The Auk 115(1):105–118, 1998

PHYLOGENETIC PATTERNS IN THE TROCHILIDAE

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ABSTRACT.—Although many aspects of hummingbird biology have been studied, few recent analyses of higher-level systematic relationships exist. Based on morphology, it has been hypothesized that the Trochilidae includes six major clades. We used starch-gel electrophoresis to construct and test phylogenetic hypotheses for representatives of the six clades, using two species of swifts (Apodidae) as outgroups. Of 45 loci scored, 38 were polymorphic. The average Nei's genetic distance (D) among 14 hummingbird taxa was 0.625; D averaged 1.61 between the swifts and hummingbirds. These distances are large, and are consistent with other nonpasserine groups, suggesting that hummingbird taxa are relatively old. Phylogenetic analyses generally were consistent with the hypothesis that hermits are a sister group to all other trochilines. Within the Trochilinae, two broad groups are recognized, here called trochiline-A and B, which correspond to the morphologically determined "primitive" and "advanced" trochiline groups of Zusi and Bentz (1982). *Androdon aequatorialis* is genetically distinct but generally aligns with the trochiline-A group. Within the trochiline-B group, four radiations hypothesized by Zusi (pers. comm.), here called Bee, Amazilia ("Emeralds"), Andean, and High Andean, were corroborated by our analyses. Our distance analysis suggests a phylogenetic pattern consistent with that derived from Sibley and Ahlquists' (1990) and Bleiweiss et al.'s (1997) DNA-DNA hybridization studies. *Received 31 October 1996, accepted 20 June 1997.*

THE HUMMINGBIRDS (TROCHILIDAE) form one of the largest bird families, with approximately 325 species. Although many aspects of hummingbird biology have been studied, few higher-level studies of systematic relationships exist. Early taxonomists (Gould 1861, Boucard 1895, Simon 1921, Peters 1945, Zimmer 1950–1953) provided species descriptions and general details of geographic variation in most species, but they did not explicitly identify systematic relationships. Recently, several approaches have been used to identify phylogenetic patterns within parts of this family: comparative analysis of vocalizations and mating behavior (Schuchmann unpubl. data), external morphology and ecology (Graves 1980, 1986; Stiles 1983, 1996; Hinklemann 1989), comparative myology (Zusi and Bentz 1982), protein electrophoresis (Gerwin and Zink 1989, Gill and Gerwin 1989), mitochondrial DNA sequences (Hernandez-Banos et al. unpubl. data), and DNA-DNA hybridization (Sibley and Ahlquist 1990; Bleiweiss et al. 1994, 1997). Most of these studies were not comprehensive at the level of the family. However, the study by Bleiweiss et al. (1997), which involved 26 species, provided a molecular phylogenetic hypothesis for the major clades in the family. We report an allozymic survey of major groups of hummingbirds designed to test phylogenetic hypotheses derived from previous morphological and DNA-DNA hybridization analyses.

Our study was based in part on Zusi and Bentz's (1982) hypothesis of higher-level groups in the Trochilidae. They identified four major groupings: hermits (Phaethorninae), "primitive" trochilines, and two groups of "advanced" trochilines. The terms primitive and advanced are reserved for discussion of characters and are misleading without an in-depth phylogenetic analysis; we refer to the two groups as trochiline-A (Zusi and Bentz's [1982] primitive group) and trochiline-B (their advanced group). The widespread notion that the hermits represent the "primitive" hummingbirds is incorrect in one sense: if these are sister clades, each is by definition the same age. Phylogenetic analysis is required to show that the hermit clade retains a disproportionate number

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of plesiomorphic characters relative to the sister group of hummingbirds before it would be "primitive" in any sense. Also, within trochilines, if there are only two groups, neither can be primitive in a phylogenetic tree. From his work on comparative myology, using the swifts (Apodidae) as the outgroup, Zusi (pers. comm.) suggested as a working hypothesis: (1) that the trochiline-A group included *Androdon, Schistes, Colibri, Doryfera,* and close relatives; and (2) that four major clades exist within the trochiline-B group: Bee, High Andean, Andean, and Amazilia ("Emeralds"). By selecting at least two representatives of each major group to reflect hummingbird diversity, our phylogenetic analysis of allozyme variation provides a higher-level phylogenetic hypothesis that can be compared with those generated from other data sets.

Most modern molecular studies of phylogeny rely on DNA sequences (Hillis et al. 1996). However, phylogenetic hypotheses based on allozymes and mitochondrial DNA often are topologically consistent (Zink and Avise 1990, Zink and Dittmann 1991, Zink et al. 1991, Zink and Blackwell 1996). Thus, although there are reasons for preferring DNA data for phylogenetic inferences (Hillis et al. 1996), allozyme-based phylogenetic inferences are valuable in studies of congruence of tree topologies inferred from different data sets (Swofford et al. 1996). In our study, we compare tree topologies from allozymes and DNA-DNA hybridization (Bleiweiss et al. 1997). Although both are based on information from the nuclear genome, each is likely an independent estimate of phylogenetic relationships. Hence, congruence of tree topologies can be used as a measure of confidence in the phylogenetic hypothesis.

