

Short communication

# Old World Shrike-babblers (*Pteruthius*) belong with New World Vireos (Vireonidae)

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## 1. Introduction

Many authors have suggested through the years that the overall morphological uniformity of passerine birds, which encompass roughly half of all avian diversity, has made their classification particularly problematic (Mayr and Amadon, 1951; Beecher, 1953; Sibley and Ahlquist, 1990). With the rise of molecular systematics and the use of DNA sequences in particular, the overall relationships among the major groups of passerines are beginning to be clarified and numerous taxa are being found to have been classified erroneously (Sibley and Ahlquist, 1990; Barker et al., 2002; James et al., 2003; Barker et al., 2004; Fuchs et al., 2006). One of these latter groups is the shrike-babblers (*Pteruthius*), a small group of passerine birds endemic to southern Asia. Recently, Cibois (2003) produced the first phylogeny of the Timaliidae and discovered that *Pteruthius* did not group with other babblers—as was expected on the basis of previous classifications—but rather with corvid outgroups. Cibois (2003) did not investigate the placement of *Pteruthius* within corvids in detail, but her finding is important because it expands our phylogenetic and biogeographic view of this large passerine clade that apparently had its origin in Australasia (Barker et al., 2004).

*Pteruthius* has most often been placed within the “babbler” family Timaliidae, which has often been regarded as a “waste basket” because it includes species whose relationships to other Old World songbirds as well as to each other have been uncertain (Mayr and Amadon, 1951;

Sibley and Ahlquist, 1990). Shrike-babblers, as their name suggests, have some characteristics typical of shrike-like birds and others similar to babblers. They have strong, hooked bills like shrikes, but have short, rounded wings and relatively stout legs like typical babblers (Baker, 1922; Delacour, 1946). All species are sexually dimorphic in plumage coloration, a trait that is not typically found in babblers. Their habits and nesting behavior, however, are similar to true timaliids (Baker, 1922).

The classificatory history of *Pteruthius* is complex. Because they possess certain shrike-like characteristics, especially a sharply hooked bill, the first species to be described was originally named a shrike (*Lanius erythropterus*; Vigors, 1830–31). Later, emphasis on other features, such as wing and leg morphology, prompted their placement into a separate genus, *Pteruthius*, by Swainson (1832). Avian classifications since have variously grouped this genus with babblers, shrikes, and various corvids (e.g., Swainson, 1832; Oates, 1889; Sharpe, 1903; Baker, 1922). Subsequent classifications have followed Baker (1922) in placing *Pteruthius* within the Timaliidae. Delacour (1946), in his revision of the babblers, partitioned genera into five tribes based on external morphological similarities and differences. He placed *Pteruthius* into his last tribe, the Turdoidini, whose members shared the characteristic of being the most widely differentiated taxa among the Timaliidae (Delacour, 1946). Later authors, including Deignan (1964) and Sibley and Monroe (1990), continued to place *Pteruthius* within muscicapoid higher-taxa (e.g., Timaliidae or Sylviidae, respectively).

In order to understand corvid history it is important to resolve the relationships of *Pteruthius* within corvids. The main objective of this study is to build on the results of Cibois (2003) and find the closest relatives of *Pteruthius*.

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We address this using sequences of two nuclear genes, RAG-1 and RAG-2, for *Pteruthius* and analyze these sequences within the largest available nucleotide dataset for passerines (i.e. Barker et al., 2004), which includes representatives of all but one passerine family. Additionally, in order to make inferences about resulting biogeographic and temporal patterns we estimate the timing of divergence of this group from its closest relatives using both non-parametric and semi-parametric methods.

