

Phylogeny of the Aphnaeinae: myrmecophilous African butterflies with carnivorous and herbivorous life histories

JOHN H. BOYLE^{1,2}, ZOFIA A. KALISZEWSKA^{1,2}, MARIANNE ESPELAND^{1,2,3}, TAMARA R. SUDERMAN^{1,2}, JAKE FLEMING^{2,4}, ALAN HEATH⁵ and NAOMI E. PIERCE^{1,2}

¹Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, MA, U.S.A., ²Museum of Comparative Zoology, Harvard University, Cambridge, MA, U.S.A., ³Museum of Natural History and Archaeology, Norwegian University of Science and Technology, Trondheim, Norway, ⁴Department of Geography, University of Wisconsin, Madison, WI, U.S.A. and ⁵Iziko South African Museum, Cape Town, South Africa

Abstract. The Aphnaeinae (Lepidoptera: Lycaenidae) are a largely African subfamily of 278 described species that exhibit extraordinary life-history variation. The larvae of these butterflies typically form mutualistic associations with ants, and feed on a wide variety of plants, including 23 families in 19 orders. However, at least one species in each of 9 of the 17 genera is aphytophagous, parasitically feeding on the eggs, brood or regurgitations of ants. This diversity in diet and type of symbiotic association makes the phylogenetic relations of the Aphnaeinae of particular interest. A phylogenetic hypothesis for the Aphnaeinae was inferred from 4.4 kb covering the mitochondrial marker *COI* and five nuclear markers (*wg*, *H3*, *CAD*, *GAPDH* and *EF1 α*) for each of 79 ingroup taxa representing 15 of the 17 currently recognized genera, as well as three outgroup taxa. Maximum Parsimony, Maximum Likelihood and Bayesian Inference analyses all support Heath's systematic revision of the clade based on morphological characters. Ancestral range inference suggests an African origin for the subfamily with a single dispersal into Asia. The common ancestor of the aphnaeines likely associated with myrmicine ants in the genus *Crematogaster* and plants of the order Fabales.

Introduction

The subfamily Aphnaeinae (Lepidoptera: Lycaenidae) consists of 278 species of butterflies with an unusual diversity of life histories. Several of the 17 genera are endemic to southern Africa, but many of the genera are distributed throughout Africa. The majority (88%) of the described species are found in Africa and/or Arabia, with the exception of 33 of the 71 species of *Cigaritis* Donzel, which are found in Asia as far east as Japan. Aphnaeines occur in many habitats and consume and/or lay eggs on a wide range of host plants (Table 1). Like most known lycaenid species, aphnaeine larvae form associations with ants (Pierce *et al.*, 2002). These associations are typically mutualistic: ants defend the larvae from predators

and parasitoids, and larvae produce nutritious secretions for the ants. In some cases, however, larvae parasitize ants, either by inducing trophallaxis from ant workers or by consuming ant brood. Interactions can range from facultative associations in which lycaenid larvae intermittently associate with many species of ants, to specialized, obligate symbioses in which larvae are never found without ants, often associating with only one or a few closely related species (Pierce *et al.*, 2002).

The Aphnaeinae are notable among the Lycaenidae for exhibiting considerable variability in feeding strategies within a single subfamily, especially considering the relatively small size of the group. Although 8 of the 17 genera consist of species that are phytophagous and mutualistically associated with ants, the remaining 9 genera contain at least one species that is 'aphytophagous' (i.e. feeding obligately on substances other than plants during at least some portion of the lifetime) and parasitically associated with ants (Pierce *et al.*, 2002, A. Heath, personal

Correspondence: Naomi Pierce, Museum of Comparative Zoology, Harvard University, 26 Oxford St., Cambridge, MA 02138, U.S.A. E-mail: npierce@oeb.harvard.edu

Table 1. Taxa included in this study, with voucher identification, and information about diet, range, larval ant association and gene regions sequenced.

Taxon	Voucher	Locality	Aphyto- phagy ^a	Range ^b	Ant associate ^c	Host plant ^d	Genbank accession number					
							COI	CAD	EF1 α	GAPDH	H3	Wg
<i>Pseudaletris</i> : 24 species worldwide												
<i>P. agrippina</i> Druce	SC-99-T500	Ebogo, Cameroon		Congolian	<i>Crematogaster</i> (M)	Algae	KF787284	KF787554	KF787472	^e	KF787202	KF787400
<i>P. clymenus</i> Druce	RD-98-U087	Beni, D.R. Congo		Congolian	–	Algae	KF787285	KF787555	KF787473	^e	KF787203	KF787401
<i>Lipaphysa</i> : 4 species worldwide												
<i>L. aderna</i> Plötz	AJG-07-E105	Mundwiji, Zambia		Zambezi, Congolian	<i>Crematogaster</i> (M)	Myrsinaceae	KF787278	KF787548	–	–	KF787196	KF787394
<i>L. leonina</i> bitje Druce	AJG-07-N567	Nchila Wildlife Reserve, Zambia		Congolian	<i>Crematogaster</i> (M)	–	KF787279	KF787549	KF787467	–	KF787197	–
<i>L. losura</i> Rebel	RD-98-U152	Beni, D.R. Congo		Zambezi, Congolian	<i>Crematogaster</i> (M)	–	KF787280	KF787550	KF787468	KF787333	KF787198	KF787395
<i>Chloroselas</i> : 13 species worldwide												
<i>C. azurea</i> Butler	SC-99-T196	Pangani, Tanzania	Yes	E. Africa, Saudi Arabia	<i>Crematogaster</i> (M)	Fabaceae	–	–	–	–	–	–
<i>C. mazoensis</i> Trimen	AJG-07-D625	Mosa Hill, Zambia		Zambezi, Congolian	–	–	KF787253	KF787523	KF787442	KF787322	KF787180	KF787366
<i>C. overlaeti</i> Stempffer	AJG-07-D616	Mosa Hill, Zambia		S. African, Zambezi, Zambezi	–	Fabales	KF787254	KF787524	KF787443	KF787323	KF787181	KF787367
<i>C. pseudozertis</i> Trimen	DJM-07-A075	Laikipia, Kenya	Yes, A	S. African, Zambezi, Congolian, Somali	<i>Crematogaster</i> (M)	Fabales	KF787255	KF787525	KF787444	KF787324	KF787182	KF787368
<i>Vansomerentia</i> : 1 species worldwide												
<i>V. cesa</i> : 1 species worldwide				E. Africa	<i>Crematogaster</i> (M)	Fabaceae	KF787256	KF787526	KF787445	KF787325	KF787183	KF787369
<i>Cnidaria</i> : 3 species worldwide												
<i>C. capensis</i> van Son	AAM-98-W799	Gamka Mt. Nature Res., South Africa	Yes, D	Southern African	<i>Anoptolepis</i> (F)	Zygophyllales	KF787273	KF787543	KF787462	–	KF787191	KF787388
<i>C. leroma</i> Wallengren	AH-95-Y658	Leeu-Gamka, South Africa		S. African, Zambezi	<i>Anoptolepis</i> (F)	Fabales	KF787274	KF787544	KF787463	KF787331	KF787192	KF787389
<i>C. wykehami</i> Dickson	SQ-02-X473	Wimpos, South Africa		Southern African	<i>Anoptolepis</i> (F)	–	KF787275	KF787545	KF787464	–	KF787193	KF787390
<i>Chrysoritis</i> : 42 species worldwide												
<i>C. aethon</i> Trimen	AH-99-T262	Graskop, South Africa	Yes	Southern African	<i>Crematogaster</i> , <i>Myrmecaria</i> Saunders (M)	Fabaceae, Amaranthaceae, Anacardiaceae, Apiaceae, Asteraceae, Bruniaceae, Crassulaceae, Ebenaceae, Euphorbiaceae, Myrsinaceae, Santalaceae, Zygophyllaceae	KF787257	KF787527	KF787446	–	–	–
<i>C. aureus</i> van Son	AH-95-Z444	Greylingstad, South Africa		Southern African	<i>Crematogaster</i> (M)	Saxifragales	KF787258	KF787528	KF787447	–	–	–
<i>C. brooksi</i> Riley	AH-95-Z415	Worcester, South Africa		Southern African	<i>Crematogaster</i> (M)	Malpighiales	KF787259	KF787529	KF787448	–	–	–
<i>C. chrysantus</i> Trimen	AH-95-Z431	Wallekrall, South Africa		Southern African	<i>Crematogaster</i> (M)	Zygophyllales	KF787260	KF787530	KF787449	–	KF787184	KF787370

