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AFFINITIES OF THE SAW-BILLED HERMIT (*RAMPHODON NAEVIUS*) DETERMINED BY CYTOCHROME-*B* SEQUENCE DATA

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ABSTRACT.—We sequenced 912 bp of the cytochrome-*b* gene to examine phylogenetic relationships of the enigmatic Saw-billed Hermit (*Ramphodon naevius*), a large and distinctive hummingbird endemic to tropical forests of southeastern Brazil. Bootstrapped maximum parsimony and maximum likelihood analyses of sequence data from 11 hummingbirds and several outgroups (two swifts, one goatsucker) support: (a) monophyly of the traditional hermit (Phaethornithinae) and nonhermit (Trochilinae) subfamilies, (b) placement of *Ramphodon* among hermits, and (c) a sister relationship between *Ramphodon* and an exemplar of the widespread polytypic hermit genus *Glaucis*. The association of *Ramphodon* with derived hermit lineages is concordant with subfamilial patterns of wing anatomy and nest architecture. However, the unusual plumages (striped underparts) and male bills (long, serrated, hooked) shared by *Ramphodon* and the Tooth-billed Hummingbird (*Androdon aequatorialis*) appear to have evolved within separate hermit and nonhermit "tooth-billed" clades. Distal placement of the *Ramphodon-Glaucis* clade within hermits implies that even distinctive Brazilian endemics such as *Ramphodon* are derived forms that evolved relatively recently. *Received 18 March 2002, accepted 6 August 2002.*

Hermit hummingbirds are common inhabitants of forest interior throughout the Neotropics. The distinctive appearance of hermits has led to their designation as a subfamily within the Trochilidae since the first systematic treatments of hummingbirds (Reichenbach 1854, Cabanis and Heine 1860, Gould 1861, Ridgway 1911). However, the intrafamilial affinities of the enigmatic Saw-billed Hermit (*Ramphodon naevius*), a large and distinctive form endemic to the humid lowlands of southeastern Brazil (Ruschi 1986, Grantsau 1988, Sick 1993), have been the subject of considerable debate (Monroe and Sibley 1993, Hinkelmann and Schuchmann 1997, Gerwin and Zink 1998, del Hoyo et al. 1999). In particular, the taxon presents a mosaic of features typical of both the hermit (Phaethornithinae)

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FRONTISPIECE. Saw-billed Hermits (*Ramphodon naevius*) perched (male) and hovering (female) among the native food plant *Centropogon cornutus* (Lobeliaceae). The remarkable bill dimorphism and other superficial characteristics of *R. naevius* combine attributes of both main hummingbird lineages (hermits and nonhermits). Analysis based on DNA sequences reveals that *R. naevius* is a surprisingly derived member of the hermit subfamily. Painting by Barry K. MacKay.

TABLE 1. Taxa and samples used in this study (see Appendix 1).

Common name	Scientific name	Material	Phylogeny reference
Saw-billed Hermit	*Ramphodon naevius*	blood	this study
White-tipped Sicklebill	*Eutoxeres aquila*	frozen tissue	Bleiweiss et al. 1994b
White-whiskered Hermit	*Phaethornis yaruqui*	frozen tissue	Bleiweiss et al. 1994b
Band-tailed Barbthroat	*Threnetes ruckeri*	frozen tissue	Bleiweiss et al. 1994b
Bronzy Hermit	*Glaucis aenea*	frozen tissue	Bleiweiss et al. 1994b
Green-breasted Mango	*Anthracothorax prevostii*	blood	Sibley and Ahlquist 1990
Green-throated Carib	*Eulampis holosericeus*	EtOH-tissue	Bleiweiss et al. 1997
Purple-crowned Fairy	*Heliothryx barroti*	frozen tissue	Bleiweiss et al. 1997
Sparkling Violet-ear	*Colibri coruscans*	frozen tissue	Bleiweiss et al. 1997, Gerwin and Zink 1998
Green-fronted Lancebill	*Doryfera ludovicae*	frozen tissue	Bleiweiss et al. 1997, Gerwin and Zink 1998
Tooth-billed Hummingbird	*Androdon aequatorialis*	EtOH-tissue	Bleiweiss et al. 1997, Gerwin and Zink 1998
Moustached Treeswift	*Hemiprocne mystacea*	EtOH-tissue	Bleiweiss et al. 1994a
White-collared Swift	*Streptoprocne zonaris*	frozen tissue	Bleiweiss et al. 1994a
Common Nighthawk	*Chordeiles minor*	frozen tissue	Bleiweiss et al. 1994a[a]

[a] Representative in same family (Apodidae) as taxon used in earlier study.

