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What are African monarchs (Aves, Passeriformes)? A phylogenetic analysis of mitochondrial genes

Éric Pasquet[a,b]*, Alice Cibois[a,b], François Baillon[c], Christian Érard[d]

[a] *Laboratoire de zoologie, mammifères et oiseaux, Muséum national d'histoire naturelle, 55, rue Buffon, 75005 Paris, France*

[b] *Systématique moléculaire, Institut de systématique (CNRS FR 1541), MNHN, 43, rue Cuvier, 75005 Paris, France*

[c] *IRD, Yaoundé, Cameroon*

[d] *Laboratoire d'écologie, (MNHN–CNRS UMR 8571), 4, av. du Petit-Château, 91800 Brunoy, France*

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Abstract – We address the phylogenetic relationships of ten passerine bird species representing the five presently supposed monarchine (family Monarchidae) genera (*Terpsiphone, Hypothymis, Elminia, Trochocercus, Erythrocercus*) from Asia and Africa, as well as three monarchs from Australasia, three representatives of the related genera *Rhipidura* and *Dicrurus*, and 20 representatives of 11 other oscine groups (including two *Culicicapa* flycatchers) and one sub-oscine, using two partial mitochondrial genes (cytochrome *b* and large sub-unit ribosomal 16S RNA). Molecular data corroborate ecological, ethological and morphological observations on the probable heterogeneity of *Trochocercus* and indicate that this genus is polyphyletic; two of its species are members of Monarchidae allied to *Terpsiphone* and *Hypothymis*; the others are more closely related to *Elminia*. *Elminia* is not a member of Monarchidae and is not related to any other sampled species, except *Culicicapa*. *Erythrocercus* is also outside the Monarchidae but inside a Sylvii-Pycnonotidae group. These results point once more to the need of a fully revised phylogeny of passerine birds. ***To cite this article: É. Pasquet et al., C. R. Biologies 325 (2002) 107–118.*** © 2002 Académie des sciences / Éditions scientifiques et médicales Elsevier SAS

molecular phylogeny / birds / flycatchers / cytochrome *b* / 16S rRNA

Résumé – Que sont les monarques africains (Aves, Passeriformes) ? Analyse phylogénétique de gènes mitochondriaux. Nous nous intéressons aux relations phylogénétiques existant entre dix espèces d'oiseaux passériformes représentant les cinq genres asiatiques et africains (*Terpsiphone, Hypothymis, Elminia, Trochocercus, Erythrocercus*), considérés jusqu'à présent comme des monarques (famille des Monarchidés), trois monarques d'Australasie, trois représentants des genres voisins *Rhipidura* et *Dicrurus*, ainsi que vingt représentants de onze autres groupes d'oscines (incluant deux gobe-mouches du genre *Culicicapa*) et un sub-oscine. Pour cela, nous avons utilisé deux gènes mitochondriaux partiels (cytochrome *b* et sous-unité 16S de l'ARNr). Les données moléculaires corroborent les observations écologiques, éthologiques et morphologiques d'une probable hétérogénéité du genre *Trochocercus* et indiquent que celui-ci est polyphylétique. Deux de ses espèces sont des Monarchidae proches des *Terpsiphone* et *Hypothymis*. Les autres sont plus étroitement apparentées aux *Elminia*, lesquels ne sont pas des Monarchidae et ne sont proches d'aucune des autres espèces échantillonnées, sauf des *Culicicapa*. Les *Erythrocercus* eux non plus ne sont pas des monarques, mais se placent dans un groupe de Sylvii-Pycnonotidés (fauvettes et bulbuls). Ces résultats soulignent une fois encore la nécessité d'une phylogénie entièrement révisée de l'ensemble des passereaux. ***Pour citer***

**Correspondence and reprints.*
E-mail address: pasquet@mnhn.fr (E. Pasquet).

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phylogénie moléculaire / oiseaux / gobe-mouches / cytochrome *b* / ARNr 16S

. Version abrégée

Les caractéristiques morphologiques et éco-éthologiques des gobe-mouches ne reflètent pas nécessairement les relations de parenté entre les espèces. Cependant, les gobe-mouches de l'Ancien Monde (du sous-ordre des Oscines) sont souvent réunis dans un nombre variable de groupes (familles ou sous-familles), dont la définition et le contenu changent au gré des auteurs. Les monarques (Monarchidae) occuperaient l'Australasie, l'Asie et l'Afrique. Dans ce dernier continent, ils sont censés être représentés par le genre *Terpsiphone*, qui est répandu jusqu'en Asie, et par trois autres genres endémiques : *Trochocercus, Elminia* et *Erythrocercus*. Les données morphologiques, écologiques et éthologiques disponibles suggèrent l'existence de deux groupes au sein des *Trochocercus*, dont un se rapprocherait des *Elminia* et l'autre des *Terpsiphone*. Par ailleurs, *Erythrocercus* se singulariserait par un certain nombre de traits biologiques.