MATERIALS AND METHODS

We used standard horizontal starch-gel electrophoresis to investigate patterns of genetic variation among 14 taxa (Zusi pers. comm.): *Glaucis* and *Phaethornis* represent the hermit, or phaethornine line; *Androdon, Colibri, Schistes,* and *Doryfera* the trochiline-A group; *Acestrura* and *Selasphorus* the "Bee" line; *Amazilia* and *Campylopterus* the "Amazilia" (Emerald) group; *Aglaeactis* and *Coeligena* the "Andean" clade; and *Metallura,* and *Oreotrochilus* the "high Andean" clade. Bleiweiss et al. (1997) renamed these clades Mangoes (Trochiline-A), Emeralds (Amazilia group), Brilliants (Andean), and Coquettes (high Andean); both Zusi and Bleiweiss et al. (1997) refer to the Bee group and the hermits. Bleiweiss et al. (1997) further referred to a Mountain Gem group, of which we had no representatives. For simplicity, we use Zusi's names, except for using Emerald in place Amazilia, because confusion results from naming a group after one of the genera in it. We included two species of swifts (Apodidae; *Reinarda squamata, Chaetura cinereiventris*) that served as outgroups (see Appendix 1). Although inferences based on low numbers of specimens have been criticized (Archie et al. 1989), in our study genetic distances were sufficiently high that our patterns likely are robust to small sample sizes of individuals. Most specimens were collected over several years during expeditions to various regions of the Neotropics. Samples of tissues were preserved in liquid nitrogen in the field until transferred to the Louisiana State University Museum of Natural Sciences (LSUMNS), where they were stored at −70°C (see Johnson et al. [1984] for details of collection and preservation methods). Voucher specimens (study skins and tissue samples) are housed at the LSUMNS.

Samples of pectoral and heart muscle and liver (total volume of tissue was approximately 0.5 cc) were placed in 0.8 mL of grinding buffer consisting of 10 mg NADP and 100 μL of 2-mercaptoethanol in 100 mL distilled water (Richardson et al. 1986) and ground for 10 to 15 s using a Tekmar Tissuemizer. These crude homogenates were then centrifuged at 36,000 × g for 30 min, and the supernatant was stored at −70°C. Six aliquots of 20 μL of each sample were stored separately and used for the first six gels, and the rest of the homogenate was stored in individual vials. Electrophoretic procedures followed Selander et al. (1971), Harris and Hopkinson (1976), Johnson et al. (1984) and Richardson et al. (1986) with slight modifications (available from author). Various gel-buffer combinations were used to optimize the resolution of banding patterns (Appendix 2).

Forty-five presumptive genetic loci were scored (Table 1), with alleles coded in reference to their mobility from the origin. The most cathodal alleles were coded "a,"with subsequently faster alleles coded as "b," "c," and so on. Multiple isozymes at a locus were also coded by mobility. The most anodally appearing isozymes were coded as "1," with the more cathodal isozymes coded as "2," "3," and so on. Acronyms for loci follow Harris and Hopkinson (1976). We entered individual genotypes into the computer program BIOSYS-1 (Swofford and Selander 1981), which produced a table of allelic frequencies, Nei's (1972, 1978) and Rogers' (1972) genetic distances, Cavalli-Sforza and Edwards' (1967) chord distance, and four UPGMA phenograms (Sneath and Sokal 1973) derived using these four distance measures. Because there is no consensus on tree-building methods, we used both distance and discrete-locus approaches and compared results. Distance-Wagner

TABLE 1. Allelic frequencies for variable loci. Numbers in parentheses are frequencies of alleles not fixed for that locus. Allelic designations by letter indicate fixation at that locus.

	Taxon[a]															
Locus	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
AB-5	B	B	B	B	B	B	B	C	B	B	B	B	B	B	A	A
ACON-2	B	A	B	B	A	B	A	A	A	A	A	A	A	A	A	C
ACP-2	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B
ADA	D	E	C	D	D	B	C	B	B	B	C	D	C	C	C	A
AK-1	F	E	D	D(.50) E(.50)	D	C	D	D	D	D	D	D	D	A	B	B
AK-2	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A	A
ALD-2	A	A	D	D	D	D	D	D	C	D	E	E	F	D	B	B
CK-1	B	B	F	E	D	E	E	E	E	E	E	E	E	E	A	C
CK-2	A	A	A	A	A	A	A	C	A	A	A	A	A	A	D	B
EAP	C	C	D	D	D	D	D	D	D	D	D	D	D	D	B	A
ENOL	C	C	D	C	B	B	C	C	C	C	C	C	C	A	D	D
EST-D	F	D	F	B(.50) E(.50)	G	E	C	C	E	E	E	E	F	E	A	E
FUM	B	B	B	C	B	C	B	B	C	B	B	C	C	B	A	D
GDA	F	F	B	A	C	D	C	C	C	C	C	C	C	C	H	G
GLUD	B	B	B	B	B	B	B	B	B	B	B	B	B	B	C	A
A-GPD	C	E	E	B	D	D	D	D	D	B	D	D	C	C	A	B
G6-PDH	D	D	F	B	D	D	D	D	D	D	D	D	A	D	E	C
GOT-1	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	A
GPI	E	B	D(.50) F(.50)	D	D	D	A	A	A	A	D	D	C	C	H	G
GR	C	C	B	C	C	C	D	C	C	C	A	C	C	C	C	C
IDH-1	B	B	A	B	B	B	E	E	C(.50) F(.50)	E	E	E	E	E	C	C
IDH-2	C	B	C	B	B	B	B	B	B	B	B	B	B	B	A	A
LA	H	F	H	J(.50) K(.50)	J	K	B	C	A	A	D	D	G	G	E	I
LDH-1	A	B	A	A	A	A	A	A	A	A	A	A	A	A	A	A
LDH-2	B	B	B	B	A	B	B	B	B	B	B	B	B	B	C	C
LGG	L(.75) M(.25)	K	H	F(.25) H(.75)	H	I	A	A	D	E	C	C	G(.75) J(.25)	B	F	F
MDH-1	B	B	B	B	B	B	B	B	B	B	B	B	B	B	A	B
MDH-2	B	E	D	D	B	E	B	B	B	B	B	B	E	B	A	A
ME-1	E	D	F	G(.75) H(.25)	H	G	J	J	G	G	G	G	I	I	A(.50) C(.50)	B
ME-2	B	A	B	B	B	B	B	B	B	B	B	B	B	B	B	B
MPI	G(.75) I(.25)	C	B	F	F	F	F	F	F	F	E	E	H	H	D	A
NP	F	B	H	A	C	D	K	K	N(.25) P(.75)	E(.25) G(.50) I(.25)	O	L	M	O	J	J
6-PGD	I	H	D	D(.25) F(.25) G(.50)	D	B	D	D	B(.50) D(.50)	B(.25) D(.50)	D	D	E	A	C	B
PGM-2	D	F	D	D	D	F	E	E	B	E	C	E	E	E	D	A
Phe-Pro	B	B	E	E	A	E	F	F	H(.50) I(.50)	E	D	C	F	F	G	G
PK-1	B	B	B	B	A	A	B	B	B	B	B	B	B	B	B	B
PK-2	A	A	A	A	A	A	A	A	A	A	A	A	A	A	B	B
SORDH	C(.25) G(.75)	C	D	D	A	D	F	F	F	F	E	E	B	E	E	E

