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Phylogeny and Life History Evolution of the Genus *Chrysoritis* within the Aphnaeini (Lepidoptera: Lycaenidae), Inferred from Mitochondrial *cytochrome oxidase I* Sequences

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Phylogenetic relationships among 26 South African species in the tribe Aphnaeini (Lepidoptera: Lycaenidae) were inferred from DNA characters of the mitochondrial gene *cytochrome oxidase I* (*COI*), using maximum-parsimony methods. The resulting phylogenetic estimate supports the systematic hypothesis made by Heath (1997, *Metamorphosis,* supplement 2), based on morphological characters, that at least three preexisting genera (*Chrysoritis, Poecilmitis,* and *Oxychaeta*) should be collapsed into the single monophyletic genus *Chrysoritis.* Two of the species groups described by Heath within *Chrysoritis* are also monophyletic, while one is paraphyletic and thus unsupported by the molecular data. Strong node support and skewed transition/transversion ratios suggest that two *Chrysoritis* clades contain synonymous species. Aphytophagy appears as a derived feeding strategy. Evolutionary patterns of ant association indicate lability at the level of ant genus, while association with different ant subfamilies may have played an ancestral and chemically mediated role in the diversification of South African aphnaeines. © 2000 Academic Press

Key Words: **taxonomy; *Chrysoritis;* Lycaenidae; transition/transversion ratios; host plant association; ant association; myrmecophily.**

INTRODUCTION

South African butterflies in the tribe Aphnaeini (Lepidoptera: Lycaenidae) provide a model system for systematic studies and comparative life history analysis. The group's component species occupy several habitat types throughout South Africa, covering a wide range of altitudes and plant communities. Like most lycaenids, these aphnaeines exhibit specialized relationships not only with their host plants but also with attendant ants. Most of these ant relationships appear to be mutualistic, with the butterfly larvae offering a nutritious secretion to the workers in return for protection from predators and parasitoids (reviewed in Fiedler, 1991; Pierce, 1987). The larvae feed on host plants from several families, although a few aphnaeine species have relinquished herbivory in favor of exploiting ants. These larvae induce trophallaxis from their ant associates, surviving exclusively on mouth-to-mouth feedings (Cottrell, 1984; Pierce, 1995).

Phylogenetic analysis offers a means of exploring such complex systems by generating evolutionary hypotheses inferred from molecular sequences. Phylogenetic estimates of species divergence can provide important insights into questions regarding taxonomy, biogeography, and coevolution. Heath (1997) revised the taxonomy of the South African Aphnaeini based on morphological characters, primarily of wing patterns and genitalic features. Here, Heath's systematic hypotheses are compared with the phylogenetic estimates derived from molecular sequences of the mitochondrial gene *cytochrome oxidase I (COI),* which has proven effective in resolving insect relationships at taxonomic levels comparable to those within the Aphnaeini (Crozier and Crozier, 1993; Brower, 1994a,b, 1996; Pierce and Nash, 1999). The implications of the molecular phylogeny for life history evolution within this tribe are also examined.

In his revision of the South African Aphnaeini, Heath (1997) made detailed morphological comparisons in order to reorganize the tribe's genus-level taxonomy. The following conclusions are most relevant to this study:

(i) The monotypic genus *Argyrocupha* was brought within the genus *Trimenia,* based on genitalic uniformity between the two taxa.

(ii) Based on similarities of wing pattern, genitalic features, and larval morphology, four genera—*Chrysoritis, Poecilmitis, Oxychaeta,* and *Bowkeria*—were

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TABLE 1

Species Groups within the Genus *Chrysoritis*, as Delineated by Heath (1997) (Species Marked with an Asterisk Were Included in This Analysis)

C. chrysaor species group	*C. thysbe* species group	
C. aethon	*C. adonis*	*C. orientalis**
C. aureus	*C. aridus**	*C. palmus**
*C. chrysaor**	*C. atlantica*	*C. pan*
*C. lycegenes**	*C. azurius*	*C. pelion*
C. lycia	*C. balli**	*C. penningtoni*
C. lyncurium	*C. bamptoni*	*C. perseus*
C. midas	*C. beaufortia*	*C. plutus*
C. natalensis	*C. beulah*	*C. psyche*
C. phosphor	*C. blencathra*	*C. pyramus*
	C. braueri	*C. rileyi*
C. dicksoni species group	*C. brooksi**	*C. stepheni*
*C. dicksoni**	*C. daphne**	*C. swanepoeli*
	C. endymion	*C. thysbe**
C. oreas species group	*C. henningi*	*C. trimeni*
*C. chrysantas**	*C. hyperion*	*C. turneri*
*C. coetzeri**	*C. irene*	*C. uranus*
*C. cottrelli**	*C. kaplani*	*C. violescens*
*C. felthami**	*C. lyndseyae*	*C. whitei*
*C. oreas**	*C. lysander*	*C. williami*
*C. pyroeis**	*C. mithras*	*C. wykehami*
*C. zeuxo**	*C. nigricans**	
*C. zonarius**		

synonymized under the genus *Chrysoritis*, described here as the "*Chrysoritis* complex."

(iii) Within the *Chrysoritis* complex, four "species groups" were delineated, based largely on genitalic and wing pattern affinities (Table 1).

MATERIALS AND METHODS

Specimen Acquisition

Butterfly specimens were freshly collected as adults, and wings were removed as vouchers for identification purposes. In order to preserve DNA sequence integrity, specimens were immediately placed in 100% ethanol and ultimately stored at −80°C. All of the material analyzed in this study has been deposited in the DNA and tissues collection of Harvard University's Museum of Comparative Zoology.

Sampling Strategy and Selection of Outgroup

Heath (1997) recognized 59 species in the *Chrysoritis* complex. For this analysis, we subsampled species representing systematic diversity as reflected by Heath's classification, as well as interesting life history diversity. Thus, the representative specimens spanned each of the "species groups" delineated by Heath (Table 1). Representative species from three of the four preexisting genera, *Chrysoritis*, *Poecilmitis*, and *Oxychaeta*, were also included. Unfortunately, the canopy-dwelling *Chrysoritis (=Bowkeria) phosphor* is extremely difficult to obtain due to its relative rarity and unique arboreal lifestyle, and we were unable to secure fresh specimens for this study. Finally, in order to examine the topology of intraspecific relationships, the subsample included conspecific pairs of *Chrysoritis nigricans nigricans* and *Chrysoritis pyroeis pyroeis*.

In order to represent the diversity of ant associations, foodplant affinities, and geographical ranges in the *Chrysoritis* complex as a whole, each species with clearly anomalous ecological characters was included. For example, we included *C. orientalis* because of its unusual eastern distribution, and *C. oreas* and *C. pyroeis* because of their atypical ant associations. Species with more common attributes, such as a Cape distribution or a *Crematogaster* association, were also well represented (Table 2). With 19 of the 59 species in the *Chrysoritis* complex, the subsample captured much of the ecological and morphological breadth of this group.

