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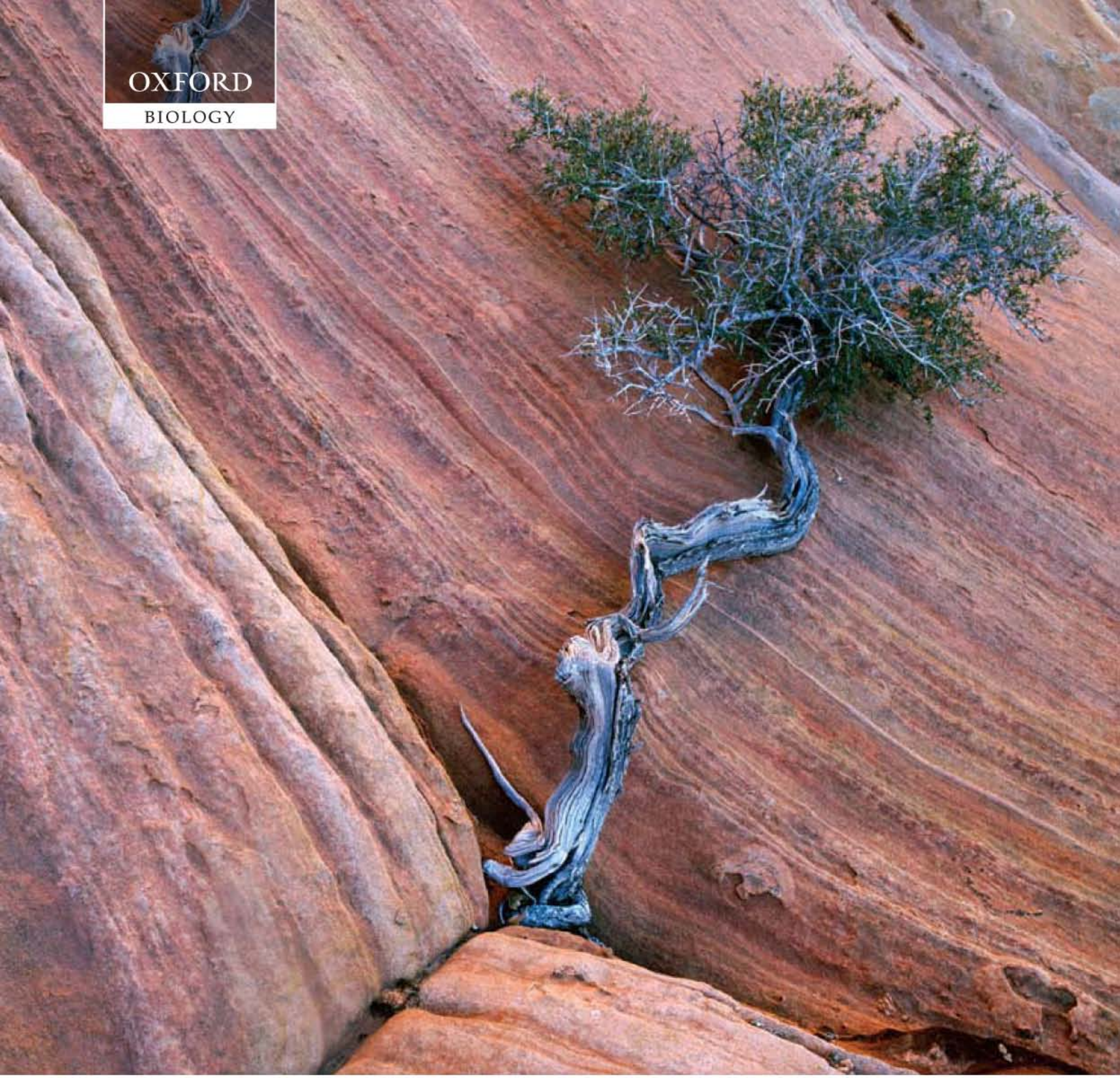

the TIMETREE of LIFE

edited by **S. BLAIR HEDGES** and **SUDHIR KUMAR**
foreword by James D. Watson

Woodpeckers, toucans, barbets, and allies (Piciformes)

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Abstract

The avian Order Piciformes comprises two major lineages, the Pici and Galbulae, which diverged as early as 70 million years ago (Ma). The jacamars and puffbirds (Galbulae) also diverged relatively early, ~53 Ma. Later diversification of the Pici gave rise to six additional families, beginning ~44–38 Ma. Molecular clock estimates for the origins of piciform clades, some estimated here, are consistent with the chronology of the sparse fossil record. With the exception of the woodpeckers, species of several piciform families were abundant on the northern continents during the Paleogene (66–23 Ma) but are now restricted to the tropics.

