

Sapayoa aenigma: a New World representative of 'Old World suboscines'

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Passerine birds are very plastic in their adaptations, which has made it difficult to define phylogenetic lineages and correctly allocate all species to these. *Sapayoa aenigma*, a member of the large group of New World flycatchers, has been difficult to place, and DNA–DNA hybridization experiments have indicated that it may have been misplaced. This is confirmed here, as base sequencing of two nuclear genes places it as a deep branch in the group of broadbills and pittas of the Old World tropics. The peculiar distribution of this lineage may be best explained in terms of a Gondwanic and Late Cretaceous origin of the passerine birds, as this particular lineage dispersed from the Antarctic landmass, reaching the Old World tropics via the drifting Indian plate, and South America via the West Antarctic Peninsula.

Keywords: avian systematics; biogeography; suboscines; *Sapayoa aenigma*

1. INTRODUCTION

Perching birds (order Passeriformes) constitute an ancient avian group (Van Tuinen & Hedges 2001) of extraordinary diversity, as the more than 5700 species show great plasticity in adaptations for locomotion and feeding leading to parallel evolution of distinctive adaptive types. Because few characters are known that confidently define lineages, and few avian systematists in the past used clear phylogenetic principles, the classification of perching birds suffers from a high degree of systematic neglect with phylogenetically isolated species 'dumped' in larger groups of similar-looking birds.

The perching birds have long been divided into two large groups of 'oscines' and 'suboscines', based on different stapes and syrinx structures. The suboscines were further subdivided into Acanthisittidae (New Zealand wrens, now known to be basal to all other Passeriformes; Ericson *et al.* 2002) and Old and New World suboscines, Eurylaimides and Tyrannides (Sibley & Ahlquist 1990, pp.576–601). However, no character may unequivocally define the latter two groups. A modification of *Musculus iliotibialis lateralis*, proposed to be a synapomorphy for

Eurylaimides (Raikow 1987, p.36), is found also in some New World suboscines (Raikow 1987, p.28), and the Old World broadbills, Eurylaimidae, resemble the New World tyrannine group Cotingidae (cotingas) in many respects. Variable and discordant characters among some tyrannines make their relationships difficult to resolve (Ames 1971; Traylor 1977; McKittrick 1985; Lanyon 1986, 1988; Prum & Lanyon 1989; Prum 1990).

One small olive-brown west Colombian bird, *Sapayoa aenigma* (broad-billed *Sapayoa*) was initially placed in the tyrannine group Pipridae (manakins) owing to fused outer toes. Lanyon (1985), Prum & Lanyon (1989) and Prum (1990) pointed out that *Sapayoa* and five other genera (*Schiffornis*, *Neopelma*, *Tyrannetes*, *Neopipo* and *Piprites*) stand out from typical manakins in their syringeal characters, and that *Sapayoa* (and *Neopipo*) differ from these other genera in lacking a (derived) enlarged femoral artery. All this indicates that *Sapayoa* is something on its own.

Sibley & Monroe (1990) proposed to place *Sapayoa* in a separate Infraorder *Incertae Sedis* in front of all New World suboscines, based on 'preliminary DNA–DNA hybridization comparisons using *Sapayoa* as a driver indicat[ing] that this species is either a relative of... broadbills, or a sister group of all other New World suboscines' (Sibley & Ahlquist 1990, p. 596). The evidence cited is melting curves for four experiments, the most remarkable being fig. 197, with a cotinga used as a tracer, where the *Sapayoa* melting curve is nearly identical to that of the broadbill *Calypomena*, and also resembles that of *Pitta* (Pittidae). However, no experiment used *Sapayoa* as a radiolabelled tracer, and Sibley & Ahlquist (1990, p.596) therefore discussed these melting curves cautiously and they never included *Sapayoa* in a phylogeny.

Since the Passeriformes are assumed to have originated in the late Cretaceous on the austral (Gondwanic) landmass (Cracraft 2001; Barker *et al.* 2002; Ericson *et al.* 2002), it would indeed be biogeographically interesting to know whether *Sapayoa* is an aberrant tyrannid, a basal New World suboscine, or closer to the Old World suboscines. We therefore included *Sapayoa* in a broader molecular study of the deeper branching of perching birds (Irestedt *et al.* 2001; Ericson *et al.* 2002; Johansson *et al.* 2002).

