

## Were bowerbirds part of the New Zealand fauna?

(cytochrome *b* sequences/phylogeny/systematics)

LES CHRISTIDIS\*†, PETER R. LEETON\*‡, AND MICHAEL WESTERMAN‡

\*Department of Ornithology, Museum of Victoria, 71 Victoria Crescent, Abbotsford, Victoria 3067, Australia; and †Department of Genetics, LaTrobe University, Bundoora, Victoria 3083, Australia

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**ABSTRACT** Bowerbirds (Ptilonorhynchidae) have previously been considered to be confined to the Australo-Papuan continental plate. We provide molecular evidence that the extinct New Zealand Piopio *Turnagra capensis* is, in fact, a bowerbird. Such a finding is surprising on biogeographical grounds. However, recent molecular evidence on the relationships of the New Zealand moas and kiwis with the Australo-Papuan flightless birds suggests the need for a reassessment of current views on the origins of New Zealand's fauna.

The advent of PCR-mediated DNA sequencing (1) has meant that taxa for which no frozen or alcohol-preserved tissue is available can now be included in molecular-based phylogeny studies by utilizing museum skins (2–4). This technology not only means that evolutionary questions involving highly endangered species can be answered without the need to obtain additional specimen material but also is particularly important for studies on families and genera from which no taxa survive today (5). One such example is the enigmatic Turnagridae. This New Zealand avian passerine family was represented by a single species, the Piopio, with two morphologically distinctive subspecies, *Turnagra capensis capensis* (South Island) and *Turnagra capensis tanagra* (North Island). The species has not been reliably recorded since 1908 and is most likely extinct (6). Considerable debate surrounds the taxonomic affinities of this species (7–10), compounded in part by a lack of sufficient specimen material for detailed morphological analysis. The two most widely held views are that it either is part of the Australasian pachycephaline assemblage (8, 11), which includes the whistlers (*Pachycephala*), or else is closer to the birds-of-paradise (Paradisaeidae) and bowerbirds (Ptilonorhynchidae) (7, 9). Limited comparisons of the skulls of *Turnagra* and *Pachycephala* did not reveal any obvious links between them (7, 9). Although comparisons with the Ptilonorhynchidae and Paradisaeidae have been more extensive, involving myology, skull osteology, and pterylography, the results have been ambiguous, suggesting links with both the bowerbirds and the “cnemophiline” birds-of-paradise. Such a link is unlikely given the concordance between different molecular data sets, which indicate that the bowerbirds and birds-of-paradise are not a monophyletic clade with respect to other corvines (10, 12–14). *Turnagra* could be associated with either one of these families but not with both.

Here we use mitochondrial DNA sequence data to examine the phylogenetic relationships of *Turnagra capensis*. Resolution of this is crucial to an understanding of the early radiations of Australo-Papuan songbirds and in particular the controversial separation of bowerbirds from the corvine radiation (10, 12). It also has implications for the origins of New Zealand's avifauna and the relative importance of vicariant versus dispersal events (5).

## MATERIALS AND METHODS

A contiguous 924-bp region within the mitochondrial cytochrome *b* gene was sequenced from a museum skin of *T. c. capensis* [Turnagridae, Museum of Victoria (MOV) accession number B19053] as well as from frozen liver samples of a golden whistler, *Pachycephala pectoralis* (Pachycephalidae, MOV accession number B433), and a bassian thrush, *Zoothra lunulata* (Turdidae, MOV accession number B214). *Zoothra* was included to provide further comparison in the event that *Turnagra* fell outside of the Australo-Papuan oscine assemblage. Sequences of other relevant taxa were obtained from the literature (14, 15, 16). These taxa were *Ptilonorhynchus violaceus*, *Ailuroedus melanotis*, *Ailuroedus crassirostris*, *Chlamydera nuchalis*, *Chlamydera maculata*, *Chlamydera cerviniventris*, *Sericulus chrysocephalus*, *Amblyornis macgregoriae*, *Amblyornis subalaris*, *Amblyornis inornatus*, *Prionodura newtoniana*, and *Scenopoeetes dentiostri* (Ptilonorhynchidae); *Epimachus fastuosus*, *Epimachus albertsi*, *Ptiloris paradiseus*, *Diphylloides magnificus*, and *Manucodia keraudrenii* (Paradisaeidae); *Gymnorhina tibicen* (Artamidae); *Cyanocitta cristata* (Corvidae); *Lanius ludovicianus* (Laniidae); *Vireo olivaceus* (Vireonidae); *Pomatostomus isidori* and *Pomatostomus temporalis* (Pomatostomidae); *Ptiloprora plumbea* (Meliphagidae); *Catharus guttatus* (Turdidae); *Parus inornatus* (Paridae); *Empidonax minimus* (Tyrannidae); and *Pitta iris* (Pittidae). Sequences of three species of parrot (*Pezoporus wallicus*, *Platycercus icterotis*, *Strigops habroptilus*) obtained from the literature (17) were used as outgroups.