The Order Piciformes is a diverse assemblage of bird species that vary greatly in size, appearance, distribution, ecology, and life history. To the extent one can make descriptive generalizations, they tend to be stocky, brightly colored birds with disproportionately large bills—taken to an extreme in the toucans—and arboreal habits (Fig. 1). Their distributions are restricted to the Asian, African, and New World tropics, with the exception of the woodpeckers, which collectively have a more expansive distribution that includes the Old and New World temperate regions. Most species are insectivorous but many eat fruit at least occasionally and the barbets are primarily frugivorous. Cavity nesting is pervasive in the order, as is particularly well known for the woodpeckers, which have adaptations of the bill, skull, and associated musculature and enervation that renders them extraordinarily effective at excavating nest cavities in wood. The honeyguides are nest parasites, but parasitize only cavity-nesting species. Here we review the relationships and divergence times of the Order Piciformes and its constituent clades to the level of families.

As many as eight nominal families have been included in the Order Piciformes under various classifications: Picidae (wrynecks, piculets, and woodpeckers; ~28 genera, 216 species), Indicatoridae (honeyguides; ~4 genera, 17 species), Megalaimidae (Asian barbets; ~3 genera, 26 species), Lybiidae (African barbets; ~7 genera, 42 species), Capitonidae (New World barbets; ~2 genera, 13 species), Ramphastidae (toucans; ~7 genera, 36 species,

Fig. 1 A Red-headed Woodpecker (*Melanerpes erythrocephalus*), Family Picidae, from North America. Photo credit: R. Moul.

W. S. Moore and K. J. Miglia. Woodpeckers, toucans, barbets, and allies (Piciformes). Pp. 445–450 in *The Timetree of Life*, S. B. Hedges and S. Kumar, Eds. (Oxford University Press, 2009).

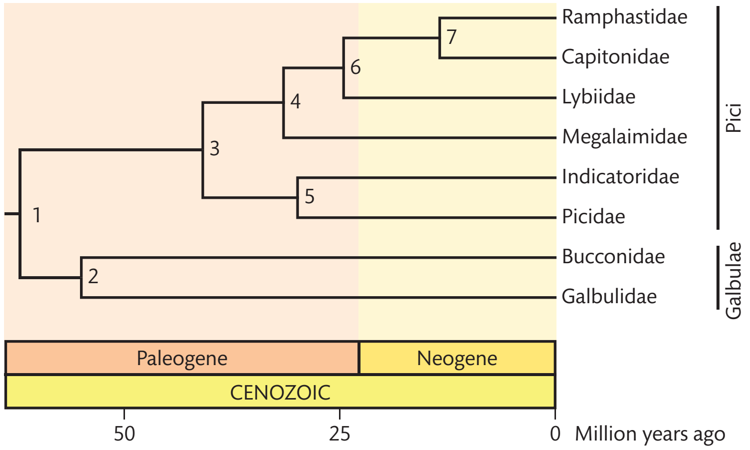

Fig. 2 The timetree of woodpeckers, toucans, barbets, and allies (Piciformes). Divergence times are shown in Table 1.

including two species of *Semnornis*), Galbulidae (jacamars; ~5 genera, 18 species), and Bucconidae (puffbirds; ~12 genera, 35 species). Collectively, the species assigned to these families group into two clades that diverged relatively early in the diversification of Neoaves (*1, 2*). One clade comprises the Galbulidae and Bucconidae and the other comprises the Picidae, Indicatoridae, and barbets and toucans, regardless of how the barbets and toucans are assigned to nominal families. These two ancient lineages are considered distinct orders, Galbuliformes and Piciformes, in some classifications, but suborders, Pici and Galbulae, of Piciformes in others. Regardless of the nomenclature, the evidence is strong that the Pici and Galbulae are both monophyletic. Until very recently, however, it was less certain whether the Pici and Galbulae were closest relatives, but three recent studies based on DNA sequence data from nuclear-encoded genes strongly support this hypothesis (*1, 2, 10*). Thus, it is reasonable to recognize the Order Piciformes comprising two major lineages, the Pici and Galbulae, totaling ~403 species.