## 2. Methods

RAG-1 and RAG-2 (2902 and 1152 base pairs, respectively) were sequenced for two species of *Pteruthius*: *P. xanthochlorus* (American Museum of Natural History DOT 5585; Nepal) and *P. melanotis* (AMNH DOT 10930; Vietnam). Standard PCR procedures were followed to amplify genomic DNA as described in Barker et al. (2002) and with previously published primers (see Barker et al., 2002; Barker et al., 2004). Sequences were deposited in GenBank (Accession Nos. DQ248105–DQ248108). Incorporation of these new sequences of protein-coding genes into the alignment of Barker et al. (2004) was straightforward and performed by eye. Amplified sequences were doubled-checked for stop codons and indels that would indicate a pseudogene.

The dataset included a total of 148 taxa (including two *Pteruthius* and two non-passerine outgroup taxa) and 4,126 aligned base pairs (see Barker et al., 2004 for more details). A maximum parsimony (MP) heuristic search using the parsimony ratchet (Nixon, 1999) was conducted by employing PAUPRat (Sikes and Lewis, 2001) in PAUP\*4.0b10 (Swofford, 1998). Five runs of 200 iterations each were conducted. All non-duplicate minimum length trees from each run were saved. Nodal support was determined by bootstrapping (100 pseudo-replicates of 10 random additions each), jackknifing (100 pseudo-replicates of 50% replacement, 10 random additions each), and calculating decay indices (using TreeRot and PAUP\*; Sorenson, 1999). Parsimony results were compared to model-based reconstructions of phylogeny, mainly maximum likelihood and Bayesian inference.

The large size of the dataset makes Maximum Likelihood (ML) analyses computationally intensive. We conducted ML analyses using three programs in order to compare the performance of these different heuristic methods: PHYML v.2.4.4 (Guindon and Gascuel, 2003) with additional NNI and TBR swapping in PAUP, as undertaken in Barker et al. (2004); GARLI v.0.942 (Zwickl, 2006 unpublished Ph.D. dissertation; <http://www.zo.utexas.edu/faculty/antisense/Garli.html>), the best of five replicates with different starting points; and PAUP, heuristic searches of 10 replicates each using SPR and NNI branch-swapping. Both PHYML and GARLI estimate a model that best fits the data during the analysis. For the PAUP search, the Akaike Information Criterion (AIC) in Modeltest v3.7 (Posada and Crandall, 1998) was used to

select the best-fit model. ML bootstrapping (100 pseudo-replicates) was conducted using GARLI. For all comparisons, likelihood scores were recalculated in PAUP using the best model chosen by Modeltest unless otherwise specified.

MrBayes v.3.11 (Ronquist and Huelsenbeck, 2003) was used to perform searches of Bayesian Inference. Settings of default prior probabilities and likelihood model settings for GTR + I + G were applied. A six-partitioned analysis using separate models for each codon position in each gene was used. Two runs of 5,000,000 generations and four heated chains each with every 500th sampled were performed utilizing a high performance parallel computing environment. Runs were checked for convergence and examined for stationarity. The first 500 saved trees of each run, a conservative cut-off, were discarded as the burn-in, while a consensus of the remaining trees was computed for the final outcome.

Divergence times were estimated using non-parametric rate smoothing (NPRS) and penalized likelihood (PL) as implemented in the program r8s v.1.70 (Sanderson, 2003). This program is designed to estimate rates of evolution and divergence times of phylogenetic trees that deviate from the assumption of the molecular clock and works by imposing a function that penalizes rates in order to smooth changes along rate-heterogeneous branches. NPRS is a non-parametric method, consisting of mainly the penalty function, while PL is semi-parametric and uses likelihood to optimize the smoothing function and data-fitting procedure (Sanderson, 2003).

The ML tree was used to calculate divergence rates. As in Barker et al. (2004), biogeographic evidence was used to calibrate the divergence of *Acanthisitta* from all remaining passerines at 82 million years ago (Cracraft, 2001). Also, *Gallus* and *Sitta*, taxa with high levels of divergence, were pruned prior to all rate estimates. In r8s, the age of the *Acanthisitta* split was fixed and the relative ages of the remaining nodes were estimated using NPRS and PL. For each method, the “checkgradient” function and the best of five iterations from different starting points were used. For PL, cross-validation was first used to select the optimal smoothing parameter, which was chosen by selecting the smoothing values with the lowest  $\chi^2$  error. Standard errors for divergence estimates were calculated by first creating 50 pseudo-replicates of the dataset by bootstrapping the characters. While constraining the topology of the ML tree, branch lengths were calculated for each replicate dataset using PAUP\*. The divergence times of these phylogenies were estimated using r8s and the distributions of nodal ages were used to derive the standard errors.

