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The phylogenetic affinities of Crossley's babbler (*Mystacornis crossleyi*): adding a new niche to the vanga radiation of Madagascar

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Crossley's babbler (*Mystacornis crossleyi*) is a passerine endemic to Madagascar. Traditionally, it has been classified as a babbler (Timaliidae), although affinities with warblers and vangas have been suggested. We investigated the phylogenetic affinities of Crossley's babbler using sequence data from two nuclear introns (myoglobin intron 2 and β-fibrinogen intron 5) and one mitochondrial gene (ND2). We present for the first time (to our knowledge) a molecular phylogeny that confidently places this enigmatic species within the vangas (Vangidae). The inclusion of Crossley's babbler within the vangas adds another foraging niche—gleaning small invertebrates from the ground—to this already large adaptive radiation of songbirds.

Keywords: *Mystacornis crossleyi*; Aves; Vangidae; adaptive radiation; Madagascar; molecular phylogeny

1. INTRODUCTION

Madagascar is the fourth largest island in the world, situated 400 km off the east coast of Africa. Madagascar originated as part of Gondwanaland, but broke off in the Late Jurassic (*ca* 160–150 Myr ago). It remained connected to India until *ca* 90–85 Myr ago when India rifted away and started to drift northwards; hence, since the Late Cretaceous, Madagascar has been isolated from other continents (Wells 2003).

The present-day biota of Madagascar has been greatly influenced by this long isolation. As with other Malagasy vertebrates, most birds appear to have arrived on Madagascar via dispersal from Africa in the Cenozoic (Yoder & Nowak 2006). The long isolation of Madagascar has, in combination with relatively few dispersals, resulted in an avifauna that is characterized by a comparably low species richness coupled with high endemism (Langrand 1990; Hawkins & Goodman 2003). Of the 209 species of birds regularly breeding on Madagascar, approximately 50 per cent are endemic. For songbirds (Passeriformes), this estimate is even greater, with 55 of the approximately 69 species (80%) being endemic (Hawkins & Goodman 2003). Furthermore, these 69 species predominantly belong to one of two large endemic radiations, the Bernieridae and the Vangidae.

The Vangidae represent the larger of these two radiations with at least 17 species; but the taxonomic circumscription of this clade remains uncertain and may include more than 20 species (Schulenberg 2003*b*). This clade represents one of the more famous examples of adaptive radiations, along with the Galapagos finches and Hawaiian honeycreepers, and shows a remarkable morphological diversification, particularly in bill size and shape. Most of the variation in bill morphology relates to foraging ecology. The vangas are primarily insectivorous, but some species also feed on fruits and a few species regularly take vertebrate prey (Schulenberg 2003*b*). The vangas have a wide range of foraging strategies, including gleaning off foliage and branches (*Calicalicus*), trunk gleaning (*Hypositta*), sallies to foliage and branches (*Leptopterus* and *Vanga*), in the air (*Pseudobias*) and to the ground (*Schetba*), and probing of trunks (*Falculea*) (Schulenberg 2003*b*).

The Bernieridae consist of at least 10 species of warbler-like birds previously classified as members of several different passerine groups (Cibois *et al.* 2001), including bulbuls, babblers and warblers. The taxonomic delimitation of this clade is poorly understood and may include a few additional species currently of uncertain phylogenetic affinity (Schulenberg 2003*a*). All species currently assigned to this clade glean for insects but segregate to some extent by diet and by the exploitation of different feeding strata (Schulenberg 2003*a*). The clade also shows variation in body size and bill shapes, although not as pronounced as in the vangas (Schulenberg 2003*a*). Other groups of songbirds on Madagascar have seemingly not undergone the same type of adaptive radiations as the vangas and Bernieridae and are represented by only one or two species.

