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Systematic affinities of the lyrebirds (Passeriformes: *Menura*), with a novel classification of the major groups of passerine birds

Per G.P. Ericson,[a,*] Les Christidis,[b] Martin Irestedt,[a,c] and Janette A. Norman[b]

[a] *Department of Vertebrate Zoology and Molecular Systematics Laboratory, Swedish Museum of Natural History, P.O. Box 50007, SE-10405 Stockholm, Sweden*

[b] *Sciences Department, Museum Victoria, P.O. Box 666E, Melbourne, Vic. 3001, Australia*

[c] *Department of Zoology, University of Stockholm, SE-106 91 Stockholm, Sweden*

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Abstract

Phylogenetic relationships of the lyrebirds are investigated using DNA sequence data. The aligned data matrix consists of 4027 bp obtained from three nuclear genes (c-*myc*, RAG-1 and myoglobin intron II) and two mitochondrial genes (cytochrome *b* and ND2). Both maximum-likelihood and parsimony analyses show that the lyrebirds unambiguously belong to the oscine radiation, and that they are the sister taxon to all other oscines. The results do not support the suggestion based on DNA–DNA hybridization data (Sibley and Ahlquist, 1990) that the treecreepers and bowerbirds are part of the lyrebird clade. Nevertheless, treecreepers and bowerbirds are sister taxa to all other oscines (except the lyrebirds) and may constitute a monophyletic group, although bootstrap support values for this clade are low. A major disagreement between the present analysis and that based on DNA–DNA hybridization data is that the Corvida (*sensu* Sibley and Ahlquist, 1990) and Passerida are not reciprocally monophyletic, as we find the latter group be nested within the Corvida. Also, the superfamilies Meliphagoidea and Corvoidea *sensu* Sibley and Ahlquist (1990), are not recovered as monophyletic in the present study. Within the oscine radiation, all taxa belonging to the earliest splits are confined to the Australo–Papuan region. This suggests strongly that the origins and early radiation of the oscines occurred in the southern supercontinent Gondwana. A new classification of the major groups of passerines is presented following from the results presented in the present study, as well as those published recently on analyses of sequence data from the nuclear c-*myc* and RAG-1 genes (Ericson et al., 2002; Irestedt et al., 2001).

1. Introduction

The order Passeriformes comprises more than half of all living species of birds. Both the monophyly of the order and its further split into two major clades, the Suboscines and Oscines, are well supported by morphological characters (Raikow, 1982), and molecular data (Edwards et al., 1991; Irestedt et al., 2001; Sibley and Ahlquist, 1990). One passerine family, the Acanthisittidae (New Zealand wrens), falls outside the suboscine and oscine clades, and thus constitutes the sistertaxon to the other passerines (Ericson et al., 2002).

The classification of major groups of oscine birds has long been a contentious issue among avian systematists, most recently reviewed by Voous (1985) and Sibley and Ahlquist (1990). DNA–DNA hybridization data suggest that the oscines consists of two major groups; the Corvida and the Passerida (Harshman, 1994; Sheldon and Gill, 1996; Sibley and Ahlquist, 1990), and morphological support for this comes from the presence of a fully developed double pneumatic fossae in the proximal end of the humerus in many oscines, but not in the taxa placed in the Corvida (Bock, 1962).

One family of birds that long has been regarded as especially difficult to place phylogenetically is the lyrebirds (Menuridae). Lyrebirds are large, terrestrial Australian passerines that are particularly renowned for their loud, elaborate songs and spectacular ability to mimic other sounds, both natural and man-made. The two extant species included in the family, the Superb Lyrebird (*Menura novaehollandiae*) and Albert's Lyrebird (*M. alberti*), are restricted to the forests of south-

* Corresponding author. Fax: +46-8-51954212.
E-mail address: per.ericson@nrm.se (P.G.P. Ericson).

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eastern Australia. The only known fossil lyrebird, *M. tyawanoides*, was identified from the Early Miocene deposits in Riversleigh, N.W. Queensland, Australia (Boles, 1995).

Anatomical differences between lyrebirds and most other passerines include features of their pterylosis (Clench, 1985; Morlion, 1985), and of their osteology and myology (Bock and Clench, 1985; Feduccia and Olson, 1982; Raikow, 1985; Rich et al., 1985; Sibley, 1974). Nevertheless, most of these characters may be correlated with the reduced flying ability of the lyrebirds (Raikow, 1985). Although Garrod (1876) confirmed Müller's (1847) conclusion that the lyrebirds possess a general syrinx morphology typical of the oscines, he also pointed out major differences from this morphology that lyrebirds only have in common with the Australian scrub-birds (Atrichornithidae).

Comparative studies of additional morphological characters have confirmed the close relationship between lyrebirds and scrub-birds (Bock and Clench, 1985), as have analyses of molecular data (Sibley and Ahlquist, 1990). The close affinity between lyrebirds and scrub-birds has rarely been contested, and they are grouped together in most classifications as Menurae. Less consensus has been reached concerning to which other passerine group the Menurae are most closely related (Bock and Clench, 1985; Feduccia and Olson, 1982; Sibley and Ahlquist, 1990).