## 3. Results

The results of the maximum parsimony, maximum likelihood, and Bayesian analyses all show that *Pteruthius* does not group with the Timaliidae but is deeply nested within the ‘core Corvoidea’ with strong support for a sister

relationship with a clade including Vireonidae and *Erpornis* (Figs. 1 and 2). Maximum parsimony analysis of the dataset resulted in 628 shortest trees of 9528 steps (Fig. 1). Bootstrap, jackknife, and decay values show strong support for the monophyly of *Pteruthius* (100/100/20), as well as the sister grouping of *Pteruthius* with the extended Vireonidae (100/100/10). Similarly, Bayesian posterior probabilities and ML bootstrap values were 100% for both these nodes.

The PHYML analysis using MP trees as starting points (as in Barker et al., 2004) gave a worse score ( $-\ln L = 59473.24$ ) than PHYML with a Neighbor-Joining starting tree ( $-\ln L = 59470.16$ ). The resulting topology of the latter analysis, however, was very different from Barker et al.'s (2004) tree although none of the conflicting nodes were well supported (i.e. >70% bootstrap). For this reason, alternative ML programs, such as GARLI and PAUP, were used. Both these programs found essentially the same tree, yet GARLI took a few hours and PAUP took a few weeks to complete the analysis. In Modeltest, both the likelihood ratio test and AIC chose the same best-fit model and

parameters: GTR + I + G using base frequencies = (0.3331 0.2055 0.2071), rate matrix = (1.2007 5.6470 0.5909 1.6301 8.2215), gamma shape = 1.0154, proportion of invariant sites = 0.3690. GARLI found the same model but with slightly different parameters: base frequencies = (0.3318 0.2063 0.2079), rate matrix = (1.1908 5.6459 0.5876 1.6238 8.1943), gamma shape = 1.0176, and proportion of invariant sites = 0.3670. The topology of the trees selected from PAUP and GARLI differed only in the resolution of *Batis mixta* in the Malaconotinae/Vangini clade, which was unresolved in the former (also in Barker et al., 2004) and sister to the clade of *Aegithina* + *Dryoscopus* + *Telophorus* in the latter. Neither topology was well supported. Both trees had the same scores using the parameters selected by Modeltest ( $-\ln L = 59469.4289$ ) and GARLI ( $-\ln L = 59469.3476$ ). A Shimodaira–Hasegawa test (Shimodaira and Hasegawa, 1999), performed using PAUP, indicates that all the trees derived from the different likelihood analyses are not significantly different (*P* values ranged from 0.54 to 0.87). The GARLI/PAUP trees were used in all further analyses. They differ from the

