GENETIC VARIATION IN PICIFORM BIRDS: MONOPHYLY AND GENERIC AND FAMILIAL RELATIONSHIPS

SCOTT M. LANYON[1] AND ROBERT M. ZINK
Museum of Zoology, Louisiana State University, Baton Rouge, Louisiana 70803 USA

ABSTRACT.—Inter- and intrafamilial relationships within the New World Piciformes were examined through an electrophoretic analysis of 20 protein-coding loci (19 of which varied between taxa). One individual from each of 26 species representing 25 genera and 5 families was analyzed; *Momotus momota* (Coraciiformes, Momotidae) was used as an outgroup. Although levels of genetic differentiation were high (the mean Nei's unbiased distance was 1.07), the data proved useful for phylogenetic inference. The jackknife technique was used to estimate the robustness of phylogenetic hypotheses. At the interfamilial level, the results suggest the following groupings: [[[(Bucconidae) (Galbulidae)]{(Picidae)[(Capitonidae) (Ramphastidae)]}]]. These results were consistent with hypotheses of familial relationships proposed by two recent cladistic analyses of morphological character complexes (Simpson and Cracraft 1981, Swierczewski and Raikow 1981). Our data challenge the currently accepted monophyly of the Piciformes, however, in much the same way as do DNA-DNA hybridization data. Agreement among independently derived hypotheses of interfamilial relationships suggests confidence in our knowledge of evolutionary patterns among piciform taxa. Hypotheses of intrafamilial relationships, some of which agreed with morphological patterns obtained in other studies, were presented. This study shows that starch-gel electrophoresis may be useful at higher taxonomic levels. *Received 10 November 1986, accepted 4 June 1987.*

RECENT years have witnessed a plethora of biochemical systematic studies (Avise and Aquadro 1982). These studies have demonstrated that molecular characters have both advantages and limitations for phylogeny reconstruction (Lanyon 1985b). There are several types of limitations. For example, molecular methods are scale dependent. That is, one would generally not use starch-gel electrophoresis of proteins to investigate relationships of higher taxa (Buth 1984) because often taxa share no alleles, which contributes no phylogenetic information. In other instances molecular methods may be unable to resolve evolutionary patterns because of the relationship between the nature and rate of character evolution (e.g. allelic substitutions occurring more or less uniformly over time) and the nature of the evolutionary history itself (Fiala and Sokal 1985). For example, if cladogenetic events are close in time and lineages are short-lived before fragmentation (speciation), then there is a low probability of the origin (and retention through evolutionary time, given a fast rate of change) of a synapomorphic character state, such as a particular allele.

Nonetheless, the potential of biochemical methods for the inference of evolutionary history is widely appreciated (Buth 1984, Wilson et al. 1985, Sibley and Ahlquist 1986) because they allow direct access to genetic information. A framework of quantitative models provides objective methods for estimating phylogenies (Felsenstein 1982). Probably few phylogenetic estimates, however, irrespective of the data or methods used to construct them, are immune to bias. Recently, statistical or quantitative tests have been developed to assess the accuracy of a particular phylogenetic pattern (e.g. Templeton 1983, Felsenstein 1985, Lanyon 1985a). Furthermore, systematic studies that compare patterns of morphological, biochemical, and behavioral variation may lead to more robust estimates of evolutionary history (Wagner 1961, Miyamoto 1981, Lanyon and Lanyon 1986). Such multidisciplinary approaches can exploit positive aspects of each suite of characters, and permit evaluation of whether consistent patterns emerge. A disadvantage of this approach is that it requires investigators to become familiar with many different techniques, which can increase greatly the time required to complete studies

[1] Present address: Division of Birds, Field Museum of Natural History, Roosevelt Road at Lake Shore Drive, Chicago, Illinois 60605 USA.

of even small assemblages. It is possible to simulate such a multidisciplinary study by integrating independently or collaboratively gathered data sets of several investigators.

We examined phylogenetic patterns in electrophoretic characters (allozymes) in the Piciformes. Recent hypotheses (Fig. 1) of piciform relationships have been based on hindlimb musculature (Swierczewski and Raikow 1981), osteological characters (Simpson and Cracraft 1981), and the anatomy of the feeding apparatus (Burton 1984). We compare phylogenetic patterns derived from analysis of our allozyme data set with those derived from analyses of muscle and skeletal variation. We illustrate the importance of examining the robustness of phylogenetic estimates through the use of the jackknife technique (Lanyon 1985a). Furthermore, this is one of the few systematic applications of protein electrophoresis at higher taxonomic levels in birds (Lanyon 1985b); we suggest that the limits of the technique for avian systematics are unknown.

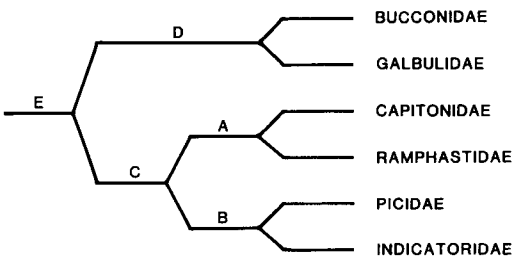

Fig. 1. Cladogram of higher-level relationships supported by Simpson and Cracraft (1981) and Swierczewski and Raikow (1981). A = Ramphastoidea, B = Picoidea, C = Pici, D = Galbulae, and E = Piciformes. Derived characters support each node.