In order to test the monophyly of the *Chrysoritis* complex, which forms the primary hypothesis in Heath's revision (1997), one species was selected from each of seven available non-*Chrysoritis* genera in the tribe Aphnaeini; these taxa served as outgroups for the analysis. The representative species selected in this way were *Phasis clavum clavum*, *Tylopaedia sardonyx peringuey*, *Crudaria leroma*, *Aloeides pierus*, *Argyraspodes argyraspis*, *Trimenia argyroplaga argyroplaga*, and *Trimenia (=Argyrocupha) malagrida maryae*. The remaining genera of the Aphnaeini, for which representative species were not available, include *Axiocerces*, *Aphnaeus*, *Liphaphnaeus*, *Spindasis*, *Pseudaletis*, *Chloroselas*, *Vansomerenia*, *Cesa*, *Zeritis*, and *Erikssonia* (Heath, 1997).

DNA Preparation and Sequencing

In order to extract genomic DNA from the preserved tissue, approximately three anterior abdominal segments were homogenized in SDS buffer (2%), digested with proteinase K (0.2 g/L), and purified through successive ethanol precipitations of cellular debris and nucleic acids. Approximately 1 kb of the mitochondrial gene *cytochrome oxidase I* was amplified and sequenced in two fragments, framed by two primer pairs (numbered according to corresponding *Drosophila yakuba* sequence in Clary and Wolstenholme, 1985): "Ron" (5′-GGATCACCTGATATAGCATTCCC-3′; 1829–1851) pairs with "Nancy" (5′-CCCGGTAAAATTAAAATATAAACTTC-3′; 2316–2291), while "Tonya" (5′-GAAGTTTATATTTTAATTTTACCGGG-3′; 2291–2316) pairs with "Hobbes" (5′-AAATGTTGNGGRAAAAATGTTA-3′; 2856–2835). The two fragments were amplified using polymerase chain reaction (PCR) techniques (30–35 cycles: 45 s at 94°C, 60 s at 42°C, and 90 s at 72°C). PCR products were purified using either phenol/chloroform washes, gel separation, or QiaQuick columns. After undergoing a dye terminator cycle sequencing reaction with the above primer pairs, samples were loaded onto a polyacrylamide gel and analyzed by an Applied Biosciences 370A DNA sequencer.

TABLE 2

Life History Characters and Specimen Identification Numbers for All Aphnaeines Analyzed in This Study[a]

Butterfly species	Specimen No.	GenBank Accession No.	Ant associate	Host plant	Geographical range
Aloeides pierus	AH-95-Y614	AF279221	*Lepisiota* sp.	*Aspalathus* (Fabaceae), *Hermannia* (Sterculiaceae), *Sida* (Malvaceae), *Zygophyllum* (Zygophyllaceae)	Genus concentrated in WC, extending north to Kenya
Argyraspodes argyraspis	AH-95-Z422	AF279223	Unknown	Possibly aphytophagous	Genus in NC inland, including borders with WC, Namibia, and Botswana
Crudaria leroma	AH-95-Y658	AF279220	*Anoplolepis* sp.	*Acacia karroo, A. sieberiana* and other *Acacia* spp. (Fabaceae)	Genus throughout Southern Africa to Tanzania
Phasis clavum clavum	AH-95-Y643	AF279232	*Crematogaster peringueyi*	*Melianthus* (Melianthaceae), *Rhus* (Anacardiaceae)	Genus in NC, WC, EC
Trimenia argyroplaga argyroplaga	AH-95-Y631	AF279226	*Anoplolepis* sp.	Possibly aphytophagous	Genus in NC, WC, EC
Trimenia malagrida maryae	AH-96-Y733	AF279233	*Anoplolepis* sp.	Possibly aphytophagous	Genus in NC, WC, EC
Tylopaedia sardonyx peringueyi	AH-97-Y711	AF279219	*Crematogaster* sp. nr. *melanogaster*	*Phylica oleaefolia* (Rhamnaceae), *Aspalathus spinosa* (Fabaceae)	Genus in NC, WC, EC
Chrysoritis aridus	AH-95-Z406	AF279231	Possibly *Crematogaster* sp.	*Chrysanthemoides incana* (Asteraceae)	NC inland
Chrysoritis balli	DR-98-U629	AF279237	*Crematogaster* sp. 3	*Dimorphotheca montana* (Asteraceae), *Thesium* (Santalaceae)	WC Kammanassie Mountains
Chrysoritis brooksi brooksi	AH-95-Z415	AF279240	*Crematogaster peringueyi*	*Thesium* (Santalaceae), *Zygophyllum* (Zygophyllaceae), *Aspalathus spinosa* (Fabaceae)	WC w. coastal/inland margin
Chrysoritis chrysantas	AH-95-Z431	AF279218	*Crematogaster melanogaster*	*Salsola tuberculata* (Chenopodiaceae)	NC inland
Chrysoritis chrysaor	AH-95-Z454	AF279241	*Crematogaster* sp. nr. *liengmei*	*Rhus* (Anacardiaceae), *Cotyledon orbiculata* (Crassulaceae), *Acacia karoo* (Fabaceae), *Zygophyllum retrofractum* (Zygophyllaceae)	Throughout NC, WC, EC, Free State, and Lesotho
Chrysoritis coetzeri	AH-95-Z423	AF279222	*Crematogaster peringueyi*	*Chrysanthemoides incana* (Asteraceae)	NC near Nieuwoudtville
Chrysoritis cottrelli	AH-95-Y674	AF279229	*Crematogaster* sp. 7	*Chrysanthemoides monilifera* (Asteraceae)	WC s. coastal near Knysna
Chrysoritis daphne	DR-98-U620	AF279244	*Crematogaster* sp. 8	*Thesium* (Santalaceae)	WC Kammanassie Mountains
Chrysoritis dicksoni	AH-95-Z971	AF279230	*Crematogaster peringueyi*	Aphytophagous; ant trophallaxis	Two local populations in WC w. and s. coastal
Chrysoritis felthami felthami	AH-95-Z460	AF279224	*Crematogaster peringueyi*	*Zygophyllum sessilifolium, Z. flexuosum* (Zygophyllaceae)	NC coastal, WC w. coastal
Chrysoritis lycegenes	AH-95-Z921	AF279243	*Crematogaster* sp. nr. *liengmei* & *C.* sp. 1	*Myrsine africana* (Myrsinaceae), *Diospyros lycioides, D. austro-africana* (Ebenaceae), *Rhus* (Anacardiaceae)	KZN midlands including borders with Free State and Mpumalanga
Chrysoritis nigricans nigricans	AH-95-Z442 (A) AH-95-Z451 (B)	AF279228 (A) AF279242 (B)	*Crematogaster* sp. 7, 4, 1	*Thesium* (Santalaceae), *Osteospermum polygaloides* (Asteraceae), *Zygophyllum* (Zygophyllaceae)	WC w. and s. coastal
Chrysoritis oreas	AH-95-Z911	AF279236	*Myrmicaria* sp. nr. *nigra*	*Thesium* (Santalaceae)	KZN highlands
Chrysoritis orientalis	AH-95-Z903	AF279234	*Crematogaster* sp. 7	*Thesium* (Santalaceae)	KZN highlands
Chrysoritis palmus palmus	AH-95-Z427	AF279235	*Crematogaster peringueyi*	*Aspalathus sarcantha* (Fabaceae), *Chrysanthemoides monilifera* (Asteraceae), *Berzilia intermedia, B. lanuginosa, B. abrotanoides* (Bruniaceae)	WC inland
Chrysoritis pyroeis pyroeis	AH-95-Y627 (A) AH-95-Y628 (B)	AF279225 (A) AF279239 (B)	*Myrmicaria nigra*	*Zygophyllum flexuosum* and other Z. spp. (Zygophyllaceae), *Thesium* (Santalaceae)	NC coastal, WC w. and s. coastal with some inland patches
Chrysoritis thysbe f. *thysbe*	AH-95-Z447	AF279238	*Crematogaster peringueyi*	*Zygophyllum* (Zygophyllaceae), *Lebeckia plukenetiana, Aspalathus* (Fabaceae), *Chrysanthemoides incana, Osteospermum polygaloides* (Asteraceae), *Thesium* (Santalaceae)	WC s. coastal
Chrysoritis zeuxo	AH-95-Z917	AF279217	*Crematogaster* sp. 1	*Chrysanthemoides monilifera* (Asteraceae)	WC w. and s. coastal
Chrysoritis zonarius	AH-95-Z919	AF279227	*Crematogaster peringueyi*	*Chrysanthemoides incana* (Asteraceae)	WC w. coastal