2. MATERIAL AND METHODS

(a) *Samples used*

One *Sapayoa aenigma* sample from Esmeraldas, Ecuador (ZMUC 126529) was analysed, along with other representatives of the suboscine radiation. Two oscines (*Passer montanus* and *Campephaga flava*) were used as an outgroup (see Ericson *et al.* 2002).

(b) *Molecular methods*

We used in the analyses nucleotide sequence data from two nuclear introns, one in the myoglobin gene (intron 2, 745 bp) and one in the glyceraldehyde-3-phosphate dehydrogenase (G3PDH) gene (intron 11, 396 bp). Details about DNA extractions, primer sequences, PCR and sequencing conditions, and alignments are given in electronic Appendix A (available on The Royal Society's Publications Web site), together with accession numbers for new sequences and for alignments.

(c) *Phylogenetic analysis*

The transversion model (TVM) for nucleotide substitutions, with a discrete (six rate categories) gamma distribution model of among-site rate heterogeneity, was used in the maximum-likelihood and Bayesian inference analyses. This model was selected for both the individual genes and for the combined dataset using the likelihood-ratio test implemented in MODELTEST v. 3.06 (Posada & Crandall 1998). Likelihood trees were also calculated by iterations using a

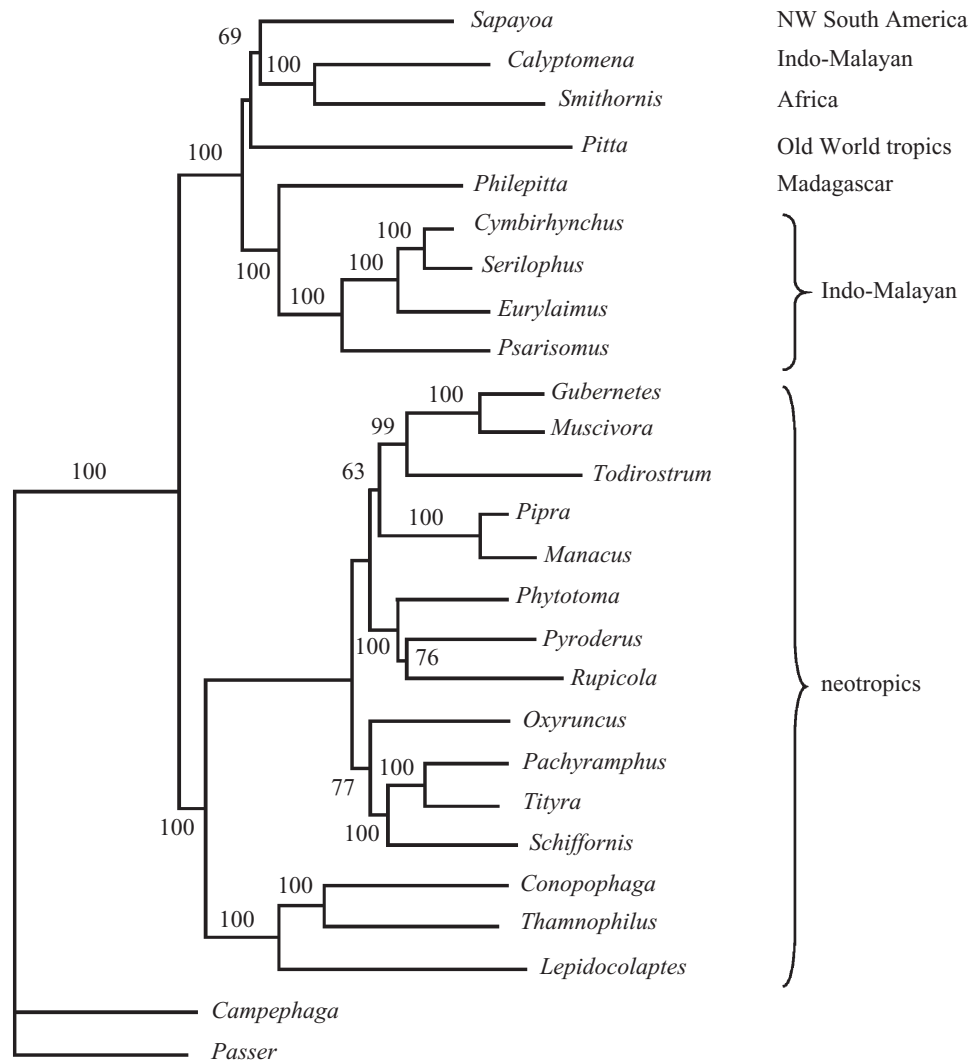


Figure 1. Phylogenetic relationships based on analyses of the combined DNA sequences obtained from two introns in the nuclear genes myoglobin and G3PDH. The maximum-likelihood tree was calculated using the TVM for nucleotide substitutions with a discrete (six rate categories) gamma distribution model of among-site rate heterogeneity with $\alpha = 1.4182$. Posterior probabilities (greater than 50%) for nodes were calculated by Bayesian inference analysis using the same model for nucleotide substitutions.

Bayesian inference of phylogeny with the program MRBAYES v. 2.01 (Huelsenbeck *et al.* 2001). Posterior probabilities for clades were calculated as described in Ericson & Johansson (2003), and indicated as branch supports in the maximum-likelihood tree. Competing tree topologies were compared with the Shimodaira–Hasegawa test (Shimodaira & Hasegawa 1999), implemented in PAUP* (Swofford 1998), using 1000 bootstrap replicates. Maximum-parsimony tree(s) were identified in PAUP* using heuristic searches with 500 random taxon additions and tree bisection–reconnection branch swamping. All characters were given equal weight.

3. RESULTS

