	ONA, PAS, TUR	DQ922865	DQ922897	DQ922833
	<i>Agraulis vanillae</i> (Linnaeus, 1758)	TS-24	Sarasota Co., Florida, USA	PAS	DQ922841	DQ922873	AF169921
Vagrantini	<i>Heliconius hecale</i> (Fabricius, 1775)	NW70-6	Stratford Butterfly Garden, UK ^b	PAS	AY090202	AY090168	AY090135
	<i>Vindula arsinoe</i> (Cramer, [1777])	NW69-4	Cairns, Queensland, Australia ^b	PAS	AY090204	AY090170	AY090137
Cethosiini	<i>Cupha prosope</i> (Fabricius, 1775)	NW71-7	Cairns, Queensland, Australia	EUP, FLA	DQ922839	DQ922871	DQ922808
	<i>Cethosia cyane</i> (Drury, 1773)	NW100-12	Sylhet Div., Bangladesh	PAS	DQ922870	DQ922902	DQ922838
	<i>Cethosia cydippe</i> (Linnaeus, 1767)	NW137-4	Cairns, Queensland, Australia	PAS	EU275513	EU275620	EU275409
	<i>Cethosia myrina</i> C. & R. Felder, 1867	NW106-8	Sulawesi, Indonesia	UNK	EU275514	EU275621	EU275410
	<i>Cethosia penthesilea</i> (Cramer, [1777])	NW118-13	West Java, Indonesia	PAS	EU275515	EU275622	EU275411
<i>Ingroup</i>							
Acraeini	<i>Abananote erinome erinome</i> (C. & R. Felder, 1861)	AC34	Junin, Peru	UNK	EU275516	—	—
	<i>Abananote radiata</i> (Hewitson, [1868])	NW90-12	Macas-Guamote, Ecuador	UNK	EU275517	EU275623	EU275412
	<i>Acraea abdera</i> Hewitson, 1852	NW160-13	Gola Forest E, Sierra Leone	FLA	EU275518	EU275624	EU275413
	<i>Acraea acerata</i> Hewitson, 1874 (ind1)	NW81-6	Arba Minch, Ethiopia	AST, CON, PAS, POA, SOL	EU275519	EU275625	EU275414
	<i>Acraea acerata</i> (ind2)	NW160-6	Bumbuna area, Sierra Leone	AST, CON, PAS, POA, SOL	EU275520	EU275626	EU275415
	<i>Acraea alciope</i> Hewitson, 1852	NW146-3 (AC49)	Ghana	URT	EU275521	EU275627	EU275416
	<i>Acraea andromacha</i> (Fabricius, 1775)	NW115-8	Australia	PAS, VIO	EU275522	EU275628	EU275417
	<i>Acraea bonasia</i> (Fabricius, 1775)	NW160-15	Bumbuna area, Sierra Leone	MAL, TIL	EU275523	EU275629	EU275418
	<i>Acraea camaena</i> (Drury, 1773)	NW160-12	Gola Forest E, Sierra Leone	PAS, VER	EU275524	EU275630	EU275419
	<i>Acraea circeis</i> (Drury, 1782)	GH-096	Bobiri Forest Preserve, Ghana	URT	EU275525	—	EU275420
	<i>Acraea egina</i> (Cramer, 1775)	NW160-14	Bumbuna area, Sierra Leone	FLA, PAS	EU275526	EU275631	EU275421
	<i>Acraea encedana</i> Pierre, 1976	NW160-8	Bumbuna area, Sierra Leone	FAB	EU275527	EU275632	EU275422
	<i>Acraea endoscoia</i> Le Doux, 1928	NW160-18	Bumbuna area, Sierra Leone	VIO	EU275528	EU275633	EU275423
	<i>Acraea eponina</i> (Cramer, 1780)	NW146-11 (AC57)	Ghana	EHR, MAL, SOL, STE, TIL	EU275529	EU275634	EU275424
	<i>Acraea igola</i> Trimen, 1889	JP22	SE Mbigou, Gabon	URT	EU275530	EU275635	EU275425
	<i>Acraea issoria</i> (Hübner, [1819])	NW108-22	Vietnam	URT	EU275531	EU275636	EU275426
	<i>Acraea jodutta</i> (Fabricius, 1793)	NW146-12 (AC52)	Ghana	URT	EU275572	EU275637	EU275427
	<i>Acraea johnstoni</i> Godman, 1885	NW116-18	Tanzania	URT	EU275532	EU275638	EU275428
	<i>Acraea lycoa</i> Godart, [1819]	NW146-7 (AC53)	Ghana	URT, STE	EU275533	EU275639	EU275429
	<i>Acraea meyeri</i> (Kirsch, 1877)	NW115-10	Papua New Guinea	UNK	EU275534	EU275640	EU275430
	<i>Acraea oberthueri</i> Butler, 1895	JP35	SE Mbigou, Gabon	TIL	EU275535	—	EU275431
	<i>Acraea parrhasia</i> (Fabricius, 1793) (ind1)	NW146-10 (AC54)	Ghana	URT	EU275536	EU275641	EU275432
	<i>Acraea parrhasia</i> (ind2)	NW116-11	Conakry, Guinea	URT	EU275537	EU275642	EU275433
	<i>Acraea peneleos</i> Ward, 1871	NW146-14 (AC55)	Ghana	URT	EU275538	EU275643	EU275434
	<i>Acraea perenna</i> Doubleday, 1847 (ind1)	NW159-3	Doumo, Cameroun	AST, EUP, PAS, MEN	EU275539	EU275644	EU275435
	<i>Acraea perenna</i> (ind2)	NW160-10	Bumbuna area, Sierra Leone	AST, EUP, PAS, MEN	EU275540	EU275645	EU275436