Table 1. Continued

Taxon	Voucher	Locality	Aphyto- phagy ^a	Range ^b	Ant associate ^c	Host plant ^d	Genbank accession number					
							COI	CAD	EF1α	GAPDH	H3	Wg
<i>C. chrysaor</i> Trimen	AH-95-Z454	Balfour, MP, South Africa		Southern African	<i>Crematogaster</i> (M)	Asterales, Fabales, Sapindales, Saxifragales, Zygophyllales	KF787261	KF787531	KF787450	KF787326	KF787185	KF787371
<i>C. dicksoni</i> Gabriel	AH-98-Y698	Cape Infanta, South Africa	Yes, A	Southern African	<i>Crematogaster</i> (M)	–	KF787262	KF787532	KF787451	–	KF787186	KF787372
<i>C. endymion</i> Pennington	AH-95-Y759	Franschoek, South Africa		Southern African	<i>Crematogaster</i> (M)	Santalales	KF787263	KF787533	KF787452	–	–	–
<i>C. felthami felthami</i> Trimen	AH-95-Z460	Cape Town, South Africa		Southern African	<i>Crematogaster</i> (M)	Zygophyllales	KF787264	KF787534	KF787453	–	–	–
<i>C. lycogenes</i> Trimen	AH-95-Z921	Buumba Cloud-lands, South Africa		Southern African	<i>Crematogaster</i> (M)	Asterales, Ericales, Sapindales	KF787265	KF787535	KF787454	–	–	–
<i>C. lycarium</i> Trimen	SJ-98-U639	Xolobe, South Africa		Southern African	<i>Crematogaster</i> (M)	Ericales	KF787266	KF787536	KF787455	–	–	–
<i>C. nigricans</i> Aurivillius	AH-95-Z442	Cape Town, South Africa		Southern African	<i>Crematogaster</i> (M)	Asterales, Santalales, Zygophyllales	KF787267	KF787537	KF787456	–	–	–
<i>C. oreas</i> Trimen	AH-95-Z911	Buumba Cloud-lands, South Africa		Southern African	<i>Myrmecaria</i> (M)	Santalales	KF787268	KF787538	KF787457	KF787327	KF787187	KF787373
<i>C. pyramus pyramus</i> Pennington	AH-95-Z951	Oudtshoorn, South Africa		Southern African	<i>Crematogaster</i> (M)	Asterales, Santalales	KF787269	KF787539	KF787458	–	–	–
<i>C. pyrois pyrois</i> Trimen	AH-95-Y627	Worcester, South Africa		Southern African	<i>Camponotus</i> (F), <i>Myrmecaria</i> (M)	Santalales, Zygophyllales	KF787270	KF787540	KF787459	KF787328	KF787188	KF787374
<i>C. thysbe thysbe</i> Linnaeus	AH-95-Z447	Cape Town, South Africa		Southern African	<i>Crematogaster</i> (M)	Asterales, Fabales, Santalales, Zygophyllales	KF787271	KF787541	KF787460	KF787329	KF787189	KF787375
<i>C. zonarius coetzleri</i> Dickson & Wykeham	AH-95-Z423	Nieuwoudtville, South Africa		Southern African	<i>Crematogaster</i> (M)	Asterales	KF787272	KF787542	KF787461	KF787330	KF787190	–
<i>Trimenia</i> : 5 species worldwide												
<i>T. argyropilaga</i> Dickson	AH-95-Y631	Calvinia, South Africa	Yes	Southern Africa	<i>Anoplolepis</i> (F)	Aphytophagous	KF787294	KF787564	KF787482	KF787344	KF787212	KF787402
<i>T. macnasteri</i> Dickson	NP-99-T474	Calitzdorp, South Africa	Yes: A, B	Southern African	<i>Anoplolepis</i> (F)	–	KF787295	KF787565	KF787483	KF787345	KF787213	KF787403
<i>T. mclagrida mclagrida</i> Dickson & Henning	AH-96-Y733	DeHoop Nature Res., South Africa	Yes: A, B	Southern African	<i>Anoplolepis</i> (F)	–	KF787242	KF787512	KF787431	KF787314	KF787170	KF787404
<i>T. m. cedrusmontana</i> Dickson & Stephen	AH-98-U487	Skurweberge, WC, South Africa	Yes: A, B	Southern African	–	–	KF787296	KF787566	KF787484	ε	KF787214	KF787405
<i>T. m. paarlensis</i> Dickson	AH-98-U492	Paarl, South Africa	Yes: A, B	Southern African	–	–	KF787297	KF787567	KF787485	–	KF787215	KF787406
<i>T. wykehami</i> Dickson	AH-99-U458	Komsberg, South Africa		Southern African	–	–	KF787298	KF787568	KF787486	–	KF787216	KF787407

Table 1. Continued

Taxon	Voucher	Locality	Aphytophagy ^a	Range ^b	Ant associate ^c	Host plant ^d	Genbank accession number					
							COI	CAD	EF1 α	GAPDH	H3	Wg
Argyropodes: 1 species worldwide												
<i>Argyropodes argyropis</i> Trimen	AH-95-Z422	Wallekraal, South Africa	Yes, D	S.W. Africa	Unknown	Unknown	–	KF787513	KF787432	–	KF787171	KF787363
Cigaritis: 71 species worldwide												
<i>C. crastaria</i> Holland	RD-98-U045	Beni, D.R. Congo	Yes	Africa, Asia, Middle East	<i>Crematogaster, Pheidole</i> (M)	Fabaceae, Anacardiaceae, Loranthaceae, Polygonaceae, Rubiaceae, Verbenaceae, Zygophyllaceae	–	KF787286	KF787474	KF787336	KF787204	KF787376
<i>C. ella</i> Hewitson	AH-04-B546	Witsand Nature Res., South Africa	Yes, D	S. African, Zambesian, Somalian	<i>Crematogaster, Pheidole</i> (M)	Fabales, Santalales	–	KF787250	KF787439	–	KF787177	KF787377
<i>C. epargyros</i> Eversmann	NK-00-P791	Balia-Kul, Kazakhstan	Yes	Asia Minor west to China	–	Fabales	–	KF787236	KF787506	–	KF787165	KF787378
<i>C. katu</i> Corbet	NP-95-Y326	Pahang, Malaysia	Yes, D	SE Asia	–	–	–	KF787287	KF787475	KF787337	KF787205	KF787379
<i>C. lohita senama</i> Fruhstorfer	MWT-93-A022	Kuala Lumpur, Malaysia	Yes	India and SE Asia	–	Dioscoreales, Fabales, Myrtales, Solanales	–	KF787288	KF787476	KF787338	KF787206	KF787380
<i>C. mozambica</i> Bertolini	AJG-07-N619	Zambia	Yes	S. African, Zambesian, Congolian, Somalian	–	Fabales	–	KF787251	KF787440	KF787320	KF787178	KF787381
<i>C. namaqua</i> Trimen	AAM-98-U299	Garies, South Africa	Yes	Southern African	<i>Crematogaster</i> (M)	Zygophyllales	–	KF787289	KF787477	KF787339	KF787207	KF787382
<i>C. natalensis</i> Westwood	AH-01-T355	Heidelberg, South Africa	Yes	S. African, Zambesian	<i>Crematogaster</i> (M)	Fabales, Gentianales, Lamiales, Santalales	–	KF787290	KF787478	KF787340	KF787208	KF787383
<i>C. phanes</i> Trimen	AH-04-B550	Witsand Nature Res., South Africa	Yes	S. African, Zambesian	<i>Crematogaster</i> (M)	Fabales, Santalales	–	KF787252	KF787441	KF787321	KF787179	KF787384
<i>C. syama terana</i> Fruhstorfer	MWT-93-A039	Kuala Lumpur, Malaysia	Yes: A, B	SE Asia	–	Fabales	–	KF787291	KF787561	KF787341	KF787209	KF787385
<i>C. takanotis</i> Matsumura	JCC-01-P022	Seoul, South Korea	Yes: A, B	Japan	<i>Crematogaster</i> (M)	–	–	KF787292	KF787480	KF787342	KF787210	KF787386
<i>C. tavetensis</i> Lathy	DJM-07-A081	Ngong Hills, Kenya	Yes	Zambesian, Congolian, Somalian	<i>Pheidole</i> (M)	Fabales	–	KF787293	KF787563	KF787343	KF787211	KF787387
Zeritis: 6 species worldwide												
<i>Z. sorhagenii</i> Dewitz	AJG-07-D630	Kabweluma Falls, Zambia	Yes	Zambesian	Unknown	Unknown	–	KF787301	KF787489	KF787348	KF787219	KF787408
Axiocerses: 20 species worldwide												
<i>A. amanga</i> Westwood	AJG-07-N758	Zambia	Yes	S. African, Zambesian, Congolian, Somalian	<i>Crematogaster, Pheidole</i> (M)	Fabaceae, Loranthaceae, Oleaceae	–	KF787244	KF787514	KF787433	KF787315	KF787172
<i>A. bambana</i> Grose-Smith	RD-98-U072	Beni, D.R. Congo	Yes	Zambesian	–	Fabales	–	KF787245	KF787515	KF787434	–	KF787173