Methods

Taxon selection.—The 27 tissue samples (representing 25 genera and 5 families and 1 outgroup; see Table 1) analyzed in this study were collected over a period of 3 yr by personnel of the Louisiana State University Museum of Zoology. From 2 to 7 individuals, each in a different genus, were selected to represent each of the five traditionally recognized New World piciform families [Bucconidae (puffbirds), Galbulidae (jacamars), Capitonidae (barbets), Ramphastidae (toucans), and Picidae (woodpeckers)]. We maximized the number of species-level taxa used, instead of individuals, to estimate levels and patterns of genetic variation within and among families. The use of a single individual/taxon to estimate genetic distances is sufficient when investigating relationships at higher taxonomic levels (Gorman and Renzi 1979, Lanyon 1985b). The outgroup (*Momotus momota*, Momotidae) was selected from the presumed sister group, the Coraciiformes (Simpson and Cracraft 1981, Swierczewski and Raikow 1981; but see Olson 1983).

Electrophoresis.—Homogenates were prepared from pooled samples of liver and pectoral muscle: 0.5–1.0 g of minced tissue and an equal volume of Cleland's Reagent were centrifuged at 12,000 rpm for 60 min, the supernatant removed and preserved at −70°C, and the pellet discarded. Homogenates were applied to 12% horizontal starch gels using filter-paper wicks. Two discontinuous and two continuous buffer systems were used to isolate proteins using horizontal starch-gel electrophoresis [buffers 8–10 of Aquadro and Avise (1982); and Tris-maleate of Selander et al. (1971), adjusted to pH 6.5]. Twenty proteins (see Table 1), presumed homologous across taxa, were identified using protein-specific assays outlined by Harris and Hopkinson (1976). During the initial survey of loci, the mobility of each character (=electromorph) across the 27 taxa was recorded relative to a standard. All alleles of similar mobility were then compared in side-by-side tests on additional gels to ensure the accuracy of character-state designations.

Data analysis.—We used the computer program BIOSYS-1 (Swofford and Selander 1981) to compute Rogers' (1972) and Nei's (1978) genetic distances and to estimate phylogenetic patterns using the UPGMA and distance Wagner procedures. The computer program PHYLIP (Felsenstein 1985) was used to produce Fitch-Margoliash (F-M) trees. These distance analyses yield phylogenetic information because of the predominantly stochastic manner in which the characters evolve (Kimura 1983). We note, however, that rates of nucleotide substitutions may differ between lineages (Avise and Aquadro 1982, Britten 1986), which results in reduced accuracy of phylogenetic hypotheses derived using distance analyses (Felsenstein 1978, 1982).

To estimate the degree of error in phylogenies constructed by our analyses of genetic distances, we employed a jackknife analysis (Lanyon 1985a), in which pseudoreplicate distance matrices were created (each consisting of all possible combinations of $n - 1$ taxa, where n = the total number of taxa under consideration), and F-M trees generated. A single strict consensus tree was then produced to identify the nodes that were consistent with all analyses.

Values reported are means ± SD.

Results

Allelic frequencies.—Inspection of the pattern of electromorphic variation at the 20 loci (Table 1) reveals considerable variation among the taxa. An average of 5.3 ± 2.1 alleles/locus was ob-

TABLE 1. Distribution of electromorphs (denoted by lowercase letters) in 26 piciform taxa and 1 outgroup (*Momotus momota*, Momotidae). Taxa 2–9 represent the family Bucconidae, 10–13 the Galbulidae, 14–20 the Picidae, 21–22 the Capitonidae, and 23–27 the Ramphastidae.