DNA was extracted (17) and amplified as a series of small segments by using the universal primer pairs L14841/H15149 (2), L15114/H15547, and L15424/H15767 (15). Double-stranded DNA was amplified in 35 cycles (92°C for 60 s, 52°C for 60 s, and 72°C for 60 s). The double-stranded DNA was used as a template for single-stranded amplifications, with one of the appropriate primers (the addition of a limiting primer was not necessary because of small amounts of “carry over” after filtration). Asymmetric products were sequenced directly by the dideoxy method (18) with a commercial kit (Sequenase, United States Biochemical). Sequences were obtained for the entire light strand and 80–90% of the heavy strand. There was complete congruence between the heavy- and light-strand sequences.

The sequences obtained in the present study and deposited in GenBank along with those obtained from the literature were analyzed qualitatively using parsimony and maximum-likelihood methods, as well as with distance-based algorithms (using Kimura two-parameter distances). The computer packages PAUP 3.1.1 (19), PHYLIP 3.5c (20), and MEGA (21) were used for these procedures. In the first instance, all 31 species were included in the analyses to identify the relative position

Abbreviations: CI, consistency index; RI, retention index.

†To whom reprint requests should be addressed.

§The sequences reported in this paper have been deposited in the GenBank data base [accession nos. U51734 (*T. c. capensis*), U51735 (golden whistler), and U51736 (bassian thrush)].

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of *Turnagra* within the broad passerine radiation. Global and transversion maximum parsimony trees were obtained as well as a "best tree" by the neighbor-joining metric (transition/transversion ratio 4.4 as calculated by MEGA). The more specific hypotheses that *Turnagra* is linked with bowerbirds, birds-of-paradise, or pachycephalines, were examined in more detail by concentrating on a limited taxa set involving only representatives from those lineages. For this restricted taxa set, global and transversion parsimony analyses were performed by using both the branch and bound and exhaustive search options of PAUP. Neighbor-joining, Fitch, and maximum-likelihood analyses were undertaken with PHYLIP. Because of computer-time constraints, only 100 bootstrap replicates were performed for the maximum-likelihood analysis, but 1000 replicates were used for the others. For several of the species of bowerbirds, the published sequences (16) had a large number of ambiguities at the 3' end of the gene. By truncating the complete data set to 857 bp, most of the ambiguities were eliminated. There were no topological differences between analyses based on the 924-bp and 857-bp data sets. However, inclusion of the ambiguities (926 bp) reduced the bootstrap support for most nodes by 10%. Consequently, the bootstrap values reported here are based on the 857-bp data set. Lake's invariant analysis (22) was also undertaken to specifically test the associations between quartets comprising (i) *Turnagra*, (ii) *Pachycephala*, (iii) bowerbirds, and (iv) birds-of-paradise. Synonymous and nonsynonymous nucleotide substitutions were estimated using the computer program MEGA.