[a] Geographical ranges are abbreviated as follows: NC, Northern Cape; WC, Western Cape; EC, Eastern Cape; KZN, KwaZulu-Natal; w. and s. coastal, western and southern coastal belts. (From Heath, 1997; Pringle *et al.*, 1994).

TABLE 3

Summary Statistics for the *cytochrome oxidase I* Molecular Data Matrix (28 taxa, 983 base pairs)

Character state status	First position	Second position	Third position	All sites
Constant	204	235	69	508
Variable and uninformative	93	82	96	271
Informative	31	10	163	204
Nucleotide bias	A	T	G	C
Mean base pair frequencies	0.31750	0.38228	0.15360	0.14663

Sequencher version 3.0 (1995) was used to align and compare reverse complementary sequences from the Ron/Nancy or Tonya/Hobbes primers. Where double-stranded corroboration was not available, as with the ends of most Tonya/Hobbes alignments, only the most unequivocal chromatograms were used for sequence data. Any ambiguities were designated as "N," which PAUP* (Swofford, 1998) treats as missing data that do not affect tree topology.

Fragments were aligned against the published *COI* sequence of *D. yakuba* (Clary and Wolstenholme, 1985). Gaps and false base pairs within each butterfly sequence were removed, primer sequences were cropped, and ambiguities were designated as "N," bringing the total sequence length of both fragments to a standard 983 characters. All 28 aphnaeine sequences were then aligned as the data matrix for phylogenetic analysis.

Phylogenetic Analyses

Phylogenetic trees were generated from the flat-weighted and unordered data matrix using heuristic search algorithms in PAUP* version 4.0b2 (Swofford, 1998). Trial searches under different weighting schemes (2:1 first and second vs third codon position) and step matrices (2:1 transversions vs transitions) revealed few significant topological differences. The data were analyzed with maximum parsimony as the optimization criterion, using simple taxon addition with four trees held during each step. Subsequent branch-swapping utilized tree bisection-reconnection (TBR), with both MULPARS and "steepest descent" invoked. These conservative search criteria promoted a thorough exploration of tree space for the most-parsimonious phylogeny. In order to assess whether or not simple taxon addition yielded only a local optimum, this heuristic search was repeated using 100 random taxon addition replicates, which would be more likely to reveal any disparate islands of parsimonious trees.

A g1 statistic for the entire data set was calculated from 100,000 randomly generated trees evaluated for total parsimony length (Hillis and Huelsenbeck, 1992). Clade robustness in the inferred phylogeny was evaluated using two independent techniques: bootstrap resampling (Felsenstein, 1985) and decay indices (Bremer, 1988). These methods followed the same PAUP* parameters as the original heuristic search for maximum parsimony (simple taxon addition, four trees held at each step, TBR branch swapping, MULPARS, and steepest descent).

Patterns of host plant utilization and ant association were examined across the phylogeny. Three life history characters were delineated for each species: phytophagy (aphytophagous, 0; phytophagous, 1), attendant ant genus (four possible states), and attendant ant subfamily (two possible states). Using MacClade version 3.0.1 (Maddison and Maddison, 1992), these life history characters were mapped onto the strict consensus of most-parsimonious trees in order to find the most parsimonious reconstruction of characters. The cladistic permutation tail probability (PTP) test was employed as an autocorrelation test of whether or not a pattern of discrete life history characters is phylogenetically constrained along a given tree topology (Kelley and Farrell, 1998). A topology was constructed using MacClade, based on the strict consensus of most-parsimonious trees, with one representative for each species. Using the "shuffle" utility in MacClade, each of these character distributions was randomly permuted along the entire constrained tree, with 1000 replicates. The resulting *P* values indicated the probability of incorrectly rejecting the null hypothesis that the observed life history patterns are phylogenetically labile products of chance.

RESULTS AND DISCUSSION

Of the 983 character states in the 28-taxon data matrix, 475 (48%) are variable and 204 (21%) are informative. These informative character states occur far more frequently in relatively unconstrained third codon positions (163) than in first (31) or second (10) positions. The sequences possess a high bias of A–T pair frequency (70%) over C–G pairs (30%; Table 3).

A plot of transition/transversion ratios against total nucleotide changes for every possible species pair reveals a clear threshold of transition saturation (Table 4; Fig. 1). The number of transitions between closely related species increases linearly with distance, but

TABLE 4

Uncorrected *P* Distances (below Diagonal) and Transition/Transversion Ratios (above Diagonal) for All Pairwise Comparisons (Species Pairs with No Differences Due to Transversions Are Marked with an Asterisk)