Genus	Locus[a,b]																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1. *Momotus momota*	g	b	b	e	c	f	b	e	b	g	e	f	h	cd	c	c	b	ab	b	a
2. *Bucco capensis*	e	a	a	d	c	f	c	cf	f	e	e	d	gh	a	e	c	ab	c	b	a
3. *Nystalus radiatus*	e	a	a	d	c	f	c	e	g	e	e	d	i	a	e	c	d	bc	b	a
4. *Malacoptila fusca*	e	a	d	d	a	f	c	e	d	e	e	d	i	c	e	a	d	c	b	a
5. *Micromonacha lanceolata*	e	a	c	d	c	f	c	e	f	e	f	d	i	c	e	a	d	c	b	a
6. *Nonnula rubecula*	e	a	d	d	c	a	c	e	f	e	e	d	h	c	e	a	d	c	b	a
7. *Hapaloptila castanea*	e	a	d	d	c	f	a	e	f	e	e	d	i	c	e	a	d	bc	c	a
8. *Monasa nigrifrons*	e	a	a	d	c	f	c	e	f	e	c	d	i	c	d	b	d	c	b	a
9. *Chelidoptera tenebrosa*	e	a	a	d	c	f	d	e	f	e	c	d	i	b	d	c	d	c	b	a
10. *Galbula albirostris*	f	a	d	d	c	g	a	e	e	e	f	f	d	c	e	a	d	b	b	a
11. *Jacamerops aurea*	f	b	e	d	c	b	a	e	a	d	g	g	h	d	e	a	d	b	b	a
12. *Brachygalba salmoni*	f	a	d	e	c	e	a	e	e	g	g	d	j	c	e	a	d	b	b	a
13. *Galbalcyrhynchus leucotis*	f	a	a	d	c	b	a	g	g	e	e	f	f	ac	e	a	d	b	b	a
14. *Campephilus haematogaster*	b	c	f	f	b	g	c	b	e	a	b	d	g	c	f	c	c	d	b	a
15. *Picoides scalaris*	b	c	f	c	b	g	c	e	h	f	a	k	c	c	d	c	c	c	a	a
16. *Sphyrapicus varius*	b	c	f	c	c	g	c	b	h	f	ad	f	a	c	d	c	c	c	b	a
17. *Picoides villosus*	a	c	f	c	b	g	c	bd	h	f	a	k	c	c	d	c	c	c	b	a
18. *Melanerpes aurifrons*	ab	c	f	c	d	g	d	b	h	b	ac	d	bc	c	d	c	c	c	b	a
19. *Colaptes auratus*	b	c	f	c	d	g	c	g	h	f	a	g	cd	c	e	c	c	c	b	a
20. *Picumnus borbae*	b	c	d	c	b	g	c	g	h	h	b	f	d	d	f	c	bc	c	b	a
21. *Capito niger*	h	c	f	d	b	g	c	g	f	f	c	k	i	c	b	a	b	c	b	a
22. *Eubucco bourcierii*	h	c	f	d	b	g	c	g	i	a	c	k	h	c	b	b	b	c	b	a
23. *Selenidera spectabilis*	h	c	f	b	a	g	c	g	c	a	e	d	d	c	b	a	b	c	b	a
24. *Aulacorhynchus prasinus*	h	c	f	b	a	g	c	g	f	c	c	f	d	d	b	a	b	c	b	a
25. *Andigena hypoglauca*	dh	c	f	b	a	g	e	g	c	c	ce	f	f	c	b	b	b	c	b	a
26. *Pteroglossus castanotis*	h	c	f	b	a	g	c	g	c	c	c	g	d	c	b	a	b	c	a	a
27. *Ramphastos sulfuratus*	h	c	d	a	a	g	c	g	c	e	c	f	d	a	b	b	b	c	b	a

[a] Loci in order are: Pgm-1 (E.C. 2.7.5.1), Mdh-1,2 (1.1.1.37), Got-1 (2.6.1.1), Ck-2 (2.7.3.2), Eap (3.1.3.2), Pgi (5.3.1.9), Est "D" (3.1.1.1), Sod-1 (1.15.1.1), Gda (3.5.1.2), 6-Pgd (1.1.1.44), Peptidase C, B (3.4.11), Mpi (5.3.1.8), Ldh-1,2 (1.1.1.27), Idh-1,2 (1.1.1.42), Fum (4.2.1.2), and Lap (3.4.11.1).

[b] Two letters signify a heterozygous genotype.

served; only one locus (Lap) was monomorphic across all taxa. Ten other loci were examined but were too variable to warrant continued analysis from a logistical standpoint.

Genetic distances.—A summary of Nei's (1978) genetic distances (Table 2) shows average genetic distances within and between major groups (the complete matrix is available from the authors). Because the distance values in Table 2 were based on conservative loci, they are underestimates of the actual level of genetic differentiation in piciform birds; this should be considered when comparing our results with values reported in the literature. The distances are, nevertheless, greater than those reported for comparisons at comparable taxonomic levels in other birds (Barrowclough 1980). The average Nei (1978) genetic distance among all piciform taxa was 1.07.

Distance analysis and interfamilial phylogenetic patterns.—The UPGMA and distance Wagner analyses (Fig. 2) and Fitch-Margoliash analysis (not shown) produced the same branching sequences for the five families. Furthermore, the branching sequence was completely stable to the jackknife analysis. Three hypotheses of interfamilial relationships proposed independently by Swierczewski and Raikow (1981) and by Simpson and Cracraft (1981) were supported. The Bucconidae and Galbulidae were identified as sister taxa, supporting a hypothesis previously defined by four osteological characters (Simpson and Cracraft 1981), six myological characters (Swierczewski and Raikow 1981), and similarities in conalbumins and ovalbumin (Sibley and Ahlquist 1972). Although these taxa have consistently been identified as close relatives, the evidence presented here reveals marked genetic dissimilarity ($D = 0.80 \pm 0.22$), and a considerable amount of time probably has elapsed since they shared a common ancestor. This is in contrast to the Capitonidae and Ram-

TABLE 2. Nei's (1978) genetic distance (±SD) between major groupings of piciform taxa. Entries in the diagonal are comparisons between taxa within each grouping. Sample sizes are the number of pairwise comparisons.