Table 1. Continued

Taxon	Voucher	Locality	Aphyto- phagy ^a	Range ^b	Ant. associate ^c	Host plant ^d	Genbank accession number					
							COI	CAD	EF1 α	GAPDH	H3	Wg
<i>A. coelestis</i> Henning & Henning (?)	SC-99-T171	Pangani, Tanzania		S. African, Zambebian	–	–	KF787246	KF787516	KF787435	KF787316	KF787174	–
<i>A. erosus</i> Trimen	AH-05-B559	Middleton, EC, South Africa		Southern African	–	–	KF787247	KF787517	KF787436	KF787317	KF787175	–
<i>A. harpax</i> Fabricius	TL-96-Y950	Cape Coast, Ghana	Yes: A	Zambebian, Congolian, Somalian, Arabia	<i>Crematogaster</i> (M), <i>Pheidole</i> (M)	Fabales	KF787248	KF787518	KF787437	KF787318	KF787176	KF787365
<i>A. ijoane</i> Wallengren	AJG-07-N847	Arcturus, Zimbabwe		S. African, Zambebian	–	Fabales	KF787249	KF787519	KF787438	KF787319	–	–
<i>Aloeides</i> : 57 species worldwide			Yes	S.E. Africa	<i>Lepisiota</i> (F), <i>Monomorium</i> Mayr, <i>Pheidole</i> (M)	Fabaceae, Malvaceae, Zygophyllaceae						
<i>A. aranda</i> Wallengren	AP-98-W757	Gourits R. Valley, South Africa		S. African, Zambebian	<i>Pheidole</i> (M)	Fabales	KF787223	KF787493	KF787412	KF787302	KF787152	KF787349
<i>A. arida</i> Tite & Dickson	AAM-98-Y996	Carolusberg, South Africa		Southern African	–	–	KF787224	KF787494	KF787413	–	KF787153	KF787350
<i>A. baupioni</i> Tite & Dickson	AH-06-M542	Steinkopf, South Africa		Southern African	<i>Lepisiota</i> (F)	Malvales	KF787225	KF787495	KF787414	–	KF787154	–
<i>A. barklyi</i> Trimen	AH-06-M543	Kamieskroon, South Africa		Southern African	–	–	KF787226	KF787496	KF787415	–	KF787155	KF787351
<i>A. carolynnae</i> Dickson	AH-00-T122	Rawsonville, South Africa		Southern African	–	Fabales	KF787227	KF787497	KF787416	KF787303	KF787156	–
<i>A. lutescens</i> Tite & Dickson	AH-00-T154	Worcester, South Africa		Southern African	–	Fabales	KF787228	KF787498	KF787417	KF787304	KF787157	KF787352
<i>A. margaretae</i> Tite & Dickson	AH-07-T064	Lamberts Bay, South Africa		Southern African	–	–	KF787229	KF787499	KF787418	KF787305	KF787158	KF787353
<i>A. nollothi</i> Tite & Dickson	AH-06-M556	Hondeklip Bay, South Africa		Southern African	<i>Lepisiota</i> (F)	Malvales, Zygophyllales	KF787230	KF787500	KF787419	KF787306	KF787159	KF787354
<i>A. pallida</i> Tite & Dickson	AH-07-C215	Beaufort West, South Africa	Yes: C	Southern African	<i>Lepisiota</i> (F)	Fabales	KF787231	KF787501	KF787420	KF787307	KF787160	–
<i>A. penningtoni</i> Tite & Dickson	AH-07-P536	Durban, South Africa		Southern African	–	–	KF787232	KF787502	KF787421	ε	KF787161	KF787355
<i>A. pierus</i> Cramer	AH-95-Y614	Redhill, South Africa		Southern African	<i>Lepisiota</i> (F)	Fabales, Malvales, Zygophyllales	KF787233	KF787503	KF787422	–	KF787162	KF787356
<i>A. simplex</i> Trimen	AH-04-B549	Witsand Nature Res., South Africa		Southern African	–	–	KF787234	KF787504	KF787423	KF787308	KF787163	KF787357
<i>A. thya</i> Linnaeus (?)	AAM-98-U293	Lamberts Bay, South Africa		Southern African	<i>Lepisiota</i> (F)	Fabales, Malvales	KF787235	KF787505	KF787424	–	KF787164	KF787358

Table 1. Continued

Taxon	Voucher	Locality	Aphytophagy ^a	Range ^b	Ant associate ^c	Host plant ^d	Genbank accession number					
							COI	CAD	EF1 α	GAPDH	H3	Wg
<i>Erikssonia</i> : 3 species worldwide												
<i>E. acraeina</i> Trimen	AJG-07-D583	Zambia		S. African	<i>Lepistota</i> (F)	Fabaceae, Thymelaeaceae						
<i>E. cooksoni</i> Druce	AJG-07-D644	Mundwiji, Zambia		S. African, Zambesian	<i>Lepistota</i> (F)	Malvales						
				Zambesian	–	–						
<i>Aphnaeus</i> : 22 species worldwide												
<i>A. erikssoni mashunae</i>	AH-99-U517	Arcturus, Zimbabwe	Yes	Africa	<i>Crematogaster</i> (M)	Fabaceae, Anacardiaceae, Euphorbiaceae, Lomathaceae, Oleaceae, Sapindaceae						
Stempfler				S. African, Zambesian	<i>Crematogaster</i> (M)	Fabales, Solanales						
<i>A. flavescens</i>	AJG-07-D629	Mosa Hill, Zambia		Zambesian, Somalian	–	–						
Stempfler												
<i>A. marshalli</i> Neave	AH-99-U518	Arcturus, Zimbabwe		Zambesian	–	Fabales						
<i>A. orcas</i> Drury	RD-98-U020	Beni, D.R. Congo		Zambesian, Congolian	–	Fabales, Malpighiales, Santalales						
<i>A. questuaxi</i>	AJG-07-N614	Zambia		Zambesian	–	–						
Aurivillius												
<i>Tytopaedia</i> : 1 species worldwide												
<i>T. sardonyx sardonyx</i> Trimen	NP-99-T463	Gamka Mountain, South Africa		Southern Africa	<i>Crematogaster</i> (M)	Fabaceae, Ebenaceae						
<i>T. s. peringueyi</i> Dickson	AH-97-Y711	Citrusdal, South Africa		Southern African	<i>Crematogaster</i> (M)	Fabales, Rosales						
<i>Phasis</i> : 4 species worldwide												
<i>P. clavum</i> Murray	AH-95-Y643	Calvinia, South Africa		Southern African	<i>Crematogaster</i> (M)	Anacardiaceae, Melianthaceae						
<i>P. pringlei</i> Dickson	AAM-98-W262	Swaarweeberg, South Africa		Southern African	<i>Crematogaster</i> (M)	Geraniales, Sapindales						
<i>P. lthero</i> Linnaeus	AAM-98-U295	Lamberts Bay, South Africa		Southern African	<i>Crematogaster</i> (M)	Geraniales, Sapindales						
<i>Fenisecca tarquinii</i> Fabricius ^f	MWT-96-Y333	Petersham, MA, USA		–	–	–						
<i>Omphalidotos peucetia</i> penningtoni Riley ^f	SJ-97-Y842	Enseleni Nature Res., South Africa		–	–	–						
<i>Simiskina pheretia</i> Hewitson ^f	NP-95-Y140	Pekan, Malaysia		–	–	–						

^aFor genera, a 'yes' indicates that one or more species within the genus exhibit aphytophagy. Form of aphytophagy, where applicable, is signalled by a letter: A, trophallaxis; B, feeds on ant brood; C, feeds on ant eggs; D, presumed aphytophagous, but form is unknown.