<i>Acraea pharsalus</i> Ward, 1871	NW146-2 (AC56)	Ghana	MOR, STE	EU275541	EU275646	EU275437
<i>Acraea polis</i> Ward, 1871	NW116-10	Conakry, Guinea	MOR, URT	EU275542	EU275647	EU275438
<i>Acraea pseudegina</i> Westwood, 1852 (ind1)	NW116-16	Uganda	PAS	EU275543	EU275648	EU275439
<i>Acraea pseudegina</i> (ind2)	NW160-9	Bumbuna area, Sierra Leone	PAS	EU275544	EU275649	EU275440
<i>Acraea quirina</i> (Fabricius, 1781)	NW116-14	Tanzania	VIO	EU275545	EU275650	EU275441
<i>Acraea rahira</i> Boisduval, 1833	NW107-13	Zimbabwe	AST, POL	EU275546	EU275651	EU275442
<i>Acraea vesperalis</i> Grose-Smith, 1890	NW160-16	Bumbuna area, Sierra Leone	URT	EU275547	EU275652	EU275443
<i>Acraea zetes</i> (Linnaeus, 1758)	NW160-5	Gola Forest E, Sierra Leone	ACA, FLA, PAS, STE	EU275548	EU275653	EU275444
<i>Actinote alalia</i> (C. & R. Felder, 1860) (ind1)	AC9	Campos do Jordão, SP, Brazil	AST	EU275574	EU275654	EU275445
<i>Actinote alalia</i> (ind2)	AC36	Pindamonhangaba, SP, Brazil	AST	EU275575	EU275655	EU275446
<i>Actinote bonita</i> Penz, 1996	NW137-24	Itatiaia, RJ, Brazil	AST	EU275549	—	EU275447
<i>Actinote brylla</i> Oberthür, 1917	AC4	Santos, SP, Brazil	AST	EU275576	EU275656	EU275448
<i>Actinote canutia</i> (Hopffer, 1874)	AC86	Itirapina, SP, Brazil	AST	EU275577	EU275657	EU275449
<i>Actinote carycina</i> Jordan, 1913 (ind1)	AC79	Paranapiacaba, SP, Brazil	AST	EU275578	EU275658	EU275450
<i>Actinote carycina</i> (ind2)	AC88	Campos do Jordão, SP, Brazil	AST	EU275550	EU275659	EU275451
<i>Actinote conspicua</i> Jordan, 1913 (ind1)	AC1	Itamonte, MG, Brazil	AST	EU275579	EU275660	EU275452
<i>Actinote conspicua</i> (ind2)	AC10	Campos do Jordão, SP, Brazil	AST	EU275580	EU275661	EU275453
<i>Actinote dalmeidai</i> Francini, 1996 (ind1)	AC6	São Luis do Paraitinga, SP, Brazil	AST	EU275581	EU275662	EU275454
<i>Actinote dalmeidai</i> (ind2)	AC12	Paranapiacaba, SP, Brazil	AST	EU275582	EU275663	EU275455
<i>Actinote discrepans</i> D'Almeida, 1935	AC7	Campos do Jordão, SP, Brazil	AST	EU275583	EU275664	EU275456
<i>Actinote genitrix</i> D'Almeida, 1922 (ind1)	AC65	Passa Quatro, MG, Brazil	AST	EU275584	EU275665	EU275457
<i>Actinote genitrix</i> (ind2)	AC66	Passa Quatro, MG, Brazil	AST	EU275551	EU275666	EU275458
<i>Actinote guatemalena</i> (Bates, 1864)	NW155-1	Chiapas, Bonam Park, Mexico	AST	EU275585	EU275667	EU275459
<i>Actinote mamita mamita</i> (Burmeister, 1861) (ind1)	AC35	Campinas, SP, Brazil	AST	EU275586	EU275668	EU275460
<i>Actinote mamita mamita</i> (ind2)	NW141-8	São Bernardo do Campo, SP, Brazil	AST	EU275552	EU275669	EU275461
<i>Actinote melanisans</i> Oberthür	AC84	São Bernardo do Campo, SP, Brazil	AST	EU275587	EU275670	EU275462
<i>Actinote morio</i> Oberthür, 1917 (ind1)	AC92	Peti, MG, Brazil	UNK	EU275588	EU275671	EU275463
<i>Actinote morio</i> (ind2)	AC93	Peti, MG, Brazil	UNK	EU275589	EU275672	EU275464
<i>Actinote paraphelus</i> Jordan, 1913 (ind1)	AC3	Passa Quatro, MG, Brazil	AST	EU275590	EU275673	EU275465
<i>Actinote paraphelus</i> (ind2)	AC23	Santos, SP, Brazil	AST	EU275591	EU275674	EU275466
<i>Actinote pellenea calymma</i> Jordan, 1913 (ind1)	AC68	Buenos Aires, Argentina	AST	EU275592	EU275675	EU275467
<i>Actinote pellenea calymma</i> (ind2)	AC69	Buenos Aires, Argentina	AST	EU275593	EU275676	EU275468
<i>Actinote pellenea epiphaea</i> Jordan, 1913 (ind1)	AC47	Abancay Anpay, Apurimac, Peru	UNK	EU275594	EU275677	EU275469
<i>Actinote pellenea epiphaea</i> (ind2)	AC71	Abancay Anpay, Apurimac, Peru	UNK	EU275595	EU275678	EU275470
<i>Actinote pellenea giffordi</i> Paluch, Casagrande and Mielke, 2006	AC67	Brasília, DF, Brazil	AST	EU275596	EU275679	EU275471
<i>Actinote pellenea hyalina</i> Jordan, 1913	AC40	Marechal Thaumaturgo, AC, Brazil	AST	EU275597	EU275680	EU275472
<i>Actinote pellenea mucia</i> (Hopffer, 1874)	AC75	Ancash, Peru	UNK	EU275598	EU275681	EU275473
<i>Actinote pellenea pellenea</i> Hübner, [1821] (ind1)	AC5	São Vicente, SP, Brazil	AST	EU275599	EU275682	EU275474
<i>Actinote pellenea pellenea</i> (ind2)	AC37	Santos, SP, Brazil	AST	EU275600	EU275683	EU275475
<i>Actinote pellenea pellenea</i> (ind3)	AC74	Ituberá, BA, Brazil	AST	EU275601	EU275684	EU275476
<i>Actinote pratensis</i> Francini, Freitas and Penz, 2004	NW125-1 (AC78)	Águas da Prata, SP, Brazil	AST	EU275602	EU275685	EU275477
<i>Actinote pyrrrha crucis</i> Jordan, 1913 (ind1)	AC72	Viçosa do Ceará, CE, Brazil	AST	EU275603	EU275686	EU275478
<i>Actinote pyrrrha crucis</i> (ind2)	AC73	Ibiapina, CE, Brazil	AST	EU275604	EU275687	EU275479
<i>Actinote pyrrrha crucis</i> (ind3)	AC94	Nova Xavantina, MT, Brazil	AST	EU275605	EU275688	EU275480
<i>Actinote pyrrrha pyrrrha</i> (Fabricius, 1775)	AC20	Santos, SP, Brazil	AST	EU275606	EU275689	EU275481
<i>Actinote quadra</i> (Schaus, 1902)	AC80	Campos do Jordão, SP, Brazil	AST	EU275607	EU275690	EU275482
<i>Actinote rhodope</i> D'Almeida, 1922	AC8	Campos do Jordão, SP, Brazil	AST	EU275608	EU275691	EU275483
<i>Actinote surima perisa</i> Jordan, 1913	AC48	Yala, Jujuy, Argentina	AST	EU275609	EU275692	EU275484
<i>Actinote surima surima</i> Schaus, 1902 (ind1)	AC85	São Paulo, SP, Brazil	AST	EU275610	EU275693	EU275485
<i>Actinote surima surima</i> (ind2)	NW136-19	Alto da Serra, SP, Brazil	AST	EU275553	EU275694	EU275486

(continued on next page)

Table 1 (continued)

Tribe	Species	Voucher code	Source of specimen	Host plant family ^c	GenBank Accession No.		
					COI	EF-1a	Wingless
	<i>Actinote surima surima</i> (ind3)	AC89	Morro do Osso, Porto Alegre, RS, Brazil	AST	EU275611	EU275695	EU275487
	<i>Actinote thalia anteas</i> (Doubleday, [1847]) (ind1)	AC13	El Carmen, Antioquia, Colombia	AST	EU275612	EU275696	EU275488
	<i>Actinote thalia anteas</i> (ind 2)	AC14	El Carmen, Antioquia, Colombia	AST	EU275613	EU275697	EU275489
	<i>Actinote thalia anteas</i> (ind 3)	AC17	Santa Rosa de Osos, Antioquia, Colombia	AST	EU275614	EU275698	EU275490
	<i>Actinote thalia crassinia</i> (Hopffer, 1874)	AC43	San Ramon-Catarata Tirol, Junin, Peru	AST	EU275615	EU275699	EU275491
	<i>Actinote zikani</i> D'Almeida, 1951	AC83	Paranapiacaba, SP, Brazil	AST	EU275616	EU275700	EU275492
	<i>Actinote</i> sp1	AC77	Curitiba, PR, Brazil	AST	EU275617	EU275701	EU275493
	<i>Actinote</i> sp1	AC90	São Francisco de Paula, RS, Brazil	AST	EU275618	EU275702	EU275494
	<i>Actinote</i> sp2	AC95	Napo, Ecuador	UNK	EU275619	EU275703	EU275495
	<i>Altinote alcione sodalis</i> (Buttler, 1877)	AC28	Junin, Peru	UNK	EU275554	EU275704	EU275496
	<i>Altinote dicaeus albofasciata</i> (Hewitson, 1869)	E-51-18	Napo, Ecuador	AST	EU275555	—	—
	<i>Altinote dicaeus callianira</i> (Geyer, 1837) (ind1)	AC25	Junin, Peru	AST	EU275556	EU275705	EU275497
	<i>Altinote dicaeus callianira</i> (ind2)	AC58	Rodriguez de Mendonza, Peru	AST	EU275557	EU275706	EU275498
	<i>Altinote dicaeus flavibasis</i> (Jordan, 1913)	C-17-3	Putumayo, Colombia	AST	EU275558	—	—
	<i>Altinote eresia</i> (C. & R. Felder, 1862)	AC87	Cochabamba, Bolivia	UNK	EU275559	EU275707	EU275499
	<i>Altinote momina</i> Jordan	RV-03-V240	Cuzco, Peru	UNK	EU275560	—	EU275500
	<i>Altinote negra demonica</i> (Höpffer, 1874) (ind1)	AC45	Sta. Teresa, Cuzco, Peru	AST	EU275573	EU275708	EU275501
	<i>Altinote negra demonica</i> (ind2)	AC46	Coroico, Yungas, Bolivia	AST	EU275561	EU275709	EU275502
	<i>Altinote negra euclia</i> (Dognin, 1887)	AC64	Cajamarca, Peru	AST	EU275562	EU275710	EU275503
	<i>Altinote neleus</i> (Latreille, [1811])	AC16	Sabaneta, Antioquia, Colombia	UNK	EU275563	EU275711	EU275504
	<i>Altinote rubrocellulata</i> (Hayward, 1960)	AC76	Ancash, Peru	UNK	EU275564	EU275712	EU275505
	<i>Altinote stratonice</i> (Latreille, [1813])	NW90-14	La Bonita, Ecuador	AST	AY218233	AY218252	DQ018892
	<i>Altinote tenebrosa</i> (Hewitson, 1868)	NW90-15	La Bonita, Ecuador	UNK	EU275565	EU275713	EU275506
	<i>Bematistes alcinoe</i> (Felder, 1865)	NW116-19	Ghana	PAS	EU275566	EU275714	EU275507
	<i>Bematistes epaea</i> (Cramer, 1779)	NW146-16 (AC51)	Ghana	PAS	EU275567	EU275715	EU275508
	<i>Bematistes macaria</i> (Fabricius, 1793)	NW160-7	Bumbuna area, Sierra Leone	PAS	EU275568	EU275716	EU275509
	<i>Bematistes umbra</i> (Drury, [1782])	NW160-17	Bumbuna area, Sierra Leone	PAS	EU275569	EU275717	EU275510
	<i>Bematistes vestalis</i> (Felder and Felder, 1865)	NW160-11	Bumbuna area, Sierra Leone	PAS	EU275570	EU275718	EU275511
	<i>Pardopsis punctatissima</i> (Boisduval, 1833)	NW154-2	NE Madagascar, Sambava Airport	VIO	EU275571	EU275719	EU275512