Crossley's babbler (*Mystacornis crossleyi*) is endemic to Madagascar and the only member of the genus *Mystacornis*. It is primarily terrestrial and distributed in the humid forests of the eastern part of the island. It is fairly common where suitable habitat remains, from sea level up to 1800 m (Dee 1986). As with several of the other endemic songbirds on Madagascar, Crossley's babbler has, as the name implies, traditionally been considered to be a babbler (Timaliidae). However, recent molecular studies have shown that the other 'babblers' have erroneously been placed in that clade. Instead, the oxylabes (*Oxylabes madagascariensis* and *Crossleyia xanthophrys*) and wedge-tailed Jery ('*Neomixis*' *flavoviridis*) belong to the Bernieridae (Cibois *et al.* 2001), a clade which in turn appears to be closely related to the acrocephaline/megalurine warblers in Sylvioidea (*sensu stricto*; Johansson *et al.* 2008), and the jeries (*Neomixis tenella*, *Neomixis viridis*

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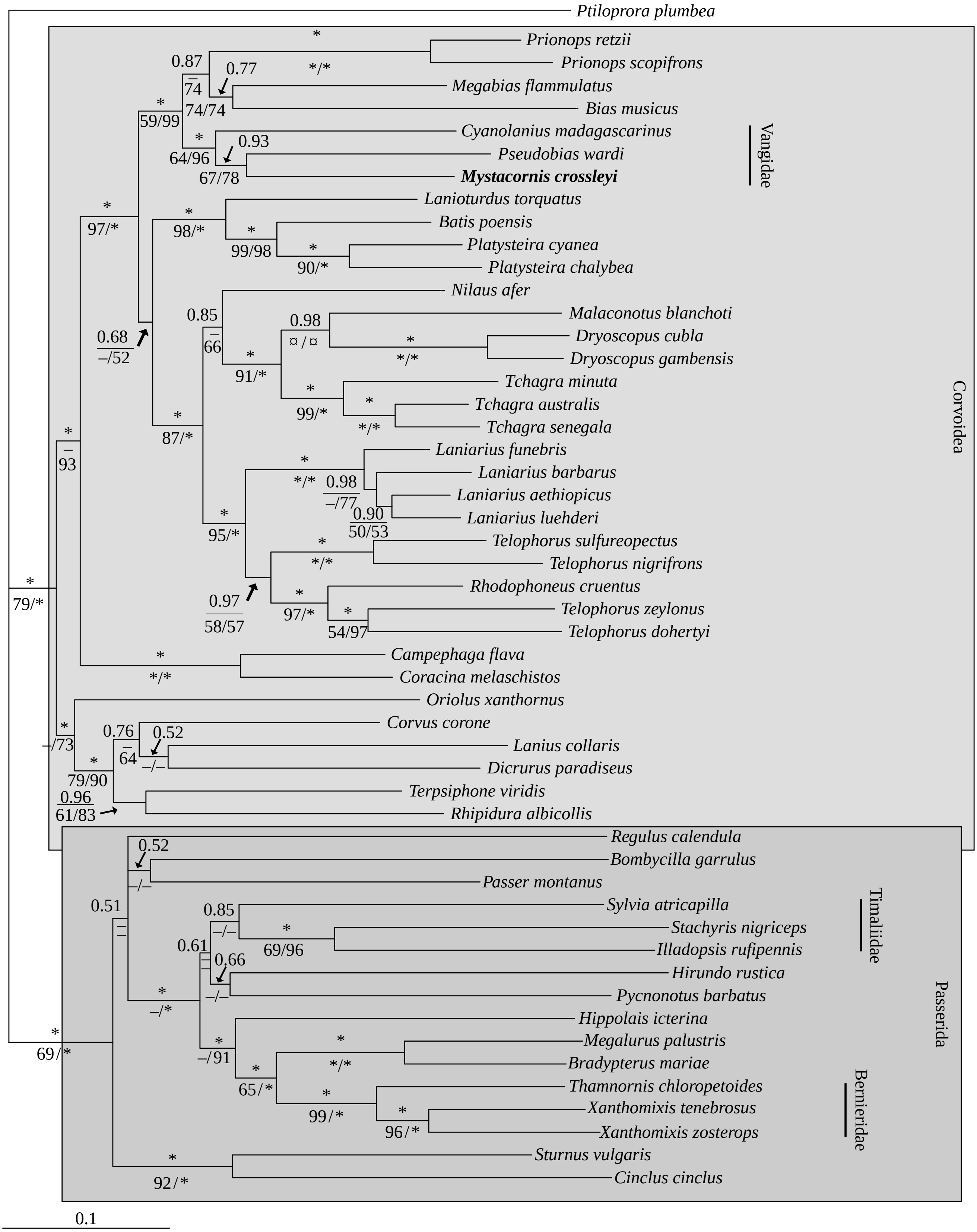