Sapayoa groups with Old World suboscines in all analyses and always with high support. It is either sister to all Old World suboscines (G3PDH using parsimony, maximum-likelihood and Bayesian likelihood) or nested with pittas (myoglobin using parsimony), or with the broadbill genera *Smithornis* and *Calyptomena* (myoglobin using likelihood; both genes using parsimony and likelihood; figure 1). A basal position of *Sapayoa* relative to the Old World suboscines is not significantly worse fit ($p = 0.502$, Shimodaira–Hasegawa test), and only one step less parsimonious, than its position in figure 1. However,

to force *Sapayoa* to group with the New World suboscines results in a significantly ($p = 0.024$) worse fit tree that is 10 steps longer than the most parsimonious tree. The nodes separating *Sapayoa*, *Pitta* and *Smithornis/Calyptomena* may be densely packed, according to the distance values. However, this assemblage is always well separated from the well-resolved group comprising the remaining broadbills (including *Philepitta*, in agreement with Prum 1993).

New World suboscines comprise two well-defined groups of tyrannids and tracheophones (Furnariida), in full agreement with other molecular studies (Sibley & Ahlquist 1990; Irestedt *et al.* 2001; Barker *et al.* 2002; Ericson *et al.* 2002).

4. DISCUSSION

These results provide strong support for placing *Sapayoa* near broadbills and pittas, and therefore supports the findings of Sibley & Ahlquist (1990). Their DNA–DNA hybridization is ambiguous, *per se*, as it could reflect an unusually fast rate of genomic evolution in *Sapayoa*, or low thermostability of heteroduplex DNA caused by some

peculiarity of the *Sapayoa* sample, such as short fragment size. The latter would increase the distances to any tracer, although the melting curves would still appear 'normal'—just shifted down towards lower temperatures (B. Slikas, personal communication). Although most experiments may have been well controlled, those involving *Sapayoa* could have gone wrong.

Among molecular techniques, direct nucleotide sequencing, as used by us, is operationally and conceptually very different from DNA–DNA hybridization: it provides information from a limited number of linked nucleotide positions but alternatively gives precise qualitative character states suitable for phylogenetic analysis, whereas the hybridization technique provides a composite summary of multitudinous genes but generates only quantitative distance values (Avise 1996). Using parsimony, deep branch attraction could give misleading results, but this should not be the case with our model-based approach, which placed *Sapayoa* as a very deep branch in the radiation of Eurylaimides.