Pour éclaircir cette apparente hétérogénéité des monarques africains, nous avons établi une phylogénie moléculaire basée sur les séquences partielles (déposées dans GenBank) de deux gènes mitochondriaux (cytochome *b* et ARNr 16S) d'un échantillon représentatif des Monarchidae africains, asiatiques et australasiens, des familles actuellement considérées comme étant leurs plus proches parents, ainsi que des principales familles de passereaux caractérisant les grands groupes reconnus chez les Oscines: les Corvoidea, les Sylvioidea, les Muscicapoidea et les Passeroidea. Les résultats sont figurés par deux arbres phylétiques établis par l'analyse combinée de jeux de données sur les deux gènes considérés. L'un a été obtenu par la méthode des distances (*neighbour-joining*, NJ), l'autre par la méthode de parcimonie (MP) avec enracinement à l'aide d'un gobe-mouche néotropical (Tyrannidae, Sub-Oscine); leur robustesse a été testée par *bootstrap analysis* avec 1000 réplicats.

Les monarques africains sont indiscutablement polyphylétiques. Aucun n'apparaît proche des Muscicapoidea, ni des Passeroidea. Le genre *Trochocercus* est clairement divisé en deux groupes. L'un comprend les deux espèces *cyanomelas* et *nitens*; il est étroitement associé aux *Terpsiphone* et autres Monarchidae (genres *Hypothymis, Pomarea, Myiagra*) dans les Corvoidea. L'autre, représenté par les espèces *nigromitratus* et *albonotatus*, est le groupe-frère de *Elminia*. Ce second clade [deuxième groupe de *Trochocercus* + *Elminia*] est très nettement séparé des premiers. Les espèces qui le composent ne sont donc pas des monarques. Ce clade apparaît comme le groupe-frère des Sylvioidea, pris en compte dans cette étude. Il n'est non plus aucunement proche parent des Rhipiduridae (qui sont des Corvoidea asiatiques, sans doute pas aussi proches des monarques, comme on a pu parfois le croire), comme certains morphologistes ou éco-éthologistes l'ont suggéré. Le genre *Erythrocercus*, quant à lui, ne s'inscrit pas non plus parmi les monarques, mais se place dans les Sylvioidea et, curieusement, près des bulbuls (Pycnonotidae). Les gobe-mouches asiatiques du genre *Culicicapa*, que certains taxinomistes ont rapproché des monarques africains, se placent plutôt près du clade incluant les *Elminia*.

Il est donc certain qu'une partie des *Trochocercus*, les *Elminia* et les *Erythrocercus* ne sont pas des Monarchidae. Si les deux premiers sont bien de la même famille, qui reste d'ailleurs à déterminer, les *Erythrocercus* appartiennent à une autre qui, elle aussi, requiert des études complémentaires pour son identification. Il est probable qu'un matériel plus conséquent et une couverture taxinomique plus large montreraient la nécessité de caractériser des familles nouvelles pour ces oiseaux. Les ressemblances morphologiques et étho-écologiques qui existent entre ces oiseaux et les monarques relèveraient de phénomènes de convergence dans l'occupation du milieu et l'utilisation de ses ressources. Manifestement, les familles de passereaux Oscines demandent des révisions. D'autres études comparatives, basées sur un échantillonnage plus important dans un éventail taxinomique beaucoup plus large, sont nécessaires avant que l'on puisse prétendre commencer à posséder une image fiable de l'évolution et des liens de parenté entre les familles qui constituent cet ordre, qui regroupe la moitié des espèces d'oiseaux actuels.

1. Introduction

Flycatchers are generally easily distinguished by their broad flat bills, with bristles around the nostrils, and by their characteristic hunting behaviour. However, the similarity of these characteristics does not necessarily reflect their phylogenetic relationships [1]. New World or sub-oscine flycatchers (Tyrannidae), for example, have been distinguished from others by their particular syringeal morphology, shared by many other endemic South-American bird families.