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
1	*C. nigricans* A		*	9.500	*	*	*	11.000	13.500	4.500	1.194	1.161	1.233	1.241	1.167	1.161	0.947	1.227	1.440	1.088	1.034	0.882	0.714	0.784	0.720	0.667	0.638	0.591	0.818
2	*C. nigricans* B	0.008		7.500	*	*	*	9.000	12.000	4.500	1.194	1.161	1.233	1.241	1.200	1.194	1.026	1.182	1.440	1.059	1.069	0.912	0.732	0.745	0.700	0.649	0.638	0.591	0.795
3	*C. aridus*	0.024	0.019		9.000	9.000	8.000	5.667	14.000	6.000	1.156	1.125	1.226	1.161	1.233	1.258	1.027	1.125	1.500	1.226	1.222	0.941	0.691	0.875	0.765	0.702	0.745	0.672	0.844
4	*C. palmus palmus*	0.018	0.013	0.023		*	*	13.000	12.500	5.250	1.226	1.129	1.133	1.207	1.233	1.200	0.919	1.227	1.520	1.182	1.034	0.912	0.643	0.745	0.700	0.614	0.667	0.591	0.814
5	*C. balli*	0.018	0.017	0.024	0.017		*	13.000	10.500	4.750	1.172	1.100	1.179	1.143	1.138	1.172	0.833	1.045	1.478	1.129	0.929	0.788	0.771	0.860	0.680	0.607	0.673	0.621	0.825
6	*C. brooksi brooksi*	0.017	0.012	0.020	0.013	0.016		10.000	11.500	4.750	1.258	1.226	1.267	1.345	1.267	1.258	0.974	1.227	1.480	1.118	1.069	0.971	0.727	0.760	0.780	0.667	0.702	0.631	0.841
7	*C. daphne*	0.015	0.012	0.025	0.017	0.018	0.013		7.333	4.000	1.286	1.143	1.296	1.462	1.250	1.333	1.000	1.350	1.522	1.241	0.963	0.903	0.642	0.720	0.745	0.679	0.623	0.556	0.850
8	*C. orientalis*	0.032	0.028	0.035	0.030	0.028	0.027	0.030		6.000	1.355	1.355	1.367	1.483	1.467	1.467	0.949	1.409	1.520	1.121	1.407	1.029	0.732	0.878	0.840	0.719	0.702	0.703	0.826
9	*C. thysbe* f. *thysbe*	0.028	0.028	0.036	0.031	0.030	0.029	0.026	0.035		1.393	1.172	1.214	1.286	1.360	1.458	0.912	1.042	1.545	1.207	1.120	0.758	0.761	0.818	0.708	0.712	0.756	0.745	0.780
10	*C. zeuxo*	0.077	0.077	0.083	0.078	0.080	0.079	0.079	0.083	0.087		1.500	8.000	10.000	1.133	1.133	0.944	0.794	1.125	1.071	0.738	0.756	0.636	0.523	0.526	0.525	0.614	0.515	0.735
11	*C. cottrelli*	0.074	0.073	0.079	0.073	0.078	0.076	0.074	0.081	0.081	0.011		2.333	5.500	0.938	0.938	0.919	0.727	1.156	0.932	0.614	0.628	0.571	0.484	0.466	0.516	0.583	0.493	0.640
12	*C. zonarius*	0.074	0.074	0.081	0.072	0.077	0.076	0.077	0.079	0.081	0.010	0.011		*	1.069	1.069	0.886	0.618	1.061	0.929	0.643	0.585	0.561	0.446	0.500	0.500	0.603	0.493	0.653
13	*C. coetzeri*	0.075	0.075	0.081	0.074	0.077	0.079	0.080	0.083	0.083	0.013	0.015	0.007		1.036	1.071	0.800	0.706	1.094	0.975	0.675	0.615	0.582	0.438	0.446	0.559	0.544	0.433	0.633
14	*C. pyroeis pyroeis* A	0.072	0.072	0.078	0.074	0.078	0.075	0.078	0.082	0.077	0.074	0.069	0.068	0.067		0.000	1.030	0.676	0.882	1.057	0.590	0.692	0.586	0.541	0.481	0.526	0.554	0.457	0.436
15	*C. pyroeis pyroeis* B	0.073	0.073	0.080	0.072	0.079	0.075	0.078	0.081	0.076	0.073	0.068	0.067	0.067	0.001		1.094	0.706	0.857	1.143	0.615	0.692	0.579	0.557	0.509	0.554	0.564	0.485	0.455
16	*C. felthami felthami*	0.085	0.088	0.090	0.083	0.083	0.086	0.086	0.088	0.084	0.086	0.083	0.079	0.079	0.079	0.077		0.711	0.750	0.977	0.628	0.521	0.609	0.567	0.603	0.524	0.583	0.547	0.475
17	*C. chrysantas*	0.057	0.056	0.061	0.057	0.058	0.057	0.059	0.062	0.063	0.073	0.068	0.066	0.070	0.068	0.068	0.082		0.833	0.844	0.485	0.441	0.510	0.421	0.471	0.421	0.397	0.460	0.489
18	*C. oreas*	0.071	0.070	0.073	0.073	0.074	0.072	0.073	0.073	0.075	0.079	0.081	0.079	0.080	0.075	0.075	0.088	0.067		1.185	0.926	0.800	0.625	0.566	0.491	0.526	0.458	0.460	0.538
19	*C. dicksoni*	0.077	0.076	0.079	0.079	0.082	0.078	0.080	0.077	0.082	0.099	0.094	0.091	0.092	0.080	0.082	0.101	0.070	0.069		0.875	1.074	0.672	0.610	0.648	0.607	0.515	0.613	0.612
20	*C. chrysaor*	0.065	0.066	0.069	0.065	0.069	0.066	0.066	0.072	0.069	0.084	0.079	0.079	0.078	0.069	0.070	0.083	0.058	0.061	0.067		0.762	0.604	0.520	0.375	0.451	0.433	0.545	0.533
21	*C. lycegenes*	0.072	0.072	0.078	0.073	0.076	0.075	0.073	0.078	0.075	0.083	0.080	0.075	0.074	0.075	0.074	0.089	0.058	0.063	0.063	0.042		0.538	0.417	0.404	0.519	0.344	0.438	0.511
22	*Tylopaedia sardonyx per.*	0.115	0.116	0.118	0.111	0.114	0.114	0.111	0.116	0.110	0.109	0.107	0.107	0.106	0.112	0.108	0.096	0.095	0.096	0.123	0.093	0.096		1.185	0.652	0.660	0.708	0.881	0.517
23	*Phasis clavum clavum*	0.102	0.100	0.104	0.101	0.100	0.099	0.107	0.104	0.104	0.116	0.109	0.108	0.110	0.108	0.107	0.112	0.096	0.100	0.107	0.086	0.099	0.073		0.439	0.492	0.778	0.820	0.468
24	*Trimenia argyroplaga ar.*	0.107	0.106	0.113	0.107	0.106	0.111	0.106	0.116	0.109	0.112	0.107	0.106	0.106	0.102	0.100	0.117	0.098	0.103	0.112	0.085	0.096	0.102	0.103		0.920	0.730	0.646	0.640
25	*Trimenia malagrida maryae*	0.112	0.110	0.121	0.110	0.113	0.112	0.114	0.117	0.116	0.114	0.112	0.108	0.116	0.105	0.103	0.114	0.104	0.109	0.116	0.090	0.102	0.108	0.115	0.060		0.566	0.696	0.441
26	*Aloeides pierus*	0.103	0.103	0.109	0.105	0.103	0.105	0.107	0.107	0.102	0.105	0.105	0.105	0.102	0.097	0.094	0.110	0.104	0.101	0.109	0.095	0.097	0.099	0.108	0.080	0.099		0.673	0.463
27	*Argyraspodes argyraspis*	0.119	0.119	0.123	0.120	0.118	0.120	0.123	0.125	0.124	0.119	0.119	0.117	0.116	0.118	0.115	0.120	0.110	0.112	0.114	0.098	0.108	0.099	0.104	0.099	0.096	0.093		0.577
28	*Crudaria leroma*	0.096	0.095	0.100	0.094	0.094	0.097	0.095	0.100	0.098	0.103	0.100	0.098	0.097	0.097	0.097	0.113	0.082	0.099	0.096	0.083	0.087	0.112	0.110	0.106	0.112	0.095	0.099	