^bFor genera, the locations listed are the countries or regions where species of that genus may be found. For species, the range is described by the biogeographical regions of Linder *et al.* (2012).

^cA dash indicates that ant association is unknown for this species.

^dFor genera, a selection of important host-plant families are listed. For species, all recorded host-plant orders are listed. A dash indicates that plant association is unknown for this species.

^eA gene fragment less than 200 bp in length was used for this specimen, because Genbank does not accept fragments of this size, the sequence is included in the supporting information (Table S8).

^fOutgroups: these taxa were not included in the ancestral range reconstruction, and their character states were marked as 'unknown' in the ancestral state reconstructions.

Sources: Ackery *et al.* (1995), Chou (1994), Corbet & Pendlebury (1992), D'Abreu (1986), Gardiner & Terblanche (2010), Heath (1997), Heath *et al.* (2002, 2008), Kroon (1999), Larsen (1991), Lewis (1973), Pierce *et al.* (2002), Pringle *et al.* (1994), Tuzov (1997) and Williams (2012).

Table 2. Summary of the major taxonomic rearrangements of Aphnaeinae.

Swinhoe (1911)	Stempffer (1967)	Heath (1997)	Current genera
Aphnaeus	<i>Pseudaletis</i>	<i>Pseudaletis</i>	<i>Pseudaletis</i>
	<i>Lipaphnaeus</i>	<i>Lipaphnaeus</i>	<i>Lipaphnaeus</i>
	<i>Chloroselas</i>	<i>Chloroselas</i>	<i>Chloroselas</i>
	<i>Desmolycaena</i>		
	<i>Crudaria</i>	<i>Crudaria</i>	<i>Crudaria</i>
	<i>Chrysoiritis</i>	<i>Chrysoiritis</i>	<i>Chrysoiritis</i>
	<i>Poecilmitis</i>		
	<i>Phasis</i>	<i>Phasis</i>	<i>Phasis</i>
		<i>Trimenia</i>	<i>Trimenia</i>
		<i>Argyraspedes</i>	<i>Argyraspedes</i>
		<i>Tylopaedia</i>	<i>Tylopaedia</i>
	<i>Spindasis</i>	<i>Jacksonia</i>	<i>Cesa</i>
		<i>Cigaritis</i>	
		<i>Spindasis</i>	
	<i>Apharitis</i>		
	<i>Zeritis</i>	<i>Zeritis</i>	<i>Zeritis</i>
<i>Axiocerses</i>	<i>Axiocerses</i>	<i>Axiocerses</i>	
<i>Aloeides</i>	<i>Aloeides</i>	<i>Aloeides</i>	
<i>Erikssonina</i>	<i>Erikssonina</i>	<i>Erikssonina</i>	
<i>Aphnaeus</i>	<i>Aphnaeus</i>	<i>Aphnaeus</i>	
<i>Paraphnaeus</i>			

Horizontal rows demonstrate how sets of species changed generic name over time. When *Spindasis* and *Cigaritis* were synonymized, *Cigaritis* was found to be the older name, even though it is not one of Stempffer's genera.

observation). This enormous range in diet – among the most diverse for a group of its size within the Lepidoptera – makes Aphnaeinae of particular interest in analysing factors contributing to life-history evolution and the loss of herbivory. By determining how such aphytophagous, parasitic species and genera are distributed throughout the phylogeny, we can gain information about the evolutionary dynamics of this parasitic behaviour: it allows us to ask does aphytophagy evolve frequently, but fail to survive or diversify across evolutionary time; or do aphytophagous species evolve more rarely, but persist long enough to speciate?

The systematics of this group has been revised several times (Table 2). Distant (1882–1886) proposed the name Aphnaria for a grouping of 24 diverse lycaenid genera based upon similarities in wing venation. This grouping remained unchanged until Swinhoe (1911) erected the subfamily Aphnaeinae based on specific morphological characters. Swinhoe made no mention of Distant or the 24 genera placed in Aphnaria; instead he proposed only one genus with 27 species, *Aphnaeus* Hübner, in his subfamily, having subsumed *Cigaritis*, *Spindasis* Wallengren and *Amblypodia* Westwood under *Aphnaeus*.

Swinhoe's one-genus subfamily remained unchanged until Stempffer (1967) proposed the adoption of 16 genera within it, namely: *Aphnaeus*, *Paraphnaeus* Thierry Mieg, *Apharitis* Riley, *Spindasis*, *Lipaphnaeus* Aurivillius, *Chloroselas* Butler, *Zeritis* Boisduval, *Desmolycaena* Trimen, *Axiocerses* Hübner, *Phasis* Hübner, *Aloeides* Hübner, *Poecilmitis* Butler, *Chrysoiritis*

Butler, *Crudaria* Wallengren, *Erikssonina* Trimen and *Pseudaletis* Druce. Stempffer's selection was based on adult morphological characters, particularly the male genitalia.

Eliot (1973) relegated this same group of genera to tribal level within Theclinae, separating *Pseudaletis* into one section within the tribe and the remainder into an *Aphnaeus* section. During the following decade several new genera were added to the tribe.

Heath (1997) reviewed the Aphnaeini based on morphological characters, and abandoned Eliot's two sections, although he considered *Pseudaletis* to be sister to the remaining genera. He did not provide a formal phylogenetic analysis as part of his revision, but arranged the taxa in his systematic classification to reflect what he considered to be the most likely evolutionary relationships among groups. He also synonymized several of the existing genera as follows: *Apharitis* with *Spindasis*, and *Poecilmitis* (as well as two additional genera erected since Eliot's revision – *Bowkeria* Quickeburge and *Oxychaeta* Tite & Dickson) with *Chrysoiritis*, and *Argyrocupha* Tite & Dickson with *Trimenia* Tite & Dickson. He proposed several new combinations by shifting all but one of the species within *Desmolycaena* into the genus *Chloroselas* and creating a new genus, *Vansomerenia* Heath, for the remaining species. In a later work, Heath *et al.* (2002) synonymized the genus *Spindasis* with *Cigaritis*. The resulting genera were thus: *Pseudaletis*, *Lipaphnaeus*, *Chloroselas*, *Vansomerenia*, *Jacksonia* Heath, *Crudaria*, *Chrysoiritis*, *Trimenia*, *Argyraspedes* Tite & Dickson, *Cigaritis*, *Zeritis*, *Axiocerses*, *Aloeides*, *Erikssonina*, *Aphnaeus*, *Tylopaedia* Tite & Dickson and *Phasis*. *Jacksonia* was subsequently renamed *Cesa* Seven after it was found to be a previously occupied name.

With respect to higher level relationships, Eliot (1973) had difficulty placing Aphnaeini within the Theclinae, originally assigning it to a somewhat derived position near the Iolaini and Cheritriini, but he later placed it – along with Polyommataini and Lycaenini – as sister to Theclinae. More specialized (and presumably derived) characters that made initial diagnosis of this group difficult include the hind wing shape, which is often tailed and lobed; the absence of a precostal vein on the hind wing; absence of tibial spurs; and an ungriddled pupa. Instead of the male fore tarsus being segmented with double claws, it is fused into a single segment ending in a single claw (except for *Aphnaeus* where it is blunted). Eliot also remarked upon the extraordinary tufts of highly specialized scales found at the tip of the female abdomen (1973). These are found in certain genera of the aphnaeines and several genera of the Hesperidae, in which he presumed they must have evolved convergently. Some of these scales adhere to the freshly laid egg, appearing to serve as camouflage for the egg. Newly hatched larvae of some species have been observed to consume these scales, presumably gaining some benefit from doing so (Heath, 1997). The tribe Aphnaeini was later placed in subfamily Lycaeninae by Scott (1985). We refer to the clade as a subfamily here in light of a forthcoming phylogeny of the Lycaenoidea which recovers the aphnaeines as a basally branching clade along with other established subfamilies (N.E. Pierce *et al.*, in preparation).