DNA–DNA hybridization data (Sibley and Ahlquist, 1990) suggested an affinity of the Menurae with the bowerbirds (Ptilonorhynchidae) and the Australian treecreepers (Climacteridae). This finding was particularly unexpected as bowerbirds were long believed to be closely related to the birds-of-paradise (Paradisaeidae). A close affinity between lyrebirds, treecreepers, and bowerbirds could not be supported using protein allozyme data (Christidis and Schodde, 1991). The lyrebirds were consistently placed in a basal position relative to the other oscines, but the systematic positions of the treecreepers, and bowerbirds varied considerably depending on which method the data were analyzed.

Christidis et al. (1996b) used cytochrome *b* sequence data to investigate the relationship between lyrebirds, treecreepers and bowerbirds. Some support was found for a grouping of lyrebirds and treecreepers, and a possible link between these and bowerbirds or honeyeaters (Meliphagidae). However, none of these phylogenetic hypotheses received bootstrap support.

Lyrebirds and treecreepers were recovered as sister-taxa in an analysis of DNA sequence data from the nuclear genes c-*myc* and RAG-1 (Ericson et al., 2002). The sampling of Australo–Papuan taxa in that study was sparse and no bowerbirds were included. The analysis unambiguously placed the clade of lyrebirds and treecreepers basal relative to the other oscines. The DNA sequence data also indicated that the lineages leading to lyrebirds and several other groups of Australo–Papuan oscines had diverged in the late Cretaceous and early Tertiary (Ericson et al., 2002). Furthermore, short internodal distances in the phylogenetic trees showed that these phylogenetic lineages branched from each other rather rapidly.

In this paper we use DNA sequence data to study the earliest evolution of oscine passerines, with the particular aim of determining the systematic position of the lyrebirds. Sequences from five genes (three nuclear and two mitochondrial) with different rates of substitution, have been combined to enhance the possibility of resolving both ancient and younger evolutionary branching patterns.

2. Materials and methods

Taxonomic nomenclature follows Christidis and Boles (1994). Apart from the lyrebird, *M. novaehollandiae*, the ingroup consists of 18 taxa of which 16 belong to the Australo–Papuan radiation (parvorder Corvida *sensu* Sibley and Ahlquist, 1990) (Table 1). The taxa were chosen to include representatives from the entire span of the corvid radiation. Two representatives of the parvorder Passerida (*Hirundo rustica* and *Sitta europea*) were included as this taxon appears to be nested within the Corvida (*sensu* Sibley and Ahlquist, 1990) according to a recent nuclear DNA sequence study (Ericson et al., 2002). It has been demonstrated that the suboscines are the sistertaxon to the oscines (including the lyrebird (e.g., Irestedt et al., 2001)), and three suboscine representatives were used as outgroups: *Furnarius cristatus* (Furnariidae), *Elaenia flavogaster* (Tyrannidae), and *Pitta* spp. (Pittidae).

For certain taxa, the cytochrome *b* sequence was obtained from GenBank. All other taxa and genes were sequenced in this study (Table 1). In four cases (*Epimachus*, *Ptiloris*, *Ailuroedus*, and *Malurus*), the cytochrome *b* sequence in GenBank derive from species that are congeneric to the one from which we had obtained the other gene sequences. In the analyses these genera are thus represented by composite species sequences.

Genomic DNA was prepared from tissue or blood specimens using the QIAamp DNA Mini Kit (QIAGEN) or by salt-chloroform extraction as described in Norman et al. (1998). Nucleotide sequences were obtained for the c-*myc*, RAG-1, and cytochrome *b* genes as described in Ericson et al. (2000), Irestedt et al. (2001), and Johansson et al. (2001). Myoglobin intron II was amplified as a single fragment and sequenced using the *f* mol Thermal Cycle Sequencing Kit (Promega) as described in Norman et al. (1998) using primers and conditions described in Heslewood et al. (1998). The ND2 gene was amplified and manually sequenced as a single 1.2 kb fragment using primers ND2.1 (or pND2-L: 5′-