and nonhermit (Trochilinae) subfamilies (Monroe and Sibley 1993; Bleiweiss et al. 1994a, 1994b, 1997; Gerwin and Zink 1998). The dull plumage, modest sexual dichromatism, and long bill of *Ramphodon* all are features typical of hermits (Sazima et al. 1995, Hinkelmann and Schuchmann 1997). However, the striking tooth-like serrations and terminal hook on the bill of male *Ramphodon*, as well as bold streaking on the underparts of both sexes, are features atypical for hermits but found to varying degrees among nonhermits (del Hoyo et al. 1999; see frontispiece). Indeed, *Ramphodon* bears a striking resemblance in plumage and bill form to the Tooth-billed Hummingbird (*Androdon aequatorialis*), a monotypic genus that several studies based on molecular (Gill and Gerwin 1989, Monroe and Sibley 1993, Bleiweiss et al. 1997, Gerwin and Zink 1998) or behavioral (Schuchmann 1995) data place among the nonhermits.

Recent morphological studies based on the patagial muscle complex of the wing (Zusi and Bentz 1982) and on cladistic analysis of a suite of external features (Hinkelmann and Schuchmann 1997) suggest that *Ramphodon* is a hermit. However, they differ in placing *Ramphodon* as a basal (external morphology) or more derived (wing anatomy) member of that subfamily. By contrast, some standard linear classifications place *Ramphodon* among the nonhermits, probably because of its morphological similarities to *Androdon* (Monroe and Sibley 1993). In this paper, we use cytochrome-*b* sequence data to assess relationships of *Ramphodon*. In particular, we address three questions about *Ramphodon*, including (a) subfamily membership, (b) placement as a basal or derived taxon, and (c) affinities with respect to similar-looking hermits (*Eutoxeres*) and putative nonhermits (*Androdon*).

MATERIALS AND METHODS

Study materials.—Considerable background information on hummingbird systematics allows judicious selection of taxa for the purpose of comparing hypotheses based on DNA sequencing with those suggested by earlier studies; except for *Ramphodon*, all taxa examined herein were included in earlier studies based on different molecular methods (Table 1). We chose single representatives from each of the four traditional hermit genera (*Eutoxeres*, *Phaethornis*, *Threnetes*, *Glaucis*; Monroe and Sibley 1993) as well as five species from the basal group of nonhermits (mangoes, subfamily Trochilinae; Bleiweiss et al. 1997). We used two levels of outgroups (Table 1) to root the phylogeny, including two representatives from the sister group to hummingbirds (swifts), and one representative from the next most distant clade, a goatsucker (Common Nighthawk, *Chordeiles minor*; Bleiweiss et al.