The Old World or oscine flycatchers have traditionally been grouped into one large family, which does not form a natural group. Among them, it is possible to distinguish the muscicapine flycatchers (Muscicapidae) by their spotted juvenile plumage [2–4] and the distinctive "turdine thumb" like pattern of their syrinx. These characters are also shared by the thrushes, Turdidae [5].

The non-muscicapine Old World flycatchers were split into two groups. The first one is the Platysteiridae (shrike-, black-and-white and puff-back flycatchers, wattle-eyes), endemic to Africa and supposedly related to the African bush-shrikes, Malaconotidae [1, 6]. A second group of non-muscicapine flycatchers is composed mainly of Australo-Papuan species, which were defined by external morphology and behaviour [7–9]. Most have been arranged into two main sub-families, Rhipidurinae (2 genera, 40 species) and Monarchinae (19 genera, 95 species), or tribes or families depending on the authors [2, 3, 8, 10–12]. For convenience, we refer to these two groups respectively as rhipidurine and monarchine birds. Rhipidurine birds (fantails) are easily defined because of their characteristic tail shape and fanning or wagging movements. However, a rigorous definition of the monarchine group is impossible [9]; they comprise rather varied genera (18 by [12]; 19 by [11]), some with numerous species like *Monarcha* and *Myiagra*, some with very few species like *Arses* (two species), *Clytorhynchus* (four species), *Peltops* (two species), or some that are monotypic like *Chasiempis*, *Mayrornis* or *Neolalage*. Several species bear glossy plumage and coloured steel blue-gray bills, some have peculiar crests, some have sexual dimorphism (*Myiagra*), and some have elongated tail streamers (*Terpsiphone*). The family name Monarchidae is an accepted synonym of Myiagridae, formed originally around the type genus *Myiagra* Vigors and Horsfield, 1827 [13].

The taxonomic position of the four non-Australasian genera (*Trochocercus*, *Hypothymis*, *Terpsiphone*, *Erythrocercus*) has been long debated [1, 8, 14–17], but they are generally all believed to be monarchine birds [11, 12]; this hypothesis has to be verified. Moreover, beside this problem of family limit, another question is to establish whether the genus *Trochocercus* is polyphyletic, as it has been reported by several authors [18–22]. These questions will be addressed with the following taxonomic sampling. Ten members of these following putative monarchine genera from Africa and Asia are included in our study: four *Trochocercus* (crested flycatchers, five species in Africa), two *Hypothymis* (blue monarchs, three species in Asia), two *Terpsiphone* (paradise flycatchers, 14 species both in Africa and in Asia), one *Elminia* (blue flycatchers, two species in Africa) and one *Erythrocercus* (three species in Africa). In order to clarify the position of the target groups of this study, we sequenced DNA for 27 additional species from various passerine families (see § *Material and Methods*, Table 1), including some other monarchines and species from the related genera *Rhipidura* and *Dicrurus* [1], as well as two species of the Asian flycatcher genus *Culicicapa*, whose relationships are unclear.

We present molecular data obtained from partial sequences of two mitochondrial genes: cytochrome *b* (cytb) and the large sub-unit ribosomal RNA (16S rRNA). Numerous previous molecular studies have provided evidence for the phylogenetic effectiveness of cytb at various taxonomic levels [e.g. 23], whereas the 16S has not often been used for resolving phylogenies among the Passeriformes (see [24–26]).

2. Material and methods

2.1. Species and source of DNA

Our samples (Table 1) of monarchine birds include representatives of all the African and the Asian genera (10 species) and two typical Australasian genera (*Myiagra*, two species, *Pomarea* one species) as well as representatives of the related genera *Rhipidura* (two species) and *Dicrurus* (one species) [1]. We include also in our analysis one species (or more, as specified) of more or less related passerines families or tribes (following the taxonomy given by [12]): Corvini, Oriolini, Malaconotini (two species), Laniidae, Petroicidae (correct family name for Eopsaltriidae, because of priority [27]), Sittidae, Muscicapidae (five species, including the two *Culicicapa*), Pycnonotidae, Cisticolidae, Sylviidae (five species), and Passeridae (two species). *Tyrannus melancholicus* (Tyrannidae) was used as the outgroup; morphological and molecular studies have shown that it is without doubt outside the oscine radiation (e.g. [1, 28]).