Table 1
Specimens included in the study

Species	Family	Sample no.	Genes sequenced	GenBank Acc. no.
Climacteris rufa	Climacteridae	MV 155	c-myc, rag-1, cyt *b*, nd2, myoglobin	AY037839, AY037846, U58501, AY064746, AY064733
Cormobates placens	Climacteridae	MV E309	c-myc, rag-1, cyt *b*, nd2, myoglobin	AY064282, AY064260, AY064278, AY064748, AY064731
Corcorax melanoramphos	Corcoracidae	AM LAB1059	c-myc, rag-1, cyt b, nd2, myoglobin	AY037842, AY037849, AY064274, AY064747, AY064737
Struthidea cinerea	Corcoracidae	AM LAB1115	c-myc, rag-1, cyt *b*, nd2, myoglobin	AY064291, AY064270, AY064277, AY064757, AY064738
Gymnorhina tibicen	Cracticidae	AM LAB1107	c-myc, rag-1, nd2, myoglobin	AY064284, AY064263
Gymnorhina tibicen	Cracticidae	MV AC78	nd2, myoglobin	AY064756, AY064741
Gymnorhina tibicen	Cracticidae	Cracraft and Feinstein (2000)	cyt *b*	AF197867
Malurus amabilis	Maluridae	MV C803	c-myc, rag-1, nd2, myoglobin	AY037840, AY037847, AY064752, AY064729
Malurus cyaneus	Maluridae	Cracraft and Feinstein (2000)	cyt b	AF197845
Manorina melanocephala	Meliphagidae	MV F593	c-myc, rag-1, nd2, myoglobin	AY064285, AY064264, AY064753, AY064734
Manorina melanocephala	Meliphagidae	Cracraft and Feinstein (2000)	cyt *b*	AF197859
Ptiloprora plumbea	Meliphagidae	MV C173	c-myc, rag-1, nd2, myoglobin	AY037841, AY037848, AY064760, AY064736
Ptiloprora plumbea	Meliphagidae	Edwards et al. (1991)	cyt b	X60943
Menura novaehollandiae	Menuridae	AM LAB1112	c-myc, rag-1, cyt b	AF295169, AF295191, AY064276
Menura novaehollandiae	Menuridae	MV F722	nd2, myoglobin	AY064754, AY064744
Orthonyx temminckii	Orthonychidae	MV B831	c-myc, rag-1, cyt *b*, nd2, myoglobin	AY064286, AY064265, AY064275, AY064755, AY064728
Pachycephala pectoralis	Pachycephalidae	MV 1419	c-myc, rag-1, nd2, myoglobin	AY064287, AY064266, AY064751, AY064727
Pachycephala pectoralis	Pachycephalidae	Christidis et al. (1996a)	cyt *b*	U51735
Epimachus albertisi	Paradisaeidae	MV C148	c-myc, rag-1, nd2, myoglobin	AF377278, AY037850, AY064745, AY064735
Epimachus fastuosus	Paradisaeidae	Helm–Bychowski and Cracraft (1993)	cyt *b*	X74253
Ptiloris magnificus	Paradisaeidae	MV C784	c-myc, rag-1, nd2, myoglobin	AY064290, AY064269, AY064761, AY064740
Ptiloris paradiseus	Paradisaeidae	Helm–Bychowski and Cracraft (1993)	cyt *b*	X74254
Eopsaltria australis	Petroicidae	MV 1390	c-myc, rag-1, cyt *b*, nd2, myoglobin	AY064283, AY064262, AY064273, AY064749, AY064732
Pomatostomus temporalis	Pomatostomidae	MV D257	c-myc, rag-1, nd2, myoglobin	AY064288, AY064267, AY064758, AY064730
Pomatostomus temporalis	Pomatostomidae	Edwards et al. (1991)	cyt *b*	X60936
Ailuroedus crassirostris	Ptilonorhyncidae	MV C310	c-myc, rag-1, nd2, myoglobin	AY064281, AY064259, AY064750, AY064739
Ailuroedus melanotus	Ptilonorhyncidae	Helm–Bychowski and Cracraft (1993)	cyt *b*	X74257
Ptilonorhynchus violaceus	Ptilonorhyncidae	AM LAB1099	c-myc, rag-1	AY064289, AY064268
Ptilonorhynchus violaceus	Ptilonorhyncidae	MV B836	nd2, myoglobin	AY064759, AY064742
Ptilonorhynchus violaceus	Ptilonorhyncidae	Helm–Bychowski and Cracraft (1993)	cyt *b*	X74256
Hirundo rustica	Hirundinidae	NRM 976238	c-myc, rag-1, myoglobin	AF377270, AY064271, AY064258
Hirundo rustica	Hirundinidae	Sheldon et al. (1999)	cyt *b*	AF074577
Sitta europea	Sittidae	NRM 976163	c-myc, rag-1, cyt *b*, myoglobin	AF377267, AY064272, AF378102, AY064257
Elaenia flavogaster	Tyrannidae	NRM 966970	c-myc, rag-1, cyt *b*, myoglobin	AF377279, AY064261, AY064763, AY064254
Furnarius cristatus	Furnariidae	NRM 966772	c-myc, rag-1, cyt *b*, myoglobin	AF295165, AF295187, AY064279, AY064255
Pitta baudii	Pittidae	ANSP 1224	c-myc, rag-1, cyt *b*	AF295177, AF295198, AY064280
Pitta versicolor	Pittidae	MV C534	nd2, myoglobin	AY064762, AY064743

Abbreviations: AM—Australian Museum, Sydney; ANSP—Academy of Natural Sciences of Philadelphia; MV—Museum Victoria, Melbourne; NRM—Swedish Museum of Natural History, Stockholm.

TCA GCT AAC TAA GCT ATC GGG C-3′) and ND2.2 (Norman et al., 1998), or as two smaller fragments using the following primer combinations: pND2-L with H5578 (Hackett, 1996) and L5575 (5′-AAA CTA GGA CTA GTG CCA TTC CA-3′) with ND2.2 using L5944 (5′-ACT ATA ATA TCA GCA TGA AC-3′), amy ND2int.c.1 (5′-CTA GCC CCA TTY CAC TTY CAC TTY TG-3′), HEND2int.c (5′-CTA TCA ACA YTA ATA ACY GCA T-3′) and H6015 (5′-AGT CAT TTA GGT AGG AAT CC-3′) as internal sequencing primers. For some species it was necessary to amplify the L5575-ND2.2 and L5944-ND2.2 fragments from the initial full-length 1.2 kb amplification product.