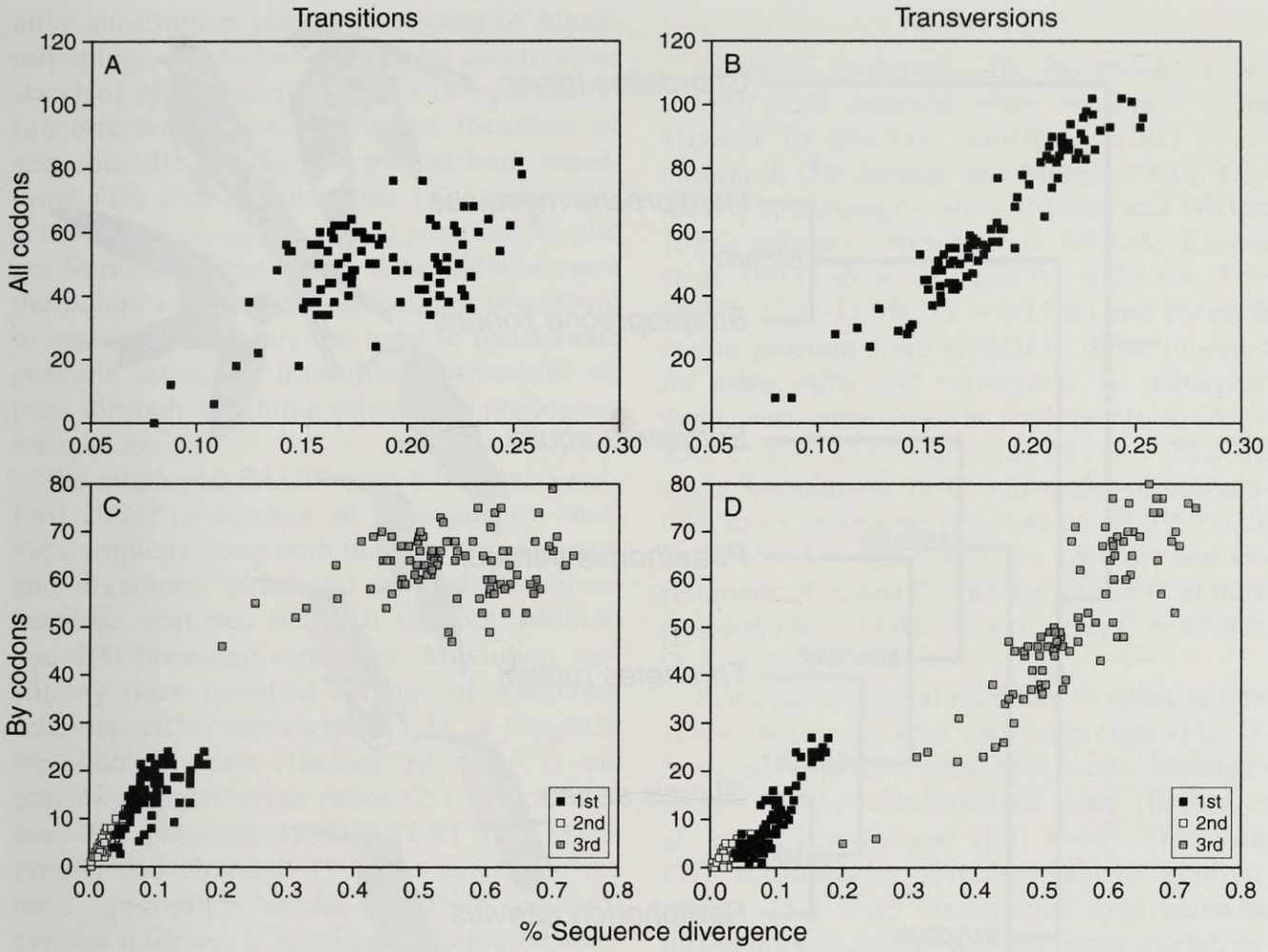

FIG. 1. Plot of the empirical numbers of transitions and transversions (y axis) versus total uncorrected (Kimura two-parameter) pairwise percentage differences estimated in MEGA (Kumar et al. 1993) for all (A, B), and individual (C, D), codon positions across all variable sites across all hummingbird and outgroup taxa.

1994b). Most authors conducted Brazilian field work (RB, MEB, YOW, and EOW), whereas collection of remaining materials was as described previously (Table 1; see also Bleiweiss et al. 1994a, 1997; Appendix). Subsets of authors executed laboratory work (RB and MEB) and analyses (RB and SLH). Specimen information and sequences have been deposited in GenBank (accession numbers AY150649–62).

Mitochondrial cytochrome-b gene isolation and sequencing.—We extracted DNA using Qiagen Tissue Kits (Valencia, California), following manufacturer's protocols. We sequenced single individuals from all taxa, using the polymerase chain reaction (PCR) to amplify the target fragment of the cytochrome-*b* gene with L14841 [5′-CCATCCAACATCTCAGCCATGATGAAA-3′] as forward (Kocher et al. 1989), and H15767 [5′-ATGAAGGGATGTTCTACTGTTG-3′] as reverse (Edwards et al. 1991) primer (numbers follow those for the human mitochondrial genome; see Edwards et al. 1991). The 100-μl reaction volume used for initial PCR comprised 1.0 μg of template, 2.5 units of Taq DNA polymerase (Promega Corporation), and concentrations of 200 μM dNTPs, 1.5 mM $Mg,^{2+}$ and 0.2 μM primer. We amplified the target sequence over 40 cycles in a Perkin-Elmer thermal cycler programmed to optimize yield of the desired product. We visualized reaction products by preparative-gel electrophoresis in TAE buffer, followed by staining with ethidium bromide. Then we used Wizard™ PCR preps to purify amplified DNA either cut from the gel or aliquoted directly from the reaction mixture. Finally, we submitted the amplified DNA product for automated sequencing (Iowa State DNA Sequencing and Synthesis facility) by dye-terminator reactions.