Genomic DNA was extracted from either frozen or alcohol-preserved tissues (muscle, liver, blood) or small

Table 1. List of taxa studied, geographic origin, number of the samples used and Genbank accession numbers.

Family or tribe S&M[1]	Family H&M[2]	Species		Origin	Number and Collection[6]	Genbank accession numbers[7] Cytb	16S
Monarchini	Monarchidae	*Terpsiphone*	*viridis*	Cameroon	MNHN, n° 2-23	AF094616	AF094646
‘’	‘’	‘’	*paradisi*	Laos	MNHN, n° 5-53	AF096466	AF096497
‘’	‘’	*Hypothymis*	*azurea*	Thailand	MNHN, n° 4-10B	AF096467	AF096496
‘’	‘’	‘’	*helenae*	Philippines	ZMC, n° 03728	AF096468	AF096495
‘’	‘’	*Trochocercus*[3]	*cyanomelas*	Tanzania	ZMC, n° 03874	AF096469	AF096494
‘’	‘’	‘’[3]	*nitens*	Cameroon	MNHN, n° 3-28	AF096470	AF096493
‘’	‘’	‘’[4]	*nigromitratus*	Kenya	MNHN, CG 1976-989	AF096472	AF096492
‘’	‘’	‘’[4]	*albonotatus*	Tanzania	ZMC, n° 02939	AF096471	AF096491
‘’	‘’	*Elminia*	*longicauda*	Cameroon	MNHN, n° 1-03	AF096474	AF096490
‘’	‘’	*Erythrocercus*	*mccallii*	Cameroon	MNHN, n° 3-25	AF096465	AF096489
‘’	‘’	*Myiagra*	*caledonica*	Loyalty Isl.	MNHN, CG 1979-922	AF096463	AF096488
‘’	‘’	‘’	*cyanoleuca*	Australia	AMNH, n° FB1048	AF096464	AF096487
‘’	‘’	*Pomarea*	*iphis*	Marquises	MNHN n° D41	AF135053	AF135054
Rhipidurini	‘’	*Rhipidura*	*albicollis*	Laos	MNHN, n° 5-48	AF096462	AF096486
‘’	‘’	‘’	*cyaniceps*	Philippines	ZMC, n° 01876	AF096461	AF096485
Dicrurini	Dicruridae	*Dicrurus*	*paradiseus*	Laos	MNHN, n° 5-57	AF096473	AF096475
Malaconotini	Platysteiridae	*Platysteira*	*cyanea*	Cameroon	MNHN, n° 2-22	AF096452	AF096483
Malaconotini	Laniidae	*Telophorus*	*sulfureopectus*	Malawi	MNHN, n° 29	AF096456	AF096476
Corvini	Corvidae	*Corvus*	*corone*	France	MNHN, n° 13-16	AF094613	AF094643
Oriolini	Oriolidae	*Oriolus*	*xanthornus*	Thailand	MNHN, n°4-10D	AF094615	AF094645
Laniidae	Laniidae	*Lanius*	*collaris*	Thailand	MNHN, n° 2-26	AF094614	AF094644
Eopsaltriidae[5]	Eopsaltriidae[5]	*Eopsaltria*	*australis*	Australia	AMNH, n° FB1550	AF096455	AF096484
Sittidae	Sittidae	*Sitta*	*europaea*	France	MNHN, n° 9-15	AF135049	AF135055
Muscicapidae	Turdidae	*Phoenicurus*	*phoenicurus*	France	MNHN, n° 22-43	AF135050	AF135057
‘’	Muscicapidae	*Muscicapa*	*striata*	France	MNHN, n° 13-1A	AF096458	AF096480
‘’	‘’	*Culicicapa*	*ceylonensis*	Thailand	MNHN, n° 4-9G	AF096453	AF096482
‘’	‘’	‘’	*helianthea*	Philippines	ZMC, n° 0783	AF096454	AF096481
Pycnonotidae	Pycnonotidae	*Andropadus*	*latirostris*	Cameroon	MNHN, n° 2-52	AF096457	AF096477
Cisticolidae	Sylviidae	*Camaroptera*	*brevicaudata*	Cameroon	MNHN, n° 2-15	AF094626	AF094654
Sylviidae	‘’	*Orthotomus*	*sutorius*	Thailand	MNHN, n° 4-8E	AF094622	AF094652
‘’	‘’	*Acrocephalus*	*aedon*	Thailand	MNHN, n° 4-8D	AF094623	AF094653
‘’	‘’	*Sylvia*	*melanocephala*	France	MNHN, n° S4	AF135052	AF135056
‘’	Timaliidae	*Garrulax*	*leucolophus*	Thailand	MNHN, n° 4-6E	AF094627	AF094655
‘’	‘’	*Pellorneum*	*ruficeps*	Thailand	MNHN, n° 4-6F	AF094632	AF094660
Passeridae	Motacillidae	*Anthus*	*pratensis*	France	MNHN, n° 13-5A	AF096460	AF096479
Passeridae	Ploceidae	*Passer*	*domesticus*	France	MNHN, n° 13-5C	AF094639	AF094667
Tyrannidae	Tyrannidae	*Tyrannus*	*melancholicus*	S. America	MNHN, n° 12-33	AF135051	AF135058