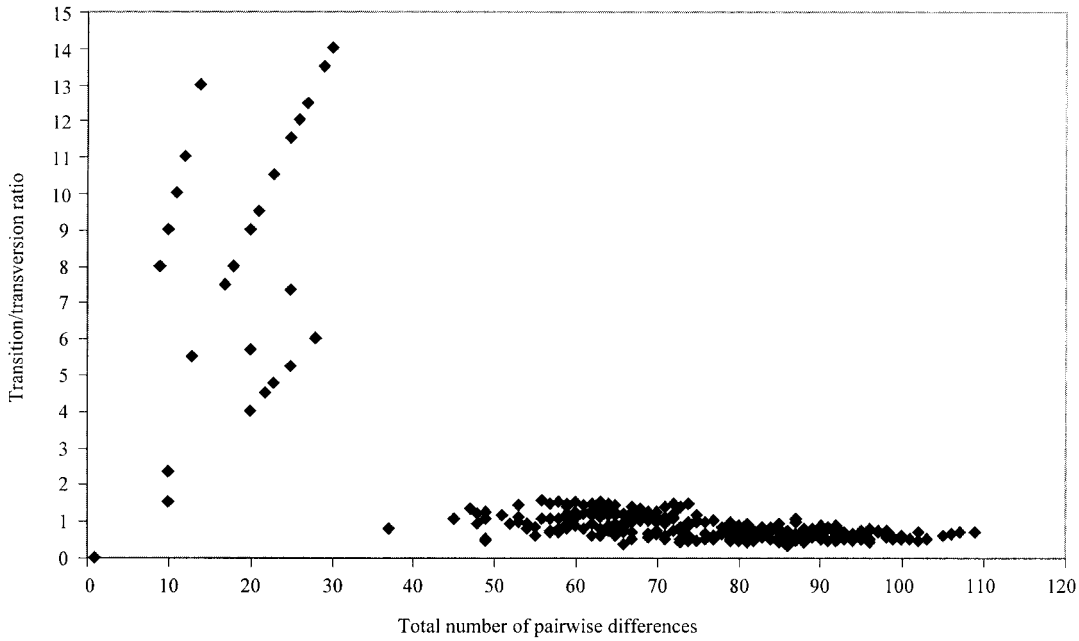

FIG. 1. The transition/transversion ratio vs the total number of pairwise differences between any two species. All of the points with 30 or fewer pairwise differences represent species pairs from the *Chrysoritis thysbe* group and the *C. coetzeri–zonarius–zeuxo–cottrelli* group, except for the intraspecific *C. pyroeis* pair near the origin. Within these two species groups, 11 pairs lack any differences due to transversions, and thus do not appear on this graph. (See Table 4 for raw transition/transversion ratios and uncorrected *P* distances.)

this pattern abruptly disintegrates for all species pairs with a total difference of more than 30 changes.

The g1 statistic for 100,000 randomly generated trees (−0.610978) is far below the critical value (−0.09; $P = 0.01$) for a matrix of these dimensions. This degree of skew indicates that the data are more structured than purely random data (Hillis and Huelsenbeck, 1992), although this approach has been criticized in some situations (Kallersjo *et al.,* 1992).

Heuristic searches, whether with simple or random taxon addition, yielded the same 17 most-parsimonious trees (983 steps), which were combined in a strict consensus tree along with decay indices (Fig. 2). Excluding uninformative characters, these trees have a consistency index (CI) of 0.457 and a retention index (RI) of 0.616, indicating that the data fit the trees reasonably well. This CI is higher than the expected value (0.290) for this number of taxa (Sanderson and Donoghue, 1989).

As with the decay indices, the bootstrap consensus tree (Fig. 3) offers an independent perspective on clade robustness. The *C. thysbe–daphne–balli–orientalis–aridus–palmus–brooksi–nigricans* clade, the *C. zeuxo–cottrelli–zonarius–coetzeri ("zczc")* clade, and the *C. pyroeis* sister group occur in every tree derived from the resampled searches.

The autocorrelation test for life history characters (Fig. 4) provides statistical support for constrained association with ants at the level of ant subfamily ($P = 0.045$). The distributions of attendant ant genera ($P = 0.289$) and phytophagy ($P = 0.340$) appear to be relatively random, however, with several possible reconstructions possessing a more parsimonious pattern of character change.

Genus Monophyly

The phylogenetic trees, inferred from molecular evidence, serve as an independent evaluation of the morphology-based systematic conclusions published by Heath (1997). Although Heath made no explicit phylogenetic claims in delineating these morphological groupings, his designated species groups and genera were intended to reflect common ancestry via monophyly.

The molecular phylogeny supports Heath's description of the *Chrysoritis* complex, previously broken into *Chrysoritis, Poecilmitis, Oxychaeta,* and *Bowkeria.* While *Oxychaeta* and *Bowkeria* were both monotypic genera, the former *Chrysoritis* and *Poecilmitis* composed paraphyletic groups (Fig. 3). This discordance between systematics and phylogeny is resolved by Heath's morphologically based, methodologically independent redefinition of the *Chrysoritis* complex, which appears here as a monophyletic group. This species-rich clade is moderately well-supported by the bootstrap consensus (72) and decay index (2). The current lack of sequence data for *Chrysoritis (=Bowkeria) phosphor* renders this corroboration incomplete, however.

Heath's synonymization of *Argyrocupha malagrida* into the genus *Trimenia* is not rejected by the molecu-