Using Peters (*3*) classification as a reference list for species usually included in an order called Piciformes, the history of systematic groupings of those species into families, superfamilies, and suborders is a kaleidoscope through the nineteenth and much of the twentieth centuries. The early systematic history has been thoroughly reviewed (*1,4–6*). Considerable stability of inferred relationships was established with the cladistic studies of Swierczewski and Raikow (*5*), and Simpson and Cracraft (*6*) based on myological and osteological characters. These studies reached identical conclusions regarding relationships among piciform families, which were subsequently corroborated in a study by Lanyon and Zink (*7*) based on protein electromorph characters, although the latter study did not include a honeyguide (Indicatoridae). The tree hypothesized in these studies is ((((Indicatoridae, Picidae), (Ramphastidae, Capitonidae)), (Bucconidae, Galbulidae)), Outgroup). Although these studies produced congruent results, some intraordinal details remained to be determined, and monophyly of the order as well as identification of its closest relative remained contentious.

Olson (*8*), Burton (*9*), and Sibley and Ahlquist (*4*) argued that Piciformes is polyphyletic with the Galbulae (Bucconidae and Galbulidae) related to the coraciiforms. Lanyon and Zink (*7*) were not able to test the hypothesis of monophyly of Piciformes directly, but they noted that their distance data supported a closer relationship of Galbulae to a coraciiform (*Momotus*) than to other piciforms, which suggests paraphyly. However, recent DNA sequence-based studies consistently support monophyly of Piciformes at statistically significant levels (*1, 2, 10*). Johansson and Ericson's (*1*) study, based on exon segments from the nuclear *RAG1* and c-*myc* genes and intron II from the *myoglobin* gene totaling 3400 nucleotides, was specifically designed to test the close relationship of Pici and Galbulae and thus monophyly of Piciformes. Combining all sequences from the three genes, monophyly was supported by maximum parsimony (90% bootstrap) and Bayesian (100% posterior probability) analyses. The maximum likelihood analysis based on the "Early Bird" data set also inferred a monophyletic Piciformes with 100% BS support (*2*). The "Early Bird" data set is more comprehensive and includes

Table 1. Divergence times (Ma) and their confidence/credibility intervals (CI) among woodpeckers, toucans, barbets, and allies (Piciformes).

Timetree		Estimates				
Node	Time	Ref. (*10*)	Ref. (*17*)		Ref. (*22*)	
		Time	Time	CI	Time	CI
1	61.8	53	70.5	89–57	93.6	107–80
2	55.0	53	56.9	74–44	73.6	92–53
3	40.9	43.6	38.1	51–28	73.2	89–59
4	31.5	32.5	30.5	42–21	–	–
5	29.9	30.9	28.8	40–20	–	–
6	24.6	24.6	24.6	–	50.9	65–36
7	13.4	13.4	13.4	–	48	63–34

Note: Node times in the timetree represent the mean of time estimates from two studies (*10, 17*). PATHd8 analysis of DNA sequences from five nuclear genes was conducted in ref. (*10*). Ref. (*17*) presents a reanalysis of the same data using the Bayesian program Multidivtime. Some divergence times were estimated here, which is detailed in the text.

~32,000 nucleotides from 19 nuclear gene regions and 169 species. Thus the inferred descent of species, listed by Peters, from a common ancestor is probably true; that is, Piciformes is monophyletic and, thus, a valid taxon.

With regard to relationships within and among families, the greatest uncertainty has involved the barbets and toucans. Traditionally the barbets, which occur in the African, Asian, and New World tropics, were considered a single family, the Capitonidae, and the toucans, which are restricted to the New World, were put in a separate family, the Ramphastidae (*3*). A series of recent studies, however, have been consistent in showing that the traditional Capitonidae is paraphyletic with a close relationship between the New World barbets and toucans and that the Asian and African barbets, each a distinct clade, are more distantly related. These recent studies are based on anatomical (*9, 11*) as well as molecular characters (*4, 12–14*).