ACA, Acanthaceae; AST, Asteraceae; BRA, Brassicaceae; CON, Convolvulaceae; EHR, Ehretiaceae; ERI, Ericaceae; EUP, Euphorbiaceae; FAB, Fabaceae; FAG, Fagaceae; FLA, Flacourtiaceae; MAL, Malvaceae; MEN, Menispermaceae; MOR, Moraceae; ONA, Onagraceae; PAS, Passifloraceae; POA, Poaceae; POL, Polygonaceae; ROS, Rosaceae; SOL, Solanaceae; STE, Sterculiaceae; TIL, Tiliaceae; TUR, Turneraceae; URT, Urticaceae; VER, Verbenaceae; VIO, Violaceae; UNK, Unknown.

^a Wahlberg et al. (2005).

^b Wahlberg et al. (2003).

^c Data obtained from DeVries (1987), Ackery (1988), Teshigori (2003), Markku Savela's web page (<http://www.nic.funet.fi/pub/sci/bio/life/insecta/lepidoptera/ditrysia/papilionoidea/nymphalidae/index.html>), Lee Dyer's web page (<http://www.tulane.edu/~ldyer/lacat/index.htm>), AVLF and DL pers. com.

ria Natural of Universidade Estadual de Campinas (Unicamp), and those of African and Asian species are in the Department of Zoology of Stockholm University, in the American Museum of Natural History (AMNH), in Harvard MCZ, in the African Butterfly Research Institute (ABRI) Nairobi, Kenya and in Natural History Museum at London (BMNH).

Previously published sequences of other Nymphalidae, including additional tribes of Heliconiinae and the subfamily Limenitidinae, were used as outgroups, as well as four species of *Cethosia*, a genus with an uncertain position within the subfamily Heliconiinae (Penz and Peggie, 2003). The final matrix has 117 terminals representing 80 species, including 68 species of Acraeini and 12 species used as outgroups (Table 1). The South American species were better sampled than the Palearctic species: from the 31 species of South American *Actinote* (*s. str.*), 22 were sampled in this study (plus two additional undescribed taxa—Table 1), and nine out of the 15 species of *Altinote* and two out of the five described species of *Abananote* were also analyzed, while only 28 out of the 220 species of *Acraea* were sampled and five out of the 24 species of *Bematistes*.

2.2. Molecular techniques

Total genomic DNA was extracted following the protocol of DNeasy[®] Tissue Kit (Qiagen) from legs of individual species. For materials older than 2 years, the protocol of DNeasy Tissue Kit modified for ancient material was used: samples were lysed overnight and recovered with 50 μ L of dilution buffer, as suggested by the manufacturer's technical support. DNA extractions were stored in TE buffer at -20°C . For each of the specimens the entire mitochondrial gene cytochrome oxidase I (COI—1508 bp) and the nuclear genes elongation factor-1 α (EF-1 α —1240 bp) and wingless (wgl—403 bp) were amplified using the primer combinations listed in Table 2. These genes have become the standard molecular markers for studies of butterflies of the family Nymphalidae (Brower and DeSalle, 1998; Brower, 2000; Wahlberg and Nylin, 2003; Wahlberg et al., 2003a,b, 2005). Amplification of DNA was performed using two methods: a direct method for COI and wgl, using primers that amplified three fragments of about 600 bp of COI (k698 + nancy, ron + mtD11 and jerry + patII), and one fragment of \approx 400 bp of wgl (lepwg1 + lepwg2). For the amplification of EF-1 α a semi-nested PCR method was used to amplify 500 bp (hillary + monica) and 700 bp (al + tipper) regions from an original template previously amplified with hillary + tipper. The following thermal cycling protocol was used to amplify COI: 94°C for 2 min, 35 cycles of 94°C for 1 min, 45°C for 1 min, 72°C for 1:30 min, and a final extension period of 72°C for 5 min. The cycling profile for EF-1 α was 95°C for 3 min, 35 cycles of 95°C for 1 min, 49°C for 1 min, 72°C for 1:50 min, and a final extension period of 72°C for 4 min. To amplify wgl we used 95°C for 7 min, 35

Table 2

Primers used in this study

Gene Name	F/ R	Sequence (5' \rightarrow 3')	
COI K698 ^a	F	TAC AAT TTA TCG CCT AAA CTT CAG CC	
	R	CCT GGT AAA ATT AAA ATA TAA ACT TC	
	Ron-mod ^a	F	GGT TCA CCT GAT ATA GCA TTC CC
	MtD11	R	ACT GTA AAT ATA TGA TGA GCT CA
	Jerry ^a	F	CAA CAT TTA TTT TGA TTT TTT GG
PatII ^a	R	TCC ATT ACA TAT AAT CTG CCA TAT TAG	
EF-1 α	Hillary ^b	F	CAC ATY AAC ATT GTC GTS ATY GG
	Monica ^b	R	CAT RTT GTC KCC GTG CCA KTC C
	Al ^b	F	GAG GAA ATY AAR AAG GAA G
	Tipper ^b	R	ACA GCV ACK GTY TGY CTC ATR TC
wgl	LepWG1	F	GAR TGY AAR TGY CAY GGY ATG TCT GG
	LepWG2	R	ACT ICG CAR CAC CAR TGG AAT GTR CA

Y, C/T; S, C/G; R, G/A; K, G/T; V, C/G/A.

^a Primers obtained from Caterino and Sperling (1999).

^b Cho et al. (1995).

cycles of 95°C for 1 min, $45\text{--}55^{\circ}\text{C}$ for 1 min, 72°C for 2 min, and a final extension period of 72°C for 10 min. PCRs were done in a 25 μ L final volume and the PCR products were cleaned by using a Gel Purification Kit (Qiagen), and then sequenced by ABI Prism BigDye Kit protocol. PCR regions were sequenced in ABI 377 or 3700 automated sequencers. All regions were sequenced in both directions. Sequences were analyzed with the program FinchTV v. 1.4.0 (Geospiza Inc.), and aligned manually using BioEdit v. 7.0.5.3 (Hall, 1999). GenBank Accession numbers are shown in Table 1.

2.3. Phylogenetic analyses

The phylogenetic analyses were performed with TNT (Goloboff et al., 2003), using Maximum Parsimony; Bayesian analysis was carried out with MrBayes v. 3.1 (Huelsenbeck and Ronquist, 2001). The purpose of performing Bayesian analysis was to investigate the effect of more restrictive assumptions on the results.