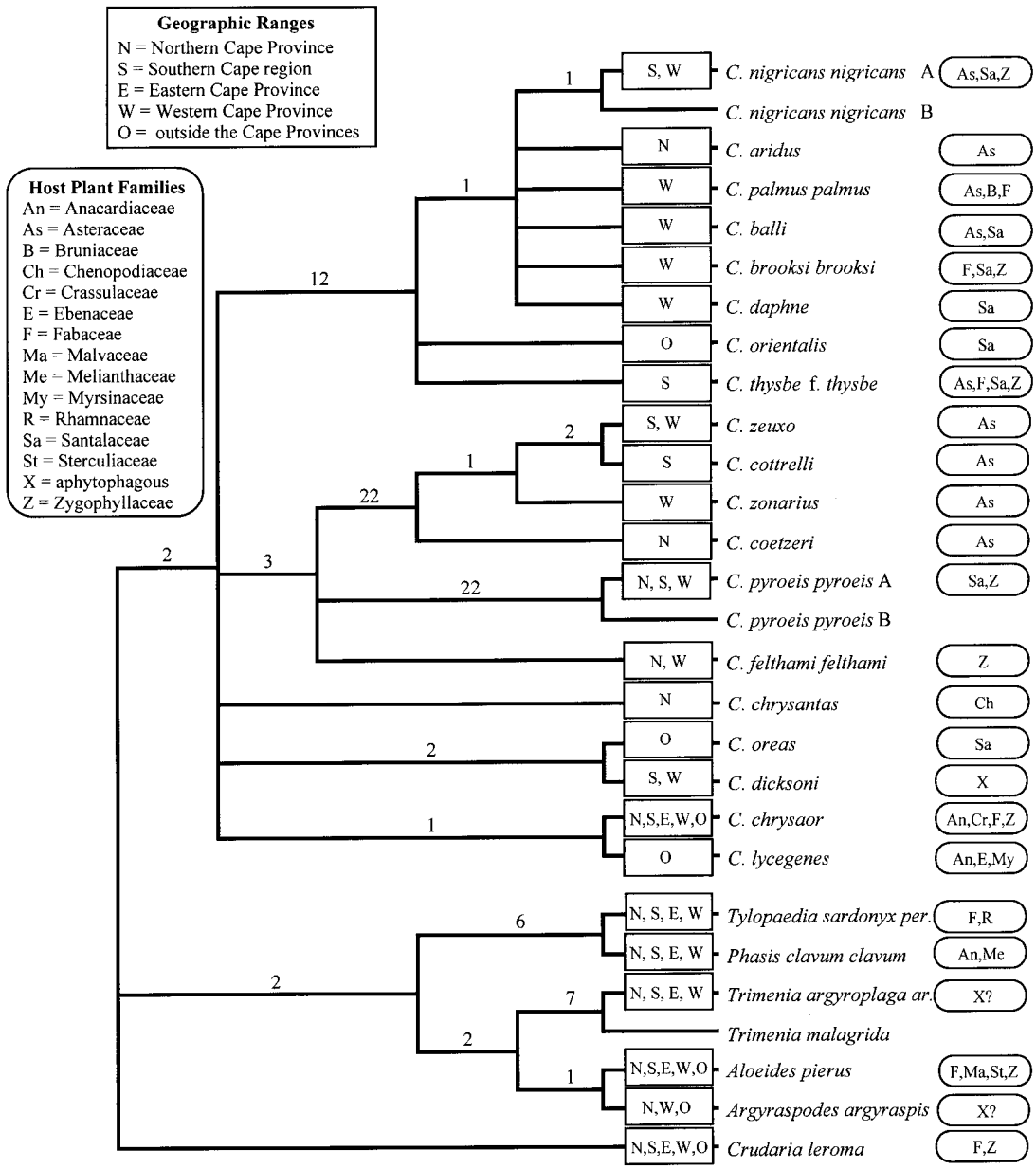

FIG. 2. Strict consensus of 17 most parsimonious trees, based on flat-weighted, unordered *cytochrome oxidase I (COI)* sequence data (983 total steps; 204 informative steps; CI = 0.457; RI = 0.616). Decay indices appear above each resolved node, while geographic distribution and host plant association are noted alongside each unique taxon. Life history information within the *Chrysoritis* complex applies to individual species; for the other aphnaeines, this life history information applies to the entire genus. "Southern Cape region" refers to the southern coastal belt within the Western Cape Province as an independent range.

lar phylogeny (Fig. 2): the monophyly of the synonymized *Trimenia* is upheld by a strong bootstrap value (90) and decay index (7). While robust monophyly can support an argument for synonymy between genera, it does not necessarily demand such revision. The *Phasis–Tylopaedia* clade is also well-supported by its decay index (6) and bootstrap value (93), though Heath retained the distinction between these two genera. Species of *Phasis* possess some genitalic similarities to those of *Tylopaedia,* but other genitalic traits and wing patterning are unique to *Phasis* among the Aphnaeini (Heath, 1997). Further morphological scrutiny and a more comprehensive molecular sampling scheme would be necessary to investigate taxonomic relationships within *Trimenia* and between *Phasis* and *Tylopaedia.*

Species Group Integrity

Within the *Chrysoritis* complex, Heath (1997) delineated four "species groups" based on similarities of

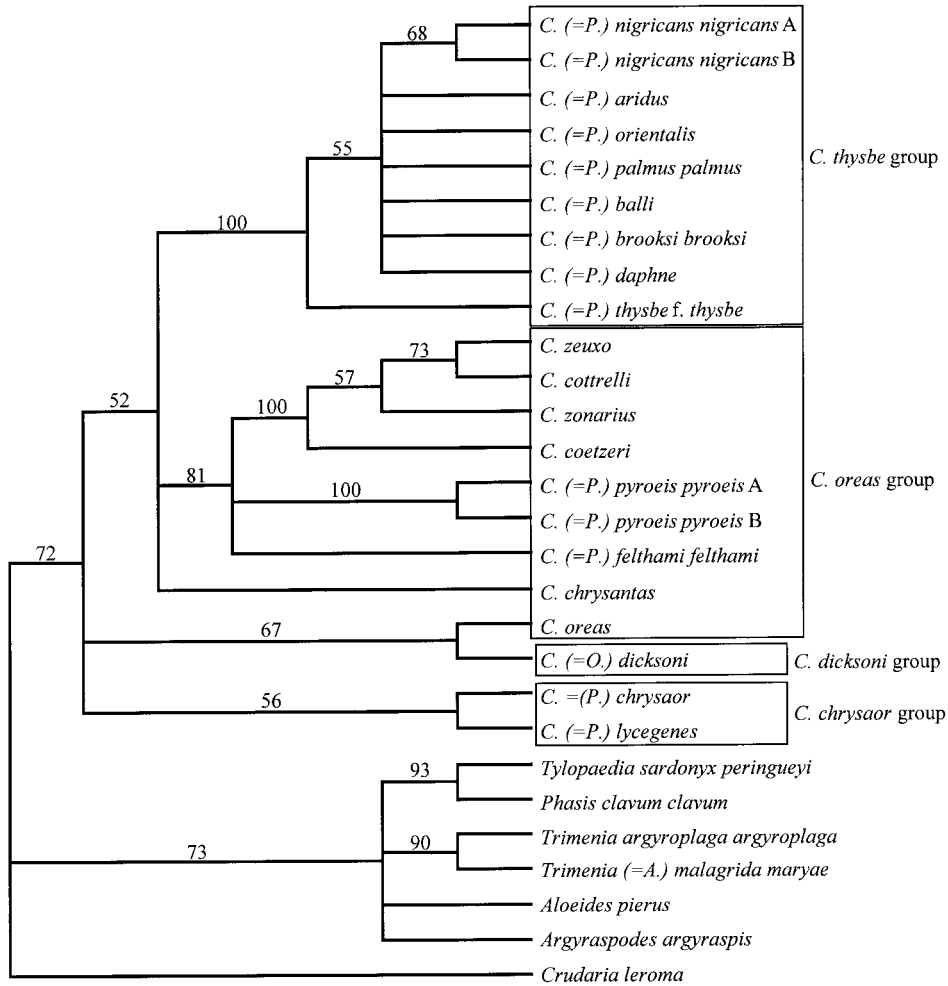

FIG. 3. Bootstrap consensus tree based on 100 heuristic search replicates, with support indices over each node that was upheld in over 50% of the replicates. Abbreviated generic names in parentheses indicate former taxonomic groupings before Heath's 1997 revision of the Aphnaeini (*P., Poecilmitis; O., Oxychaeta; A., Argyrocupha*). Heath's four "species groups" within the *Chrysoritis* complex are also indicated.

genitalia and wing pattern (Table 1; Fig. 3). This analysis presents a comprehensive phylogeny of only the *C. oreas* assemblage and the monotypic *C. dicksoni* group. Nevertheless, the included taxa yield strong support for the monophyly of the *C. thysbe* group, whether measured by bootstrap value (100) or decay index (12). Although only 8 of the 41 species in this group were included in the analysis, this subsample incorporates a high degree of within-group diversity in terms of range, ecology, and morphology. Thus, one would expect the monophyly of the *C. thysbe* group to be supported by the inclusion of other representative taxa. Support for the *C. chrysaor* group is not as strong, with a low bootstrap value (56) and decay index (1). It remains to be evaluated whether or not the addition of more taxa from this group, including *C. (=B.) phosphor*, will resolve the clade with greater clarity.