Apart from a phylogenetic study of the genus *Chrysoiritis* which used several outgroups from other aphnaeine genera

(Rand *et al.*, 2000), no work has been done to elucidate the relationships within Aphnaeinae using molecular characters. Using data from mitochondrial DNA, Rand and his colleagues found *Aloeides* + *Argyraspodes* to be weakly supported sister genera, with *Trimenia* sister to that clade. Sister to *Trimenia* + (*Aloeides* + *Argyraspodes*) was the grouping *Tylopaedia* + *Phasis*. The relationships among these, *Chrysoritis* and *Crudaria* were not determined.

Phylogenetic analyses are important prerequisites for understanding the evolution of life-history variation, and the Aphnaeinae are of particular value in this regard because of the remarkable variability among species in both feeding strategy and ant association. The focus of this study is to use molecular characters from both mitochondrial and nuclear markers to infer relationships within and among the main lineages of Aphnaeinae, providing a rigorous framework for further exploration of life-history evolution. We also make the first contribution toward such exploration by using ancestral state reconstructions to infer biogeographical history and the evolution of host plant associations in the group.

Materials and methods

Taxon sampling

Samples studied here included 79 ingroup taxa representing 15 of the 17 genera recognized in Heath's review of the aphnaeines (Heath, 1997; Heath *et al.*, 2002). Unfortunately, no specimens of the rare monotypic genera *Vansomerenia* and *Cesa* could be obtained. Three taxa from the closely related lycaenid subfamilies Poritiinae and Miletinae (*sensu* Eliot, 1973) were used as outgroups. Specimens were collected fresh into 90–100% ethanol and stored at –20 or –80°C prior to DNA extraction. Wings were kept separately in glassine envelopes, and vouchers of all samples are deposited in the DNA and Tissues collection of the Museum of Comparative Zoology.

Molecular protocols

DNA was extracted from butterfly legs or thoracic tissue using a Qiagen DNEasy Blood and Tissue Kit (Qiagen, Inc., Valencia, CA, U.S.A.). Fragments were amplified from a mitochondrial gene, *cytochrome oxidase I (COI)*, and five nuclear gene regions, *histone 3 (H3)*, *elongation factor 1 alpha (EF1 α)*, *wingless (wg)*, *glyceraldehyde-3-phosphate dehydrogenase (GAPDH)* and *carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD)*. These mitochondrial and nuclear genes were selected due to their utility in reconstructing the phylogeny of other insect groups of similar age and diversity (e.g. Wahlberg *et al.*, 2005; Vila *et al.*, 2011; Talavera *et al.*, 2013).

Amplifications were done by standard polymerase chain reactions (PCR) mostly using published primers (Table S1). For each sample, 25- μ L reactions were subjected to a 3 min initial denaturation at 94°, then cycled through a 50 s denaturation at 94°,

followed by a variable annealing phase (Table S2), and an 80 s extension phase at 72°. The last cycle was followed by a final extension phase of 5 min at 72°.

PCR products were purified by adding 1.0 μ L Antarctic Phosphatase, 1.0 μ L Antarctic Phosphatase buffer and 0.6 μ L Exonuclease I (New England Biolabs, Ipswich, MA, U.S.A.) and incubating at 37°C for 35 min followed by 20 min at 80°C. Samples were then amplified for sequencing using BigDye chemistry v3.1 (Applied Biosystems, Foster City, CA, U.S.A.). Amplified fragments were sequenced in both directions on an Applied Biosystems 3130xl Genetic Analyzer using specified reaction conditions (Table S3).

Phylogenetic analyses

Sequences were edited in Sequencher 4.8 (Gene Codes, Ann Arbor, MI, U.S.A.) and manually aligned. No indels were found in any of the six markers.

Phylogenetic analyses were performed using Maximum Parsimony, Maximum Likelihood and Bayesian Inference-based methods. Bayesian inference was performed using MrBayes v3.2 (Ronquist & Huelsenbeck, 2003). Molecular data were partitioned as in the maximum likelihood analysis (below). For each partition, a model was chosen using jModelTest (Table S4), using that program's Akaike information criterion method to select the highest-weighted model that is provided for in MrBayes (Posada, 2008). We used two runs, each with four chains, and ran the analysis for 25 million generations, sampling every 10 000 generations. Likelihoods were viewed using Tracer v1.5.0 (Rambaut & Drummond, 2007) and a burn-in set at 1 million generations before summarizing the sampled trees.

We also performed a parsimony analysis in TNT v1.1 (Goloboff *et al.*, 2008). The data included concatenated sequences of all six markers. We used a New Technology Search with Sectorial Search and Tree Fusing, driving the search until a minimum length had been found 100 times. We then made a strict consensus tree of all the most parsimonious trees and calculated nodal support with 1000 standard bootstraps.

Maximum likelihood analysis was performed using RAxML v7.7.5 (Stamatakis, 2006; Stamatakis *et al.*, 2008). The data were partitioned by first, second or third codon position for each of the six markers, for a total of 18 partitions. RAxML determined the tree with the highest likelihood using a general time-reversible model with the Gamma model of rate heterogeneity and an estimated proportion of invariant sites, and performed a rapid bootstrap analysis with 100 bootstraps.

Ancestral range reconstruction

The *chronopl* function in the ape package in R (Paradis *et al.*, 2004) was used to produce an ultrametric version of the summary tree produced by MrBayes, setting lambda = 0, and removing the three outgroup taxa before transforming the tree. Ancestral ranges were estimated on this tree using LAGRANGE v20130526 (Ree & Smith, 2008). Each species was coded as

belonging to one or more of four of the biogeographical regions of sub-Saharan Africa as defined in Linder *et al.* (2012; we provide an illustration in Fig. 2), and/or as having a range outside of sub-Saharan Africa (see Table 1). The Southern African region was set as nonadjacent to the Somalian region and to the non-African region; all other possible area combinations were permitted.

Ancestral state reconstruction

We used BayesTraits v1.3 to estimate ancestral ant associates and host plants (Pagel *et al.*, 2004). Species were coded as associating with one or more of six genera of ants, and with one or more of 18 orders of host plants (Table S5). The reversible-jump MCMC algorithm of the BayesMultiStates model was used along with an ultrametric phylogeny (transformed as for LAGRANGE, but with the outgroup taxa included) and priors chosen to produce an acceptance rate around 30%. For the analysis of ant associates, the *ratedev* parameter was set to 1.2 and a reversible-jump hyperprior set to *exp 0 30*. For the analysis of host plants, the same hyperprior was used with a *ratedev* parameter of 0.103. The analysis was run for 5.05 million generations, sampling every hundred generations after a 50 000 generation burn-in.

Results and discussion

Phylogenetic hypotheses of Aphnaeinae and Systematics

Monophyly of genera. With one exception, the genera described in Heath's (1997) organization of the aphnaeinae were recovered as monophyletic with strong support in all three analyses, although only a single representative of the genus *Zeritis* was included in the phylogeny (see Fig. 1; Figures S1 and S2). The single exception was *Aloeides*. In his 1997 review, Heath suggested that *Aloeides* and *Erikssonina* may be congeneric, although he did not find sufficient reason to synonymize them. Our analysis is similarly ambiguous as to the relationship between the two genera. *Aloeides* was recovered as monophyletic, but the support for this arrangement was weak in the maximum likelihood and Bayesian analyses. Based on our data, we cannot rule out the possibility that *Erikssonina*, although a monophyletic group itself, is nested within *Aloeides*. Additional markers and/or sample taxa will be needed to confirm that the two groups are indeed reciprocally monophyletic.

Our analysis also provides support for several earlier synonymizations. When Riley (1925) erected the genus *Apharitis*, he noted that it differed from *Spindasis* only in its coloration; he acknowledged that structurally – in venation and other characters – the genera were similar. Stempffer described and illustrated all the structural features in both genera and provided no distinguishing characteristics (1967). Heath illustrated the male genitalia of *Cigaritis*, *Spindasis* and *Apharitis*, showing all three to be of the same type and arguing that their wing markings were also of a common pattern. He synonymized *Apharitis* with

Spindasis on structural grounds, considering coloration to be an insufficient basis for retaining *Apharitis* (1997). In a later publication, Heath *et al.* (2002) synonymized *Spindasis* with *Cigaritis* for similar reasons. Our analysis provides support for this, as *Cigaritis epargyros*, a representative of the former genus *Apharitis*, is found nested within the rest of the genus *Cigaritis*. Heath (1997) also synonymized the genus *Argyracupha* with *Trimenia*. He found the male genitalia, the wing markings and other characteristics to be similar, differing only in the shape of the male hind wing. In this molecular phylogeny, *T. malagrida* Wallengren (formerly *Argyrocupha*) is closely related to other (original) *Trimenia* species.