[a]1, *Glaucis hirsuta*; 2, *Phaethornis philippii*; 3, *Androdon aequatorialis*; 4, *Colibri coruscans*; 5, *Schistes geoffroyi*; 6, *Doryfera johannae*; 7, *Acestrura mulsant*; 8, *Selasphorus sasis*; 9, *Amazilia viridicauda*; 10, *Campylopterus largipennis*; 11, *Aglaeactis castelnaudii*; 12, *Coeligena violifer*; 13, *Metallura tyrianthina*; 14, *Oreotrochilus estella*; 15, *Reinarda squamata*; 16, *Chaetura cinereiventris*.

trees (Farris 1972, 1981; Swofford 1981) were produced using Rogers' (1972) distance measure. Distance-Wagner trees were generated by specifying the Multiple Addition Criterion and allowing for 30 partial networks to be used during each successive step. Both Prager and Wilson's "F" value (1976) and the Fitch and Margoliash (1967) percent standard deviation (% SD) were used to determine which partial networks to save. Distance-Wagner trees were rooted by designating the two swift taxa as outgroups. To complement the distance-Wagner trees, we used PHYLIP to perform a Fitch-Margoliash (1967; F-M) distance analysis according to the following parameters: global search option, no negative branch lengths, and 10 random addition sequences. Both distance-Wagner and F-M analyses permit variable evolutionary rates whereas UPGMA assumes rate constancy. We are aware of the criticisms of distance approaches (Farris 1981, 1985, 1986; Nei et al. 1983; Felsenstein 1986); however, in our experience there is often little difference between distance and discrete-character (locus) approaches.

We conducted a cladistic analysis using loci as characters and alleles as unordered character states (Baverstock et al. 1979; Patton and Avise 1983; Buth 1984). For polymorphisms, the most frequent allele was considered the state, an approach that ignores frequency information; if there were two alleles at 0.50 frequency at a locus, we assigned the state that matched other taxa, if at all (other methods of coding did not alter conclusions). These data were analyzed with the computer program PAUP 3.1.1 (Swofford 1993), using heuristic searches with 50 random addition replicates. Multiple equally parsimonious trees were summarized as strict consensus trees. A bootstrap analysis (Felsenstein 1985) was performed using heuristic searches and 100 replications. The computer program MacClade (Maddison and Maddison 1992) was used to evaluate alternative tree topologies derived from the literature.

RESULTS

Genetic variation.—Of the 45 loci scored, 38 exhibited some polymorphism (at least two alleles found across all taxa; Table 1). Seven loci were monomorphic and fixed for the same allele in all taxa (Gapdh, Got-2, HK, Lap, Odh-1, SOD-1,2). Of the 38 polymorphic loci, five were nearly monomorphic and exhibited only two alleles: AcP-2, AK-2, Ldh-1, Mdh-1, ME-2. Three loci (Glud, Got-1, PK-2) were monomorphic and fixed in the Trochilidae for an allele that differed from the outgroup (Apodidae).

Genetic distances.—Nei's (1978) genetic distance averaged 0.625 ± SD of 0.215 (Table 2) among the 14 hummingbird taxa. Values range

TABLE 2. Matrix of Nei's (1978; above diagonal) and Rogers' (1972; below diagonal) genetic distances for taxa analyzed.

Taxon	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 *G. hirsuta*	—	0.636	0.741	0.702	0.811	0.936	0.871	0.936	0.838	0.793	0.871	0.811	0.865	0.811	1.685	1.852
2 *P. philippii*	0.476	—	1.086	0.871	0.956	0.891	0.891	0.956	0.858	0.813	0.891	0.891	0.885	0.891	1.859	2.054
3 *A. aequatorialis*	0.527	0.663	—	0.508	0.733	0.788	0.706	0.878	0.877	0.702	0.733	0.847	0.811	0.817	1.440	1.705
4 *C. coruscans*	0.511	0.586	0.424	—	0.509	0.363	0.629	0.681	0.493	0.419	0.616	0.476	0.635	0.694	1.489	1.443
5 *S. geoffroyi*	0.557	0.615	0.526	0.424	—	0.573	0.528	0.573	0.562	0.522	0.573	0.528	0.824	0.668	1.571	1.872
6 *D. johannae*	0.608	0.590	0.551	0.329	0.436	—	0.719	0.719	0.473	0.500	0.668	0.573	0.767	0.719	1.859	1.584
7 *A. mulsant*	0.582	0.590	0.509	0.479	0.410	0.513	—	0.173	0.353	0.253	0.368	0.405	0.479	0.405	1.571	1.872
8 *S. sasin*	0.608	0.615	0.586	0.505	0.436	0.513	0.128	—	0.353	0.253	0.486	0.445	0.566	0.486	1.571	1.718
9 *A. viridicauda*	0.569	0.577	0.590	0.416	0.439	0.388	0.311	0.311	—	0.195	0.392	0.316	0.533	0.539	1.538	1.490
10 *C. largipennis*	0.550	0.557	0.511	0.369	0.412	0.399	0.233	0.233	0.197	—	0.360	0.323	0.504	0.388	1.554	1.422
11 *A. castelnaudii*	0.582	0.590	0.526	0.479	0.436	0.487	0.308	0.385	0.337	0.310	—	0.167	0.566	0.405	1.453	1.584
12 *C. violifer*	0.557	0.590	0.577	0.402	0.410	0.436	0.333	0.359	0.285	0.284	0.154	—	0.479	0.445	1.453	1.466
13 *M. tyrianthina*	0.579	0.587	0.558	0.481	0.562	0.536	0.382	0.433	0.421	0.401	0.433	0.382	—	0.325	1.447	1.711
14 *O. estella*	0.557	0.590	0.561	0.509	0.487	0.513	0.333	0.385	0.423	0.326	0.333	0.359	0.280	—	1.348	1.466
15 *R. squamata*	0.809	0.843	0.762	0.767	0.791	0.843	0.791	0.791	0.779	0.785	0.766	0.766	0.763	0.740	—	0.655
16 *C. cinereiventris*	0.839	0.872	0.817	0.762	0.846	0.795	0.846	0.821	0.773	0.758	0.795	0.769	0.818	0.769	0.484	—