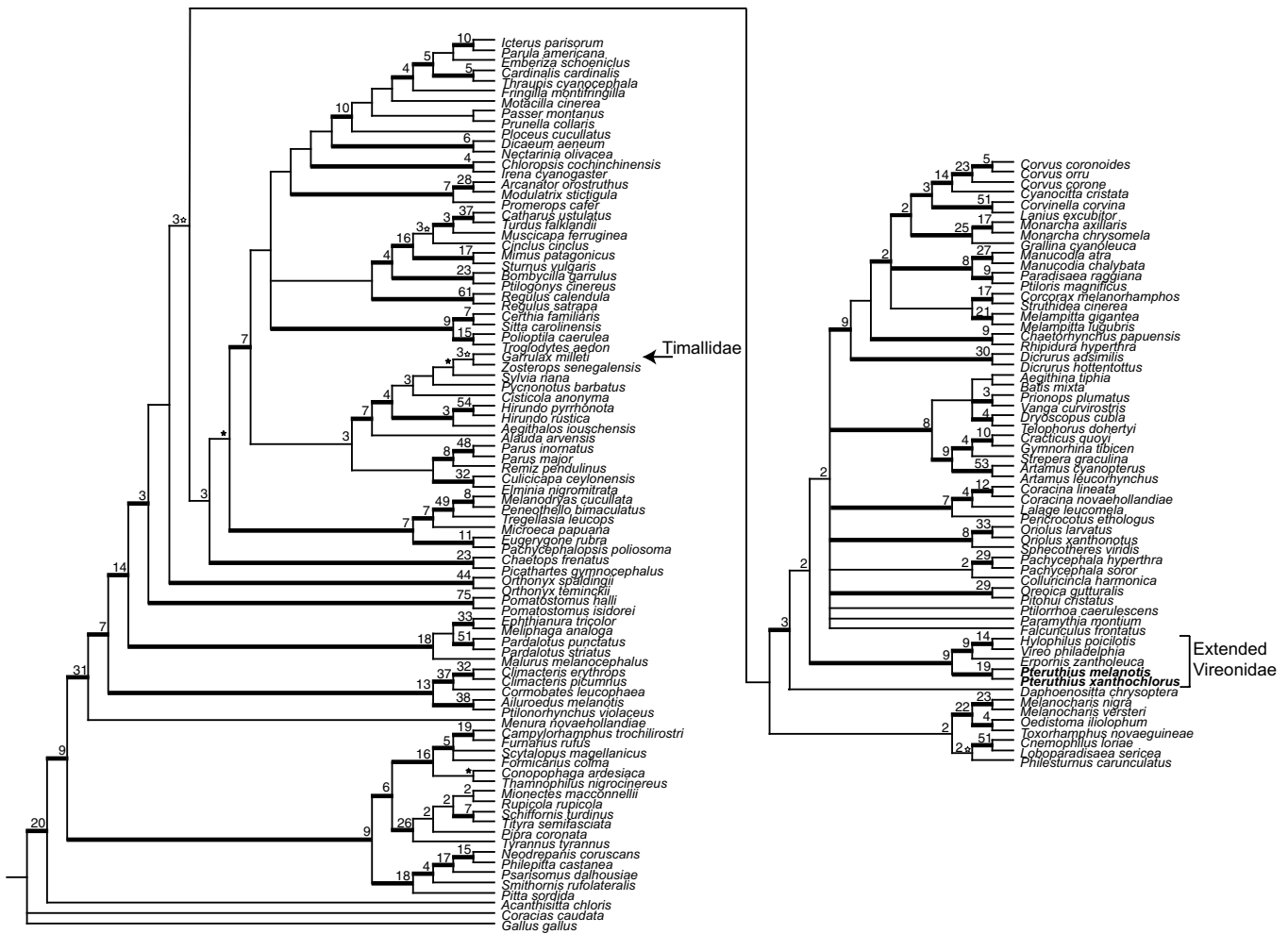


Fig. 1. Strict consensus of 628 most parsimonious trees (9528 steps). *Pteruthius* taxa are in bold. Arrow points to true Timaliidae representatives. Thicker branches indicate nodes where both bootstrap and jackknife values  $\geq 70\%$ . Nodes where only bootstrap (solid stars) or only jackknife (open stars) values are  $\geq 70\%$  are indicated with symbols. Decay indices (when  $> 1$ ) are listed at nodes.