[1] The taxonomy presented here follows Sibley and Monroe [11]; the listed tribes belong to Corvidae.
[2] The taxonomy follows here Howard and Moore [12].
[3] These two species are under generic name *Terpsiphone* in Howard and Moore.
[4] These two species are under generic name *Elminia* in Howard and Moore.
[5] Family name Petroicidae is used in the text as it is the correct family name for Eopsaltriidae, because of priority.
[6] **AMNH**: American Museum of Natural History, New York, USA; **MNHN**: National Museum of Natural History, Paris, France; **ZMC**: Zoological Museum and Institute of Zoology, Copenhagen, Denmark.
[7] Genbank accession numbers like AF094xxx correspond to DNA sequences presented by Cibois et al. [26].

pieces (0.5 to 1 cm^2) of museum skins (labelled MNHN, CG in Table 1), using CTAB buffer containing proteinase K (0.1 mg/ml) [29–30]. In the case of museum skins, protein digestion time was expanded from 2 to 24 h.

2.2. DNA amplification and sequencing

Polymerase Chain Reaction (PCR) was performed on DNA samples for 35–40 cycles. For each cycle, denaturation was done at 93 °C for 30 s, annealing at 50–55 °C for 40 s, and extension at 72 °C for 40 s. Most amplifications were performed with primers 2 and 4 (Table 2); one or two other pairs of primers were used for all other amplifications (see legend of Table 2). Sequencing was performed with amplification primers and internal primers 5 and 6. For phylogenetic analysis, we used two homologous fragments, including 836 bases from cytb and 503 bases located at the 3’-end of the 16S gene. These are positions 15025-15860 and

Table 2. Primers used in this study. Most of the samples were amplified with primer pair 2 & 7. For *Terpsiphone viridis*, *Rhipidura cyaniceps* and *Muscicapa striata*, we used primer pair 2 & 8; for *Hypothymis helenae*, *Trochocercus cyanomelas* and *Pellorneum ruficeps*, we used primer pairs 1 & 4 and 3 & 7; for *Trochocercus nigromitratus*, *Pomarea iphis* and *Elminia longicauda*, we used primer pair 3 & 7, which delimit a shorter sequence of 636 bases. Primers 5 and 6 were used for internal sequencing.

Primers	References
cytb:	
1. L14827 (ND5) (5'-CCA-CAC-TCC-ACA-CAG-GCC-TAA-TTA-A-3')	[49]
2. L14990 (5'-CAT-CCA-ACA-TCT-CTG-CTT-GAT-GAA-A-3')	[50, modified]
3. L15206 (5'-CAC-ATC-GGC-CGA-GGA-ATC-TAC-TA-3')	[26]
4. H15298 (5'-CAG-CCC-CTC-AGA-ATG-ATA-TTT-GTC-CTC-A-3')	[50, modified]
5. L15383 (5'-GGA-CAA-ACA-CTA-GTA-GAA-TG-3')	[26]
6. H15487 (5'-GAT-CCT-GTT-TCG-TGG-AGG-AAG-GT-3')	[51]
7. H15916 (5'-ATG-AAG-GGA-TGT-TCT-ACT-GGT-TG-3')	[52]
8. H16065 (tRNA-thr) (5'-GGA-GTC-TTC-AGT-CTC-TGG-TTT-ACA-AGA-C-3')	[49]
16S:	
L3214 (5'-CGC-CTG-TTT-ATC-AAA-AAC-AT-3')	[53]
H3783 (5'-CCG-GTC-TGA-ACT-CAG-ATC-ACG-T-3')	[54]