Maximum Parsimony analyses (MP) were performed on the entire data set using the New Technology Search implemented in TNT, employing all four search methods—ratchet, tree-fusing, tree-drifting, and sectorial (Goloboff, 1999)—and 1000 random taxon addition replicates, followed by traditional search using TBR branch-swapping, with all characters equally weighted. A strict consensus tree was computed whenever multiple equally parsimonious trees were obtained, using the program Winclada (Nixon, 1999). The consistency index (CI) and the retention index (RI) were also calculated in Winclada. The stability of each branch was determined using the non-parametric bootstrap test (Felsenstein, 1985), with 1000 replicates and 100 random taxon additions. Bremer support and Partitioned Bremer support values (to obtain the contribution of each data set to the Bremer support values of the combined analysis)

(Bremer, 1988, 1994; Baker and DeSalle, 1997; Baker et al., 1998) were calculated using TNT (Goloboff et al., 2003). The analysis was conducted with 100 random taxon addition replicates, TBR branch-swapping and 100 trees held in each replicate.

The program Modeltest v. 3.06 (Posada and Crandall, 1998) was used to determine the available substitution model with the best fit to each separated data set. The best fit model was found to be the most complex available, i.e., the GTR + G + I [General Time-Reversible model (Rodríguez et al., 1990), with gamma distribution (G) and with proportion of invariable sites (I)]. However, it has been noted that the G shape parameter and the I parameter are highly correlated and are considered to be pathological when estimated together (Ren et al., 2005), thus Bayesian analyses (Huelsenbeck and Ronquist, 2001; Huelsenbeck et al., 2001, 2002) were carried out for the combined data set under the model GTR + G. Analysis of combined data by Bayesian methods permits partition-specific substitution models and parameters (Nylander et al., 2004). For that reason, all substitution model parameters (gamma shape, character state frequencies, substitution rates of GTR model) were allowed to vary across partitions (=genes). Four simultaneous chains were conducted for 5.0×10^6 generations, sampling trees every 500 cycles for a total of 10,000 sampled trees. Stability of the process was assessed by plotting the likelihood scores against generation time (Lin and Danforth, 2004). The first 2500 trees were discarded as “burn in”. For all analyses, *Adelpha bredowii* was used as outgroup to root the tree.

2.4. Evolution of host plant use

The evolution of host plant use was investigated by superimposing the host plant families used by each species of Acraeini onto the phylogenetic hypothesis proposed for them. The analysis were performed over the MP consensus tree due to the nature of the optimizations algorithms, which are based on parsimony, and all analyses of character evolution used the same outgroups as the phylogenetic analyses. Character states were treated as unordered and of equal weights, and were optimized on the MP tree using MacClade v. 3.08 (Maddison and Maddison, 1999). Polymorphic taxa were coded as polymorphic (“0 and 1”), and missing data was coded as “?”, meaning that the state to this taxon is unknown because of incomplete information (Maddison and Maddison, 1999).

To test whether there is a phylogenetic signal in the characters traced, the methodology proposed by Wahlberg (2001), modified from the PTP test described by Faith and Cranston (1991), was used. The test consists of comparing the number of steps of the tree constructed with the actual data with the number of steps obtained for each random reshuffling of the states of each separated character. We performed 300 random reshufflings of character states among the fixed terminal taxa, with the equally weighted data set, using the program Mesquite (Maddison and

Maddison, 2006). The probability (P) that the observed pattern does not differ from a random pattern is given by the number of replications as short as or shorter than the tree obtained with the actual data, plus one, divided by the number of replications. Following Faith and Cranston (1991), a significant phylogenetic signal is observed when P is less than 0.05, and here, the minimal value should be 0.003 (number of trees as short or shorter than the original tree + 1/300).

3. Results

The full data set contained 3151 nucleotides, 1508 from the mitochondrial DNA, 1240 from EF-1 α and 403 from wgl. The alignment of COI and EF-1 α did not show indels, and a single-codon insertion was found in wgl sequences of the group of species belonging to the “Old World *Actinote*” clade.

3.1. Phylogenetic analyses

Parsimony searches over the equally weighted combined data set resulted in 280 most parsimonious trees, with 8041 steps (CI = 26; RI = 68) (Fig. 3). According to this phylogenetic hypothesis, *Pardopsis* is not part of the tribe Acraeini, but appeared with weak support in the Argynnini clade (Fig. 3 and Table 3). In this topology, the genus *Cethosia* is the sister group of Heliconiini, and both tribes are sister of Acraeini (excluding *Pardopsis*).

The monophyly of the node 100 (see Fig. 3 for number of nodes), which joins *Acraea* + *Bematistes* + *Altinote* + *Abananote* + *Actinote* (*s. str.*) (thereafter Acraeini *s. str.*), is supported by strong bootstrap and Bremer values (Fig. 3 and Table 3). The genus *Acraea* is paraphyletic, and includes *Bematistes* and the monophyletic “Old World *Actinote*”; this last clade is the sister group to all Neotropical species. The clade *Altinote* + *Abananote* + *Actinote* (*s. str.*), including all Neotropical Acraeini (node 96), has high bootstrap stability and Bremer support (Table 3). The genus *Altinote* is not monophyletic, since *Abananote* (polyphyletic) appeared as part of a clade within this genus, and *Altinote eresia* emerged as an isolated branch sister to all Neotropical Acraeini. The clade including *Abananote* + *Altinote* (except *Alt. eresia*) has strong support (node 55). The genus *Actinote* (*s. str.*) appeared as a monophyletic group supported by strong bootstrap and Bremer values (Table 3), and sister to *Abananote* + *Altinote*. Most of the relationships among the species of *Actinote* (*s. str.*) are not well supported, particularly in the “*Act. pellenea* clade” (especially among the morphologically similar species *Act. pellenea*, *Act. pyrrrha* and *Act. carycina*).

The contribution of each gene to the combined tree, assessed by Partitioned Bremer support, was almost the same for all genes. Several nodes however presented conflicts: the nuclear gene wgl conflicted at 16 of 102 nodes, followed by EF-1 α in 17 nodes and COI in 18 (Table 3). On the whole however, the three genes contributed posi-