The sequences obtained from the nuclear protein-coding genes correspond in the published chicken sequences to the regions between, respectively, positions 759 and 1235 (c-*myc* exon 3) (Watson et al., 1983), and 1054 and 1983 (RAG-1) (Carlson et al., 1991). The myoglobin intron II sequences correspond to the region between positions 303 (exon 2) and 400 (exon 3) of humans (Genbank Accession XM009949). The analyzed mitochondrial ND2 and cytochrome *b* sequences correspond in the chicken to the regions between, respectively positions 5241 and 6263, and 15,088 and 15,915 (Desjardin and Morais, 1990). No unexpected start, stop, or nonsense codons, which could indicate the presence of a nuclear copy, were observed in the mitochondrial genes. All sequences are deposited in GenBank (Table 1).

For each taxon multiple sequence fragments obtained by sequencing with different primers were assembled to complete sequences with SeqMan II (DNASTAR). The sequences of all genes were aligned by eye. Most indels in the myoglobin intron II were either autapomorphic, or could readily be aligned across taxa. Statistics for nucleotide variation were computed with MEGA version 2.0 (Kumar et al., 2001) and MacClade 3.0 (Maddison and Maddison, 1992).

Parsimony and maximum-likelihood analyses were performed using the heuristic search option in PAUP* 4.0b8 (Swofford, 1998), with TBR branch-swapping and random additions of taxa. The model of nucleotide substitutions for the maximum-likelihood analysis was selected using the likelihood-ratio test implemented in Modeltest 3.06 (Posada and Crandall, 1998). With this method the simplest model of evolution that cannot be rejected in favor of a more complex model is chosen. A transversion model (a variant of the general-time reversal model with transversions variable and transitions equal) was selected. This included an estimate of the proportion of invariable sites and a discrete (six rate categories) gamma distribution model of among site rate heterogeneity. Initially, the gamma shape parameter, proportion of invariable sites, and substitution rate parameters were estimated from a neighbor-joining tree. These estimates were then used with the empirical base frequencies in a heuristic search with TBR branch-swapping. On the resulting tree, the gamma parameter, proportion of invariable sites and substitution rate parameters were again estimated, and a new heuristic search with TBR branch-swapping was employed. A third estimation of the parameters was done based on this tree and these were then used in the final search for the best-fit tree. Nodal supports were estimated with 100 bootstrap replicates for the maximum-likelihood tree, and with 1000 bootstrap replicates for the maximum parsimony tree.

Searches for maximum parsimony trees were performed with all characters coded as unordered. To reduce the risk of finding local optima only, multiple analyses were performed with taxa added in a randomized order. Trees were rooted using an outgroup (Farris, 1972; cf. Nixon and Carpenter, 1993). Bootstrap support values for internal nodes were calculated from 1000 replicates.

The number of transitions and transversions in different partitions of the mitochondrial genes were plotted against an estimate of time to explore levels of saturation due to multiple substitutions at single sites. Pairwise genetic distances calculated from the combined c-*myc* and RAG-1 sequences served as the time estimates, as both genes have been shown to accumulate substitutions in a clock-like fashion during the time span of when the taxa studied here are believed to have evolved (Ericson et al., 2002). The two-parameter method of Kimura (1980) was used to calculate the genetic distances as it corrects for multiple substitutions at sites, and thus may provide better time estimates than other methods.

3. Results

3.1. Molecular variation, base compositions, and patterns of substitution

A total of 4027 bp of homologous DNA sequence was obtained from the individual samples. Of this, 516 bp derive from c-*myc*, 930 bp from RAG-1, 730 bp from myoglobin intron II, 828 bp from cytochrome *b*, and 1023 bp from ND2. Sequence length variation between taxa was observed in the three nuclear genes. Sequence length in c-*myc* ranged from 486 bp (*Ptilonorhynchus*) to 513 bp (*Manorina*), in RAG-1 from 927 bp (*Ailuroedus* and *Ptilonorhynchus*) to 930 bp (all other taxa), and in myoglobin intron II from 708 bp (*Cormobates*) to 729 bp (*Ptilonorhynchus*).

In c-*myc* 20% of the positions were variable between taxa examined with almost half (9%) being phylogenetically informative. In RAG-1 26% of the positions were variable (11% informative), and in myoglobin intron II 47% of the positions were variable (16% informative). In cytochrome *b* 45% of the positions were variable (38%

informative) and this decreased to 21% (14% informative) when third codon positions were excluded. The corresponding figures for ND2 were 60% (48% informative), and 42% (27% informative), respectively.

The observed base compositions at different codon positions for coding genes agree well with those previously reported for these genes in passerine birds (Edwards et al., 1991; Hackett, 1996; Helm–Bychowski and Cracraft, 1993; Irestedt et al., 2001).

The degree of saturation at different codon positions was investigated for the protein coding genes. No signs of saturation could be observed in the nuclear c-*myc* and RAG-1 genes (data not shown). This agrees with previous observations in analyses of avian data sets (Groth and Barrowclough, 1999; Irestedt et al., 2001; Johansson et al., 2001). For cytochrome *b* third codon positions were saturated for both transitions and transversions (Fig. 1), with transversions largely outnumbering transitions. Transitions at third codon positions in ND2 also were clearly saturated. Saturation in transversions was less marked although the accumulation of substitutions appeared to level off at 3–4% sequence divergence (Fig. 1). To diminish the influence on the results of multiple hits at the fastest evolving sites, all third codon positions in the cytochrome *b* and ND2 genes were excluded from the analyses. This perhaps overly conservative decision was supported by the observation that when third position transversions were included in the analyses, overall tree topology remained largely the same but the number of nodes receiving bootstrap support above 50% decreased.