positions 3254-3756 respectively in the chicken mitochondrial genome [31]. For *Elminia longicauda*, *Trochocercus nigromitratus* and *Pomarea iphis*, cytb sequences were 200 bases shorter at the 5' end, because we could not amplify this section with either L primers number 1 or 2; some other sequences are 1 to 8 bases shorter. Sequencing reactions were performed by direct cycling PCR with the 'Thermo Sequenase Cycle Sequencing' kit from Amersham Pharmacia Biotech. Electrophoresis was performed on polyacrylamide gels with manual sequencers. Autoradiographs were read twice independently and managed with the MUST package [32].

2.3. 16S gene alignment

Like other rRNA, 16S has a specific secondary structure with stems (paired regions) and loops (unpaired regions). Alignment of the sequences was obtained in two different ways. Firstly by eye, minimizing the number of gap positions. Secondly, we used the computer program MALIGN (version 1.9, [33]), which aligns by optimising the length of the sequences with the shortest tree via heuristic searching and branch swapping. We used the following options, quick, iter, score 3, tresswap, alignswap, changecost 2 and internal 3, which gave consistent results [see also 26]. Gaps in 16S sequences were treated as a fifth character, with or without coding the multigaps using BARCOD [34]. All analyses gave very similar results.

2.4. Phylogenetic analyses

For both methods used (neighbour-joining and parsimony), we first analysed cytb and 16S data sets separately and then combined both data sets into a single analysis. The incongruence between cytb and 16S datasets was also evaluated by ILD test [35]. Neighbour-joining (NJ) topologies were obtained with uncorrected distances calculated with PAUP* from full sequences for both separate and combined datasets [36]. Parsimony analyses (MP) were also performed with PAUP* using the heuristic algorithm, TBR swapping, and 100 random addition-sequence replicates. Cytb data were also weighted for transitions versus transversions using a step matrix in PAUP*. The weight applied to transversions was determined via saturation analysis (not shown; [37]). No weighting was used with 16S data, as there was no evidence of saturation (not shown). The robustness of NJ and MP trees was tested by bootstrap analysis with 1000 replicates [38]. The polyphyly of the monarchine birds was evaluated by the Kishino–Hasegawa test [39, 40] by comparing the resulting optimal tree topologies with the most parsimonious trees using constrained topologies in PAUP*. Sequences were deposited in Genbank (Table 1).

3. Results

3.1. Sequences variation and saturation

As expected for a protein-coding gene, our cytb sequences showed no insertions or deletions and their base composition and pattern of variability are typical of avian cytb sequences, suggesting that they are actual cytb and not nuclear pseudogene sequences.

Among the 836 sites analysed, a total of 425 sites (50.8%) were variable and 331 (39.6%) were potentially phylogenetically informative. As with many other cytb studies, comparison of transitions and transversions percentages, plotted for each pair of species, indicated saturation with increasing sequence divergence (not shown). From the initial slope of this

Table 3. Bootstrap support indices (only values >50% shown) obtained with various analyses. ‘ Tv*4 ’ indicates that a weight of 4 is given to transversions. The 16S analyses based on different alignments, with coding the gaps or not, gave similar results; only one set of result is presented.