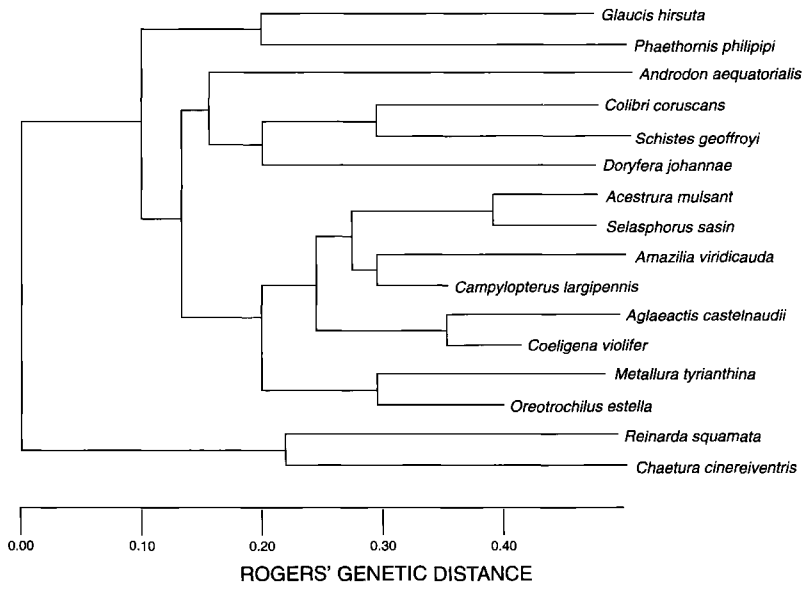

FIG. 1. Distance-Wagner tree rooted by the outgroup method (Farris 1972). Units are in Rogers' (1972) D. The % SD equals 13.635 (unoptimized).

from 0.137 (*Selasphorus sasin* vs. *Acestrura mulsant*) to 1.086 (*Phaethornis philippii* vs. *Androdon aequatorialis*). The average genetic distance between the families (two species in the Apodidae, 14 in the Trochilidae) was 1.61 ± 0.180 (n = 28). Interfamilial values range from 1.348 (*Oreotrochilus estella* vs. *Reinarda squamata*) to 2.054 (*Phaethornis philippii* vs. *Chaetura cinereiventris*). Each swift species shows a similar range of values to the hummingbirds (1.4 to 2.0, *C. cinereiventris*; 1.4 to 1.9, *R. squamata*). The genetic distance between the two swifts was 0.655.

Distance analyses.—Because most distance analyses use metric measures (Farris 1972, Sneath and Sokal 1973; but see Nei 1987), we used Rogers' (1972) distances. UPGMA phenograms (not shown) generated with various distance measures (Cavalli-Sforza and Edwards 1967; Nei 1972, 1978; Rogers 1972) yielded the same topology and differed only in branch lengths. These phenograms exhibited a nearly identical topology to the "best" distance-Wagner tree (Fig. 1), as judged by the minimum value of %SD (60 trees) or Prager and Wilson's "F" (15 trees). Low values of each of these goodness-of-fit measures indicate that the dendrogram faithfully portrays the distances in the original matrix. We emphasize the distance-Wagner trees because of the potential bias caused by using a phenogram when rates vary (Felsenstein 1986).

The hermits (*Glaucis, Phaethornis*) were most similar to the trochilines. The placement of *Androdon* differed between the UPGMA phenogram (not shown) and distance-Wagner tree. In the UPGMA phenogram it is a sister group to the trochilines, whereas in the distance-Wagner analysis it is placed basally within the trochiline-A assemblage, although it was relatively divergent. The F-M tree (not shown) differs from the distance-Wagner tree only in suggesting that *Androdon* was sister to all other hummingbirds, and in that the remainder of the trochiline-A group was paraphyletic, but placed between the hermits and the rest of the trochilines (as in the distance-Wagner topology). Thus, the F-M tree suggests more than two major lineages of hummingbirds. The trochiline-A assemblage also includes *Colibri* and *Schistes* as sister taxa, with *Doryfera* a distant member; however, in the F-M tree, *Doryfera* and *Schistes* were sister taxa. The distance analyses are inconclusive concerning the monophyly and relationships of the trochiline-A group.