	Motmot	Puffbirds	Jacamars	Barbets	Toucans	Woodpeckers
Motmot	—					
Puffbirds (Bucconidae)	1.15 ± 0.10 $n = 7$	0.32 ± 0.09 $n = 28$				
Jacamars (Galbulidae)	1.08 ± 0.08 $n = 4$	0.80 ± 0.22 $n = 32$	0.52 ± 0.11 $n = 6$			
Barbets (Capitonidae)	1.57 $n = 2$	1.12 ± 0.24 $n = 16$	1.50 ± 0.20 $n = 8$	0.22 $n = 1$		
Toucans (Ramphastidae)	1.55 ± 0.25 $n = 5$	1.38 ± 0.29 $n = 40$	1.59 ± 0.37 $n = 20$	0.48 ± 0.07 $n = 10$	0.32 ± 0.07 $n = 10$	
Woodpeckers (Picidae)	1.55 ± 0.19 $n = 7$	1.38 ± 0.22 $n = 56$	1.75 ± 0.36 $n = 28$	1.26 ± 0.49 $n = 14$	0.98 ± 0.19 $n = 40$	0.44 ± 0.18 $n = 21$

phastidae, also recognized as sister taxa, between which the average intergeneric distance was considerably smaller ($D = 0.48 \pm 0.07$).

The monophyly of the Capitonidae and Ramphastidae, supported by two myological characters (Swierczewski and Raikow 1981) and one osteological synapomorphy (Simpson and Cracraft 1981), was also supported by the electrophoretic analysis. We suggest that capitonids and ramphastids are as closely related as are the jacamar genera ($D = 0.52 \pm 0.11$).

The electrophoretic data supported a monophyletic assemblage consisting of the Picidae, Capitonidae, and Ramphastidae. This hypothesis has been supported previously by four osteological characters, six myological synapomorphies, and the mobility of an s-Mdh zymogram (Avise and Aquadro 1987). A close relationship between capitonids and picids was suggested by analysis of egg-white proteins (Sibley and Ahlquist 1972), but ramphastids were not analyzed. In contrast, Peters (1964) placed the capitonids and ramphastids with the bucconids and galbulids rather than with the picids. Sibley and Ahlquist (1985) suggested that the New World capitonids were more closely related to ramphastids than to Old World capitonids. We had tissue samples only from New World capitonids, and we cannot comment on the monophyly of the Capitonidae.

To evaluate the familial relationships supported by this study, one specimen from each of the five families (plus *Momotus*) was selected at random and a Fitch-Margoliash analysis conducted. Ten iterations were performed, and in all cases the results (not shown) supported the branching sequence of families shown in Fig. 2.

Intrafamilial phylogenetic relationships.—To investigate relationships within piciform families, we assumed that each family represented a monophyletic assemblage. A jackknifed F-M strict consensus tree was produced for each family and was rooted using the remaining taxa. At this lower level of taxonomic investigation, we detected tree instability. Jackknifing allowed us to expose inconsistencies within the distance matrix, identified as unstable nodes, and to isolate reliable nodes within families (Figs. 3–5).

Four nodes were retained for the eight bucconid genera (Fig. 3a). The hypothesis that *Bucco* and *Nystalus* represent sister taxa was supported [Peters (1964) and Cottrell (1968) considered them to be congeners]. Swierczewski and Raikow (1981) identified *Nystalus* and *Chelidoptera* as being more closely related to each other than either is to *Malacoptila* or *Nonnula* (Fig. 3b). This was neither supported nor refuted by the electrophoretic investigation, which failed to resolve the branching sequence for these taxa. The electrophoretic characters also identified *Malacoptila, Nonnula, Hapaloptila,* and *Micromonacha* as a monophyletic assemblage within which *Micromonacha* is the sister taxon to the remaining three genera. Finally, we note that the distance between *Bucco* and *Nystalus,* which are currently considered congeneric (A.O.U. 1983), is comparable to the distance between *Chelidoptera* and *Monasa* ($D =$ 0.418 and 0.387, respectively). The latter two taxa have not previously been suggested as sister taxa (or congeners) but have been placed