**RESULTS**

Global parsimony analysis produced a single tree of 2296 steps (excluding autapomorphies) with a consistency index (CI) of 0.32 and retention index (RI) of 0.42. The suboscines, *Pitta* and *Empidonax*, were linked with the Turdidae and Paridae. *Turnagra* was linked with the Ptilonorhynchidae, and this clade, in turn, was linked to a corvine assemblage comprising Artamidae, Paradisaeidae, Lanidae, and Corvidae. In all analyses, *Manucodia* did not cluster with the other Paradisaeidae but was associated instead with the Laniidae (see also ref. 14). Removal of *Lanius* from the data led to *Manucodia* being included with the Paradisaeidae. Apart from branches either defining families or within established families (Ptilonorhynchidae, Paradisaeidae, Turdidae, and Pomatostomidae), all other nodes were not supported by bootstrap values > 50%. Transversion parsimony (24 trees, 909 steps, CI = 0.32, RI = 0.55) produced a similar topology. *Turnagra* again associated with the Ptilonorhynchidae, but the corvines now were linked with the remaining passerines. Bootstrap support was comparable to the global parsimony analysis. The neighbor-joining tree (Fig. 1) separated the two suboscines from the remaining passerines. *Turnagra* grouped with the Ptilonorhynchidae, but bootstrap support for this was 48%. Branching patterns were not altered when several transition/transversion ratios (from 4.0 to 12.0) were used.

Resolution of relationships between *Turnagra*, Ptilonorhynchidae, and the corvines was improved when analysis was restricted to these 21 taxa (Turdidae, Paridae, Laniidae, Vireonidae, Pomatostomidae, Meliphagidae, Pittidae, and Tyrannidae were excluded). Global parsimony (1437 steps, CI = 0.42, RI = 0.50), transversion parsimony (529 steps, CI = 0.45, RI = 0.63), neighbor-joining, Fitch, and maximum-likelihood analyses all aligned *Turnagra* as the sister group to the Ptilonorhynchidae (Fig. 2). Bootstrap support for this node ranged from 54% (transversion parsimony) to 78% (Fitch). Support was 74% in the neighbor-joining tree. The significance of the branch lengths in the neighbor-joining tree was also examined by a standard error test (23) using the confidence probability (CP) program of MEGA. When CP exceeds 0.95, the branch length is considered to be highly significant. According

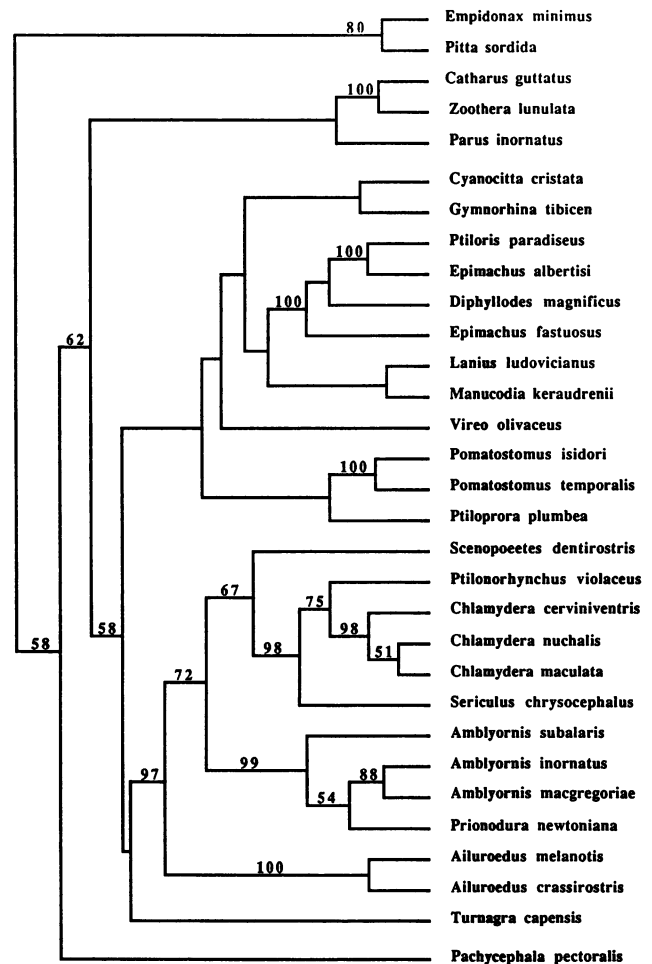


FIG. 1. Neighbor-joining analysis using a transition/transversion ratio of 4.4. Bootstrap values based on 1000 replicates are indicated above the nodes.