3.2. Phylogenetic analysis

Maximum-likelihood (ML) and maximum parsimony analyses (MP) yielded almost identical phylogenetic trees, where the lyrebird *Menura* has a basal position among the oscine ingroup taxa (Fig. 2). The two bowerbirds *Ailuroedus* and *Ptilonorhynchus* unambiguously group together, as do the two treecreepers *Climacteris* and *Cormobates*. These latter four taxa form a monophyletic group, which receives only weak bootstrap support (ML: 61% and MP: 54%). Monophyly of the remaining oscines has slightly better support (ML: 71% and MP: 74%). Within that clade the highest bootstrap supports are given to the intra-familial groupings of honeyeaters (*Manorina* and *Ptiloprora*), mud-nesters (*Corcorax* and *Struthidea*), and birds-of-paradise

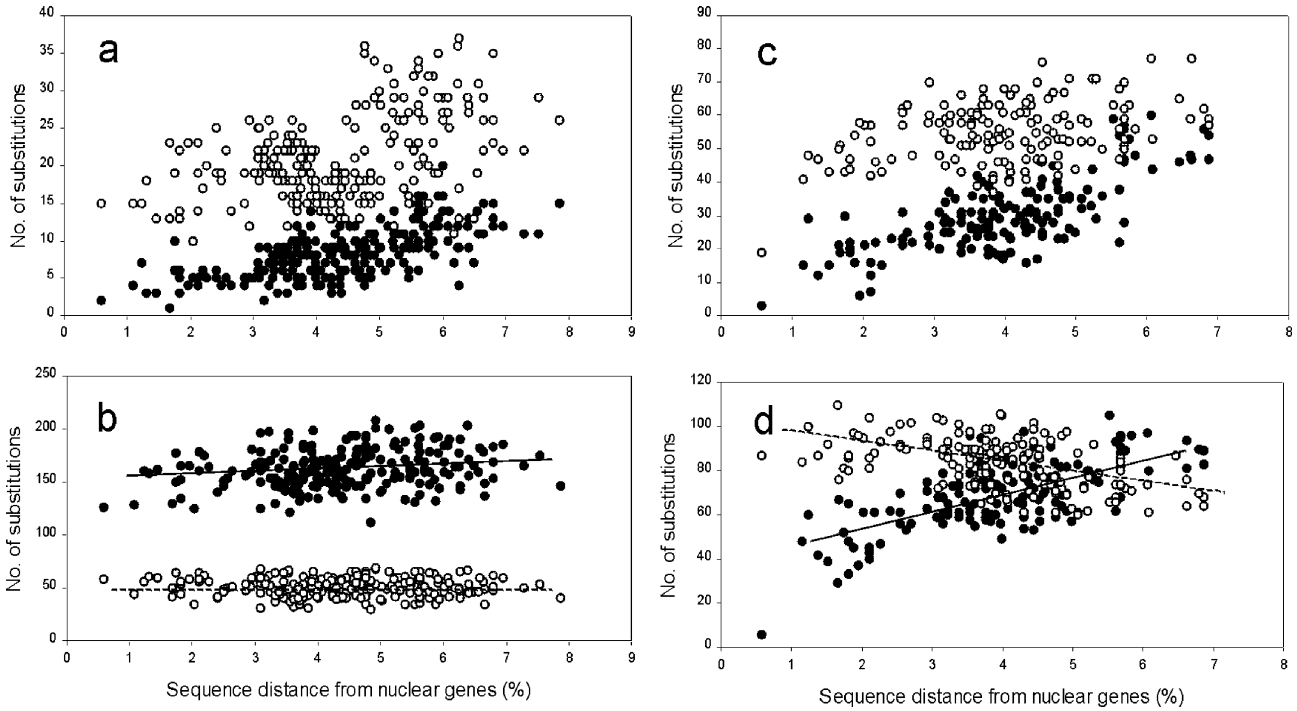

Fig. 1. The observed number of transitions (open circles) and transversions (filled circles) in the mitochondrial genes cytochrome *b* and ND2 genes, plotted against the pairwise sequence divergences calculated for the combined c-*myc* and RAG-1 genes. The plots show (a) first and second codon positions combined for cytochrome *b*; (b) third codon positions for cytochrome *b*; (c) first and second codon positions combined for ND2; and (d) third codon positions for ND2. The lines (solid lines for transversions and broken lines for transitions) are included only to indicate trends in the data sets, and are not statistically calculated. Saturation of the gene is indicated when the observed number of substitutions no longer increases with increasing pairwise sequence distances, i.e., when the imposed curve is leveling off. No sign of this is noted for the first and second codon positions in the two genes. At the third codon positions transitions in both genes and transversions in cytochrome *b* clearly reach saturation in the present data set, while transversions in ND2 exhibits a lesser degree of saturation. Note that transversions are considerably more common than transitions at third codon positions in cytochrome *b*.