Phylogenetic analysis.—We examined rel-

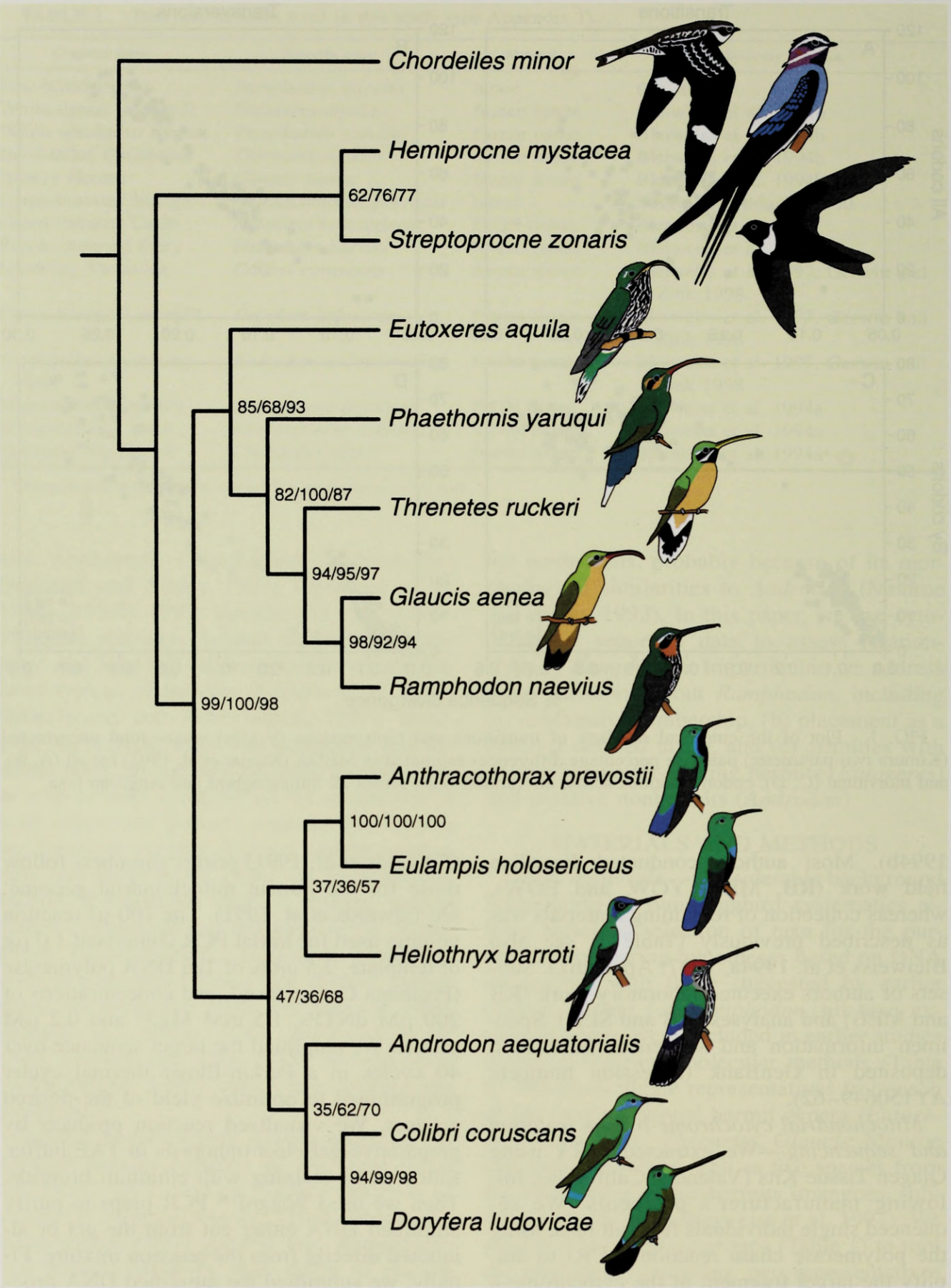

FIG. 2. Bootstrapped topology obtained by maximum parsimony and maximum likelihood for cytochrome-*b* gene sequences for all 14 taxa (rooted to the outgroup goatsucker, *Chordeiles minor*). Numbers at internal nodes indicate percentage bootstrap support out of 100 resamplings: unweighted parsimony (tree length = 1045)/ weighted (codon 1–3 positions = 2:4:1; Ti:Tv = 3:1) parsimony (tree length = 2,300)/likelihood (log likelihood

ative substitution rates with respect to substitution type and codon position by constructing standard plots against Kimura two-parameter (uncorrected) pairwise distance measures of sequence divergence. We plotted both transitions (Ti) and transversions (Tv) separately across all sites, and by codon position. We did not fit regressions through these plots because the points are not independent, but used them to assess qualitatively the need to incorporate possible saturation (multiple substitution) effects through weighting schemes in phylogeny estimation.

We employed PAUP* (ver. 9.0 Beta; Swofford 2002) to conduct all phylogenetic analyses, implementing both maximum parsimony and maximum likelihood methods based on heuristic searches with 10 random addition and 100 bootstrap replicates. Maximum parsimony trees specified a range of weighting schemes: either unweighted (1:1), or weighted for codon position (1st:2nd:3rd = 2:4:1) and transition-transversion ratios (2:1 or 3:1). We used the program MODELTEST (ver. 3.06; Posada and Crandall 1998) to determine the most appropriate model of DNA sequence evolution for use in maximum likelihood analysis. MODELTEST computes likelihood ratio tests (Kishino and Hasegawa 1989; Huelsenbeck and Crandall 1997) first on nested models of DNA substitution from the simplest (Jukes and Cantor 1969) to more complex (Rodríguez et al. 1990) and then on models of the most appropriate of these substitution matrices nested with respect to addition of parameters for invariant sites (I) and the gamma distribution of rates for variable sites (G). Through these series of steps, the program identifies the model of DNA sequence evolution that minimizes parameters without a significant (here based on the program's default $P < 0.01$) improvement in likelihood scores.