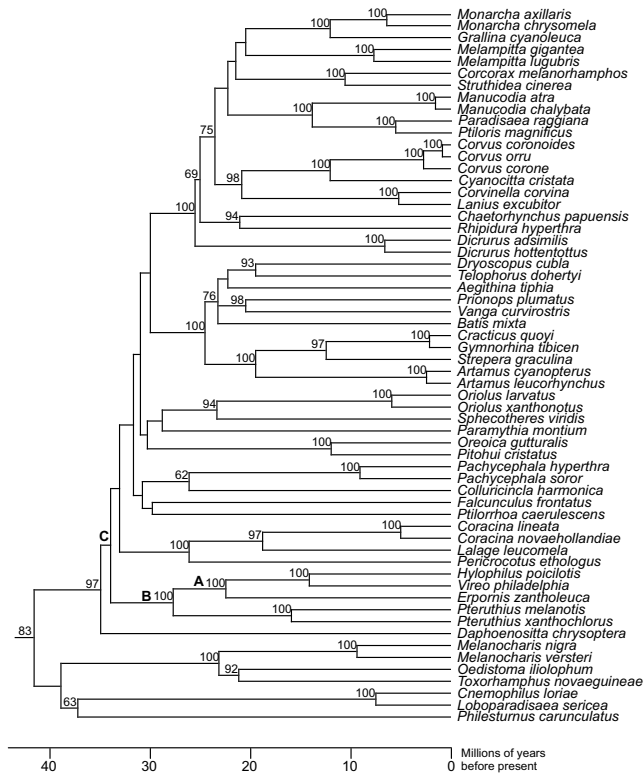


Fig. 2. Penalized likelihood chronogram generated using r8s. Only the clade of the “core Corvoidea” and allies is shown. Numbers at nodes indicate ML bootstrap values greater than 50% from GARLI. Letters refer to nodes mentioned in Table 1.

Barker et al. (2004) tree only in the placement of *Batis* (see above) and of *Mionectes*, which here is sister to the clade of *Tityra* + *Schiffornis* + *Rupicola*.

The extended Vireonidae + *Pteruthius* clade is consistently among the most basal splits of the ‘core’ corvoids. In the parsimony and likelihood analyses, *Daphoenositta* is the most basal split and the Vireonidae + *Pteruthius* clade diverges next and is sister to the remaining Corvoidea. All basal relationships of the Corvoidea, however, are not well supported. In the Bayesian analysis, *Daphoenositta* splits first but the relationships of the Vireonidae + *Pteruthius* clade and the remaining Corvoidea are not resolved in the 50% majority consensus tree.

The chronogram generated by r8s is shown in (Fig. 2). Divergence dates and bootstrap standard errors for nodes of the *Pteruthius* + Vireonidae clade are listed in Table 1. Estimates using both NPRS and PL are very similar. Both the estimated timing of the split of the extended Vireonidae (including *Erpornis* and *Pteruthius*) from the remaining Corvoidea and the divergence between Vireonidae and *Erpornis* are younger than the estimate reported in Barker et al. (2004). We tested whether this difference was due to the addition of new taxa or changes in the model parameters and hence the branch lengths inferred. We found only slight differences in the divergence time estimates of these two events with the pruning of *Pteruthius* taxa and when using other ML analyses with different model parameters

and branch length estimates (see Table 1). However, all these estimates are not significantly different from one another because they overlap when their standard errors are taken into account.

#### 4. Discussion

The results of this study corroborate the findings of Cibois (2003) that *Pteruthius* does not belong in the Timaliidae (Passerida). Instead, this genus falls within the Corvoidea, with a highly supported sister relationship to the Vireonidae + *Erpornis*. *Erpornis* (née *Yuhina zantholeuca*) was recently discovered to be another aberrant taxon formerly placed in the Timaliidae (Cibois et al., 2002; Barker et al., 2004). An extended Vireonidae (including *Erpornis* and *Pteruthius*) is consistently among the basal-most divergences within the core Corvoidea, although the nodal support for these groupings is low.

The relationships of the vireos have been confounding avian systematists for a long time. For many years, they were suggested to be closely related to wood-warblers (Parulidae) because of their similar coloration, body size, and distribution in the New World, or to shrikes (Laniidae) due to their comparable bill morphology (Cicero and Johnson, 2001). DNA evidence has consistently placed vireonids within the Corvoidea, but an explicit statement of relationships has been challenging to pinpoint (Sibley and Ahlquist, 1990; Cicero and Johnson, 2001; Barker et al., 2002). Now, with better taxonomic sampling, we have identified at least two close relatives of the Vireonidae.