The other major branch leads to four pairs of taxa comprising the trochiline-B group, three of which are characterized by long branch lengths. The length of these branches, however, could be a function of the low number of taxa

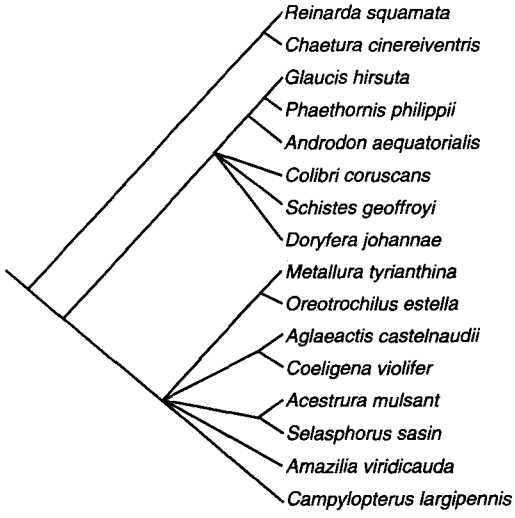

FIG. 2. Strict consensus of 40 equally parsimonious trees.

used per clade. The two members of each of Zusi's (pers. comm.) four clades grouped together, and topologies did not differ between F-M and distance-Wagner trees. The high Andean taxa (*Metallura, Oreotrochilus*) were basal, followed by the Andean group (*Aglaeactis, Coeligena*) and the remaining two lines, the Emeralds (*Amazilia, Campylopterus*) and Bee (*Acestrura, Selasphorus*) groups, the latter two of which are sister-groups. All distance approaches produced congruent topologies for the trochiline-B group.

Parsimony analysis.—Various alleles (Table 1) support phylogenetic groupings: Hermits (ALD-2, CK-1, EAP, Phe-Pro, SORDH), trochilines (EAP), Emeralds (LA), Bee (EST-D, LGG, ME-1, NP), Andean (ALD-2, LA, LGG, MPI), and High Andean (GPI, LA, ME-1, MPI). We found 40 equally parsimonious trees (length 178, CI = 0.87, RI = 0.71). Using PAUP, we found that the shortest of 1,000 random trees was 204, and the negative g_1-statistic (−0.88) is significant, suggesting signal in the data set (Hillis 1991, Kallersjo et al. 1992). The strict consensus tree (Fig. 2) indicates that: *Androdon* is a sister taxon to the hermits and these plus the trochiline-A group are a sister clade to the trochiline-B group. No clear pattern of sister-group relationships exists within the trochiline-A group. Within the trochiline-B group, the Bee, Andean, and High Andean groups each are monophyletic, whereas the two representatives of the Emerald group form a clade in only 90% of the 40 trees; there is no strong cladistic support for sister-group relationships in the trochiline-B group, although the Bee and Emerald groups are a clade in 90% of the 40 trees. Bootstrap analysis (not shown) supports (at ≥70%) the same groupings in the trochiline-A group, and *Androdon* plus hermits, but no other major groupings.

Overly distant outgroups can bias tree topology and interpretation by altering the ingroup topology or by placing the root randomly along the longest branch within the ingroup (Smith 1994). Because of the relatively great distance from the outgroups to the hummingbirds, we excluded the swifts and recomputed maximum parsimony trees. We found 102 equally parsimonious trees (length 127, CI = 0.84, RI = 0.68), the strict consensus of which supports the two trochiline groups, but places *Androdon* sister to them. However, this topology would result from excluding the swifts from Fig. 2 and rerooting at one of the hermits. Inclusion of the swifts (Fig. 2) therefore did not alter the phylogenetic patterns within the hummingbirds, but the placement of the root could be problematic owing to the high level of swift-hummingbird divergence. Thus, cladistic analyses do not support a hermit-Trochiline dichotomy.

DISCUSSION

Levels of genetic divergence.—Protein electrophoresis found widespread application in avian systematics, although it was applied infrequently at higher taxonomic levels (Dittmann et al. 1989). The major emphasis was on congeneric passerines, and in particular Nearctic passerines (Zink 1991). These studies found that birds were less differentiated at comparable taxonomic levels than many other vertebrates (Avise 1994). However, non-passerine groups and tropical passerines showed greater levels of divergence (Gutiérrez et al. 1983; Johnson and Zink 1983; Lanyon and Zink 1987; Gerwin and Zink 1989; Gill and Gerwin 1989; Hackett and Rosenberg 1990; Christidis et al. 1991; Randi et al. 1991, 1992; Hackett 1995; Brumfield and Capparella 1996). Levels of allozymic differentiation in hummingbirds were high, 0.625 (± 0.215) within families and 1.61 (± 0.180) between families, consistent with

other nonpasserines (e.g. Randi et al. 1991, 1992).

The absolute age of hummingbirds is unknown: "There is no fossil record of the Trochilidae other than of modern species from a few Quaternary cave deposits, mostly in the West Indies . . ." (Olson 1985). True swifts appear in the fossil record in the early Miocene of France (Olson 1985). If the Trochilidae is the sister group of swifts (as found by Sibley and Ahlquist 1990), then hummingbirds are of at least this vintage. Based on DNA-DNA hybridization studies, the split between the two groups is an ancient one, occurring approximately 95 million years ago (Sibley et al. 1990). Applying a calibration of genetic distances suggested by Marten and Johnson (1986), namely one unit of Nei's (1978) 1D equals 20 million years (MY), the hummingbird-swift split was 32 MY ago; whereas, if we use the calibration of Gutiérrez et al. (1983), 1D = 26.3 MY, this split occurred 43 MY ago (range 36 to 55 MY ago). Conversely, if one assumes a split of 95 million years for swifts and hummingbirds, the corresponding allozymic rate calibration for hummingbirds would be 1D = ca. 50 million years, one of the slowest suggested rates for vertebrates (Avise 1994). These rate calibrations obviously conflict, and additional data are needed to resolve them. We agree with Avise (1994) that rate calibrations must be interpreted cautiously.

Intrafamilial Phylogenetic Relationships

The lack of previous explicit hypotheses of higher-level relationships in hummingbirds might be due to the complex nature of morphological variation. Morphological patterns of variation, especially plumage patterns and coloration, may be inadequate indicators of phylogenetic relationships because of convergence or parallelism (homoplasy), sexual selection, or extreme anagenesis (Sibley and Ahlquist 1990), factors probably widespread in hummingbirds. For example, approximately 60 monotypic genera occur in the Trochilidae, a likely signal of taxonomic uncertainty in general (Platnick 1977) and among hummingbirds in particular (Gill and Gerwin 1989), owing to a lack of synapomorphies at higher levels. Our work permits evaluation of previous hypotheses of hummingbird relationships based on morphological variation, as well as the recent DNA-DNA hybridization study by Bleiweiss et al. (1997). Because our distance and cladistic analyses differ, below we point out discrepancies.