RESULTS

Sequence evolution.—No insertions or deletions were detected when sequences were aligned to chicken (*Gallus gallus*) cytochrome-*b* (Desjardins and Morais 1990). Observed base compositional (Prager and Wilson 1988) patterns (estimated in MEGA; Kumar et al. 1993) across all sites (A = 27.5%, T = 25.5%, C = 33.5%, G = 13.6%) and for each codon position were similar to those reported for other birds and vertebrates. In particular, there was little bias at first position (A = 25.2%, T = 23.6%, C = 28.7%, G = 22.5%), slight enrichment in C and under-representation in G at second position (A = 19.5%, T = 39.6%, C = 26.7%, G = 14.1%), and enrichment of A and C with very little G at third position (A = 37.6%, T = 13.2%, C = 45.0%, G = 4.2%).

Pairwise empirical numbers of substitutions across hummingbirds and outgroups (Fig. 1) revealed expected substitution-rate heterogeneity for this mitochondrial gene (Brown et al. 1982, Lanyon and Hall 1994). Transition (Ti) substitutions saturated above approximately 15% level uncorrected total pairwise differences (Kimura two-parameter model; estimated in MEGA) whereas transversions (Tv) remained linear over the observed range (Fig. 1A–B). Among codons, base substitutions accumulated fastest at third position and slowest at second position for both Ti and Tv (Fig. 1C–D). Most of the departure from linearity was contributed by sites at the more rapidly evolving third codon position, for which Ti divergence plateaued or even dropped beyond about 30% divergence (Fig. 1C). The nonlinear portions of curves were comprised almost exclusively of the hummingbird-to-outgroup (swifts, goatsucker) comparisons. Thus, models that incorporate weightings and distinct rate categories are justified.

Specific features of the amplified DNA and

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= −5572.63). Number of parsimony-informative sites ranges from 272 to 282, depending upon the model. Cartoons are based on the following sources: *Chordeiles minor*, *Hemiprocne mystacea*, *Ramphodon naevius*, *Anthracothorax prevostii*, *Eulampis holosericeus*, *Androdon aequatorialis* (del Hoyo et al. 1999); *Streptoprocne zonaris*, *Eutoxeres aquila*, *Phaethornis yaruqui*, *Threnetes ruckeri*, *Glaucis aenea*, *Colibri coruscans*, *Doryfera ludovicae* (Hilty and Brown 1986). Note scattered placement of large-bodied species (*Ramphodon*, *Eutoxeres*, *Androdon*) with large, specialized bills and striped underparts. Birds are not drawn to scale.

its genetic composition also argue that the mitochondrial sequences reported herein do not include ones that underwent atypical change after translocation to the nucleus (Arctander 1995). Only a single gene product of the predicted size ever resulted from our amplifications. Moreover, variation at first and second codon positions did not indicate a relaxation of constraints on the acceptance of substitutions, as would otherwise be expected for a noncoding pseudogene translocated to the nucleus. Rather, the aligned sequences revealed patterns of codon specific and base specific (transition bias) substitution patterns typical of avian mtDNA sequences. Furthermore, the gene sequences were translated in full using the chicken mitochondrial code (Desjardins and Morais 1990), without nonsense or intervening stop codons. The possibility remains that some of the sequences could be recent translocations to the nucleus, in which case insufficient time may have passed to alter the mitochondrial characteristics of the pseudogene. Nevertheless, congruence between sequence and nuclear (DNA hybridization) data in regard to phylogenetic placement of most taxa (see Discussion) provides strong collateral evidence against nuclear contaminants among the mitochondrial sequences.

Phylogenetic analysis.—Bootstrapped 50% majority-rule consensus trees (plus other groups compatible with that tree) for unweighted and weighted parsimony analyses produced the same topology (Fig. 2). An identical topology was obtained for the bootstrapped 50% majority-rule consensus tree for likelihood analysis based on model selection and parameter values determined by MODELTEST; likelihood ratio tests indicated that our data are best fit by a general-time-reversible substitution matrix (6 substitution types, R[A-C] = 0.9109, R[A-G] = 5.4224, R[A-T] = 1.1972, R[C-G] = 0.5601, R[C-T] = 9.5556, R[G-T] = 1.0000), in which the proportion of sites assumed not to vary was 0.4745, and in which rates for variable sites followed a gamma distribution with a shape parameter of 0.9175 (model = GTR + I + G). Bootstrap values for both parsimony and likelihood generally were similar in terms of absolute and relative support among nodes (Fig. 2). Support for internal nodes was higher overall for likelihood, and for hermits compared to nonhermits, but all analyses agreed on level of support and placement of *Ramphodon*.