We present here the Bayesian phylogenetic hypothesis, which we used for ancestral range and state reconstructions. The maximum-likelihood and Bayesian trees recover the same well-supported relationships among genera, although with a different branching order at one weakly-supported node, and with minor differences in branch length and node support throughout the tree (Fig. 1; Figure S1). The parsimony tree (Figure S2) differs in several key respects from the likelihood-based methods, but many taxa show the same relationships under all three methods. The most important difference among the trees is that parsimony recovers *Aphnaeus* as sister to the rest of the subfamily, and *Axiocerses* + *Zeritis* as sister to the remainder, although these clades are nested within one of two larger clades in the Bayesian and likelihood analyses. These three genera have among the longest branch lengths of any genera in the Bayesian and likelihood analyses. The different arrangement of the parsimony tree may be influenced by long branch attraction, and so we focus here on the Bayesian tree.

The six genera *Pseudaletis*, *Chrysoritis*, *Crudaria*, *Cigaritis*, *Chloroselas* and *Lipaphnaeus* form a single clade (marked Clade J on Fig. 2). These data support Heath's (1997) decision to abandon Eliot's (1973) placement of *Pseudaletis* in a section separated from the other genera. *Pseudaletis* is sister to the other five genera, and *Chrysoritis* sister to the remaining four. The relationships among the remaining four genera are not well supported. The Bayesian phylogeny places *Crudaria* + *Cigaritis* sister to *Chloroselas* + *Lipaphnaeus*, but the only well-supported relationship is *Lipaphnaeus* + *Chloroselas* forming a single clade. This *Lipaphnaeus* + *Chloroselas* clade is also supported by similarities of the genitalia, as is the grouping of those two genera with *Pseudaletis* and *Crudaria* (Heath, 1997). Heath also grouped these four genera with *Chrysoritis*, as is seen in our phylogenies. Although he did not include *Cigaritis*, he did include the monotypic genera *Vansomerenia* and *Cesa* in this grouping.

Clade J is sister to the remaining lineages, marked as Clade B. Among these, *Tylopaedia* + *Phasis* is sister to the rest of Clade B, but with poor support. Within this clade, *Aphnaeus* is sister to *Zeritis* + *Axiocerses*, *Trimenia* + *Argyraspodes* + (*Aloeides* + *Erikssonina*) form a single clade, and *Trimenia* is sister to *Argyraspodes* + *Aloeides/Erikssonina*. However, there is no strong support for the relationships between these groupings. This clade was not predicted by the morphological analyses of either Eliot (1973) or Heath (1997), but was recovered by Rand *et al.* (2000), with low

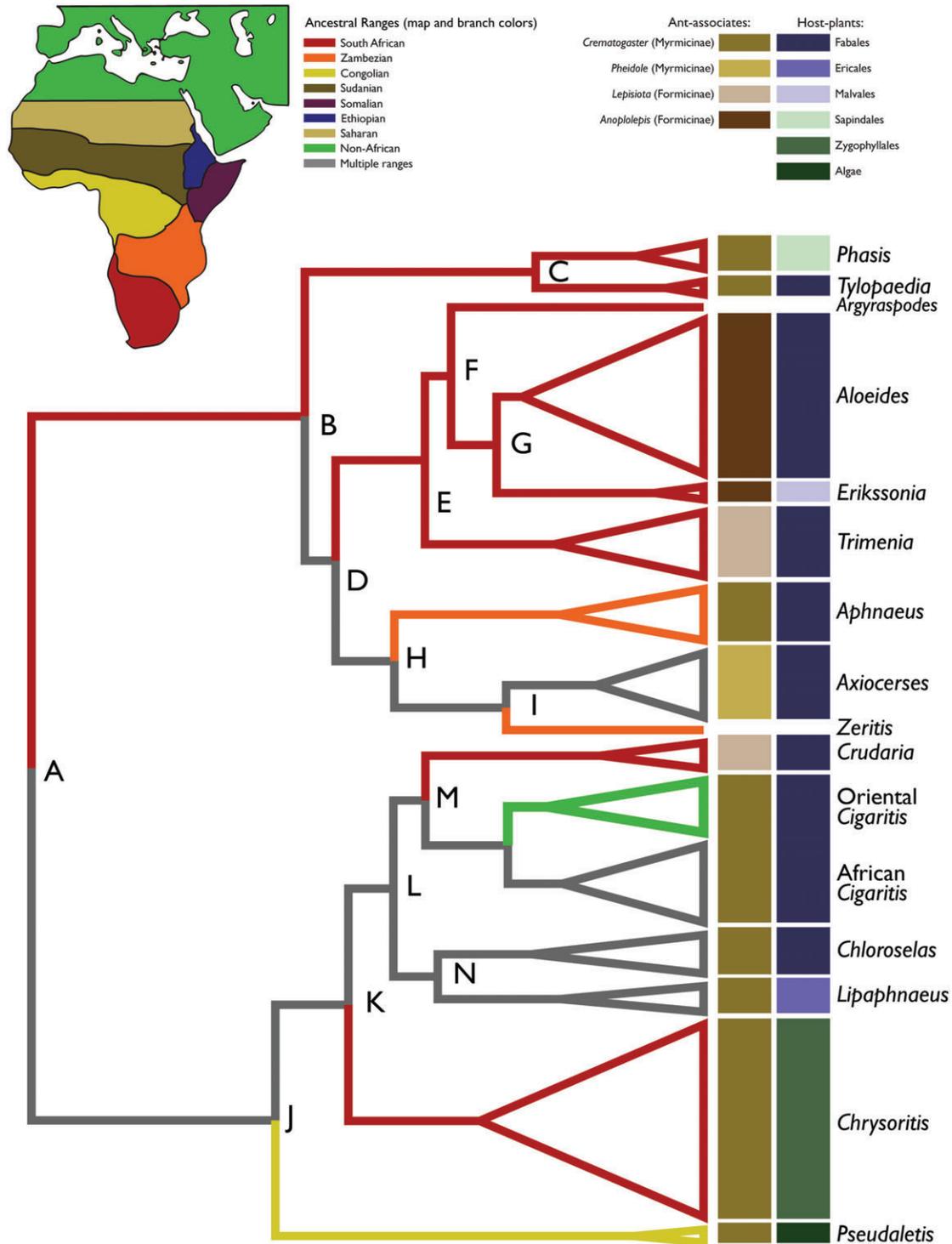


Fig. 2. Life-history evolution of Aphnaeinae. The colour of the first block to the right of each butterfly genus indicates which ant genus had the highest probability of association with the common ancestor of the given aphnaeine genus, as determined by the BayesTraits reconstruction. The colour of the second block indicates the order of host plant BayesTraits assigned as most likely to have associated with the common ancestor of the same aphnaeine genus. The colour of each branch indicates the most plausible ancestral range along that branch. The inset map of Africa (after Linder *et al.*, 2012) shows the boundaries of each region. Note that aphnaeines are not found in several regions of Africa. Grey branch lines indicate that LAGRANGE recovered multiple zones with a >0.2 probability that the range of the common ancestor contained at least that zone. For specific detail regarding each node, see Tables S5–S7.

support. Within the group, the clades of *Tylopaedia* + *Phasis* and *Zeritis* + *Axiocerses* are recovered with strong support. Genitalic similarities further support *Zeritis* + *Axiocerses*, and argue for the clustering of *Trimenia* and *Argyraspodes* (Heath, 1997); the clade of *Tylopaedia* + *Phasis* was also recovered by Rand *et al.* (2000).

Ancestral range reconstruction

LAGRANGE provided evidence for a Southern African distribution of the ancestor of the taxon labelled Clade B in Fig. 2, with a 0.81 probability that the ancestral distribution included Southern Africa, and a 0.21 probability of ancestors distributed outside Southern Africa (Fig. 2 and Table S5).