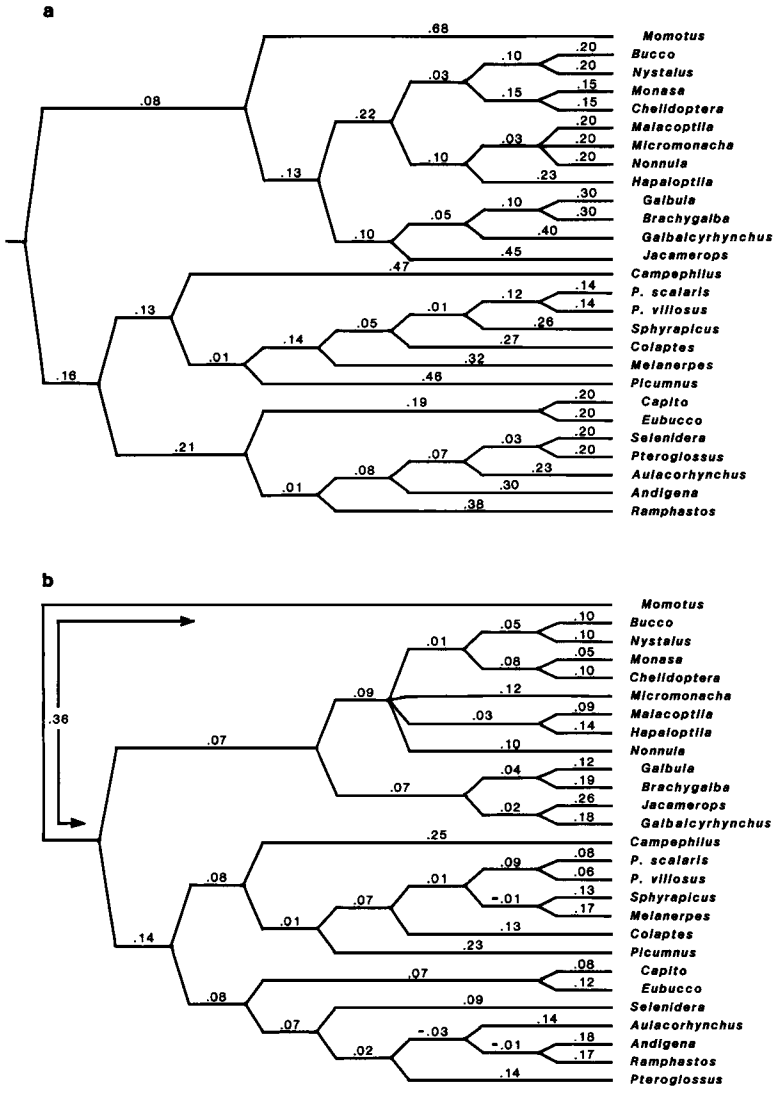

Fig. 2. UPGMA (a) and optimized distance Wagner (b) trees derived using Rogers' genetic distance measures calculated from the matrix of electromorphs (Table 1). The cophenetic correlation coefficient for the UPGMA phenogram is 0.93, indicating a reasonably good fit of the branching diagram to the distance matrix. Farris' "f" for the distance Wagner tree is 14.2. The values on the diagrams represent branch lengths. The distance Wagner tree was rooted using *Momotus* as an outgroup. See legend to Table 1 for family membership of taxa.

together at the end of the family in recent classifications (Peters 1964, A.O.U. 1983). Although genetic distance cannot be an absolute measure of taxonomic status, our data suggest scrutiny of the postulated close relationship between *Bucco* and *Nystalus*.

None of the galbulid phylogenetic hypotheses generated by the distance analyses (Fig. 2) was retained after jackknifing. Despite this potential lack of confidence in the branching pattern depicted (it might, after all, be correct), the allelic frequency data do provide insight into galbulid evolution. The relatively great genetic distance between galbulid genera, 0.52 ± 0.11, suggests that the four genera analyzed herein are from distinct, relatively old lineages. These lineages might have originated close in time.

Two nodes were consistently supported for

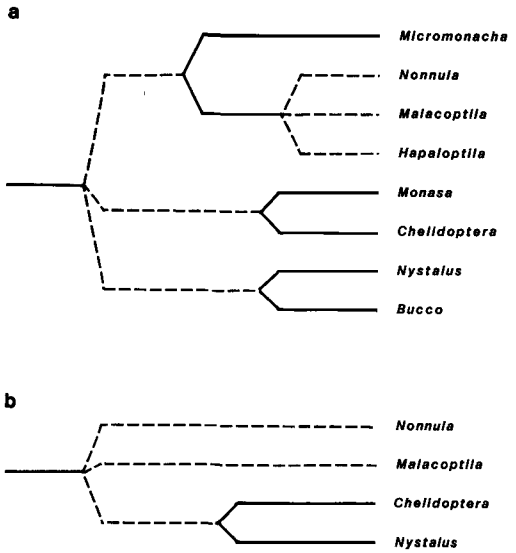

Fig. 3. (a) Jackknifed strict consensus Fitch-Margoliash tree for the Bucconidae. All other taxa were used as a composite outgroup to root the tree. Dotted lines indicate portions of the topology that were unstable to the jackknife manipulation. (b) Phylogenetic relationships within the Bucconidae supported by Swierczewski and Raikow's (1981) analysis of hindlimb musculature.