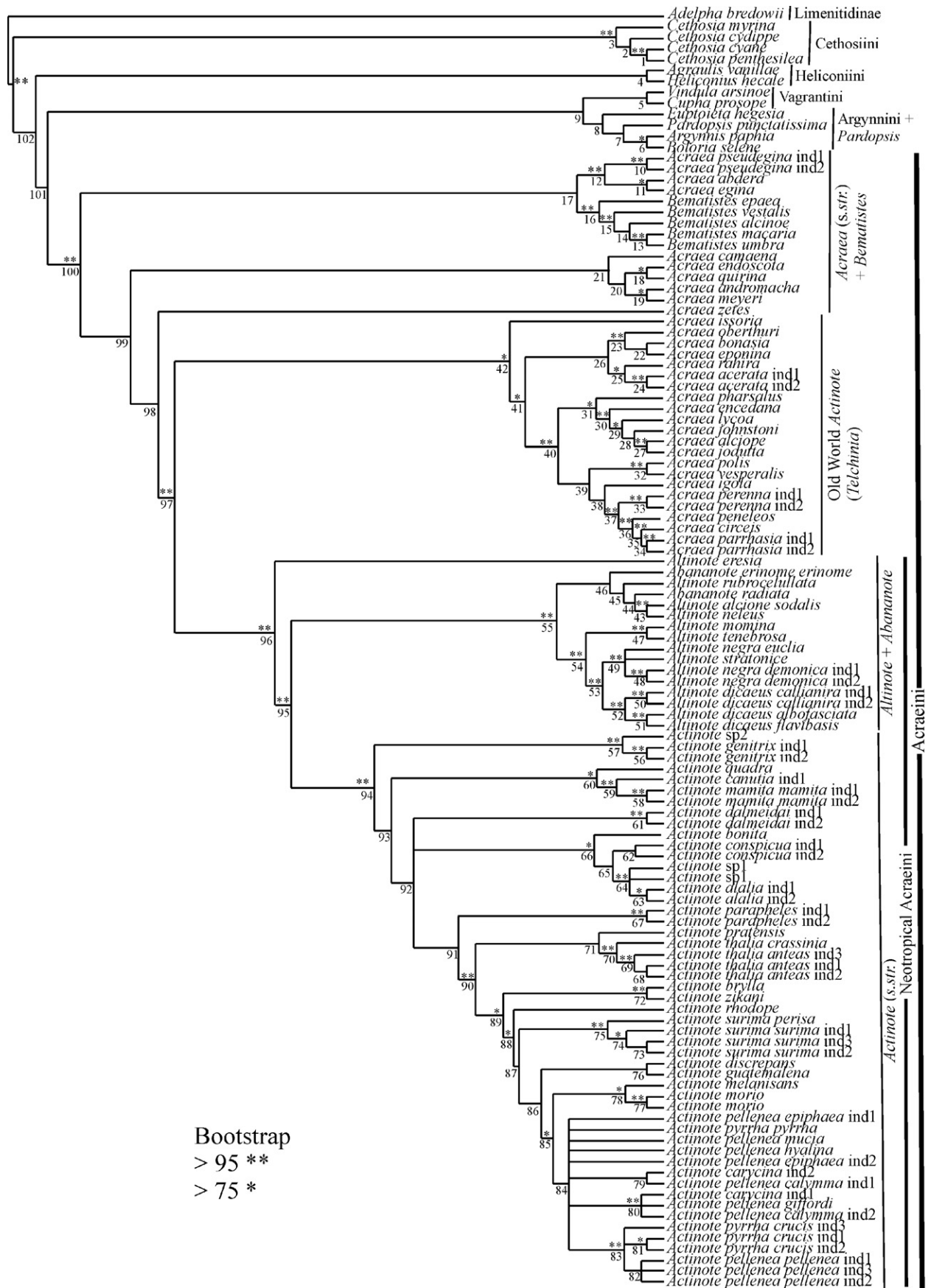


Fig. 3. Consensus tree of the 280 most parsimonious trees based on combined data analyses of COI, EF-1 α and wgl. Values on the branches indicate the node numbers. **Bootstrap >95; *Bootstrap >75.

Table 3

Partitioned Bremer support (PBS), Bremer support (BS), and Bootstrap values of 1000 replications (where it exceeds 50%) for the nodes of the Maximum Parsimony consensus tree (Fig. 3)

Node	PBS			BS	Bootstrap
	COI	EF-1 α	wgl		
1	13	8	2	23	100
2	1	-1	1	1	—
3	36.5	17	11.5	65	100
4	-12	11	10	9	68
5	17	3	-11	9	69
6	55	4	2.5	12	93
7	-10	12	2	4	—
8	-8.1	10.9	0.1	2.9	—
9	-9.5	12	0.5	3	—
10	38.8	26.2	6	71	100
11	5	2	0	7	82
12	-0.5	15	9.5	24	100
13	6	5	2	13	99
14	1	0	1	2	50
15	9.2	8.3	3.5	21	100
16	24	10	8	42	100
17	4	-0.5	-1.5	2	—
18	2	5	2	9	92
19	-6.7	20	-5.3	8	87
20	-4.7	12	-4.3	3	—
21	4.5	-5	5.5	5	71
22	1	0	2	3	54
23	8	0	5	13	94
24	41	23	22	86	100
25	11	0.5	2.5	14	88
26	1.5	-0.5	1	2	—
27	2	5	8	15	100
28	-0.5	0	1.5	1	—
29	-1	2	6	7	89
30	3	5	9	17	98
31	0	4	4	8	87
31	6	18	16	40	100
33	9.5	20.5	14	44	100
34	3	0	2	5	99
35	7	2	2	11	97
36	11	9	4	24	99
37	-3	20	4	21	100
38	0	2	0	2	—
39	1	-1	2	2	59
40	2.5	-0.5	16	18	97
41	2	-0.5	6.5	8	75
42	15.5	0.5	1	17	93
43	-1	4.4	7.7	11.1	100
44	-2	1	2	1	—
45	2.5	0.5	1	4	64
46	2.5	0.5	1	4	67
47	28	0	1	29	100
48	11	0	-1	10	99
49	7	2	-1	8	96
50	17	0	0	17	100
51	13	0	0	13	100
52	13	0	0	13	100
53	10	6.5	2.5	19	100
54	9.5	0.5	1	11	98
55	9	0	0	9	98
56	8	0	0	8	99
57	37	0	-1	36	100
58	23	2	1	26	100
59	26	4	4	34	100
60	3	5	0	8	85

Table 3 (continued)

Node	PBS			BS	Bootstrap
	COI	EF-1 α	wgl		
61	26	3	1	30	100
62	4	3	3	10	99
63	2	0	0	2	78
64	13	2.1	2	17.1	100
65	2	0	1	3	66
66	7	-1	0	6	93
67	43	0	4	47	100
68	0	1	0	1	61
69	8	0	0	8	99
70	8	3	0	11	100
71	2	0	0	2	—
72	7.5	4.5	0	12	98
73	0	1	0	1	59
74	5	-1	0	4	94
75	14	-1	0	13	99
76	1.4	-0.3	-0.1	1	—
77	3.4	2.8	1.7	7.9	99
78	8.3	-1.2	-0.1	7	93
79	-0.6	0.7	0.9	1	68
80	5	-0.1	0.1	5	99
81	2.1	1.9	-1	3	86
82	1.1	0.8	-0.9	1	55
83	6.9	0.2	-0.1	7	99
84	-1.6	1.7	1.9	2	59
85	4.4	-0.3	-0.1	4	83
86	3.4	-1.3	-0.1	2	—
87	0	1	0	1	—
88	5	-1	0	4	77
89	0.3	2.7	1	4	79
90	12.6	3.4	1	17	100
91	-3.5	6.5	0	3	—
92	-2	7	0	5	66
93	3	0	1	4	70
94	14.8	4.2	1	20	100
95	11.5	0.5	1	13	98
96	21.5	0.5	1	23	100
97	32	0	0	32	100
98	3.5	-0.5	-1	2	—
99	7	1	-5	3	—
100	6.7	8.3	6	21	99
101	-2.7	4.8	0.9	3	—
102	-8	10.9	0.2	3.1	—

tively to most nodes and the combined analysis appears to be justified.

Bayesian analyses for the combined data set reached stationarity well before generation 300,000. The topology of the tree was quite similar to that obtained by MP (Fig. 4), and both the tribe Acraeini (*s. str.*) and the South-American Acraeini appeared as a monophyletic group. The monophyletic *Bematistes* appeared as sister group of all tribe Acraeini (*s. str.*), while *Acraea* is paraphyletic. *Actinote* (*s. str.*) appeared as a monophyletic group with very short branches separating the species. The genera *Altinote* and *Abananote* are both polyphyletic, and *Alt. eresia* arose isolated in a branch sister to all Neotropical Acraeini. The Bayesian tree differed from the MP consensus tree in the position of *Cethosia*, which joined the Heliconiini clade (*Agraulis* + *Heliconius*) with a high posterior probability (PP) value. In this topology, the monotypic