A morphology-based phylogeny of broadbills (Prum 1993) differed slightly from the topology presented in figure 1, but this is because broadbills and asities were defined, *a priori*, as a monophyletic group sister of pittas (following the analysis of Raikow (1987) of leg muscles). Morphological and molecular trees agree in placing *Smithornis* and *Calyptomena* apart from the other, more derived genera of broadbills and asities (with *Neodrepanis*, *Pseudocalyptomena* and *Corydon* in addition to those included in figure 1; see Prum 1993).

Sapayoa resembles a small, flat-billed tyrannid, especially *Neopelma*, which has similar colours, shape (except for a less broad bill) and yellow coronal patch. However, parallel evolution of a broad flycatcher bill is seen in several lineages of perching birds and the coronal patch is a kind of 'flash colour' that has evolved independently to enhance short-distance communication in birds inhabiting dense foliage. Although we know *Sapayoa* anatomy only from the literature and in communications from S. Olson, the phylogeny presented in figure 1 does not seem to cause serious character conflicts. Thus, the principal problem is understanding the disjunction between New and Old World distributions.

Considering the age of the South Atlantic, direct dispersal between the Old and New World tropics is highly unlikely (Goldblatt 1990). Mid-Tertiary suboscine fossils are known from Europe but not from North America, so dispersal from Europe across the North Atlantic and onwards to Colombia via North America and the eastern Caribbean island arc does not seem a very plausible hypothesis. Therefore the recent idea of a Gondwanic origin of the perching birds (see references above) provides a better explanation. We suggest that the early isolation of New World suboscines in South America was followed, around the end of the Cretaceous, by another colonization from the West Antarctic Peninsula of Antarctic suboscines of the lineage that otherwise gave rise to broadbills, asities and pittas. The African and Indian land masses were already well separated from Antarctica at that time (Smith *et al.* 1995), but stepping stones for dispersal may have existed in the form of islands within the areas of the Kerguelen and Crozet Plateaus and island chains in the wake of drifting India (along the Mascarene, Maldive and

Ninety East Ridges). Most suboscines are sedentary, but it is worth noting that pittas are migratory in the Indo-Australian archipelago and that broadbills have found their way to the Philippines. If they could distribute themselves across the Old World tropics from Africa and Madagascar to the Philippines and northern Australia, they may also have managed some degree of island-hopping during the period of the break-up of Gondwana.

The restricted distribution of *Sapayoa* at the north-western corner of South America is most easily interpreted as being relictual: the Chocó area of endemism has many species representing deep phylogenetic branches (compared, for instance, with Amazonian forest birds; Cracraft & Prum 1988).