Phaethorninae.—Distance analyses (Fig. 1) suggest that the hermits (*Glaucis* and *Phaethornis*) are a sister group to other hummingbirds, which is consistent with other evidence (Gould 1861; Zusi and Bentz 1982; Sibley and Ahlquist 1990; Bleiweiss et al. 1994, 1997; Hernandez-Banos et al. unpubl. data). Surprisingly, cladistic analyses (Fig. 2) did not support the long-standing division into hermits and trochilines. Although our use of the swifts apparently did not bias ingroup relationships, a traditional topology (Fig. 3) required 180 steps, only two steps longer than the most parsimonious trees (178). Lack of a clear signal for the basal relationships likely contributes to the different placement of the hermits in the cladistic analysis in which relatively few characters support major groupings (Fig. 3). Based on a 433-bp segment of mitochondrial cytochrome-*b*, Hernandez-Banos et al. (unpubl. data) found that the two subfamilies of hummingbirds were only slightly more distant than some of the trochilines were from each other. Nonetheless, we conclude that the weight of evidence favors a hermit-trochiline dichotomy.

Trochiline group A: Problems of classifying morphologically complex taxa.—An example of complex morphological patterns obscuring phylogenetic affinities involves the placement of *Androdon* and *Doryfera*. *Androdon* and *Doryfera* are usually placed at the beginning of the hummingbird section of checklists (Peters 1945, Meyer de Schauensee 1966, Morony et al. 1975), which might simply represent uncertainty about their relationships. Our results and those of others (Zusi and Bentz 1982; Sibley and Ahlquist 1990, Bleiweiss et al. 1994, 1997; Schuchmann pers. comm.) indicate that *Androdon* and *Doryfera* do not belong in the hermit group. Proteins of *Androdon* and *Doryfera* were analyzed electrophoretically along with several *Phaethornis* species, both species of *Eutoxeres* and *Threnetes*, and *Glaucis hirsuta*, and were found to be distinct from phaethornine taxa (Gill and Gerwin 1989).

We suspect that the placement of *Androdon* near hermits in traditional checklists is the result of several factors. It is morphologically similar to the hermits in two ways. *Androdon*

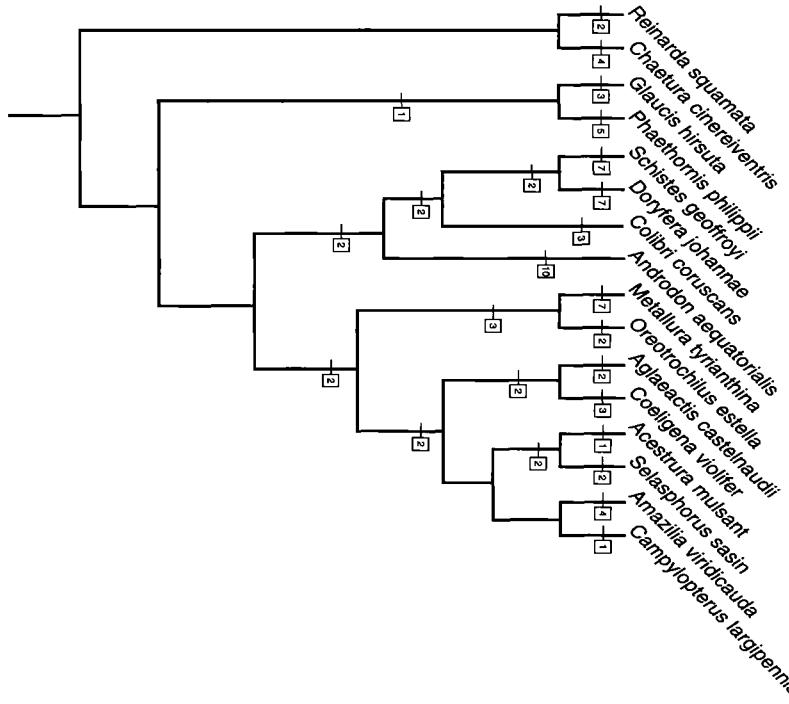

FIG. 3. Topology suggested by Sibley and Ahlquist (1990) and distance-Wagner approach (Fig. 1). Numbers indicate unambiguous synapomorphies along branches. Note the relatively weak support for major clades.

has a long (45 mm) and straight or slightly upturned bill, similar to some species in the genus *Phaethornis*. On its underparts (throat, breast and abdomen), *Androdon* shows a pattern of dull gray-white with dark brown/black streaks, similar to the hermit genus *Eutoxeres*. Conversely, *Androdon* is similar to many trochilines in dorsal color (green), tail shape (rounded) and pattern (broad white tips), although all three characters are shown by some Phaethorninae. The bills of *Androdon* and *Ramphodon* are unique among trochilids in possessing small, comb-like serrations along both tomia, and in being hooked (Ornelas 1994). A number of other species have minute bill serrations, involving either the maxilla or tomia, and some additionally have a hook at the end of the bill; however, no other species share the *Androdon-Ramphodon* pattern. In addition to serrate tomia, *Androdon* and *Ramphodon* share a similar pattern of streaking underneath. Thus, morphologically *Androdon* shares traits with different groups of hummingbirds, and its placement depends on which set of traits is emphasized.

Ornelas (1994) hypothesized that bill serrations aid in nectar robbing. Unfortunately, our sampling of taxa is insufficient to determine precisely the coevolutionary relationship between this behavior and bill serrations, but our tree agrees with Ornelas (1994:708) in suggesting that "the complex of features of the bill for nectar robbery has evolved more than once in birds with such morphology." Thus, bill serrations in taxa such as *Androdon* might indeed be homoplasious and unlikely to provide a reliable phylogenetic signal.