The most definitive study is Moyle's (*14*) based on a data set that combines 1045 nucleotides from the mitochondrial encoded *cyt b* gene with 938 nucleotides from intron 7 of the nuclear-encoded *β-fibrinogen* gene (*β-fibint7*). Combining the rapidly evolving *cyt b* sequences with the more slowly evolving *β-fibint7* sequences produced a data set with phylogenetically informative characters at low levels of homoplasy across the full time spectrum of barbet evolution. Moyle also had a relatively complete representation of taxa. The combination of genes evolving at appropriate rates, analytical methods based on detailed nucleotide substitution models (maximum likelihood and Bayesian) and a dense taxon sample enabled him to resolve nearly complete relationships among species comprising the traditional Capitonidae and Ramphastidae, including enigmatic basal lineages and/or convergent lineages such as *Gymnobucco*, *Calorhamphus*, *Semnornis*, and *Trachyphonus*.

Moyle's analyses strongly support the existence of three geographically defined clades: Asian barbets, African barbets, and New World barbets, the last one including the toucans. This is consistent with the earlier finding of Sibley and Ahlquist (*4*), and their recognition of three families is warranted: Lybiidae (African), Megalaimidae (Asian), and Capitonidae (New World). Recognition of the Family Ramphastidae also seems warranted, but the placement of the toucan-barbet, *Semnornis ramphastinus*, then becomes an issue. This species, which appears very much like what one might imagine as the common ancestor of toucans and New World barbets, is not strongly supported as the closest relative of either. In Moyle's model-based analyses, *Semnornis* was closest to the toucans but the ML bootstrap and estimated Bayesian posterior probabilities supporting this node were only 54% and 67%, respectively. The morphological intermediacy of the toucan-barbet, of course, is not the cause of uncertainty in the molecular analyses, but rather the internode that represents the common ancestor of *Semnornis*, with either the toucans or New World barbets, is short and deep in the tree. Regardless of the exact placement of *Semnornis*, it, along with the toucans and New World barbets, comprise a clade supported by

100% ML bootstrap and posterior probabilities (*14*). For simplicity of presentation, we will consider the toucans (including *Semnornis*) a family, Ramphastidae, and similarly we consider the New World barbets (Capitonidae), Asian barbets (Megalaimidae), and African barbets (Lybiidae) as individual families.

Monophyly of the other families and their inferred relationships have been more certain. Bucconidae (puffbirds) and Galbulidae (jacamars) are clearly monophyletic and related (*4–7, 15*). The DNA–DNA hybridization data support the close relationship of Galbulidae and Bucconidae but indicate that the divergence between these two lineages is ancient (*4*). The Picidae includes the wrynecks, piculets, and woodpeckers, each comprising a subfamily, and is closest to the Indicatoridae (*5, 6, 16*).

In summary, the Order Piciformes as defined here is monophyletic, comprising eight families (Fig. 2). The closest relative of the Piciformes is probably a clade comprising a subset of species traditionally assigned to the Order Coraciiformes (*2*). Thus, Coraciiformes is paraphyletic but Piciformes is not. The deepest split within Piciformes separates the Suborder Pici from Galbulae. The Galbulae soon bifurcated to give rise to two families, the Bucconidae and Galbulidae. In the Pici, the first bifurcation gave rise to two clades, one comprising the Indicatoridae and Picidae and the other the geographical clades of barbets plus toucans. Within the latter clade, the Asian barbet lineage (Megalaimidae) is basal and the next split gave rise to the common ancestor of African barbets (Lybiidae) and New World barbets plus toucans. Finally, the toucan lineage (Ramphastidae) diverged from the New World barbets (Capitonidae). To date, no study including a molecular clock analysis has focused specifically on Piciformes. However, the broad-based phylogenetic study of 75 families representing essentially all of Neoaves by Ericson *et al.* (*10*), and the ensuing comment (*17*) and reply (*18*) include molecular clock estimates for five of the seven piciform nodes (Table 1); the remaining two nodes can be estimated from relevant sequences archived in the National Center for Biological Information (NCBI) nucleotide data base.