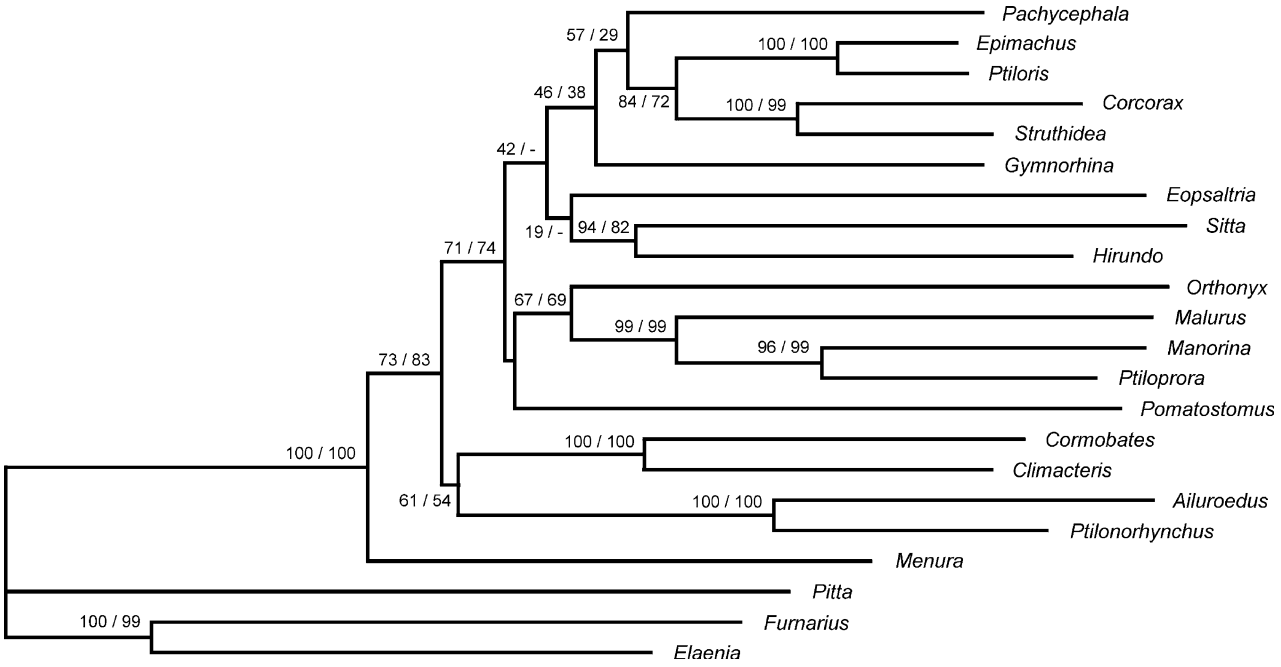

Fig. 2. Tree resulting from maximum-likelihood analysis of combined sequences obtained from the nuclear genes c-*myc*, RAG-1 and myoglobin intron II, and the mitochondrial genes cytochrome *b* and ND2. All genes except myoglobin intron II are protein coding. Only first and second codon positions for the mitochondrial genes were included in the analyses to reduce the influence of saturation upon the results. The maximum-likelihood tree is largely congruent with the most parsimonious tree calculated from the same data set, the only differences occur in the relative positions of *Eopsaltria*, *Gymnorhina*, and *Pachycephala*, which internal relationships must be regarded as unresolved. Nodal support values are calculated from 100 replicates of the maximum-likelihood analysis (left), and 1000 replicates of the parsimony analysis (right).

(*Epimachus* and *Ptiloris*). The fairy-wren *Malurus* is strongly grouped with the honeyeaters (ML and MP: 99%), and this clade in turn is linked to the logrunner *Orthonyx* (ML: 67% and MP: 69%). The birds-of-paradise and mud-nesters form a monophyletic group that receives good bootstrap support (ML: 84% and MP: 72%). Although the position of the whistler *Pachycephala* and the Australian Magpie *Gymnorhina* associate with this assemblage, there is little or no bootstrap support. Similarly, the positions of the Australasian robin *Eopsaltria* and Australo–Papuan babbler *Pomatostomus* lack bootstrap support. All differences between the topology of the most parsimonious tree and that for the best-fit maximum-likelihood tree, concern the relationships among these four taxa which thus must be regarded as unresolved. The two representatives of the Passerida, the nuthatch *Sitta* and the swallow *Hirundo*, group together with a high bootstrap support (ML: 94% and MP: 82%), but they have no close relatives among the other taxa examined here.

4. Discussion

4.1. Insertions and deletions in the protein coding genes

Several occurrences of indels in the nuclear coding genes were observed and some of these are potentially useful for phylogenetic purposes. Ericson et al. (2000) reported on an insertion of one amino acid between positions 789 and 790, relative to the published chicken sequence, in the c-*myc* gene that occurred in all species of Passerida but not in the other passerine or non-passerine taxa they examined. From the present study, an additional 14 genera of non-Passerida have been observed to lack this insertion. Furthermore, in *Ptilonorhynchus* a deletion of four amino acids occurs in the c-*myc* gene at position 777 (relative to the published chicken sequence). This deletion is not shared by *Ailuroedus*, the other bowerbird species studied. An insertion of five amino acids in the c-*myc* sequence occurs in *Manorina* (but not the other honeyeater *Ptiloprora*) at position 975 (relative to the published chicken sequence).

Only one occurrence of an indel was observed in the RAG-1 gene. It is a deletion of one amino acid between positions 1126 and 1128 (relative to the published chicken sequence), shared by the two bowerbirds examined in this study, *Ailuroedus* and *Ptilonorhynchus*.

4.2. Phylogenetic relationships

A basal position of the lyrebirds within the oscine radiation is well corroborated by the present data. This also supports a recent analysis indicating that the entire oscine clade is of Australian origin (Ericson et al., 2002).