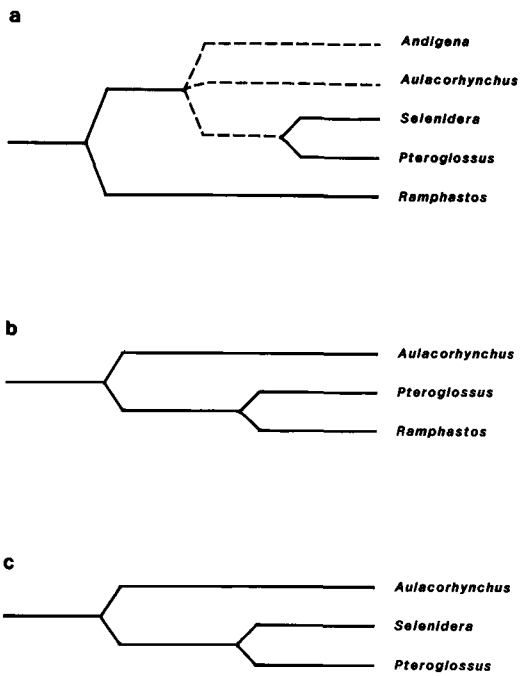

Fig. 4. (a) Jackknifed strict consensus Fitch-Margoliash tree for the Ramphastidae. All other taxa were used as a composite outgroup to root the tree. Dotted lines indicate portions of the topology that were unstable to the jackknife manipulation. (b) Phylogenetic relationships within the Ramphastidae supported by Swierczewski and Raikow's (1981) analysis of hindlimb musculature. (c) Phylogenetic relationships within the Ramphastidae supported by Haffer's (1974) analysis of plumage, vocalizations, and biogeography.

the five ramphastid taxa examined (Fig. 4a). *Ramphastos* was identified as the outgroup to the remaining genera, and *Selenidera* and *Pteroglossus* were identified as sister taxa (note, however, that these results differ from the distance Wagner tree shown in Fig. 2b). Our findings conflict with those of Swierczewski and Raikow (1981), who concluded that *Pteroglossus* and *Ramphastos* were sister taxa (Fig. 4b), but are consistent with Haffer's (1974) phylogeny for part of the family (Fig. 4c). Haffer (1974) suggested that the lowland genera *Selenidera* and *Pteroglossus* were sister taxa on the basis of vocal similarities.

Three apparently robust phylogenetic hypotheses were generated for the seven woodpecker taxa analyzed (Fig. 5a). The identification of *Picumnus* as the sister group to the remaining forms is consistent with Short's (1982) classification and the findings of Swierczewski and Raikow (1981) (Fig. 5b). *Campephilus* was shown to be the next lineage to arise (relative to those surveyed), as suggested by Swierczewski and Raikow (1981). In the classification of Short (1982) the relationships among the Melanerpini, Campetherini, and Campephilini were unresolved. Lastly, *Picoides villosus* and *P. scalaris* were identified as sister taxa, as one would expect from their congeneric status. Several hypotheses suggested by Short's classification and the cladogram developed by Swierczewski and Raikow (1981) cannot be addressed here. Specifically, we lack a consistently supported branching sequence for *Melanerpes, Sphyrapicus, Colaptes,* and *Picoides.* Additional study of patterns of protein variation promises insights into picid relationships.

Discussion

Polyphyly of the Piciformes.—Sibley and Ahlquist (1972, 1985), Olson (1983), and Burton (1984) have suggested that the Piciformes might be polyphyletic. These authors suggest that the

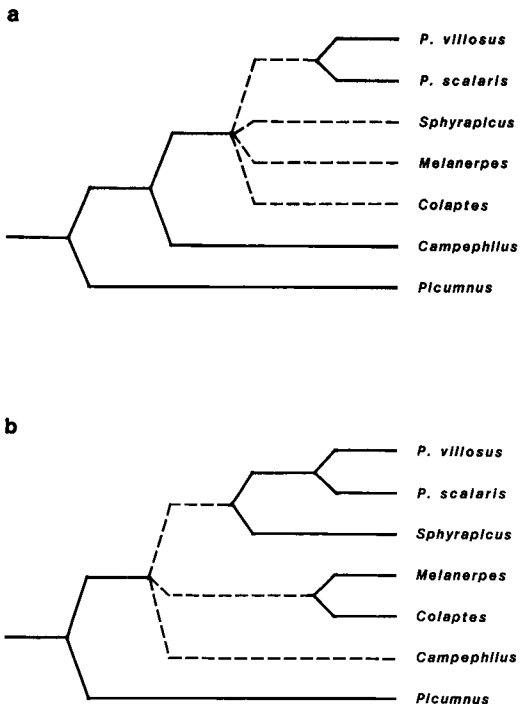

Fig. 5. (a) Jackknifed strict consensus Fitch-Margoliash tree for the Picidae. All other taxa were used as a composite outgroup to root the tree. (b) Phylogenetic relationships within the Picidae supported by Swierczewski and Raikow's (1981) analysis of hindlimb musculature.

Galbulae (Bucconidae and Galbulidae) might be more closely related to some Coraciiformes than either is to the Picae (Picidae, Ramphastidae, and Capitonidae). We did not specifically address this question. We can consider the question of monophyly, however, under the assumption of a strong positive correlation between genetic distance and time since divergence. The UPGMA phenogram, which assumes a strong time correlation, places *Momotus* as the sister taxon to the Galbulae. The reason for this topology can be seen from the summary of genetic distances (Table 2). The average distances between *Momotus* and the galbulid and bucconid genera (1.08 ± 0.08 and 1.15 ± 0.10, respectively) are considerably smaller than the average distance between the Picae genera and *Momotus* ($D = 1.55 \pm 0.19$). Therefore, our findings are consistent with the possibility that the order Piciformes (*sensu* Peters 1964) is polyphyletic.