Ericson *et al.* (*10*) determined sequences totaling 5007 nucleotides for five nuclear gene regions: *c-myc* (exon 3), *RAG-1*, *myoglobin* (intron 2), *β-fibrinogen* (intron 7, *β-fibint7*), and *ornithine decarboxylase* (introns 6 and 7, and exon 7). They used two computer programs with distinct rate smoothing algorithms, PATHd8 (*19*) and r8s, a penalized likelihood (PL) method (*20*), to estimate divergence dates. Calibration was based on 22 fossils mapped onto the Bayesian tree estimated by Ericson *et al.* (*10*). A 47.5 million-year-old fossil representing the hummingbird stem was used as a fixed calibration point and the remaining 21 fossils established minimum ages for the ancestral lineages they were thought to represent. The PL dates averaged older than the corresponding PATHd8 dates. Moreover, the authors described a "ghost range" in the PL analysis where the origins of lineages represented by fossils averaged 21 million years older than the fossils themselves. Ericson *et al.* (*10*) thought the PATHd8 dates to be more reliable, but pointed out that the systematic disparity between the ages of nodes estimated by PATHd8 and PL left the answer to the question of whether diversification of Neoaves came before or after the Mesozoic/Cenozoic boundary (66 Ma) ambiguous, but they presented only the estimates for the PATHd8 analysis (*10*). Brown *et al.* (*17*) reanalyzed Ericson *et al.*'s (10) data using their revised fossil calibrations, a somewhat different sequence alignment, and a Bayesian methodology (Multidivtime, *21*). The contention and disparate results between Brown *et al.*'s comment (*17*) and Ericson *et al.*'s (*10*) initial study and reply (*18*) stem from some misunderstanding of details in the original paper (*18*) but also from different fossil calibrations and analytical methods. The last two illustrate how important these factors are in estimating divergence times based on molecular clocks. These estimates are summarized in Table 1.

In addition, we estimated times for the divergence of Ramphastidae, Capitonidae, and Lybiidae as follows (Fig. 2). We downloaded 63 *β-fibint7* sequences from 57 piciform species representing all eight families. The sequences were aligned and average genetic distances computed using the MEGA software (Tajima-Nei distance with gamma parameter = 1.0) for all clades shown in Table 1. Two regression lines were then determined for the *β-fibint7* distances as functions of the estimated ages inferred using (a) PATHd8 (*10*) and (b) Multidivtime (*17*) for all other nodes in Fig. 2. The slopes of the regression lines were estimated such that each line was forced through the origin (i.e., genetic distance equals zero at the time the lineages diverged). The slopes of the least-squares regression lines are the substitution rates as estimated by each of the two methods. These rates were then interpolated to estimate the ages of the divergence of the two nodes in the ((Ramphastidae, Capitonidae), Lybiida) tree using the genetic distances for *β-fibint7*.

The regression line for the Multidivtime data was a remarkably good fit with no apparent deviation from linearity or outliers (slope = 0.00456 nucleotide substitutions/million years). By this method, the estimated age for the split between Asian barbets (Megalaimidae) and the clade of African and New World barbets (common ancestor of Ramphastidae, Capitonidae, and Lybiida) is 24 and 13.4 Ma for the split between Capitonidae and Ramphastidae. The regression analysis based on the PATHd8 method is slightly more complex, because an aspect of the PATHd8 program is that it collapses internodes that are short or where there is uncertain phylogenetic resolution (*10*). Thus, the two oldest splits in Fig. 2 were collapsed to the same level and both dated at 53.0 Ma (Table 1) in Ericson *et al.*'s (*10*) PATHd8 analysis. It was apparent from the fitted regression line that the common ancestor of all piciforms is a salient outlier and its age is underestimated by the PATHd8 analysis. Discarding this point and recalculating the slope of the regression line gives, remarkably, a value identical to that fitted to the Multidivtime slope. Thus, the ages estimated for the Ramphastidae, Capitonidae, and Lybiida divergences by the two methods are identical. A better estimate for the common ancestor of Piciformes, based directly on PATHd8 calculated from the regression equation, is 70.8 Ma.