	Gene	Cytb			16S		Both genes		
	Sites	No weigthing		Tv*4	No weigthing		No weigthing		(Cytb :tv4)
Node	Method	NJ	MP	MP	NJ	MP	NJ	MP	MP
1	*Rhipidura albicollis* + *cyaniceps*	72			100	97	100	90	77
2	(1) + *Corvus*						72		
3	*Lanius* + *Dicrurus*	56	61	66			61	57	74
4	*Myiagra caledonica* + *cyanoleuca*	100	100	100	100	97	100	100	100
5	*Trochocercus cyanomelas* + *nitens*	100	100	100	98	86	100	100	100
6	*Terpsiphone paradisi* + *viridis*			66	85	85	72	66	86
7	*Hypothymis azurea* + *helenae*				57				
8	*Terpsiphone* + *Hypothymis*	85	50	86	76	77	97	86	96
9	(8) + *Trochocercus*	60	54	74			50		72
10	(9) + *Pomarea*	57		59		52	63	62	75
11	Monarchine birds	65		62	100	94	100	97	87
12	*Phoenicurus* + *Muscicapa*	93	82	79	93	74	100	97	86
13	*Anthus* + *Passer*	84	90	79	73	74	99	99	92
14	*Culicicapa ceylonensis* + *helianthea*	100	100	100	100	100	100	100	100
15	*Trochocercus albonotatus* + *nigromitratus*	85	69	59	94	86	98	94	76
16	(15) + *Elminia*	90	90	100	100	96	100	100	100
17	*Camaroptera* + *Orthotomus*	66		80	100	100	100	99	99
18	*Erythrocercus* + *Andropadus*				55	56	74		
19	*Garrulax* + *Pellorneum*				68	74	70	70	
20	(19) + *Sylvia*				60	57			
21	Sylvii-Timalii-Pycnonotidae clade				63	57	84	82	
22	(21) + (16) + (14)						52	55	
	Number of nodes > 50%	14	10	14	17	17	20	17	15
	Number of MP trees		5	1		14		4	1
	Length of the MP trees		2275	4997		717		3024	5748

transition/transversion distribution, we estimated their ratio to 4:1. This value was subsequently used to weight transversions in the cytb analyses. A few gaps were necessary for the alignment of 16S sequences: manual and MALIGN alignments led to sequence lengths of 503 (see Appendix) and 511 bases respectively (not shown). Recoding manual alignment using BARCOD added 17 extra characters. MALIGN alignment did not need to be recoded as no informative contiguous gaps were obtained. Among the 503 sites of manual alignment, 175 (35%) were variable and 124 (24%) were potentially phylogenetically informative. There was no evidence of saturation in the 16S data (not shown).

3.2. Phylogenetic results

Supported results are presented in Table 3 for separate and combined analyses (nodes bearing bootstrap proportions more than 50%) and full topologies are presented only for NJ and MP combined analyses (Figs. 1 and 2). The cytb analyses (NJ or MP) led to a poorly resolved topology, with only 10 to 14 nodes supported by bootstrap proportions of more than 50% (Table 3). The 16S analyses gave very similar results whatever the method used, NJ or MP (17 nodes at same bootstrap level, Table 3). No incongruence was evident between both datasets (ILD test not significant) and 8 to 13 of the supported nodes were common to analyses of both genes, depending of the method of analysis. The combined analyses resulted in 15 to 20 supported nodes (Table 3).

All phylogenetic results present the following well defined clades. Node # 11 (Table 3) includes *Terpsiphone*, *Hypothymis*, *Myiagra*, *Pomarea* and two of the four *Trochocercus*, *T. nitens* and *T. cyanomelas* (both members of the superspecies *T.* [*cyanomelas*]). Inside this clade, the representatives of each genus are often linked in terminal positions, but the two species of genus *Hypothymis* are in a paraphyletic or unresolved position in most of the analyses. *Terpsiphone* and *Hypothymis* form a clade (# 8 in Table 3), which is consistent in all analyses. When *Trochocercus*, *Pomarea* and *Myiagra* species are added, this clade #8 is included within deeper clades in paraphyletic positions. The second well-defined clade (# 16 in Table 3) is composed of *Elminia* and the two other *Trochocercus* (*T. albonotatus* and *T. nigromitratus*, both members of the