In the distance analyses, *Androdon* was placed in one of four positions: (1) entirely outside the other trochilids; (2) as a sister taxon to the hermits; (3) as a separate lineage between the hermits and trochiline-A group; and (4) as a sister group to the other trochiline-A members (as in Fig. 1). Maximum parsimony (Fig. 2) consistently places *Androdon* as a sister taxon to the hermits. Because Zusi and Bentz (1982), Sibley and Ahlquist (1990), Bleiweiss et al. (1994, 1997) and our distance-Wagner tree (Fig. 1) suggest that *Androdon* is a basal member of the trochiline-A group, we favor this placement pending an analysis that includes more taxa.

Androdon and *Doryfera* appear to share characteristics with several hummingbird groups,

which has no doubt contributed to taxonomic uncertainty. The placement of *Doryfera* near *Androdon* in most checklists is presumably because it resembles *Androdon* in bill morphology (long (25–35 mm) and straight or slightly upturned). *Doryfera,* however, lacks serrations on the bill (Ornelas 1994) and possesses a uniform green to dark overall plumage with a glittering frontlet (green or violet); neither characteristic resembles *Androdon* nor *Phaethornis.* The position of *Doryfera* varies in our distance trees, although it is clearly associated with other taxa in the trochiline-A group. It is placed between *Androdon* and *Schistes/Colibri* within the trochiline-A assemblage (Fig. 1). However, in the F-M tree *Doryfera* groups with *Colibri* and in our cladistic analyses it groups with *Schistes.* Zusi and Bentz (1982) and Sibley and Ahlquist (1990) placed *Doryfera* and *Colibri* within the trochiline-A assemblage. Our data agree in suggesting that four of the taxa we studied (*Androdon, Doryfera, Colibri,* and *Schistes*) are part of this group, but discrepancies between distance and cladistic analyses do not suggest a clear phylogenetic pattern (Figs. 1, 2). Bleiweiss et al. (1997) found that *Eulampis holosericeus* and *Heliothryx barroti* grouped with *Doryfera, Colibri* and *Androdon.* Other taxa are considered part of this group (Zusi and Bentz 1982), and further analyses are required to assure monophyly of the group.

Trochiline group B.—Zusi and Bentz (1982) studied hummingbird and swift musculature, particularly the *tensor patagii brevis* (TPB) muscle, including the same genera and most of the same species included in this study. They found two muscle types among the trochiline-B clade, and members of the Bee group (*Selasphorus, Acestrura*) have one or the other; unfortunately no phylogenetic conclusion can be drawn concerning the monophyly of the Bee group nor the relationships of the four groups. Our data support the four groups suggested by Zusi (pers. comm.), although support for the Emerald clade was relatively weak (< 50% bootstrap support). Hernandez-Banos et al. (unpubl. data) suggested that *Amazilia* likely was not monophyletic, although the two Bee hummingbirds formed a clade. Thus, additional sampling is needed to assure monophyly of the four groups, especially the Emeralds.

Congruence of allozymes and DNA-DNA hybridization.—Sibley and Ahlquist (1990:846) suggest the following ordering of taxa belonging to the family Trochilidae (they did not include a putative Bee representative): (Phaethorninae, (trochilinae-A plus *Androdon*), (High Andean, (Andean, Emerald)))). With the exception of the missing Bee group, this topology exactly matches our distance analysis (Fig. 1), and requires only four more steps in our cladistic analysis; our distance analysis unites the Bee and Emerald groups as sister clades. Placing the Bee group basal within the trochiline-B group results in a total tree length of 182, and moving it to the trochiline-A group (suggested as a possibility by R. Zusi) results in a tree length of 184. The study of Bleiweiss et al. (1997), which unlike the Sibley/Ahlquist study included a complete, reciprocal matrix of hybridization distances, yields a topology (their figure 2) that also matches that of our distance-Wagner tree (Fig. 1). However, Bleiweiss et al. (1997) favored their figure 3, which depicts the Andean clade (Brilliants) as basal in the Trochiline-B assemblage. The distinction between trees in figures 2 and 3 of Bleiweiss et al. (1997) involved very short internodes, and their reasons for favoring their figure 3 involved assumptions used in the analysis of DNA-DNA hybridization data. Although they concluded that additional study would likely favor the Andean (Brilliant) clade as basal, our data suggest that the high Andean clade is basal.

Of the 25 genera and 26 species used by Bleiweiss et al. (1997), eight genera and only three species were common between our studies. Thus, different groups of species were used to represent the major lineages. Although different numbers of species were used in each study, it is exceedingly unlikely that our topologies would match by chance. An obvious reason for the match is that both trees recover phylogeny; in theory, another reason might be that each analysis is biased by homoplasy in the same way. Although we found conflicting support for the relationships in Figure 1 in our alternative analyses, we suggest that the congruence between the DNA-DNA hybridization (Bleiweiss et al. 1997:figure 2) and our allozyme distance tree (Fig. 1) indicates that the relationships of the major clades of hummingbirds are nearly resolved.

ACKNOWLEDGMENTS

We thank numerous colleagues at the LSUMNS who collected tissue samples used in this study. We

thank the late J. S. McIlhenny and the late B. S. Odum for providing funds for those expeditions. We thank F. B. Gill and M. B. Robbins, of the Philadelphia Academy of Natural Sciences, for providing tissue samples. We thank F. B. Gill, K.-L. Schuchmann, C. G. Sibley, and R. Zusi for the use of unpublished data and for fruitful conversations. S. Lanyon, G. Graves, R. Zusi, J. Klicka, K. Winker, J. Weckstein, K. Johnson, A. Fry, and three anonymous reviewers provided useful comments on the manuscript. We thank C. White, N. Tague, and S. J. Hackett for preparing the figures. J. V. Remsen and M. S. Hafner provided useful comments on early drafts of the manuscript, which formed a part of a M.S. thesis by Gerwin in the Department of Zoology and Physiology at LSU. Financial support was provided by the American Museum of Natural History (Chapman Fund), and the LSUMNS.