Until recently, the grouping of the New World Vireonidae within the Corvoidea, a clade consisting mainly of Old World families, was perplexing in terms of biogeography. The discovery of *Pteruthius* and *Erpornis* as close relatives of vireonids clarifies the phylogenetic and biogeographic relationships of this enigmatic family. Two biogeographic scenarios for the history of Vireonidae in the New World have been put forward. The first proposes that these birds dispersed to North America via the Bering land-bridge in the late Tertiary (Beecher, 1953; Cicero and Johnson, 2001). A second states that an ancestral vireonid–corvoid form might have been present on the southern landmass of Australia–Antarctica–South America that a vireonid lineage diverged following the separation of South America and Antarctica around 40 million years ago (Sibley and Ahlquist, 1990). The latter hypothesis can be rejected because the closest relatives of the New World vireos are Asian and because the divergence estimates based on molecular distance estimates (Johnson et al., 1998; Barker et al., 2004; this study) are also more in line with a northern biogeographic history. The origins and appearance of the vireonid clade on mainland Asia are difficult to reconstruct because there is not yet any strong evidence for the identity of their sister-group within the more basal corvoids and relationships among these groups are still poorly understood. We can say that the vireonid clade is one of



Table 1  
Divergence dates estimated using (a) NPRS with standard errors and (b) PL for the splitting of nodes labeled in Fig. 2

Node	Barker et al. (2004)	ML (GARLI)	ML (PAUP)	ML (PHYML)	ML (GARLI) without <i>Pteruthius</i>
(A)					
A	NW Vireos vs. <i>Erpornis</i>	28 ± 2.9	23.5 ± 2.8	22.5 ± 2.9	25.0 ± 3.4
B	<i>Pteruthius</i> vs. NW Vireos + <i>Erpornis</i>	NA	29.2 ± 2.7	27.8 ± 2.9	NA
C	Corvoids vs. Vireonidae	40 ± 2.6	36.2 ± 3.8	34.6 ± 2.6	36.9 ± 2.0
(B)					
A	NW Vireos vs. <i>Erpornis</i>	25	22.4	22.4	23.1
B	<i>Pteruthius</i> vs. NW Vireos + <i>Erpornis</i>	NA	27.6	27.5	NA
C	Corvoids vs. Vireonidae	37	33.9	33.8	34.3

the earliest groups of corvoids to reach Asia, presumably from Wallacea/Australasia. The divergence time estimate for the split between the extended Vireonidae from the rest of the Corvoidea (33.97 million years ago) is much younger than other basal, noncorvoid passerines hypothesized to have dispersed out of Australasia. Thus, the African Picathartidae and primitively Laurasian Passerida were estimated to have dispersed out of Australasia ~45 and 44 million years ago, respectively (Barker et al., 2004). These findings imply multiple dispersal events of passerine groups out of Australasia, but our understanding of relationships among corvoids and of the detailed geological history of Wallacea/Australasia place limits on our biogeographic scenarios. Interestingly, the recalculated estimate for the split of the Vireonidae from its Asian relatives at 22.5 million years is concordant with other passerine dispersals into the New World (i.e. Mimini and Emberizinae both estimated at 22 million years ago (Barker et al., 2004)).

In conclusion, in light of the findings reported in this study as well as in Barker et al. (2004) and Cibois (2003), the classification of the genera *Pteruthius* and *Erpornis* should be changed. These new phylogenetic results demonstrate these genera do not belong in the Timaliidae but rather that they should be placed within the Vireonidae. As tissue samples and nucleotide data for passerine birds accumulate, especially for poorly studied Asian taxa, it is possible that other aberrant species might also be found to belong in this redefined clade.

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