to the test, the node linking *Turnagra* with the Ptilonorhynchidae was 0.85, whereas those defining the Ptilonorhynchidae and Paradisaeidae were 0.98 and 0.99, respectively. The *Turnagra*-Ptilonorhynchidae clade was also retained in parsimony analysis restricted to codon positions 1 and 2 as well as a neighbor-joining analysis based on nonsynonymous substitutions. Lake's invariant analysis identified significant support for an association between *Turnagra* and the Ptilonorhynchidae (Table 1).

**DISCUSSION**

The interior position of *Turnagra* in the various analyses demonstrates that the DNA amplified and sequenced was avian and of an oscine passerine. Within the oscines, *Turnagra* is part of the corvines and is associated most closely with the bowerbird subgroup. All analyses are consistent with the finding that *Turnagra* neither is related to the pachycephalines nor is a link between the birds-of-paradise and bowerbirds. It is, in fact, closely related to the bowerbirds lineage. Nevertheless, the limited bootstrap support for this association indicates that further molecular work is required to confirm this. The present cytochrome *b* data contradicts the two most recently held views that *Turnagra* either (i) is part of the Australian pachycephaline assemblage (8, 11) or (ii) is a link between the cnemophiline birds-of-paradise and the bowerbirds (9). Although sequence data for the cnemophiline birds-of-paradise are lacking at present, protein allozyme data show that these are clearly linked with the paradisaeines and have no close

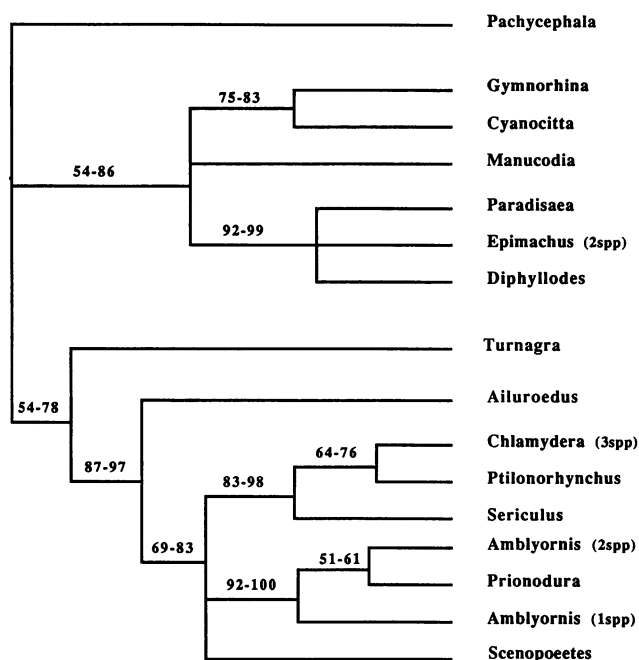


FIG. 2. Topology obtained from global parsimony, transversion parsimony, maximum-likelihood, neighbor-joining, and Fitch analyses. Taxa compared here were limited to the Ptilonorhynchidae, Paradisaeidae, Artamidae, Corvidae, *Pachycephala*, and *Turnagra*. Trees were rooted using psittaciform taxa. Ranges of bootstrap values from the various analyses are indicated above the nodes.

affinities with bowerbirds (24). For the present, it is prudent to retain Turnagridae as a family but placed in sequence next to the Ptilonorhynchidae.

The association of *Turnagra* with the bowerbirds described above clearly has major implications for the origin of bower building and brightly colored males in the Ptilonorhynchidae. There is no reported evidence of bower building in *Turnagra*, and the species was sexually monomorphic (7). Cytochrome *b* sequence data place the monomorphic, non-bower-building catbirds (*Ailuroedus*) at the base of the Ptilonorhynchidae radiation (16). Although it had been argued that this is consistent with the hypothesis that dull male plumage and lack of bower-building are ancestral traits (16), it was just as likely that colorful males and bower-building were lost secondarily in the catbirds. However, the finding that *Turnagra* is basal to both the bowerbird and catbird lineages clearly supports the former hypothesis. Such a scenario is more parsimonious in terms of changes, as it only requires a gain of characters in bowerbirds.