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- Ames, P. L. 1971 The morphology of the syrinx in passerine birds. *Bull. Peabody Mus. Nat. Hist.* **37**, 1–194.
- Avise, J. C. 1996 Three fundamental contributions of molecular genetics to avian ecology and evolution. *Ibis* **138**, 16–25.
- Barker, F. K., Barrowclough, G. F. & Groth, J. G. 2002 A phylogenetic hypothesis for passerine birds: taxonomic and biogeographic implications of an analysis of nuclear DNA sequence data. *Proc. R. Soc. Lond. B* **269**, 295–308. (DOI 10.1098/rspb.2001.1883.)
- Cracraft, J. 2001 Avian evolution, Gondwana biogeography and the Cretaceous–Tertiary mass extinction event. *Proc. R. Soc. Lond. B* **268**, 459–469. (DOI 10.1098/rspb.2000.1368.)
- Cracraft, J. & Prum, R. O. 1988 Patterns and processes of diversification: speciation and historical congruence in some Neotropical birds. *Evolution* **42**, 603–620.
- Ericson, P. G. P. & Johansson, U. S. 2003 Phylogeny of Passerida (Aves: Passeriformes) based on nuclear and mitochondrial sequence data. *Mol. Phyl. Evol.* (In the press.)
- Ericson, P. G. P., Christidis, L., Cooper, A., Irestedt, M., Jackson, J., Johansson, U. S. & Norman, J. A. 2002 A Gondwanan origin of passerine birds supported by DNA sequences of the endemic New Zealand wrens. *Proc. R. Soc. Lond. B* **269**, 235–241. (DOI 10.1098/rspb.2001.1877.)
- Goldblatt, P. (ed.) 1990 *Biological relationships between Africa and South America*. New Haven, CT: Yale University Press.
- Huelsensbeck, J. P., Ronquist, F. & Hall, B. 2001 MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**, 754–755.
- Irestedt, M., Johansson, U. S., Parsons, T. J. & Ericson, P. G. P. 2001 Phylogeny of major lineages of suboscines (Passeriformes) analysed by nuclear DNA sequence data. *J. Avian Biol.* **32**, 15–25.
- Johansson, U. S., Irestedt, M., Parsons, T. J. & Ericson, P. G. P. 2002 Basal phylogeny of the Tyrannoidea based on comparisons of cytochrome *b* and exons of nuclear *c-myc* and RAG-1 genes. *Auk* **199**, 984–995.
- Lanyon, S. M. 1985 Molecular perspective on higher-level relationships in the Tyrannoidea Aves. *Syst. Zool.* **34**, 404–418.
- Lanyon, W. E. 1986 A phylogeny of the thirty-three genera in the Empidonax assemblage of tyrant flycatchers. *Am. Mus. Novit.* **2846**, 1–64.
- Lanyon, W. E. 1988 A phylogeny of the thirty-two genera in the Elaenia assemblage of tyrant flycatchers. *Am. Mus. Novit.* **2914**, 1–57.
- McKittrick, M. C. 1985 Monophyly of the Tyrannidae (Aves): comparison of morphology and DNA. *Syst. Zool.* **34**, 35–45.
- Posada, D. & Crandall, K. A. 1998 MODELTEST: testing the model of DNA substitution. *Bioinformatics* **14**, 817–818.
- Prum, R. O. 1990 A test of the monophyly of the manakins (Pipridae) and of the cotingas (Cotingidae) based on morphology. *Occas. Pap. Mus. Zool. Univ. Mich.* **723**.
- Prum, R. O. 1993 Phylogeny, biogeography, and evolution of the broadbills (Eurylaimidae) and asities (Philepittidae) based on morphology. *Auk* **110**, 304–324.

- Prum, R. O. & Lanyon, W. E. 1989 Monophyly and phylogeny of the Schiffornis group (Tyrannoidea). *Condor* **91**, 444–461.
- Raikow, R. J. 1987 Hindlimb myology and evolution of the Old World suboscine passerine birds (Acanthisittidae, Pittidae, Philepittidae, Eurylaimidae). *Am. Ornithol. Union Ornithol. Monogr.* **41**, 1–81.
- Shimodaira, H. & Hasegawa, M. 1999 Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Mol. Biol. Evol.* **16**, 1114–1116.
- Sibley, C. G. & Ahlquist, J. E. 1990 *Phylogeny and classification of birds—a study in molecular evolution*. New Haven, CT: Yale University Press.
- Sibley, C. G. & Monroe, B. L. 1990 *Distribution and taxonomy of birds of the world*. New Haven, CT: Yale University Press.
- Smith, A. G., Smith, D. G. & Funnell, B. M. 1995 *Atlas of Mesozoic and Cenozoic coastlines*. Cambridge University Press.
- Swofford, D. L. 1998 *PAUP**. *Phylogenetic analysis using parsimony (*and other methods)*, v. 4. Sunderland, MA: Sinauer.
- Traylor Jr, M. A. 1977 A classification of the tyrant flycatchers (Tyrannidae). *Bull. Mus. Comp. Zool.* **148**, 129–184.
- Van Tuinen, M. & Hedges, S. B. 2001 Calibration of avian molecular clocks. *Mol. Biol. Evol.* **10**, 512–526.

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