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Associate Editor: A. J. Baker

APPENDIX 1. Species studied, sample sizes, and localities for specimens.

Species	Tissue no.	Locality
Glaucis hirsuta (Rufous-breasted Hermit)	103561, 103612	PERU: Loreto: S bank Maranon R. along Samiria R., Est. Biol. Pithecia
Phaethornis philippii (Needle-billed Hermit)	9020, 9168	BOLIVIA: depto. Pando; Prov. Nicolas Suarez, 12 km by road S Cobija, 8 km W on road to Mucden
Androdon aequatorialis (Tooth-billed Hummingbird)	1402	PANAMA: Darien; ca. 9 km NW Cana on slopes Cerro Pirre
Colibri coruscans (Sparkling Violetear)	5549, 5574	PERU: depto. San Martin; 15 km NE Jirillo, 1,350 m
Schistes geoffroyi (Wedge-billed Hummingbird)	8126	PERU: depto. Pasco; Playa Pampa, 8 km NW Cushi on Chaglla Trail
Doryfera johannae (Blue-fronted Lancebill)	1728	PERU: depto. Pasco; Santa Cruz; ca. 9 km SSE Oxapampa
	5402	PERU: depto. San Martin; 20 km NE Tarapoto, 1,050 m
Acestrura mulsant (White-bellied Woodstar)	6312, 6314	ECUADOR: Pichincha; Yanayacu, N Slope Pichincha, 3,500 m
Selasphorus sasin (Allen's Hummingbird)	0142	USA: Louisiana; Jefferson Parish; Metairie
	5740	USA: Louisiana, E. Baton Rouge Parish; Baton Rouge
Amazilia viridicauda (Green-and-white Hummingbird)	8136, 8158	PERU: depto. Pasco; Cushi, ca. 1,800 m
Campylopterus largipennis (Gray-breasted Sabrewing)	4474	PERU: depto. Loreto; Lower Rio Napo, E Bank Rio Yanayacu
	5577	PERU: depto. San Maritn; ca. 15 km by trail NE Jirillo on trail to Balsapuerto, 1,350 m
Aglaeactis castelnaudii (White-tufted Sunbeam)	3605, 3620	PERU: depto. Huanuco; Quebrada Shugush, 30 km on Huanuco-La Union road
Coeligena violifer (Violet-throated Starfrontlet)	3504	PERU: depto. Huanuco; Bosque Potrero, 14 km W Panao
	8218	PERU: depto. Pasco; Millpo, E Tambo de Vacas on Pozuzo-Chaglla Trail, 3,450 m
Metallura tyrianthina (Tyrian Metaltail)	8209, 8218	PERU: depto. Pasco; Millpo, E Tambo de Vacas on Pozuzo-Chaglla Trail, 3,450 m
Oreotrochilus estella (Andean Hillstar)	103834, 103835	PERU: depto. Ayacucho; Pampa Galeras, 25 km WNW of Puquio, 3,850 m
Reinarda squamata (Fork-tailed Palm-Swift)	5039	PERU: depto. Loreto; S Rio Amazonas, ca. 10 km SSW Rio Napo
Chaetura cinereiventris (Gray-rumped Swift)	9397	BOLIVIA: depto. Pando; Prov. Nicolas Suarez, 12 km by road S Cobija, 8 km W on road to Mucden

APPENDIX 2. Loci scored, gel type used and the position of bands on that particular gel type. For loci with two types listed, both have reproducible results.

Locus	E.C. number	Gel-buffer[a,b]	
GP-5	—	Poulik	A
ACON-2	4.2.1.3	AC	C
ACP-2	3.1.3.2	PC	C
ADA	3.5.4.4	TC III	A
ADH (ODH-1)	1.1.1.1	TM II	C
AK-1	2.7.4.3	TC III	A
AK-2	2.7.4.3	TM I	A
ALD-2	4.1.2.13	AC	C
CK-1,2	2.7.3.2	Poulik	A
ACP-1 (EAP)	2.7.3.2	TM I, TC II	A
ENOL	4.2.1.11	TM I	A
EST-D	3.1.1.1	Poulik	A
FUM	4.2.1.2	TC III	C
GAPDH	1.2.1.12	AC	C
GDA	3.5.4.3	TC II	A
α-GPD	1.1.1.8	AC	C
G6PDH	1.1.1.49	PC	A
GPI	5.3.1.9	AC	C
GR	1.6.4.2	TM I	A
GLUD	1.4.1.3	TM II	A
GOT-1,2	2.6.1.1	AC	C
HK	2.7.1.1	TM I	C
IDH-1,2	1.1.1.42	AC	C
PEP-A (LA)	3.4.11 or 13	AC	A
LDH-1,2	1.1.1.27	Poulik	A
PEP-B (LGG)	3.4.11 or 13	AC	A+C
MDH-1	1.1.1.37	TC II	A
MDH-2	1.1.1.37	TC II, PP	C
ME-1	1.1.1.40	TC II, TC 8.5	A
ME-2	1.1.1.40	TM 7.5	C
MPI	5.3.1.8	AC	A
NP	2.4.2.1	AC	A
6PGD	1.1.1.44	AC	A
PGM-2	2.7.5.1	Poulik, TC 8.5	A
PEP-D (PHE PRO)	3.4.11 or 13	AC	A
SORDH	1.1.1.14	PP	C
PK-1	2.7.1.40	Poulik	A
PK-2	2.7.1.40	Poulik	A

[a] AC = Amine-Citrate pH 6.1; PC = Phosphate-Citrate pH 6.2; PP = PGI-Phosphate pH 6.8; TC II = Tris-Citrate pH 8.0; TC III = Tris-Citrate pH 7.0; TC 8.5 = TC II titrated to pH 8.5 with N-3-aminopropylmorpholine; TM I = Tris-Maleate pH 7.5; TM II = Tris-edta-Maleate pH 6.5.

[b] A = anodal mobility; C = cathodal mobility.