Figure 1. Bayesian consensus tree of the combined mixed-model analysis of the three data partitions: total 2369 bp. Numbers above the branches indicate Bayesian posterior probabilities and numbers below indicate bootstrap support under the parsimony optimality criterion and maximum likelihood (ML), respectively. The asterisk indicates a posterior probability of 1.0 or 100 per cent bootstrap support. The symbol '¤' indicates that the maximum parsimony/ML analysis supports (more than 50%) a conflicting topology.

and *Neomixis striatigula*) appear to be part of another sylvioid clade, the cisticolas (Cisticolidae) (Nguembock *et al.* 2007). This leaves Crossley's babbler, together with the white-eyes (*Zosterops*), as the only putative babblers on Madagascar. Although historically associated with the babblers, *Mystacornis* also bears resemblance to several of the species currently placed in the Bernieridae, particularly *Oxylabes*. However, molecular data do not support any of these associations, but rather indicate a position outside the Passerida (Cibois *et al.* 1999, 2001). Schulenberg (2003*b*) suggested, based on preliminary data, that *Mystacornis* might be related to the vangas within the 'Corvoidea' radiation (=crown Corvoidea *sensu* Barker *et al.* 2004).

We investigate the phylogenetic affinities of *Mystacornis* using sequence data from two nuclear introns (myoglobin intron 2 and β-fibrinogen intron 5) and one mitochondrial gene (ND2). Our data support a vangid affinity of *Mystacornis*, which adds yet another feeding niche and behavioural specialization to the vanga radiation.

2. MATERIAL AND METHODS

(a) *Taxon sampling*

We compared DNA sequences of *M. crossleyi* with 51 species of passerines, using a meliphagid (Leaden Honeyeater *Ptiloprora plumbea*) as an outgroup. We sampled representative species from both the Passerida and 'crown Corvoidea' radiations, including representatives of the babblers (*Sylvia atricapilla*, *Stachyris nigriceps* and *Illadopsis rufipennis*), the endemic Malagasy warbler radiation ('*Xanthomixis*' *tenebrosus*, *Xanthomixis zosterops*, *Thamnornis chloropetoides* and *N. flavoviridis*), bulbuls (*Pycnonotus barbatus*), vangas (*Cyanolanius madagascarinus* and *Pseudobias wardi*) and several other representatives of the malaconotine radiation: a complete list of all species included (as well as GenBank accession numbers) is detailed in table 1 of the electronic supplementary material.

Laboratory procedures for the extraction, PCR amplification and sequencing of the nuclear genes follows Irestedt *et al.* (2002), Fuchs *et al.* (2004), and Johansson & Ericson (2005). Mitochondrial ND2 was amplified with primers L5219 Met and H6313 Trp (Sorenson *et al.* 1999). The concatenated alignment of the three gene segments included 2369 nucleotide positions (myoglobin: 676 bp; β-fibrinogen intron 5: 659 bp; ND2: 1034 bp). All gaps were treated as missing data in the phylogenetic analyses.

Phylogenetic relationships were estimated using Bayesian inference in MrBayes v. 3.1.1 (Ronquist & Huelsenbeck 2003). The three gene partitions were analysed both separately and in a combined mixed-model analysis. The model of sequence evolution for each partition was selected with the Akaike information criterion (Akaike 1973) calculated in MrModeltest v. 2.2 (Nylander 2004). The general time-reversible (GTR) model with gamma-distributed rate variation across sites (four rate categories: Γ_4) and proportion of invariant sites (I) was chosen for ND2, whereas GTR+Γ_4 was chosen for myoglobin intron 2 and β-fibrinogen intron 5. Default priors were used in all MrBayes analyses. Two independent runs, each with four Metropolis-coupled Markov chain Monte Carlo chains, were run for 5 million generations and sampled every 100 generations. At the end of the run, the standard deviation of split frequencies was less than 0.01. Trees sampled before the chain reached stationarity were discarded.

Nodal support was also evaluated under the parsimony optimality criterion with a non-parametric bootstrap analysis in PAUP* v. 4.0.b10 (Swofford 2002), as well as with maximum likelihood (ML) using RAxML-VI-HPC (Stamatakins 2006). For the ML analyses, a GTR+Γ_4 model was used for each gene region and support was estimated with 100 bootstrap pseudo-replicates, whereas the parsimony analysis was performed with 1000 bootstrap pseudo-replicates, each with 10 random additions of taxa.