There was less evidence for the distribution of the ancestor of the Clade J. The probability that the ancestral distribution included at least Southern Africa was 0.64, and the probability that it included at least the Congolian region was 0.55. The most likely single possibility was a strictly Southern African distribution, with a probability of 0.21.

LAGRANGE thus provides evidence for a probable Southern African origin of Aphnaeinae, with the possibility of a more extensive ancestral distribution. Aphnaeine butterflies appear to have radiated outside Africa only once, because the Oriental species of *Cigaritis* appear to form a single radiation. A few other species have also independently colonized Arabia, including *Chloroselas arabica* Riley, *C. esmerelda* Butler (Larsen, 1991), and *Axiocerses harpax* Fabricius. However, the Southern African origin – as well as the ancestral ranges at internal nodes of the Aphnaeinae phylogeny – is not well supported. Our sampled taxa represent about a quarter of the overall aphnaeine diversity (79 of 278 species, 28%), and even less in some important genera, such as *Cigaritis* (12 of 71, 17%) or *Pseudaletis* (2 of 24, 8%). Increased taxon sampling will be necessary to uncover more about the history of the apparent single colonization of Asia by *Cigaritis* and to clarify the within-Africa evolutionary history of the rest of the subfamily. However, it is also possible that Linder *et al.*'s African regions (2012) are simply too restricted in area to produce a strong signal among relatively mobile insects, whose ancestral range history could conceivably include many expansions, contractions, and reintroductions.

Ancestral state reconstruction

Ant associations. Almost all aphnaeine butterflies associate with ants: ant association has been recorded in 14 of the 17 genera, and life histories are still unknown for the remaining three, *Zeritis* and the monotypic genera *Argyraspodes* and *Cesa*. All aphnaeines with known life histories (approximately one third of the total) interact in some way with ants, and these associations are obligate in 97% of cases (Pierce *et al.*, 2002).

As to which ants have been the associates of Aphaenini, BayesTraits predicted that the aphnaeine common ancestor associated with *Crematogaster* Lund (Formicidae: Myrmicinae) species ($P = 0.70$). The most likely scenario for ant association

is an association with *Crematogaster* in the common ancestor, with a few subsequent switches of ant associate: (i) to *Pheidole* Westwood (also a myrmicine) by the common ancestor of *Zeritis* + *Axiocerses*; and (ii) to *Anoplolepis* Santschi (Formicinae) by the common ancestor of the genus *Crudaria*. In addition, the common ancestor of Clade E likely associated with one of the formicine genera *Lepisiota* Santschi ($P = 0.51$) or *Anoplolepis* ($P = 0.27$). Either of these species may have been the associates of the common ancestor of Clade D ($P = 0.31$ and 0.14, respectively), with a subsequent return to association with *Crematogaster* by the common ancestor of Clade H ($P = 0.41$); however, there is little support for any particular scenario in this part of the tree. The common ancestors of all other genera were most likely to have associated with *Crematogaster* (Table S6).

As with the ancestral range reconstructions, missing life-history data prevent us from drawing strong conclusions regarding the overall frequency of ant switching and its effect on the evolution of the group.

Plant associations. The evolution of host plant use is less clear. Most species in Aphnaeinae feed and/or oviposit on host plants in the order Fabales, and this trait was likely shared by the aphnaeine common ancestor ($P = 0.66$). As shown in Table S7, the most likely host plant order was Fabales for most common ancestors at the genus-level and higher, with the exceptions of *Phasis* (Sapindales/Geraniales, $P = 0.51/0.46$), *Phasis* + *Tylopaedia* (Sapindales/Geraniales, $P = 0.24/0.20$), *Erikssonsonia* (Malvales, $P = 0.76$), *Pseudaletis* (algae, $P = 0.96$), *Chrysoritis* (Zygophyllales, $P = 0.53$), and *Lipaphnaeus* (Ericales, $P = 0.45$). However, support for Fabales is weak in many of the internal branches, possibly because BayesTraits requires internal nodes to have a single character state. Low support values for any single character state could be a result of ancestral aphnaeines feeding on host plants from multiple orders. This is a trait certainly observed in modern species, for aphnaeines consume a broad diversity of host plants outside of Fabales. Life-history records include host plants from at least 23 families in 19 orders, 16 of which are represented in our ingroup taxa (Table 1). Species in the larger genera, such as *Aloeides*, *Chrysoritis* and *Cigaritis*, all associate with several other plant orders, and several individual species have been recorded to feed on four or more orders of host plants. This great diversity of host plants, as well as limited life-history information [host plant is unknown for 20 (25%) of our ingroup taxa] and limited taxon sampling, makes interpreting the evolutionary history of plant associations extremely difficult.

Aphytophagy

A strikingly high proportion of the genera of Aphnaeinae have species recorded as parasites of ants, either eating ant eggs and brood in the nest, or inducing trophallaxis from the ant workers ('cuckoo feeding'). This specialization on ants is in contrast to the closely related Miletinae. All of the some 120 species of miletines are aphytophagous, but the majority feed on insects associated with ants, such as aphids and scale insects,

and a smaller proportion feed on the ants themselves (Pierce *et al.*, 2002). Although aphytophagy is widespread across the Lycaenidae and Riodinidae – almost certainly as a consequence of larval associations with ants – parasitic and predaceous life histories appear to be more common in at least two of the earlier-branching lineages of the Lycaenidae, the Miletinae and Aphnaeinae. Life histories of the third early branching lineage, the Poritiinae, are still too sparse to assess. Despite its representation amongst these older lineages, this strategy does not appear to be particularly persistent over evolutionary time, as suggested by the ‘tippy’ distribution of aphytophagous species across the phylogeny of Aphnaeinae (Fig. 2) as well as throughout the Lycaenoidea as a whole (Pierce *et al.*, 2002).

Conclusion

A phylogenetic hypothesis based on molecular characters supports the classification of Heath (1997) based on morphological analysis, including the monophyly of most of the aphnaeine genera. The phylogeny also underscores the great variety of life histories within the Aphnaeinae, with many species having complex larval ant associations and diet and host-plant preferences. Plant feeding appears to have been lost multiple times within the group, typically by individual species within otherwise phytophagous genera. As we discover more information about the life histories of these butterflies, this phylogeny will provide the framework necessary to explore how ant association and loss of phytophagy may have affected subsequent diversification in the Aphnaeinae and the Lycaenidae as a whole.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12098

Figure S1. Phylogenetic hypothesis of Aphnaeinae: maximum likelihood. Nodes are labelled with bootstrap support.

Figure S2. Phylogenetic hypothesis of Aphnaeinae: maximum parsimony. Nodes are labelled with bootstrap support, with unlabelled nodes indicating less than 50% bootstrap support.

Table S1. Genes and primers used in molecular analyses.

Table S2. Annealing conditions used in PCR.

Table S3. Thermocycling program for BigDye reactions.

Table S4. Substitution models used in Bayesian Inference.

Table S5. Ancestral range reconstruction information for internal nodes (as marked in Fig. 2) above the genus level, and the first node of *Cigaritis*, which separates African from non-African species.

Table S6. Ant associate ancestral state reconstruction information for common ancestors of each genus and other interior nodes marked in Fig. 2.

Table S7. Host-plant ancestral state reconstruction information for common ancestors of each genus and other interior nodes marked in Fig. 2.

Acknowledgements

We thank A.J. Berry, J.C. Choe, S.C. Collins, C. Congdon, R. Ducarme, A.J. Gardiner, S. Joubert, N.P. Kandul, T.B. Larsen, D.J. Martins, A.A. Mignault, A. Plowes, S.P. Quek, M.W. Tan, S.E. Woodhall and the late I. Bampton for providing material and field assistance. We are grateful to a number of Nature Conservation bodies for permission to collect specimens used in this study, particularly those of the Western Cape and Northern Cape Provinces of South Africa. We took advantage of the Odyssey cluster for many of our computations, supported by the FAS Science Division Research Computing Group at Harvard University, and of the Willi Hennig Society for the use of TNT. J.H.B. was supported by a Smith Family Graduate Fellowship from the Department of Organismic and Evolutionary Biology of Harvard University; Z.A.K. was supported by an NSF Graduate Research Fellowship. T.R.S. was supported by a Radcliffe Research Partnership Grant and a Research Experiences for Undergraduates Fellowship from the National Science Foundation; M.E. was supported by a postdoctoral fellowship from the Research Council of Norway. This research was supported by grants from the Baker Foundation, the Putnam Expeditionary Fund of the Museum of Comparative Zoology, the Templeton Foundation (FQEB), and NSF DEB-0447242 to N.E.P.