In comparison, the monophyly of Heath's *C. oreas* species group is rejected by both the heuristic search and the bootstrap consensus. Morphologically, these species are held together by a single negative character: the absence of tibial spicules. *C. oreas* itself contributes to the paraphyly of the group, resolving as a sister taxon only to *C. dicksoni*. This relationship has a relatively weak bootstrap support (67) and decay index (2). These measures suggest a far more likely relationship between *C. oreas* and *C. dicksoni*, however, than between *C. oreas* and other members of its hypothetical species group. Furthermore, *C. chrysantas* does not fall within Heath's grouping, instead appearing as an unresolved basal member of the *Chrysoritis* complex in the strict consensus tree, and with similarly poor resolution in the bootstrap tree.

Excluding *C. oreas* and *C. chrysantas*, the remainder of the *C. oreas* species group does resolve into a well-supported clade, containing *C. felthami felthami, C. pyroeis, C. coetzeri, C. zonarius, C. zeuxo,* and *C. cottrelli*. This node has a moderately robust bootstrap

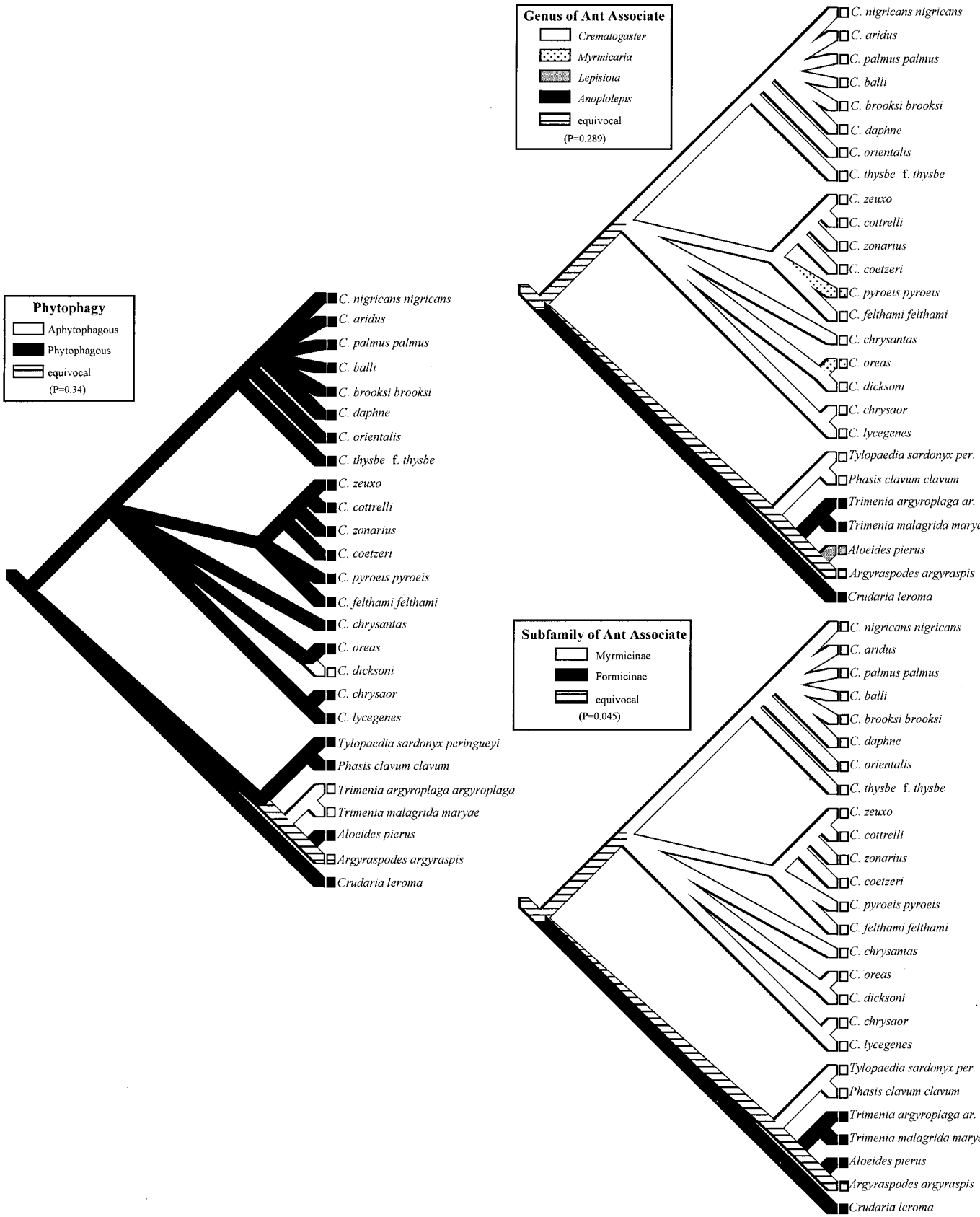

FIG. 4. Selected host plant and ant associate characters mapped onto the strict consensus of most parsimonious trees. Modified PTP autocorrelation values appear in each legend.

value (81) and decay index (3). The clade presents an interesting morphological anomaly in *C. pyroeis*, which is the only member to have blue scales on its forewings. Blue scales are common among the species in the *C. thysbe* group, but appear different enough in *C. pyroeis* that Cottrell (1978) hypothesized two evolutionary events to account for both scale types; such an explanation corresponds well with the molecular phylogeny.

Species Synonymy

At least two clades in this phylogeny present interesting implications for further revision of the genus *Chrysoritis*. The *C. thysbe* species group and the "*zczc*" clade both have 100% bootstrap support, and unusually high decay indices (12 and 22, respectively). The *C. thysbe* group contains numerous polytomies, however, and both groups have relatively low within-clade bootstrap values (55–73) and decay indices (1–2).

In and of themselves, these measures would not constitute sufficient evidence for species synonymy; for example, the *Aloeides–Argyraspodes* node exhibits a similar lack of statistical support, yet these two genera are almost certainly not the same. This intergeneric group has an entirely different data structure, however, from that of the two clades mentioned above. In Fig. 1, the pairwise differences between taxa in the *C. thysbe* group and in the "*zczc*" clade comprise all but one of the data points representing 30 or fewer total character differences. (The exception is the *C. pyroeis* interspecific comparison, described below.) The differences between these closely related species are almost entirely transitions, indicating that these divergences are too recent to have accumulated a significant number of relatively infrequent transversions.