3. RESULTS AND DISCUSSION

The combined mixed-model analysis of the three-gene dataset strongly suggested a corvoid affinity for *Mystacornis*, with no support for an association with the babblers or members of the Bernieridae (figure 1). Instead, there is strong support for placing *Mystacornis* as yet another member of the vanga radiation, as *Mystacornis* is placed as sister to *Pseudobias*, with *Cyanolanius* basal relative to them. The vangas, including *Mystacornis*, are placed with strong support in a clade together with the African helmetshrikes (*Prionops*) and the flycatcher-shrikes (*Megabyas flammulatus* and *Bias musicus*; see also Fuchs *et al.* 2004). This clade may also include the Asian woodshrikes (*Tephrodornis*), flycatcher-shrikes (*Hemipus*) and philentomas (*Philentoma*) (Moyle *et al.* 2006). A corvoid affinity of *Mystacornis* is supported in all gene trees, and both the nuclear β-fibrinogen intron 5 and mitochondrial ND2 place *Mystacornis* in the vanga clade (see figures 2 and 3 of the electronic supplementary material). Myoglobin is inconclusive in this regard, but places *Mystacornis* in a polytomy together with the vangas, helmetshrikes, batises (*Batis* and *Platysteira*) and a clade with the bushshrikes and allies (malaconotids) (see figure 1 of the electronic supplementary material).

Based on patterns of the jaw musculature, Beecher (1953) proposed that *Mystacornis* might be a vanga. However, this suggestion had little impact on later classifications and *Mystacornis* remained placed among babblers despite very little evidence for this association. The vangid affinity of *Mystacornis* leaves the white-eyes as the only representatives of the babbler radiation on Madagascar.

The addition of *Mystacornis* to the vanga clade adds more than just another species to this radiation. *Mystacornis* is primarily an understorey bird, only taking wing when threatened or during territorial fights (Langrand 1990; T. S. Schulenberg 1989–1995, personal observation). Despite the remarkable adaptive radiation among the vangas, *Mystacornis* is the only vanga species that forages on the ground, primarily by gleaning small invertebrates from either the ground or low vegetation. Three genera of vangas exclusively forage by gleaning vegetation, and six other genera frequently employ this foraging behaviour. Interestingly, some observations suggest that *Mystacornis* also probes dead wood on the forest floor (Goodman, in Schulenberg 2003*b*); probing of woody substrates is another frequently employed foraging strategy of vangas, especially in species with the most derived bill morphologies (e.g. *Falculea*, *Xenopirostris*).

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Akaike, H. 1973 Information theory as an extension of the maximum likelihood principle. In *Second Int. Symp. on information theory* (eds B. N. Petrov & F. Csaki). Budapest, Hungary: Akademiai Kiado.

Barker, F. K., Cibois, A., Schikler, P., Feinstein, J. & Cracraft, J. 2004 Phylogeny and diversification of the largest avian radiation. *Proc. Natl Acad. Sci. USA* **101**, 11 040–11 045. (doi:10.1073/pnas.0401892101)

Beecher, W. J. 1953 A phylogeny of the oscines. *Auk* **70**, 270–333.

Cibois, A., Pasquet, E. & Schulenberg, T. S. 1999 Molecular systematics of the Malagasy babblers (Passeriformes: Timaliidae) and warblers (Passeriformes: Sylviidae), based on cytochrome *b* and 16S rRNA sequences. *Mol. Phylogenet. Evol.* **13**, 581–595. (doi:10.1006/mpev.1999.0684)

Cibois, A., Slikas, B., Schulenberg, T. S. & Pasquet, E. 2001 An endemic radiation of Malagasy songbirds revealed by mitochondrial DNA sequence data. *Evolution* **55**, 1198–1206. (doi:10.1554/0014-3820(2001)055[1198:AEROMS]2.0.CO;2)

Dee, T. J. 1986 *The endemic birds of Madagascar*. Cambridge, UK: International Council for Bird Preservation (ICBP).