References

- Ackery, P.R., Smith, C.R. & Vane-Wright, R.I. (1995) *Carcasson's African Butterflies: An Annotated Catalogue of the Papilionoidea and Hesperioidea of the Afrotropical Region*. CSIRO, Canberra.
- Bogdanowicz, S.M., Wallner, W.E., Bell, J., Odell, T.M. & Harrison, R.G. (1993) Asian Gypsy Moths (Lepidoptera: Lymantriidae) in North America: evidence from molecular data. *Annals of the Entomological Society of America*, **86**, 710–715.
- Brower, A.V.Z. & DeSalle, R. (1998) Patterns of mitochondrial versus nuclear DNA sequence divergence among nymphalid butterflies: the utility of *wingless* as a source of characters for phylogenetic inference. *Insect Molecular Biology*, **7**, 73–82.
- Cho, S., Mitchell, A., Regier, J.C., Mitter, C., Poole, R.W., Freidlander, T.P. & Zhao, S. (1995) A highly conserved nuclear gene for low-level phylogenetics: elongation factor-1 alpha recovers morphology-based tree for heliothine moths. *Molecular Biology and Evolution*, **12**, 650–656.
- Chou, I. (ed) (1994) *Monographia rhopalocerorum Sinensium*, Vol. 2. Henan Science and Technology Publishing House, Zhengzhou.
- Colgan, D.J., McLauchlan, A., Wilson, G.D.F. *et al.* (1998) Histone H3 and U2 snRNA DNA sequences and arthropod molecular evolution. *Australian Journal of Zoology*, **46**, 419–437.
- Corbet, A.S. & Pendlebury, H.M. (1992) *The Butterflies of the Malay Peninsula*. United Selangor Press, Kuala Lumpur.
- D'Abrera, B. (1986) *Butterflies of the Oriental Region: Lycaenidae & Riodinidae*. Hill House Publishers, Melbourne.
- Distant, W.L. (1882–1886) *Rhopalocera Malayana: A Description of the Butterflies of the Malay Peninsula*. West, Newman, London.

- Eliot, J.N. (1973) The higher classification of the Lycaenidae (Lepidoptera): a tentative arrangement. *Bulletin of the British Museum (Natural History) Entomology*, **28**, 371–505.
- Folmer, O., Black, M., Hoeh, W., Lutz, R. & Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome *c* oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294–299.
- Gardiner, A.J. & Terblanche, R.F. (2010) Taxonomy, biology, biogeography, evolution and conservation of the genus *Erikssonia* Trimen (Lepidoptera: Lycaenidae). *African Entomology*, **18**, 171–191.
- Goloboff, P.A., Farris, J.C. & Nixon, H.C. (2008) TNT, a free program for phylogenetic analysis. *Cladistics*, **24**, 774–786.
- Heath, A. (1997) A review of African genera of the tribe Aphnaeini (Lepidoptera: Lycaenidae). *Metamorphosis*, **8**(Suppl. 2), 1–60.
- Heath, A., Newport, M.A. & Hancock, D. (2002) *Butterflies of Zambia*. Lepidopterists' Society of Africa & A.B.R.I., Nairobi, Kenya.
- Heath, A., McLeod, L., Kaliszewska, Z.A., Fisher, C.W.S. & Cornwall, M. (2008) Field notes including a summary of trophic and ant-associations for the butterfly genera *Chrysoritis* Butler, *Aloeides* Butler and *Thestor* Hubner (Lepidoptera: Lycaenidae) from South Africa. *Metamorphosis*, **19**, 127–148.
- Kroon, D.M. (1999) *Lepidoptera of Southern Africa: Host-plants & Other Associations: A Catalogue*. Lepidopterists' Society of Africa, Jukskei Park, South Africa.
- Larsen, T.B. (1991) *The Butterflies of Kenya and their Natural History*. Oxford University Press, New York, NY.
- Lewis, H.L. (1973) *Butterflies of the World*. Follett Publishing Co., Chicago, IL.
- Linder, H.P., de Klerk, H.M., Born, J., Burgess, N.D., Fjeldsa, J. & Rahbek, C. (2012) The partitioning of Africa: statistically defined biogeographical regions in sub-Saharan Africa. *Journal of Biogeography*, **39**, 1189–1205.
- Monteiro, A. & Pierce, N.E. (2001) Phylogeny of *Bicyclus* (Lepidoptera: Nymphalidae) inferred from *COI*, *COII* and *EF-1alpha* gene sequences. *Molecular Phylogenetics and Evolution*, **18**, 264–281.
- Pagel, M., Meade, A. & Barker, D. (2004) Bayesian estimation of ancestral character states on phylogenies. *Systematic Biology*, **53**, 673–684.
- Paradis, E., Claude, J. & Strimmer, K. (2004) APE: analysis of phylogenetics and evolution in R language. *Bioinformatics*, **20**, 289–290.
- Pierce, N.E., Braby, M.F., Heath, A., Lohman, D.J., Mathew, J., Rand, D.B. & Travassos, M.A. (2002) The ecology and evolution of ant association in the Lycaenidae (Lepidoptera). *Annual Review of Entomology*, **47**, 733–771.
- Posada, D. (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253–1256.
- Pringle, E.L., Henning, G.A. & Ball, J.B. (1994) *Pennington's Butterflies of Southern Africa*, 2nd edn. Struik Winchester, Cape Town.
- Rambaut, A. & Drummond, A.J. (2007) *Tracer v1.5* [WWW document]. URL <http://beast.bio.ed.ac.uk/Tracer> [accessed on 31 August 2011].
- Rand, D.B., Heath, A., Suderman, T. & Pierce, N.E. (2000) Phylogeny and life history evolution of the genus *Chrysoritis* within the Aphnaeini (Lepidoptera: Lycaenidae), inferred from mitochondrial cytochrome oxidase I sequences. *Molecular Phylogenetics and Evolution*, **17**, 85–96.
- Ree, R.H. & Smith, S.A. (2008) Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology*, **57**, 4–14.
- Riley, N.D. (1925) The species usually referred to the genus *Cigariis* Boisd. [Lepidoptera: Lycaenidae]. *Novitates Zoologicae*, **32**, 70–95.
- Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.
- Scott, J.A. (1985) The phylogeny of butterflies (Papilionoidea and Hesperioidea). *Journal of Research on the Lepidoptera*, **23**, 241–281.
- Stamatakis, A. (2006) RAXML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**, 2688–2690.
- Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A rapid bootstrap algorithm for the RAXML web servers. *Systematic Biology*, **57**, 758–771.
- Stempffer, H. (1967) The genera of the African Lycaenidae (Lepidoptera: Rhopalocera). *Bulletin of the British Museum (Natural History) Entomology*, (Suppl. 10), 1–322.
- Swinhoe, C. (1911) *Lepidoptera Indica*, Vol. 9. Lowell Reeve & Co., London.
- Talavera, G., Lukhtanov, V.A., Pierce, N.E. & Vila, R. (2013) Establishing criteria for higher-level classification using the systematics of *Polyommatus* blue butterflies (Lepidoptera: Lycaenidae). *Cladistics*, **29**, 166–192.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011) MEGA5: molecular evolutionary genetic analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, **28**, 2731–2739.
- Tuzov, V.K. (1997) *Guide to the Butterflies of Russia and Adjacent Territories: Lepidoptera, Rhopalocera*. Pensoft, Sofia.
- Vila, R., Bell, C.D., Macniven, R. et al. (2011) Phylogeny and palaeoecology of *Polyommatus* blue butterflies show Beringia was a climate-regulated gateway to the New World. *Proceedings of the Royal Society B*, **278**, 2737–2744.
- Wahlberg, N., Braby, M.F., Brower, A.V.Z. et al. (2005) Synergistic effects of combining morphological and molecular data in resolving the phylogeny of butterflies and skippers. *Proceedings of the Royal Society B*, **272**, 1577–1586.
- Williams, M.C. (2012) *Afrotropical Butterflies and Skippers – A Digital Encyclopaedia*. [WWW document]. URL <http://atbutterflies.com> [accessed on 6 July 2012].

Accepted 1 July 2014

First published online 20 October 2014