With the Common Nighthawk specified as the root, the deepest branches among the apodiform taxa indicate monophyly for swifts (two species from two families) and hummingbirds (11 species from across both subfamilies). *Ramphodon* groups with representatives of the other four traditional hermit genera (Sibley and Ahlquist 1990). Moreover, a sister-group relationship between *Ramphodon* and the single exemplar of *Glaucis* within hermits is strongly supported (92–98% bootstrap support across methods). Indeed, all nodes within the derived hermit clade receive very strong support ≥82%), thereby bolstering placement of the *Ramphodon-Glaucis* pairing as a relatively distal lineage within the subfamily. *Ramphodon* showed more distant affinities with the other two taxa (*Eutoxeres*, *Androdon*) that share some of its more distinctive attributes (in plumage and bill morphology). *Eutoxeres* always defined the first split within extant hermits, which separated it from *Ramphodon* by several other branching events within hermits. *Androdon* was placed in the nonhermit subfamily, though bootstrap support for its specific affinities generally was lower than that observed among other hummingbird taxa.

DISCUSSION

Molecular estimates of phylogeny are of particular value for groups in which strong selection has produced superficial resemblances based on shared adaptation, as has undoubtedly occurred many times among hummingbirds (Bleiweiss et al. 1997, Bleiweiss 1999). Cytochrome-*b* sequence data are unequivocal in placing *Ramphodon* with other traditional hermit genera, in demonstrating its sister relationship with an exemplar of the distal and superficially dissimilar hermit *Glaucis*, and in placing the superficially similar *Androdon* in the nonhermit subfamily. Thus, our results reinforce conclusions based on external (Hinkelmann and Schuchmann 1997) and internal (Zusi and Bentz 1982) anatomy that *Ramphodon* is a hermit. However, our data suggest that *Ramphodon* is a more recently diverged member of that clade (compare phylogenetic hypotheses summarized in Fig. 3). Nevertheless, phylogenetic inferences based on infor-

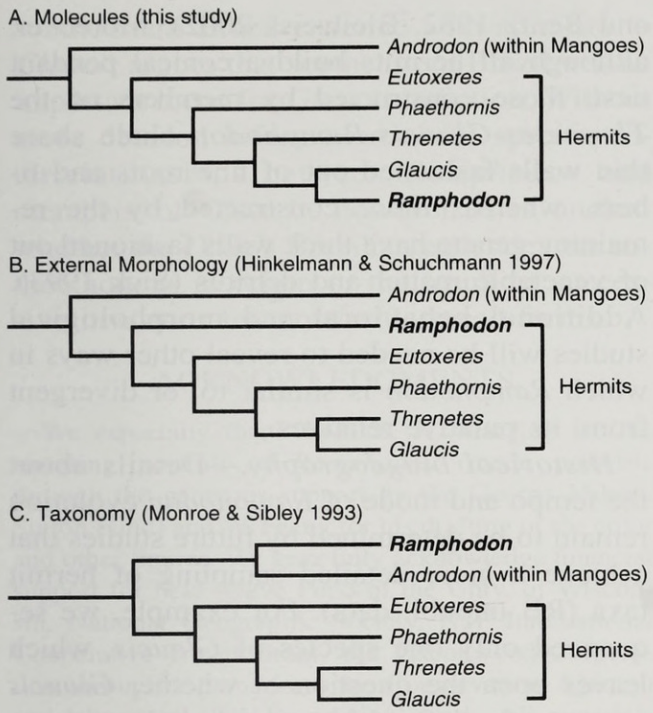

FIG. 3. Comparison of phylogenetic hypotheses proposed for *Ramphodon*. Hypothesis B is based on the study of external morphology by Hinkelmann and Schuchmann (1997). Hypothesis C is based on the placement of *Ramphodon* at beginning of the subfamily Trochilinae (nonhermits) in linear classification of Monroe and Sibley (1993). Compared to log likelihood for heuristic cytochrome-*b* topology A, log likelihoods for alternate (differing from consensus tree in Fig. 2 only in placement of *Ramphodon)* topology B (ln likelihood = −5613.40) or C (ln likelihood = −5635.30) are significantly lower by nonparametric likelihood ratio ($\chi^2_{A\text{-}B}$ = 40.76, $\chi^2_{A\text{-}C}$ = 62.67; df = 1, two-tailed $P < 0.0001$) and parameteric Kishino-Hasegawa (100,000 RELL bootstrap replicates, two-tailed $P = 0.0012$ for A versus B and $P < 0.0001$ for A versus C) tests (calculated in PAUP*; see also Swofford et al. 1996). Thus, the cytochrome-*b* data strongly favor hypothesis A over either B or C. See text for discussion.

mation from a single mitochondrial gene could be biased because topologs recover only the gene tree rather than the species tree. Here we discuss additional support for our hypothesis, as well as its implications for understanding hummingbird evolution.