Based on DNA–DNA hybridization data (Sibley and Ahlquist, 1990), the oscines could be divided into two sisterclades: Passerida and Corvida. A re-analysis of the DNA–DNA hybridization data set, employing a more rigorous statistical analysis, confirmed monophyly of the Passerida, but not of the Corvida (Harshman, 1994). Instead, the three major groups of Corvida (Meliphagoidea, Corvoidea, and Menuroidea) formed a polytomy that also included the Passerida. The protein allozyme studies of Christidis (1991) and Christidis and Schodde (1991) also suggested that the Passerida was most closely linked to the Corvoidea (i.e., crows, birds-of-paradise, drongos, and their allies). Our findings, suggesting that the Passerida is nested within the Corvida, renders that group paraphyletic, in agreement with the three last-mentioned studies. The present study did not identify a monophyletic Menuroidea. Although bowerbirds and treecreepers seemingly are sistergroups, they do not group with the lyrebird but instead constitute the sistergroup of all other oscines (excluding the lyrebird).

A basal phylogenetic position of the Menurae within the oscines, as suggested by allozyme data (Christidis and Schodde, 1991) and the present study, is concordant with the morphological analyses of Ames (1971) and Feduccia and Olson (1982). Based on a comparative osteological analysis, Feduccia and Olson (1982) concluded that the systematic position of the Menurae appear to be at the base of the oscine radiation. Ames (1971), in his extensive study of the syringeal anatomy in passerines, viewed the syrinx of other oscines to be more derived than the Menurae, not only in the number of intrinsic muscles (lyrebirds and scrub-birds have three pairs compared to four pairs in all other oscines, including bowerbirds and treecreepers), but in their general syringeal morphology. He (1971:164) stated that "no single group of oscines can be considered syringeal primitive in the sense that the [lyrebirds and scrub-birds] can be considered so." However, no character analysis accompanied this statement and further detailed studies of the syringeal anatomy in oscines is required to judge whether the morphology of the syrinx in the Menurae is primitive or derived compared to that of other oscines (Bock and Clench, 1985). The unusual syrinx of the treecreepers (Ames, 1987) is also in keeping with the present study, which indicates that they diverged early in the evolution of the oscines.

Sibley and Ahlquist (1990) placed honeyeaters, fairy-wrens, Australasian warblers, and allies, in the Meliphagoidea. The three taxa from this group included in the present analysis (*Manorina*, *Ptiloprora*, and *Malurus*), group together with a 99% bootstrap support in both the MP and ML trees. DNA–DNA hybridization data placed the Meliphagoidea as the sistergroup to the Corvoidea with Menuroidea basal to them (Sibley and Ahlquist, 1990). This pattern of relationships was not supported in the present study and could not be confirmed in the re-analysis of the DNA–DNA hybridization data by Harshman (1994).

Sibley and Ahlquist also placed the Australasian robins and logrunners as the earliest diverged lineages of the Corvoidea. Christidis (1991) and Christidis and Schodde (1991) instead aligned the robins and logrunners with the Meliphagoidea. In the present DNA sequence analysis, the logrunner (*Orthonyx*) groups with the honeyeaters and fairy-wrens, and this relationship is supported by bootstrap values near 70%. The position of the Australasian robin (*Eopsaltria*) is unresolved but there is no obvious support for its association with the core corvines examined here (*Ptiloris*, *Epimachus*, *Gymnorhina*, *Pachycephala*, *Corcorax*, and *Struthidea*). Christidis (1991) considered the Meliphagoidea, *sensu* Sibley and Ahlquist (1990) along with the logrunners and the Australasian robins to represent a core early radiation of oscines on the Australo–Papuan continental plate. An even earlier divergence resulted in the lyrebirds and treecreepers. The present study provides strong support for Christidis' (1991) scenario concerning logrunners, treecreepers, and lyrebirds and does not confirm nor deny his views on the affinities of the Australasian robins.

The relationships among the other studied taxa are compromised by the short internodes between them. Despite the use of a considerable amount of nucleotide data, spanning both fast and slowly evolving genes from both the nuclear and mitochondrial genomes, little resolution of the branching patterns was obtained. Similarly, in their DNA–DNA hybridization study, Sibley and Ahlquist (1990) observed very small delta-$T_{50}H$ values between major groups within the Corvoidea.

A further difference between our results and those based on DNA–DNA hybridization data (Sibley and Ahlquist, 1990) is that the birds-of-paradise and mud-nesters form a relatively robust clade to the exclusion of *Gymnorhina*. Based on DNA–DNA hybridization data, Sibley and Monroe (1990) separated the mud-nesters in the subfamily Corcoracinae and included *Gymnorhina* and birds-of-paradise in the Corvinae. A closer relationship between the mud-nesters and the corvinae is supported in part by micro-complement fixation of albumin (Baverstock et al., 1992). Clearly further sampling from the large corvine assemblage is warranted before meaningful systematic conclusions can be drawn.

Two statements can be made concerning the taxonomic status of the Passerida *sensu* Sibley and Ahlquist (1990). First, monophyly of the group is well corroborated by both phylogenetic analyses of nucleotide sequence data (Ericson et al., 2002), and the observation of a synapomorphic insertion of one amino acid in the c-*myc* gene (Ericson et al., 2000). Secondly, the group is clearly nested within the "Corvida," and is not the sistergroup to this taxon (*contra* Sibley and Ahlquist, 1990).