Table 1. Results of Lake's invariant analysis (22), where the quartets were: A = *Turnagra*, B = three genera of bowerbirds, C = three genera of birds-of-paradise, and D = *Pachycephala*

	Tree*		
	1	2	3
Lake's method			
Quartets favoring tree	19.3	4.8	2.8
Counts favoring tree	146	26	45
Evolutionary parsimony			
Quartets favoring tree	24.0	1.0	2.0
Counts favoring tree	587	346	333
Transversion parsimony			
Quartets favoring tree	27.0	0.0	0.0
Counts favoring tree	432	210	225

\*Tree 1 = (A,B)(C,D). Tree 2 = (A,C)(B,D). Tree 3 = (A,D)(B,C). Tree 1 is significant at  $P = 0.02$ . Trees 2 and 3 are not significant.

Further work is required to establish nearest relatives of the bowerbird-*Turnagra* lineage within the Australo-Papuan songbirds. Passerine remains have been recorded from Australia as early as 54 million years ago, but the details are insufficient to identify them with either the oscines or suboscines (25). Moreover, recent reinterpretation of the avian fossil record (26) suggests that most modern families arose in the late Eocene or early Oligocene. Divergence dates based on transversion differences in cytochrome *b* place the separation of the bowerbirds from the birds-of-paradise and other corvines at 28 million years ago and the separation of major lineages within the bowerbirds at 24 million years ago (14). According to these calculations and use of the transversion differences, *Turnagra* diverged from bowerbirds some 27 million years ago. Similar times were obtained by using estimates from the number of synonymous and nonsynonymous substitutions per site (27). This suggests that the split between the bowerbirds and *Turnagra* occurred over a short period of time soon after the "proto-bowerbirds" diverged from the "corvines." The pattern here is indicative of a star phylogeny with major branching patterns characterized by very short internodal divergence times (14). Resolution of such phylogenies may require the combination of several independent data sets in a single analysis. Given that the phylogenetic signal in cytochrome *b* sequences becomes compressed at large distances and that the differences may not be linear with time, it should be stressed that the dates of separation calculated here are speculative.

New Zealand became isolated from the Australian-Antarctic complex approximately 80 million years ago (28). This would indicate that an ancestral bowerbird reached New Zealand from the Australian-New Guinean land mass much more recently. Although bowerbirds and catbirds have been recorded on the Capricorn islands off Queensland, Australia (29), they do not appear to cross large water barriers readily and are confined to the Australo-Papuan continental plate. Even within Australo-Papua, bowerbirds are absent from islands and only occur on the New Guinea and Australian mainlands. Although there is the possibility that some fragmented land connections existed between Australia and New Zealand via the Kermadec and Colville Ridges as recently as 40 million years ago (30), the Lord Howe Rise has remained deeply submerged throughout the past 65 million years (31). Recent molecular evidence on the affinities of the New Zealand kiwi and the Australian emu-cassowary lineages suggest that these last shared a common ancestor some 40 million years ago at the end of the Eocene (5, 10). Similarly, rbcL sequence data place the separation between New Zealand and Australian *Nothofagus* trees at 50 to 60 million years ago (32), though it has been noted that certain plant genera support a connection between Norfolk Island and New Zealand well into the Eocene (33). The later entry of an ancestral bowerbird into New Zealand may have been facilitated by the storm-generated westerly gales that became prevalent during the Oligocene and Miocene as a result of the opening of the southern ocean in the late Eocene (34). The various molecular data sets suggest that dispersal needs to be considered as a mode of origin for groups of New Zealand taxa previously thought to have been vicariant relicts from the break-up of Gondwana. This is consistent with recent views of avian evolution, which suggest that the major bird radiations occurred well after the continents comprising Gondwana and Laurasia had separated (26).

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