Phylogenetic signal.—Relationships among the four traditional hermit genera obtained through analysis of cytochrome-*b* sequences agree in all respects with those indicated by earlier studies based on DNA hybridization (Bleiweiss et al. 1994b, 1997; Fig. 1). Hermits form a basal monophyletic group within hummingbirds. Moreover, *Eutoxeres* defines the first branch within this clade, followed by *Phaethornis* and then *Threnetes-Glaucis*. As DNA hybridization measures genetic divergence over the entire single-copy genome, the congruence between the DNA hybridization-based results and those obtained from our gene sequence study gives us confidence that the topology for hermits, including the additional placement of *Ramphodon*, accurately reflects phylogeny.

For nonhermits as well, cytochrome-*b* data provide support for several relationships inferred from other methodologies, including the pairing of *Colibri* with *Doryfera* (Bleiweiss et al. 1997) and of *Anthracothorax* with *Eulampis* (del Hoyo et al. 1999). Moreover, phylogenies generated from DNA sequencing (herein), DNA hybridization (Bleiweiss et al. 1997), and less strongly, allozymes (Gill and Gerwin 1989, Gerwin and Zink 1998) all suggest that *Androdon* is a nonhermit. However, the specific affinities of *Androdon* differ among (taxa in common between) studies; our sequencing places *Androdon* as a sister taxon to the *Colibri-Doryfera* clade whereas DNA hybridization places *Androdon* as a sister to *Heliothryx* rather than to *Colibri-Doryfera*. For the sequencing data, however, bootstrap values for basal branches among nonhermits in general, and for the *Androdon* node in particular, were low (<50%) in several analyses, thereby echoing lack of resolution suggested by some allozyme studies (Gerwin and Zink 1998). Thus, cytochrome-*b* may better resolve relationships among hermits than among divergent nonhermits. In any case, we consider the specific relationships of *Androdon* within nonhermits unresolved by cytochrome-*b* sequence data.

Character evolution.—The available molecular phylogenies imply that evolution of "toothed" bills in *Ramphodon* and other hummingbirds has both a phylogenetic and homoplastic component (see Fig. 2). The special relationship between *Ramphodon* and *Glaucis* serves to associate the only two hermit taxa known to develop bill serrations, although these serrations are smaller and sometimes develop only on the maxilla in *Glaucis* (*G. dohrnii* sometimes is placed in *Ramphodon*; Monroe and Sibley 1993) as compared to both tomia in male *Ramphodon* (Ornelas 1994). Moreover, males of *Glaucis dohrnii* and of *Ramphodon* also share the unusual hook at the

bill tip lacking in other hermits (Ornelas 1994). Absence of such features in more basal hermits implies that these similarities between *Ramphodon* and *Glaucis* are synapomorphic. Although our sequence data did not provide strong support for placement of *Androdon* within nonhermits, earlier DNA hybridization-based phylogenies indicate that *Androdon* belongs to a second monophyletic group (within the basal Mangoes) whose constituent species are characterized by serrated bills (see discussion in Bleiweiss et al. 1997). Thus, *Ramphodon* and *Androdon* appear to be extreme exemplars within each of two independent radiations of tooth-billed forms.

Hinkelmann and Schuchmann (1997) placed *Ramphodon* and *Eutoxeres* as the two earliest branches, respectively, in hermit phylogeny (Fig. 3B) based in part on their striped underparts, a feature otherwise unusual among hummingbirds (see frontispiece, Fig. 2). However, Hinkelmann and Schuchmann (1997) noted that the striped underparts of *Ramphodon* and *Eutoxeres* might not be "homologous" because the individual feathers differ in detail; those in *Ramphodon* are black along the rachis and have white margins whereas those in *Eutoxeres* are white along the rachis and have black margins. The pattern in *Androdon* is like that in *Eutoxeres* (RB unpubl. data). On the other hand, *Ramphodon* and *Glaucis* share similar facial patterns (dark auricular patch with light trim) and rich ochre head markings (see frontispiece, Fig. 2), features also present in related hermits (*Phaethornis*, *Threnetes*) but absent in other hummingbirds with striped underparts (including *Eutoxeres* within hermits). Thus, striped underparts of *Ramphodon* probably are homoplastic *vis à vis Eutoxeres* and *Androdon*, whereas the distinctive head pattern and color shared by *Ramphodon* and *Glaucis* probably are synapomorphic with such features in other derived hermits.

Despite the striking degree of homoplasy between *Ramphodon* and certain hermits and nonhermits with respect to external morphology, other prominent characteristics of *Ramphodon* appear to concord with its special relationships as defined by molecular data. Thus, the form of the patagial muscle in *Ramphodon* is like that in other derived hermits but unlike that in the basal *Eutoxeres* (Zusi and Bentz 1982, Bleiweiss 2002). Moreover, although all hermits build a conical pendant nest, those constructed by members of the *Threnetes-Glaucis-Ramphodon* clade share thin walls fashioned out of fine roots and fibers, whereas those constructed by the remaining genera have thick walls fashioned out of vegetable matter and detritus (Sick 1993). Additional behavioral and morphological studies will be needed to reveal other ways in which *Ramphodon* is similar to, or divergent from, its putative relatives.