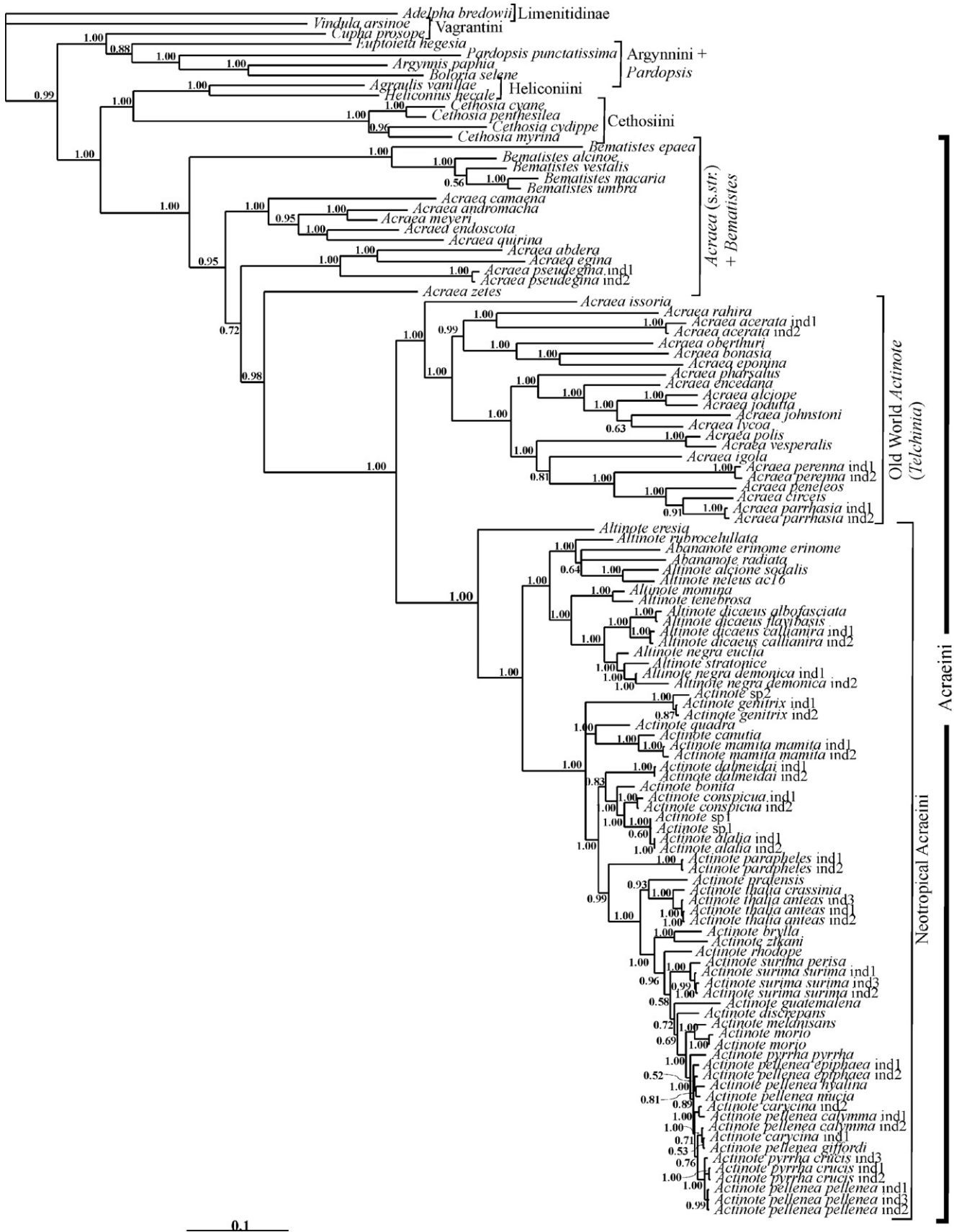


Fig. 4. Phylogenetic tree inferred by Bayesian analysis from combined data set. Values above the branches indicate Bayesian posterior probability values.

genus *Pardopsis* also appeared within the Argynnini clade with a high PP value.

3.2. Evolution of host plant use

According to the character optimization of the host plant families used by each species of Acraeini, the ancestral state of the tribe is the use of host plants of the family Passifloraceae, and most of the basal *Acraea* + *Bematistes* feed on plants of this family (Fig. 5). The species of *Acraea* of this basal clade also feed on Violaceae. The clade joining the remaining *Acraea* (“Old World *Actinote*”) is characterized by feeding mostly on Urticaceae, with some species highly polyphagous—the branch appears generally as “equivocal” to this polyphagous species. The host plant used by the ancestor of the South-American Acraeini clade is equivocal, but if we consider the whole clade but *Alt. eresia* (whose host plant is unknown) the ancestor host plant of this group may have been Asteraceae, and all Neotropical Acraeini with a well known life cycle feed on plants of this family only. Feeding on this host plant family is characteristic of the ancestor of the genus *Actinote* (*s. str.*). The phylogenetic signal obtained by the modified PTP test was significant ($P = 0.003$), suggesting that the distribution of this trait among taxa can be explained by their phylogenetic relationships.

4. Discussion

4.1. The internal relationships of Heliconiinae

Although the main subject of this study has been the relationships within the tribe Acraeini, it is worth discussing the position of the tribe within the subfamily Heliconiinae. Even with all the recent advances in the systematics of Nymphalidae, the affinities of the tribe Acraeini within the Heliconiinae continue to be undefined and controversial. The recent phylogenetic proposal of Penz and Pegg (2003) for Heliconiinae, based on characters of early stages and adult morphology, showed a polytomy among the four major clades in the trees obtained with equally weighted characters; however, the successive approximation weighting analyses resulted in a better resolved tree, with the Acraeini appearing as the sister group of Heliconiini + (Vagrantini + Argynnini). The same results were found by Freitas and Brown (2004) with independent data sets of morphological characters from adults and immatures. Alternative hypotheses were proposed by Brower (2000) and Wahlberg et al. (2003b, 2005), with the tribe Argynnini appearing as sister to Acraeini + Heliconiini. The taxonomic sampling within Heliconiinae in these studies, however, is too limited (usually one or two taxa representing each tribe) to permit further discussion on the tribal relationships. Here we found two different results in relation to the tribal relationships within Heliconiinae, although our sample does not include many representatives of other tribes outside Acraeini. The results achieved by the

Bayesian phylogenetic hypothesis are similar to that found by other studies, with Vagrantini + Argynnini (containing *Pardopsis*) sister to Heliconiini (+Cethosiini) + Acraeini. On the other hand, the MP consensus tree shows the tribe Heliconiini sister to the other three tribes.

The monophyly of the tribe Acraeini was recovered in all results of Penz and Pegg (2003) and Freitas and Brown (2004) (with *Pardopsis* as part of Acraeini in both studies), and the present study has also recovered the monophyly of the tribe Acraeini (*s. str.*) in all MP and Bayesian analyses, although all results have shown that *Pardopsis* does not belong to Acraeini. The monotypic genus *Pardopsis* has been considered as part of Acraeini and sister to all other genera of this tribe (Penz and Pegg, 2003; Freitas and Brown, 2004). However, this taxon was never included in a molecular study before, and the results of the present analyses showed that the genus is in fact part of Argynnini. The position of *Pardopsis* within Argynnini was recovered by the Bayesian analysis with a high posterior probability value (Fig. 4), and in the consensus MP tree with a weak support, and we consider it not to be within the tribe Acraeini (*s. str.*), as defined in this study. Some clues about the position of *Pardopsis* can be obtained from the immature stages (see Van Son, 1963), which share several character states with the Argynnini, as discussed in Freitas (1999). These character states include, for example, the egg with several longitudinal ridges anastomosing near half the height, and the subdorsal prothoracic scoli longer than the others (both present in *Euptoieta hegesia*—Freitas, 1999). A future study with better taxonomic sampling of Argynnini and Vagrantini, and the addition of morphological data, will be crucial to define the real affinities of this puzzling genus.

The position of *Cethosia* within the Heliconiinae has been discussed in several recent papers, but still remains controversial (Penz and Pegg, 2003). Brower (2000) shows *Cethosia* as sister to Acraeini + Heliconiini. In Freitas and Brown (2004), *Cethosia* is the sister group of *Vindula* (=Vagrantini of Penz and Pegg, 2003), and the successive weighting analysis defined this pair as sister to Heliconiini. The same general topology was also found by Penz and Pegg (2003), and the Bayesian analysis in the present study shows *Cethosia* as sister to Heliconiini (Fig. 4). Harvey (1991), however, proposes that *Cethosia* belongs to its own tribe, Cethosiini, sister to the Acraeini, based on an extremely well developed “gland sous-papillaire” in the female abdomen, although in the “Addendum” of the same work the author places *Cethosia* into Acraeini and abandons Cethosiini. Clearly a broad molecular study of the subfamily Heliconiinae is needed with a comprehensive sampling of species in the subfamily to clarify the position of *Cethosia*.