Coincidental with the final revision of this paper, Brown *et al.* (*22*) published an additional temporal analysis of Aves based on 4594 bp of mtDNA sequence from 135 avian species (*ND1*, *ND2*, *12S rRNA*, and nine tRNA genes). Although this study focused on diversification of major lineages in relation to the Mesozoic/Cenozoic boundary, estimated dates and confidence intervals for five of the seven piciform nodes can be extracted from their timetree. These have been added to Table 1. Disparities between the ages of nodes based on the mtDNA analysis and the earlier nuclear gene analyses, including those reported in the earlier Brown *et al.*'s (*17*) paper, are striking with the five mtDNA-based estimates ranging 1.3–3.6 times older. A thorough exploration of the cause of this apparent bias is beyond the scope of this paper; however, some tentative inferences are evident: The fossil calibrations used in the mtDNA study are nearly identical to those used in the nuclear gene study of Brown *et al.* (*17*), which was a modification of the set used by Ericson *et al.* (*10*). This suggests that differences in calibration are not the cause of the disparity. Differences in estimation methods suggest a second potential cause. Both sets of nodal times were estimated by Bayesian methods, but the nuclear gene estimates were computed by the program package Multidivtime; whereas, the mitochondrial-gene estimates were computed by BEAST. Brown *et al.* (*22*) tested several computational methods on the mtDNA data set: in general the BEAST estimates are higher than the Multidivtime estimates, but not as consistently, or of nearly the magnitude, as the differences between the mitochondrial and nuclear gene estimates. Thus, different estimation procedures might contribute a small amount to the discrepancy, but it is unlikely to be the major factor. This leaves the differences between the data sets, mitochondrial vs. nuclear gene sequences, as a likely cause. Based on simulation studies, Moore *et al.* (*23*) showed that the mitochondrial encoded *cyt b* gene would not perform well as a molecular clock for birds beyond ~10 Ma, whereas the nuclear-encoded *β-fibrinogen* intron 7 would perform well even to estimate time nodes older than 60 Ma. The rapid evolution of mtDNA and saturation by multiple substitutions leads to underestimation of the substitution rate and over estimation of the age of nodes. For this reason, we did not include the time estimates based on the mtDNA in calculating the average age of piciform nodes, although we did include the estimates in Table 1. It is clear that much work remains to resolve sources of uncertainty and that more attention needs to be paid to differences in the "clocking" accuracy of different genes at different time depths.

With the exception of the woodpeckers, the distributions of modern piciform species are restricted to tropical regions of both the New and Old Worlds. This might suggest that their distributions resulted from the breakup of Gondwanaland and the tectonic rifting of the southern continents. The African and South American barbets are closest relatives (*14*), for example, and their divergence may be attributed to the separation of South America from Africa ~100 Ma (*24*). However, the timetree dates are at odds with this hypothesis because the inferred dates for all nodes are much too recent; specifically, the split between African and Asian barbets occurred only 24.6 Ma. The piciforms as a whole are relatively weak fliers and dispersers. The Asian barbets, for example, have not crossed Wallace's Line, and only three species of woodpeckers have crossed the line—and then barely. Trans-Atlantic rafting of terrestrial vertebrates appears to have occurred in rare instances (*25*) but would seem highly improbable in the case of barbets because they have high metabolic rates and it is doubtful that even a large oceanic raft could support their ecological needs for a sustained journey. Nonetheless, the hypothesis of direct dispersal of an ancestral barbet from Africa to South America via rafting cannot be absolutely rejected. A more plausible hypothesis is that the ancestral piciform species dispersed across Beringia and that they were once distributed broadly across the temperate regions of Eurasia and North America, but these ancestral forms were subsequently extinguished from the northern continents.