Such conclusions are supported, albeit provisionally, by comparable phylogenetic relationships between accepted conspecifics. The *C. thysbe* group and the "*zczc*" clade are similarly well-supported compared with the node between two specimens from the same population of *C. pyroeis*, with its high bootstrap value (100) and decay index (22). On the other hand, the two specimens of *C. nigricans nigricans* within the *C. thysbe* clade exhibit relatively poor measures of sister status, with a bootstrap value of 68 and a decay index of 1. This discrepancy between the two conspecific clades suggests a comparable degree of gene flow among some members of the *C. thysbe* group and among individuals of *C. pyroeis*.

Morphological observations provide further evidence to support the synonymy of species within the given *Chrysoritis* clades. Many species in the *C. thysbe* group possess a high uniformity of genitalic features and share the same basic pattern on the underside of the hindwing (Heath, 1997). *C. coetzeri* and *C. zonarius* are also morphologically similar enough to be local variants rather than discrete species, as are *C. zeuxo* and *C. cottrelli*. These groups might therefore be regarded as primary targets for synonymy in future revisions.

A genealogical species, as defined by Baum and Shaw (1995), lies in a transitory state from the reticulate pedigrees of population genetics toward the divergent evolutionary patterns of discrete phylogenetic entities. The poor resolution of the *C. thysbe* group and the "*zczc*" clade could be evidence for a lingering reticulate pattern, and thus a lack of true genealogical species distinctions; sequences of other genes would be necessary to confirm this pattern.

Aphytophagy

Some species of Aphnaeini do not feed on plants during the larval stage, instead meeting all of their nutritional requirements through trophallaxis from ants. Pierce (1995) noted that carnivorous and otherwise exploitative lycaenid/ant relationships tend to be derived life history characters; this pattern is supported by the aphnaeine phylogeny, along which the distribution of aphytophagy is not correlated with phylogeny (PTP test, $P = 0.34$). Although the phylogeny includes no outgroup for the Aphnaeini as a whole, it is reasonable to assume that phytophagy is the ancestral life history character, since no sister tribe candidates are aphytophagous.

Trophallaxis has been well-documented in *C. dicksoni* (Heath, 1997, 1998), which appears as the only aphytophagous species among a wide array of phytophagous, mutualistic congeners. Within the Lycaenidae, it is not uncommon to see the origin of an aphytophagous species in a genus whose other members appear to maintain mutualistic associations with ants. For example, *Arhopala wildei* feeds on the brood of *Polyrhachis queenslandica*, while all known congeners are phytophagous (King and Ring, 1996). Similar examples can be observed within *Ogyris Spindasis, Anthene, Chilades, Athsanota,* and *Aloeides* (Field, 1997; Pierce, 1995; A. Heath, unpublished observations). Such patterns of convergence suggest that while carnivory has evolved on numerous occasions, it may constitute an unstable life history. This could be due to heightened life cycle complexity and/or constraints imposed by the morphological, physiological, and ecological features of ancestral herbivory (Pierce, 1995).

There are limited examples of aphytophagous lycaenid lineages, however, most notably the entire subfamily Miletinae Genera such as *Acrodipsas* and wholly carnivorous on ants. Others, including *Lepidochrysops*, *Maculinea*, and *Phengaris* (tribe Polyommatini) all have larvae that feed on plants for the first few instars, and later on ants or ant regurgitations. The monotypic *Argyraspodes* and the five species in *Trimenia* are suspected of being aphytophagous, but until these life histories and the monophyly of these two taxa are confirmed, it is unclear whether or not *Trimenia* and

Argyraspodes together represent another radiation of aphytophagy.

Ant Associations

By examining the phylogeny of myrmecophilous lycaenids, it is also possible to assess the role that ant association may have played in their cladogenesis. Figure 4 presents each species' ant associate at the generic level, mapped onto the strict consensus of most-parsimonious trees. Within the *Chrysoritis* complex, most species associate with species of *Crematogaster. C. oreas* and *C. pyroeis* are exceptional in their association with *Myrmicaria nigra,* a distinct ant genus within the Myrmicinae, the same subfamily that contains the genus *Crematogaster.* These two species of *Chrysoritis* are not sister taxa, indicating that a shift from *Crematogaster* to *Myrmicaria* has occurred at least twice in the evolution of this genus.

While closely related lycaenids may associate with ants of disparate species or genera, subfamily association may be more conservative. For example, species in the *Chrysoritis* complex, *Phasis,* and *Tylopaedia* associate with myrmicines, while the other aphnaeine genera in this study associate with ants in the subfamily Formicinae: *Aloeides* with *Lepisiota,* and both *Trimenia* and *Crudaria* with *Anoplolepis. Aloeides, Trimenia,* and *Argyraspodes* form a monophyletic group in the strict consensus of most-parsimonious trees. Although the monophyly of this clade breaks down in the bootstrap consensus tree, it is reasonable to hypothesize that *Argyraspodes* is phylogenetically closer to *Aloeides* and *Trimenia* than it is to *Phasis* and *Tylopaedia.* Although the ant associate of *Argyraspodes* is as yet unknown, the above pattern predicts that the ant will be in the Formicinae.

Since this analysis does not include an outgroup for the Aphnaeini, it is impossible to determine which subfamily association is ancestral. In either case, it appears likely that ancestral shifts have occurred between ant subfamilies, with a subsequent lability of association with various ant genera. The modified PTP autocorrelation test upholds this pattern of conservatism versus lability: association according to ant subfamily is well-supported across the aphnaeine taxa ($P = 0.045$), while association according to ant genus is not ($P = 0.289$). Perception of this pattern rests on published life history information, however, which may be incomplete or unknown for some species. For example, one of us (A.H.) has made recent observations of ants from the myrmicine genus *Monomorium* in contact with *Aloeides* caterpillars, which are usually formicine allies. The nature of this association has yet to be thoroughly observed; it may be that the *Monomorium* ants, often invasive opportunists, are relatively recent arrivals that lack a strong relationship with *Aloeides* larvae.

Conserved affiliations with ant subfamilies rather than ant genera may reflect a phylogenetic hierarchy of chemical differences relevant to caterpillars. Lycaenid larvae employ a battery of chemical cues that enable them to communicate and associate with a given ant species. If myrmicine and formicine ants each employ chemical signals unique to their subfamily, then a shift in the lycaenids' chemical mimicry would be a costly and infrequent evolutionary event. Conversely, if these chemical signals were relatively similar among ant genera within a subfamily, then one would expect relative lability of ant association at this taxonomic level. A study of behavioral and biochemical cues would be necessary to test this hypothesis.

The phylogenetic pattern of ant association might also be attributed to the coevolution of aphnaeines and attendant ants in a historical process of reciprocal speciation. This explanation seems unlikely, as the ants that associate with species of *Chrysoritis* typically have alternative food resources available, and are not dependent upon larval secretions for survival. An analysis of whether or not South African aphnaeines have diverged in tandem with their ant associates would require extensive additional investigation of these ant–lycaenid interactions, as well as a phylogeny of the ant associates.

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