4.2. The tribe Acraeini

Our results on the relationships within Acraeini are similar to the proposal of Pierre (1987) (Fig. 2), but due to the

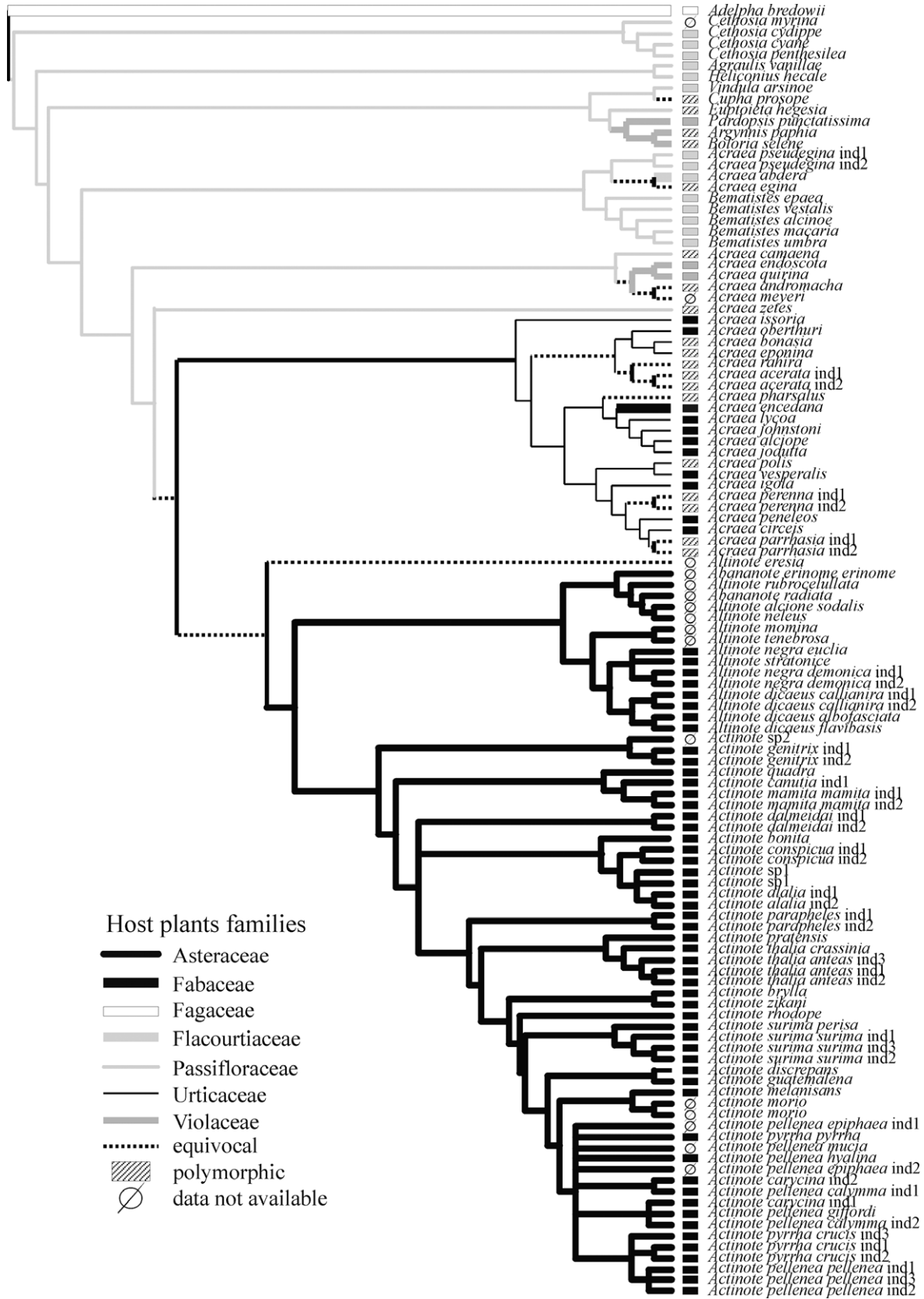


Fig. 5. Optimization of the host plants families used by each Acraeini species on the Maximum Parsimony phylogenetic hypothesis. See Table 1 for details of host plant families used by each species.

limited taxonomic sampling of Palaeotropical species, most of Pierre's species-groups could not be evaluated here. In all topologies, we recovered a monophyletic clade including all species of the subgenus *Acraea sensu* Pierre (1987), sister to the "Old World *Actinote*" + the Neotropical Acraeini. The genus *Bematistes* appeared always as monophyletic, but their position within Acraeini is not clear. The hypothesis of this clade as sister to all remaining Acraeini, as found in our Bayesian tree, was not proposed before, and should be further investigated.

Based on the above results, a different genus could be erected for the "Old World *Actinote*" clade (Figs. 3 and 4). Additionally, *Actinote* (*s. str.*) could be expanded to cover at least all South American Acraeini and, in this proposal, *Altinote* and *Abananote* are dropped. Although sampling of African Acraeini is still incomplete, some preliminary patterns were found, which will form the basis for future studies of the tribe Acraeini. There are three main taxonomic suggestions arising from the present results: (1) *Acraea* should be used temporarily at generic level for the mainly Passifloraceae-feeding African *Acraea*, until further sampling define the natural groups in this paraphyletic genus, (2) *Telchinia* Hübner, [1819] [type species: *Papilio serena* Fabricius 1775, more familiar as its junior synonym *Acraea eponina* Cramer 1780 and formerly known as *Acraea terpsichore* L. 1758; see e.g. Larsen, 2005] would be revived at generic level for the mainly Urticaceae-feeding series included by Pierre in his subgenus *Actinote* ("Old World *Actinote*" in this paper), and (3) *Actinote* Hübner, [1819] should be expanded to include all Neotropical Acraeini.

Such changes may appear radical, yet they are also pragmatic and would resolve both the current absurd incompatibility of Neotropical and Afrotropical *Acraea* parochial taxonomy, and the problem of paraphyly of the African *Acraea* (*s. lat.*). However, more complete sampling of the African Acraeini is clearly needed to resolve their relationships, and to support any such taxonomic actions as we suggest here. Moreover, we do not yet know from this analysis where exactly to place *Miyana moluccana*. Independent of the generic arrangement that will be proposed for the Acraeini, both *Pardopsis* and *Cethosia* should be excluded from the tribe.

4.3. The neotropical Acraeini

All Neotropical species form a clade well supported with high bootstrap and Bremer support values. This could be considered strong evidence of a single colonization of South America by the ancestor of the Neotropical Acraeini. Three main groups arise from the Neotropical lineage (Figs. 3 and 4): (1) the monobasic Andean species *Altinote eresia*, sister to all Neotropical species, (2) a clade of Andean species in the genera *Altinote* and *Abananote* (both polyphyletic as currently conceived), and (3) the clade of the genus *Actinote* (*s. str.*), monophyletic and with an evident center of more recent diversification in the mountains

of SE Brazil (Francini, 1989, 1992). The position of *Alt. eresia* is well supported by both parsimony and Bayesian analyses and there is no conflict among the data partitions, suggesting that its position will be stable to the addition of new data. Additionally, the position of *Alt. eresia* in the tree solves the problem of the origin of all Neotropical Acraeini, which, based on the present evidence, was in the Andean region. There is no previous available phylogenetic proposal for the Andean clade (*Altinote* + *Abananote*), and the present hypothesis is the only one on hand so far.

For the genus *Actinote* (*s. str.*), the only previously available hypothesis is based on morphology of adults and immatures (Francini, 1992); the taxonomic sampling was limited to the 17 species of SE Brazil with known life history at that time. In all analyses developed here, the clade joining *Act. genitrix* + *Actinote* sp2 (an undescribed species from Ecuador) appeared as sister to the remaining *Actinote* (*s. str.*), followed by a clade composed by *Act. quadra* + *Act. canutia* + *Act. mamita*. Francini (1992) also found *Act. canutia* and *Act. mamita* close together and sister to the remaining *Actinote* (*s. str.*), and in that study *Act. genitrix* was closely related to *Act. melanisans*.