Testing the stability of phylogenetic trees.—Several sources of bias cause phylogenetic trees to be unstable or unreliable. A particular phylogenetic estimate is dependent on the samples of individuals, characters, and taxa used in its construction; different samples of each might result in different phylogenetic estimates. In addition, the particular algorithm used has inherent assumptions, such as an average uniform rate of change (UPGMA) or parsimony (distance Wagner). It usually is not possible to know the nature of character evolution and therefore to select the appropriate algorithm. For some algorithms (e.g. distance Wagner), several equivalent or nearly equivalent trees can result. Thus, methods of testing robustness of trees are being developed (Templeton 1983, Felsenstein 1985, Lanyon 1985a).

It is sometimes not appreciated that methods of producing branching diagrams do so without any implied confidence in the branching pattern of either the entire tree or parts thereof. Often, a tree's structure depends on few characters, and sampling additional characters or individuals shifts branching patterns. Systematists should evaluate routinely the robustness of particular nodes in phylogenetic estimates. Several factors merit comment, however. The patterns obtained from the first pass of an algorithm (e.g. UPGMA) might be correct in their entirety; such a conclusion would be enhanced if a similar branching pattern was obtained from an independent data set. Because some nodes of the phylogeny might be based on a single or very few characters, such nodes might disappear wrongly with some resampling method, such as jackknifing. In other words, an ancestral branch might be very short lived, with time for few character-state transitions. If one happened to analyze the (derived) characters that define such a branch, the fact that only a few characters (could) support the branch might cause it to be collapsed in tests of robustness. In addition, other methods of testing the stability or confidence of branching diagrams, such as bootstrapping (Felsenstein 1985), might indicate instability in a consensus tree obtained by jackknifing. Therefore, we note that our branching patterns for all taxa might be correct, but we limit interpretation to those patterns for which some confidence is indicated by jackknifing. We suggest that at least one test of the robustness of branching patterns be employed, along with conservative interpretation of patterns. Few avian systematic studies have used explicit, ob-

jective methods to examine the robustness of phylogenetic hypotheses (Lanyon 1985b).

Lastly, we note the ongoing debate concerning the inference of phylogenies from distance matrices (e.g. Farris 1986, Felsenstein 1986). Resolution of this issue, we suspect, will not alter our primary conclusions.

Suggestions for further work on piciform relationships.—As a result of this study and independent analyses of two very different suites of characters (Simpson and Cracraft 1981, Swierczewski and Raikow 1981), a set of reliable hypotheses of systematic relationships has been identified for the Piciformes at the family level and above. With the exception of the Picidae, however, for which Short (1982) constructed a relatively detailed classification, relationships within the piciform families remain obscure. Using the reliable hypotheses of familial relationships to identify proper outgroups and the preliminary intrafamilial hypotheses presented here and by Swierczewski and Raikow (1981) as a foundation, studies of phylogenetic relationships within each piciform family may be designed. For example, it will be useful to determine whether New World capitonids are more closely related to ramphastids or to Old World capitonids (see Sibley and Ahlquist 1985). Several taxa representing the range of variation within the Picidae should be used as a composite outgroup.

A critical higher-level systematic question that remains to be answered definitively is whether the Piciformes are monophyletic. Allozymic data suggest that the traditional Piciformes may be polyphyletic (Fig. 2a), although further assessment of this question must await a study in which a composite outgroup is constructed from closely related orders. We note also the usefulness of electrophoresis of proteins (allozymes) for estimating phylogenetic relationships at higher taxonomic levels, levels at which the technique supposedly fails (Buth 1984). Given that this approach is less expensive than others (e.g. DNA-DNA hybridization), it deserves application at higher taxonomic levels in birds, especially when it can complement other molecular and nonmolecular data sets.

Acknowledgments

We thank the Louisiana State University Museum of Zoology for financial support and the personnel of the Museum for collecting the tissue samples used in this study. We also thank J. C. Avise and C. F. Aquadro for providing a prepublication copy of their manuscript. R. L. Cann, A. P. Capparella, J. Cracraft, J. A. Gerwin, M. S. Hafner, S. J. Hackett, and J. V. Remsen, Jr., commented constructively on the manuscript.

Literature Cited

American Ornithologists' Union. 1983. Check-list of North American birds, 6th ed. Washington, D.C., Amer. Ornithol. Union.

Aquadro, C. F., & J. C. Avise. 1982. Evolutionary genetics of birds. VI. A reexamination of protein divergence using varied electrophoretic conditions. Evolution 36: 1003–1019.

Avise, J. C., & C. F. Aquadro. 1982. A comparative summary of genetic distances in the vertebrates. Pp. 151–185 *in* Evolutionary biology, vol. 15 (M. Hecht, B. Wallace, and R. Prans, Eds.). New York, Plenum Press.