Fuchs, J., Bowie, R. C. K., Fjeldså, J. & Pasquet, E. 2004 Phylogenetic relationships of the African bush-shrikes and helmet-shrikes (Passeriformes: Malaconotidae). *Mol. Phylogenet. Evol.* **33**, 428–439. (doi:10.1016/j.ympev.2004.06.014)

Hawkins, A. F. A. & Goodman, S. M. 2003 Introduction to the birds. In *The natural history of Madagascar* (eds S. M. Goodman & J. P. Benstead), pp. 267–281. Chicago, IL: The University of Chicago Press.

Irestedt, M., Fjeldså, J., Johansson, U. S. & Ericson, P. G. P. 2002 Systematic relationships and biogeography of the tracheophone suboscines (Aves: Passeriformes). *Mol. Phylogenet. Evol.* **23**, 499–512. (doi:10.1016/S1055-7903(02)00034-9)

Johansson, U. S., Fjeldså, J. & Bowie, R. C. K. 2008 Phylogenetic relationships within Passerida (Aves: Passeriformes): a review and a new molecular phylogeny based on three nuclear intron markers. *Mol. Phylogenet. Evol.* **48**, 858–876. (doi:10.1016/j.ympev.2008.05.029)

Johansson, U. S. & Ericson, P. G. P. 2005 A re-evaluation of basal phylogenetic relationships within trogons (Aves: Trogonidae) based on nuclear DNA sequences. *J. Zool. Syst. Evol. Res.* **43**, 166–173. (doi:10.1111/j.1439-0469.2004.00292.x)

Langrand, O. 1990 *Guide to the birds of Madagascar*. New Haven, CT: Yale University Press.

Moyle, R. G., Cracraft, J., Lakim, M., Nais, J. & Sheldon, F. H. 2006 Reconsideration of the phylogenetic relationships of the enigmatic Bornean Bristlehead (*Pityriasis gymnocephala*). *Mol. Phylogenet. Evol.* **39**, 893–898. (doi:10.1016/j.ympev.2006.01.024)

Nguembock, B., Fjeldså, J., Tillier, A. & Pasquet, E. 2007 A phylogeny for the Cisticolidae (Aves: Passeriformes) based on nuclear and mitochondrial DNA sequence data, and a re-interpretation of an unique nest-building specialization. *Mol. Phylogenet. Evol.* **42**, 272–286. (doi:10.1016/j.ympev.2006.07.008)

Nylander, J. A. A. 2004 MrModeltest: program distributed by the author, Evolutionary Biology Centre, Uppsala University.

Ronquist, F. & Huelsenbeck, J. P. 2003 MrBayes 3: Bayesian phylogentic inference under mixed models. *Bioinformatics* **19**, 1572–1574. (doi:10.1093/bioinformatics/btg180)

Schulenberg, T. S. 2003*a* The radiation of passerine birds on Madagascar. In *The natural history of Madagascar* (eds S. M. Goodman & J. P. Benstead), pp. 1130–1134. Chicago, IL: The University of Chicago Press.

Schulenberg, T. S. 2003*b* Vangidae, vangas. In *The natural history of Madagascar* (eds S. M. Goodman & J. P. Benstead). Chicago, IL: The University of Chicago Press.

Sorenson, M. D., Ast, J. C., Dimcheff, D. E., Yuri, T. & Mindell, D. P. 1999 Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. *Mol. Phylogenet. Evol.* **12**, 105–114. (doi:10.1006/mpev.1998.0602)

Stamatakis, A. T. 2006 RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**, 2688–2690. (doi:10.1093/bioinformatics/btl446)

Swofford, D. L. 2002 *PAUP*: phylogenetic analysis using parsimony (*and other methods)*, v. 4.0.b10. Sunderland, MA: Sinauer Associates.

Wells, N. A. 2003 Some hypotheses on the Mesozoic and Cenozoic paleoenvironmental history of Madagascar. In *The natural history of Madagascar* (eds S. M. Goodman & J. P. Benstead). Chicago, IL: The University of Chicago Press.

Yoder, A. D. & Nowak, D. 2006 Has vicariance or dispersal been the predominant biogeographic force in Madagascar? Only time will tell. *Annu. Rev. Ecol. Evol. Syst.* **37**, 405–431. (doi:10.1146/annurev.ecolsys.37.091305.110239)