The close relationship between *Act. bonita* and *Act. alalia* found here was recovered by Francini (1992) in a phenetic analysis (he did not include *Act. conspicua* in his analyses). According to the present results, *Act. conspicua* is sister to *Act. alalia*, and shares several characters with it, in the red-orange mimetic group, or "red *Actinote*" (Francini, 1992). In our study, all "red *Actinote*" (including an undescribed taxon from South Brazil, *Actinote* sp1) are closely together. The close relationship between *Act. parapeles* and *Act. discrepans* found in Francini (1992) diverges from our results, where *Act. parapeles* is an isolated taxon branching after the "red *Actinote*", while *Act. discrepans* appeared together with *Act. guatemalena* in our MP tree or as an isolated branch, as found in our Bayesian topology. The close relationship between *Act. pellenea* and *Act. carycina* was also recovered by Francini (1992), but in his results these two taxa were always closely related with *Act. surima*, which in our results is not part of this clade. Additionally, the present study shows that the taxon *Act. pellenea* is not monophyletic, including two morphologically similar species, *Act. carycina* and *Act. pyrrrha*, in a complex of taxa separated by very short branches, suggesting recent speciation in this group. Indeed, *Act. pellenea* is the most widespread species of *Actinote* (*s. str.*), occurring in all South America, from Colombia and Venezuela to Northern Argentina, with 17 known subspecies (Paluch, 2006), all of them morphologically distinct and geographically isolated. The lack of resolution in the clade including the subspecies of *Act. pellenea* could be due to incomplete lineage sorting (Freeland, 2006), and a general study focused on the patterns of speciation in this group (together with *Act. carycina* and *Act. pyrrrha*) could provide important information to better understand the evolution of the Neotropical lineage as a whole.

The results suggest that additional evidence would be crucial to resolve the *Actinote* (*s. str.*) clade, including sequencing of other regions as well as a detailed morphological study of adults and immatures of most species. Inclusion of additional species in this clade could also add resolution and help to understand the natural groups within *Actinote* (*s. str.*). Presently we have 22 out of 31 species of *Actinote* (*s. str.*) in our taxonomic sampling (plus other two additional undescribed taxa), and at least one important missing species, *Act. lapitha*, seems different from all other species in the genus, and cannot be assigned *a priori* to any clade. The inclusion of this species is a high priority for future studies. It is important to note however that based on the available morphological data (Francini, 1989, 1992; Paluch, 2006) the genus *Actinote* (*s. str.*) is expected to remain as a compact monophyletic clade.

4.4. Evolution of host plant use

Based on our optimization of host plant families in the Acraeini phylogenetic hypothesis, a clear pattern can be observed, mostly similar to the proposal of Pierre (1987) for Acraeini. The ancestor of all Acraeini (*s. str.*) appears to have used Passifloraceae as its larval host plant, and this family may also be considered ancestral for all the Heliconiinae clade. Interestingly, species in the genus *Parthenos*, which appears to be the most basal group of the sister subfamily Limenitidinae (Wahlberg et al., 2003b; Willmott, 2003a), also feed on species of Passifloraceae, making the plant family the possible ancestral of the heliconiine clade (as defined in Wahlberg et al., 2003b). This plant family is used by *Bematistes* and is also the ancestral host plant for the *Acraea* (*s. str.*) clade. Subsequently, there is a major change in host plant use in two main lineages: the “Old World *Actinote*” clade (suggested genus *Telchinia*), showing a general tendency for using Urticaceae (including several species using up to five host plant families—Ackery, 1988), and the Neotropical clade, using Asteraceae only (the few records of *Actinote* (*s. str.*) feeding on Urticaceae, Fabaceae, Amaranthaceae, Poaceae, and Verbenaceae are now considered to be in error, after extensive field observation and laboratory experiments by AVLF, MP, and RBF—see also Francini, 1989). However, for the Andean clade there is a general lack of data on host plant use by each species, and any additional data could help to evaluate whether all species in this clade indeed feed exclusively on Asteraceae. In any case, there is strong evidence for the use of Asteraceae by all *Altinote* + *Abananote* (Brown and Francini, 1990). Based on the above patterns of host plant use, it is possible to propose a simple scenario where the South-American Asteraceae-feeding Acraeini could have been originated from an Urticaceae-feeding “Old World *Actinote*” (i.e. stem-group of suggested genus *Telchinia*) ancestor by a single colonization event. In South America, these butterflies may have colonized plants of the family Asteraceae, retaining their ability to synthesize their own cyanoglycosides. No evidence, at present, suggests that

these butterflies are able to sequester the secondary compounds found in Asteraceae (Brown and Francini, 1990). The reasons that lead to this shift to Asteraceae are still unknown, but it could be possible that the founder individual was already perfectly adapted to colonize Asteraceae (see e.g. Janz et al., 2006). Whilst Asteraceae does not today feature prominently in the host plant repertoire of African “Old World *Actinote*” (e.g. hosts database, Natural History Museum, and present paper) it is well worth considering that the colonizer may have been indeed an Asteraceae-feeder. It is notable then that three polyphagous species that we sampled in the group of “Old World *Actinote*” (*Acr. acerata*, *Acr. perenna*, and *Acr. rahira*) feed on Asteraceae besides other host plant families (Table 1). In fact, there is a single record of *Acraea perenna perenna* Doubleday, 1847 on the genus *Mikania* (Fontane, 1988; Larsen, 2005, p. 443); this butterfly is the sole representative of the morphological “Old World *Actinote*”-clade 1 in which it was placed by Pierre (1987) (Fig. 2). This butterfly is not restricted to Asteraceae [also feeding on *Olobopetalum* (Menispermaceae), *Bridelia* (Euphorbiaceae), and *Adenia* (Passifloraceae)], and so this isolated record must be viewed as evidence of ability of the “Old World *Actinote*” lineage to feed on this composite genus. Moreover, it would not be surprising if such a founder individual was such a generalist, and indeed was *r*-selected as appears characteristic of the multi-brooding Neotropical Acraeini. Whilst the SE Brazilian *Actinote* (*s. str.*) clearly favor Asteraceae, elucidation of the host plant of the Andean *Altinote eresia* would further help to confirm the ancestral host plant for the whole clade. The host plants of *Acraea mirifica* Lathy, 1906 and *Acr. odzala* Collins, 1997 would further be interesting to learn, since they have previously been placed in the same morphological clade (clade “6c”) that Pierre (1987) used for Neotropical Acraeini (Larsen, 2005, p. 443).

The Andean origin of the Neotropical Acraeini in our global phylogenetic hypothesis is an intriguing aspect of our study. In one view, this Andean origin constrained the arrival of a putative colonizer to later than the start of the Andean orogeny. Alternatively the putative ancestor could be a lowland species that arrived before the Andean orogeny. In this scenario, the radiation of Neotropical Acraeini, with a clear predominance of cold climate species (few taxa were able to colonize the coastal plains and amazon basin), could also be favored with the later orogeny of the Andes as a result of the heavy use of Asteraceae, that is clearly more abundant in montane habitats compared with the wet tropical forests. This issue, however, can only be solved after a reliable estimation of the age of origin of the Neotropical clade become available.

Acknowledgments

Andean and Paleotropical species were provided by several collaborators: Sandra Uribe, Mario Alejandro Marin, Carlos Eduardo Giraldo, Andrés Lopez (Colombia), Carlos Peña (Sweden), Torben B. Larsen (Denmark), Hara Hiroshi

(Japan), Rudi Mattoni (USA-Argentina), Renato Rogner Ramos, Eduardo Emery, Elaine Cambui Barbosa (Brazil), Roger Vila, and Andrew Brower (USA). The authors are especially grateful to the African Butterfly Research Institute and to Dr. Jacques Pierre (Museum National d'Histoire Naturelle, France) for providing specimens from Africa. Andean species were identified by Dr. Gerardo Lamas (Peru) and Brazilian species were identified by Dr. Ronaldo Francini, Dr. Márlon Paluch, and Dr. André V. L. Freitas. The authors thank Rosângela A. Rodrigues for technical assistance, and Dr. José R. Trigo, Dr. Jacques Pierre, and two anonymous referrers for discussions and comments on the manuscript. This research was supported by the Brazilian CNPq (fellowship 151004/2005-6) and by Fundação de Amparo à Pesquisa—FAPESP (Grants #06/60127-0 and #07/53919-0) to K.L.S.B., and by FAPESP to A.M.L.A.E. (Grant 05/57680-7). D.C.L. was supported by Leverhulme Trust Research Fellowship #F/00696/I (2002). N.W. was supported by a grant from the Swedish Research Council and the Academy of Finland. A.V.L.F. thanks FAPESP (Grants 00/01484-1 and 04/05269-9) and the BIOTA-FAPESP program—98/05101-8), the Brazilian CNPq (fellowship 300315/2005-8) and the National Science Foundation Grant DEB-0527441.

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