———, & ———. 1987. Malate dehydrogenase isozymes provide a phylogenetic marker for the Piciformes (woodpeckers and allies). Auk 104: 324–328.

Barrowclough, G. F. 1980. Genetic and phenotypic differentiation in a wood warbler (genus *Dendroica*) hybrid zone. Auk 97: 789–798.

Britten, R. J. 1986. Rates of DNA sequence evolution differ between taxonomic groups. Science 231: 1393–1398.

Burton, P. J. K. 1984. Anatomy and evolution of the feeding apparatus in the avian orders Coraciiformes and Piciformes. Bull. Brit. Mus. Nat. Hist. (Zool.) 47: 331–443.

Buth, D. G. 1984. The application of electrophoretic data in systematic studies. Ann. Rev. Ecol. Syst. 15: 501–522.

Cottrell, G. W. 1968. The genera of puffbirds. Breviora, No. 285.

Farris, J. S. 1986. Distances and statistics. Cladistics 2: 144–157.

Felsenstein, J. 1978. Cases in which parsimony or compatibility methods will be positively misleading. Syst. Zool. 27: 401–410.

———. 1982. Numerical methods for inferring evolutionary trees. Q. Rev. Biol. 57: 379–404.

———. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39: 783–791.

———. 1986. Distance methods: a reply to Farris. Cladistics 2: 130–143.

Fiala, K. L., & R. R. Sokal. 1985. Factors determining the accuracy of cladogram estimation: evaluation using computer simulation. Evolution 39: 609–622.

Gorman, G. C., & J. R. Renzi. 1979. Genetic distance and heterozygosity estimates in electrophoretic studies: effects of sample size. Copeia 1979: 242–249.

HAFFER, J. 1974. Avian speciation in tropical South America. Publ. Nuttall Ornithol. Club No. 14.

HARRIS, H., & D. A. HOPKINSON. 1976. Handbook of electrophoresis in human genetics. Amsterdam, North-Holland Publ. Co.

KIMURA, M. 1983. The neutral theory of molecular evolution. Cambridge, England, Cambridge Univ. Press.

LANYON, S. M. 1985a. Detecting internal inconsistencies in distance data. Syst. Zool. 34: 397–403.

———. 1985b. Molecular perspective on higher-level relationships in the Tyrannoidea (Aves). Syst. Zool. 34: 404–418.

LANYON, W. E., & S. M. LANYON. 1986. Generic status of Euler's Flycatcher: a morphological and biochemical study. Auk 103: 341–350.

MIYAMOTO, M. 1981. Congruence among character sets in phylogenetic studies of the frog genus *Leptodactylus*. Syst. Zool. 30: 281–290.

NEI, M. 1978. Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–590.

OLSON, S. L. 1983. Evidence for a polyphyletic origin of the Piciformes. Auk 100: 126–133.

PETERS, J. L. 1964. Check-list of birds of the world, vol. VI. Cambridge, Massachusetts, Harvard Univ. Press.

ROGERS, J. S. 1972. Measures of genetic similarity and genetic distance. Univ. Texas Publ. 7213: 145–153.

SELANDER, R. K., M. H. SMITH, S. Y. YANG, W. E. JOHNSON, & J. B. GENTRY. 1971. Biochemical polymorphism and systematics in the genus *Peromyscus*. I. Variation in the old-field mouse (*Peromyscus polionotus*). Univ. Texas Publ. 7103: 49–90.

SHORT, L. L. 1982. Woodpeckers of the world. Greenville, Delaware Mus. Nat. Hist.

SIBLEY, C. G., & J. E. AHLQUIST. 1972. A comparative study of the egg white proteins of non-passerine birds. Bull. Peabody Mus. Nat. Hist., Yale Univ., No. 39.

———, & ———. 1986. Reconstructing bird phylogeny by comparing DNA's. Sci. Amer. 254: 82–92.

SIMPSON, S. F., & J. CRACRAFT. 1981. The phylogenetic relationships of the Piciformes (class Aves). Auk 98: 481–494.

SWIERCZEWSKI, E. V., & R. J. RAIKOW. 1981. Hind limb morphology, phylogeny, and classification of the Piciformes. Auk 98: 466–480.

SWOFFORD, D. L., & R. K. SELANDER. 1981. A computer program for the analysis of allelic variation in genetics. J. Hered. 72: 281–283.

TEMPLETON, A. R. 1983. Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and apes. Evolution 37: 221–244.

WAGNER, W. H., JR. 1961. Problems in the classification of ferns. Recent Adv. Botany 1: 841–844.

WILSON, A. C., R. L. CANN, S. M. CARR, M. GEORGE, U. B. GYLLENSTEN, K. M. HELM-BYCHOWSKI, R. G. HIGUCHI, S. R. PALUMBI, E. M. PRAGER, R. D. SAGE, & G. M. STONEKING. 1985. Mitochondrial DNA and two perspectives on evolutionary genetics. Biol. J. Linn. Soc. 26: 375–400.