This is exactly the pattern observed in the fossil record, albeit a sparse fossil record. Moreover, dates associated with various fossil piciforms accord well with the molecular clock estimates for the chronology of nodes. The fossil record shows that early piciforms occurred in both the New and Old World temperate zones in the Lower Eocene (~54 Ma) and perhaps the Paleocene (>54 Ma). Fossils assigned to the extinct piciform Family Gracilitarsidae have been described for the Lower Eocene of Europe, North America, and the Paleocene of Brazil (*26*). Middle Eocene to Lower Oligocene European fossils assigned to the extinct Family Sylphornithidae exhibit combinations of characters representative of the two major lineages, the Pici and Galbulae, of the Piciformes, and a cladistic analysis based on osteological characters established monophyly of a group comprising Gracilitarsidae, Sylphornithidae, and the crown Piciformes (*26*). The oldest fossil representative of the Pici dates to the early Oligocene, ~34–30 Ma (*27*). These fossil dates are reasonably good matches to those inferred in the timetree. The timetree indicates that the ancestral piciform lineage bifurcated ~70.5 Ma to give rise to the Pici and Galbulae lineages. One would expect to find the earliest fossils for the lineage leading to living Piciformes and its two daughter lineages in the Paleocene, Eocene, and Oligocene, which is born out by the fossil record. The inferred bifurcation establishing the Pici and Galbulae predates the Paleocene slightly, but the confidence interval (89–57 Ma) is large and includes much of the Paleocene.

Acknowledgments

We thank P. Ericson and J. Brown for information. This work was supported in part by the U.S. National Science Foundation.

References

1. U. S. Johansson, P. G. P. Ericson, *J. Avian Biol.* **34**, 185 (2003).
2. S. J. Hackett *et al.*, *Science*, **320**, 1763 (2008).
3. J. L. Peters, *Check-List of Birds of the World, Vol VI* (Harvard University Press, Cambridge, 1948).
4. C. G. Sibley, J. E. Ahlquist, *Phylogeny and Classification of Birds: A Study in Molecular Evolution* (Yale University Press, New Haven, London, 1990).
5. E. V. Swierczewski, R. J. Raikow, *Auk* **98**, 466 (1981).
6. S. F. Simpson, J. Cracraft, *Auk* 98, 481 (1981).
7. S. M. Lanyon, R. M. Zink, *Auk* **104**, 724 (1987).
8. S. L. Olson, *Auk* **100**, 126 (1983).
9. P. J. K. Burton, *Bull. Brit. Mus. Nat. Hist. Zool.* **47**, 331 (1984).
10. P. G. P. Ericson *et al.*, *Biol. Lett.* **2**, 543 (2006).
11. R. O. Prum, *Zool. J. Linn. Soc.* **92**, 3131 (1988).
12. S. M. Lanyon, J. G. Hall, *Auk* **111**, 389 (1994).
13. F. K. Barker, S. M. Lanyon, *Mol. Phylogenet. Evol.* **15**, 215 (2000).
14. R. G. Moyle, *Mol. Phylogenet. Evol.* **30**, 187 (2004).
15. E. Hofling, H. M. F. Alvarenga, *Zool. Anzeiger* **240**, 196 (2001).
16. D. M. Webb, W. S. Moore, *Mol. Phylogenet. Evol.* **36**, 233 (2005).
17. J. W. Brown, R. B. Payne, D. P. Mindell, *Biol. Lett.* **3**, 257 (2007).
18. P. G. P. Ericson, C. L. Anderson, G. Mayr, *Biol. Lett.* **3**, 260 (2007).
19. T. Britton, C. Anderson, D. Jacquet, S. Lundqvist, K. Bremer, *PATHd8: A Program for Phylogenetic Dating of Large Trees without a Molecular Clock*, www.math.su.se/PATHd8/ (Stockholm University, Stockholm, Sweden, 2007).
20. M. J. Sanderson, *Mol. Biol. Evol.* **19**, 101 (2002).
21. J. L. Thorne, H. Kishino, *Syst. Biol.* **51**, 689 (2002).
22. J. W. Brown, J. S. Rest, J. Garcia-Moreno, M. Sorenson, D. P. Mindell, *BMC Biol.* **6**, 6 (2008).
23. W. S. Moore, S. M. Smith, T. Prychitko, in *Proceedings of 22nd International Ornithological Congress*, N. Adams, R. Slotow, Eds. (University of Natal, Durbin, 1999), pp. 745–753.
24. B. C. Storey, *Nature* **377**, 301 (1995).
25. N. Vidal, A. Azvolinsky, C. Cruaud, S. B. Hedges. *Biol. Lett.* **4**, 115 (2007).
26. G. Mayr, *Biol. Rev.* **80**, 515 (2005).
27. G. Mayr, *Auk* **122**, 1055 (2005).