Historical biogeography.—Details about the tempo and mode of *Ramphodon* evolution remain to be determined by future studies that achieve a more detailed sampling of hermit taxa (RB unpubl. data). For example, we sequenced only one species of *Glaucis*, which leaves open the question of whether *Glaucis* as currently defined is monophyletic or paraphyletic with respect to *Ramphodon*. However, the broad outlines of the origin of *Ramphodon* can be gleaned from the results of our study. For example, Stiles (1981) suggested that the hummingbird fauna of southeastern Brazil is relatively old, based in part on the occurrence there of many distinct endemic monotypic forms (e.g., *Stephanoxis*, *Leucochloris*, *Aphantochroa*, *Eupetomena*). Contrary to this hypothesis, the distal placement of *Ramphodon* within hermits suggests that even the most distinctive Brazilian endemics evolved relatively recently (and potentially rapidly). Indeed, *Ramphodon* is younger than the ancestor to the more homogeneous *Glaucis-Threnetes* pairing, which fossil-calibrated DNA hybridization distances date to the Pliocene (approximately 4 mybp; Bleiweiss 1998a).

Previous studies have argued that many species endemic to the lowland forests of southeastern Brazilian evolved there in allopatry after a more widespread ancestor invaded these habitats (Haffer 1974, Willis 1992) including hummingbirds (Bleiweiss 1998b). This hypothesis provides a plausible scenario for the origin of *Ramphodon* because the taxon's closest relatives (*Glaucis*, *Threnetes*) are widespread in the South American lowlands. Thus, the ancestors of *Ramphodon* probably resided in these same habitats. Although we were unable to secure tissues of *Glaucis dohrnii* for analysis, the existence of this second

southeastern Brazil endemic with affinities to *Glaucis* argues that southeastern Brazil was an important locus for *Glaucis* radiation. Without more detailed information about specific interrelationships between *Ramphodon* and members of *Glaucis*, however, it is unclear whether the clade's Brazilian endemics are authocthonous in origin or the result of successive invasions by more widespread forms.

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APPENDIX

Collection localities for specimens used in this study.—Ramphodon naevius: Santa Lúcia field station of the Museu de Biologia Mello Leitão, Santa Teresa, Espirito Santo, Brazil; *Eutoxeres aquila*: Cooperative Salsedo Lindo #1, road from San vicente Maldonado to encampamento de CODESA, 21.6 km from San Vicente Maldonado, Prov. de Pichincha, Ecuador; *Phaethornis yaruqui*: Cooperative Salsedo Lindo #1, road from San Vicente Maldonao to encampamento de CODESA, 21.6 km from San Vicente Maldonado, Prov. de Pichincha, Ecuador; *Threnetes ruckeri*: Centro Científico Río Palenque, 56 km SW Santo Domingo de los Colorados on Río Babo, Prov. de los Ríos, Ecuador; *Glaucis aenea*: below Cooperative Salsedo Lindo #1, road from San Vicente Maldonao to encampamento CODESA, 21.6 km from San Vicente Maldonado, Prov. de Pichincha, Ecuador; *Anthracothorax prevostii*: Central America; *Eulampis holosericeus*: trail to Muskmelon Bay just beyond trail to Crab Cove, Guana Island, British Virgin Islands; *Heliothryx barroti*: Centinella de Guayllabamba, road from San Vicente Maldonado to encampamento de CODESA, 21.6 km from San Vicente Maldonado, Prov. de Pichincha, Ecuador; *Colibri coruscans*: Calle Gonzalo Pizarro, 2.0–2.5 km from Via Interoceania, Barrio Churo Loma, Tumbaco, Prov. de Pichincha, Ecuador; *Doryfera ludovicae*: below Hacnda Santa Rosa on Río Cinto, Prov. de Pichincha, Ecuador; *Androdon aequatorialis*: Cooperative Salsedo Lindo #2, road from San Vicente Maldonao to encampamento de CODESA, 21.6 km from San Vicente Maldonado, Prov. de Pichincha, Ecuador; *Hemiprocne mystacea*: New Guinea; *Streptoprocne zonaris*: old road to Mindo at Y to Mindo, 64.8 km from Ave. Occidental, Prov. de Pichincha, Ecuador; *Chordeiles minor*: Wild Animal Rehabilitation Center, Milwaukee, Wisconsin.

Note: for material from Ecuador, the Caribbean, and the United States, voucher specimens were deposited as study skins, spirit specimens, or skeletons in the collections of the Univ. of Wisconsin Zoological Museum or Museo Ecuatoriano de Ciencias Naturales.