4.3. The earliest evolution of the oscines

Ericson et al. (2002) hypothesized that the oscine ancestor occurred in Eastern Gondwana and radiated in Australia after the separation of this continent from Antarctica (see also Christidis, 1991; Christidis and Schodde, 1991). This is corroborated in the present study by the observation that not only the most basal member of the oscine clade, the lyrebird, but almost all early oscine branches are geographically confined to the Australo–Papuan region. Considering the potential of dispersal in birds, surprisingly few early oscine groups have spread outside the Australo–Papuan region, and even fewer farther than Southeast Asia (Sibley and Ahlquist, 1985). In reality, the very large number of oscines inhabiting the rest of the world are descendants of but a few successful "escapees" from Australia. The most successful in terms of number of species and geographic coverage is the ancestor of the entire Passerida radiation.

The earliest evolution of the oscines thus seems to have taken place on the Australo–Papuan continental plate. The rifting between Australia and Antarctica started to develop 90–110 Mya, and the separation was completed around 34 Mya (Cox and Moore, 2000). Ericson et al. (2002) estimated the date of the split between the lyrebirds and other oscines to 53–59 Mya (using a "molecular clock" that was calibrated by the assumed split between the New Zealand wrens and the remaining passerines at 82–85 Mya).

4.4. A novel classification of major passerine groups

The results of the present investigation call for several changes in the taxonomy of oscines, compared to that based on DNA–DNA hybridization data (Sibley and Ahlquist, 1990; Sibley and Monroe, 1990). Most importantly, paraphyly of the Corvida renders this name poorly suited as a taxonomic unit. Furthermore, the DNA sequence analyses do not support monophyly of any of the three subdivisions of the Corvida (Menuroidea, Meliphagoidea, and Corvoidea). Based on a compilation of the present results with the works of Irestedt et al. (2001), and Ericson et al. (2002), we propose the following classification:

Passeriformes
- Acanthisittia (New Zealand wrens)
- Eupasseres (all other passerines)
  - Suboscines
    - Eurylaimides ('Old World suboscines': pittas, broadbills, asites)
    - Tyrannides ('New World suboscines')
      - Furnariida (ovenbirds, woodcreepers, tapaculos, gnateaters, antbirds, and allies)
      - Tyrannida (tyrant flycatchers, cotingas, manakins, plantcutters, sharpbill)
  - Oscines
    - Menurae (lyrebirds, scrub-birds)
    - Euoscines (all other oscines)

This classification is also consistent with the most parsimonious distribution of the anatomical characters traditionally used to infer relationships among basal passerines. Most of these characters can be used as synapomorphies for nodes in the tree (Fig. 3). Although no morphological character can be listed at several nodes, this is merely an effect of the few well-studied characters that are at hand. In fact, the distribution of character states are in conflict with the tree in Fig. 3 for only one thoroughly studied character: the shared absence of a distal belly of *M. flexor* perforatus digiti IV in oscines and New Zealand wrens (Raikow, 1987). As this belly is present in the outgroups and all suboscine taxa, the most parsimonious explanation of this character distribution is that it was lost only once. When mapped on the current phylogeny it must either first have been lost in the passerine ancestor and then have re-evolved in the suboscines, or been lost independently in oscines and New Zealand wrens. However, the value of this single, myological character can be questioned in the light of the many characters that are in agreement with the proposed classification.

The Euoscines comprises the greatest number and diversity of species. Three distinct assemblages are evident from the available DNA sequence analyses: (1) bowerbirds and treecreepers; (2) honeyeaters, fairy-wrens, Australasian warblers, and logrunners; and (3) remaining Corvida and the Passerida. However, the relationships of Petroicidae (Australasian robins), Irenidae (fairybirds from Asia), Laniidae (shrikes), and Vireonidae (Vireos) need to be established with respect

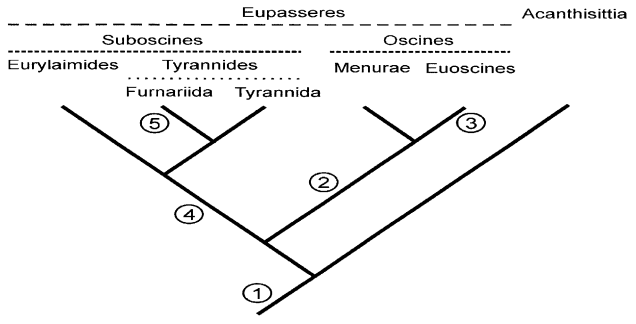

Fig. 3. Systematic relationships among major groups of passerine birds as revealed by analyses of nuclear and mitochondrial DNA sequences (this study, Ericson et al., 2002; Irestedt et al., 2001). Numbered nodes are supported by the following morphological characters. Node 1: aegithognathous palate, "passerine" tensor propatagialis brevis, bundled spermatozoa with coiled head, enlarged hallux, and type VII deep plantar tendons (Raikow, 1982). Node 2: acromyodian (oscine) syrinx (Garrod, 1876; Müller, 1847). Node 3: syrinx with four intrinsic muscles (Ames, 1971). Node 4: stapal bone (columella) with expanded foot (Feduccia, 1975). Node 5: tracheophone syrinx (Ames, 1971; Garrod, 1876).

to lineages (2) and (3) before definitive superfamilies can be proposed in the Euoscines.

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