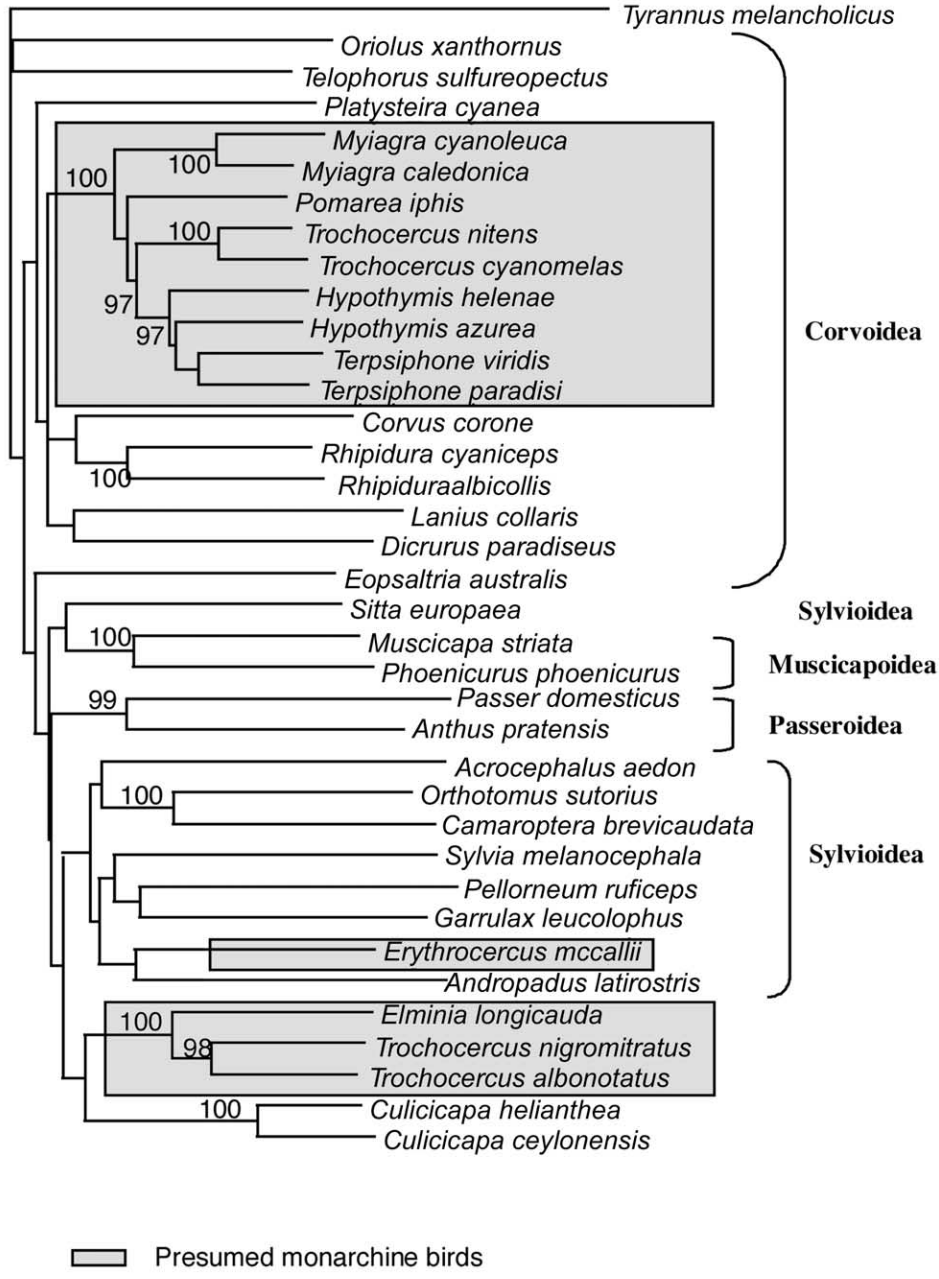

Fig. 1. Unweighted NJ tree obtained with NJ analysis of combined datasets (cytb and 16S). Bootstrap values shown are those higher than 90%.

superspecies *T.* [*albonotatus*]). Members of this clade do not appear closely related to the *Trochocercus* or any member of clade # 11, nor to any of the Corvoidea or other super-family representatives, but instead this clade is sister-group to *Culicicapa*, which is not closely related to *Eopsaltria*, monarchine birds or Muscicapoidea. We tested the monophyly of *Trochocercus* species for the most parsimonious trees of the combined analyses (all unweighted sites: length 3024; weighting cytb by Tv4: length 5748; see Table 3) with constrained topologies (Kishino and Hasegawa test): the monophyly of the *Trochocercus* is rejected for both topologies ($p < 0.0001$), either when *Elminia* is included or not. The monophyly of the monarchine birds (clade # 11 + *Erythrocercus* + clade # 16) is also rejected ($p < 0.0001$) in both topologies. If we exclude *Erythrocercus* from this test, the monophyly of monarchine birds is still rejected for both topologies, albeit weakly ($p = 0.0926$ and $p = 0.0024$). *Erythrocercus* is not closely related to any other presumed monarchine birds (from clade # 11 or clade # 16), but belongs to the main Sylvioidea clade (clade # 21 in Table 3).

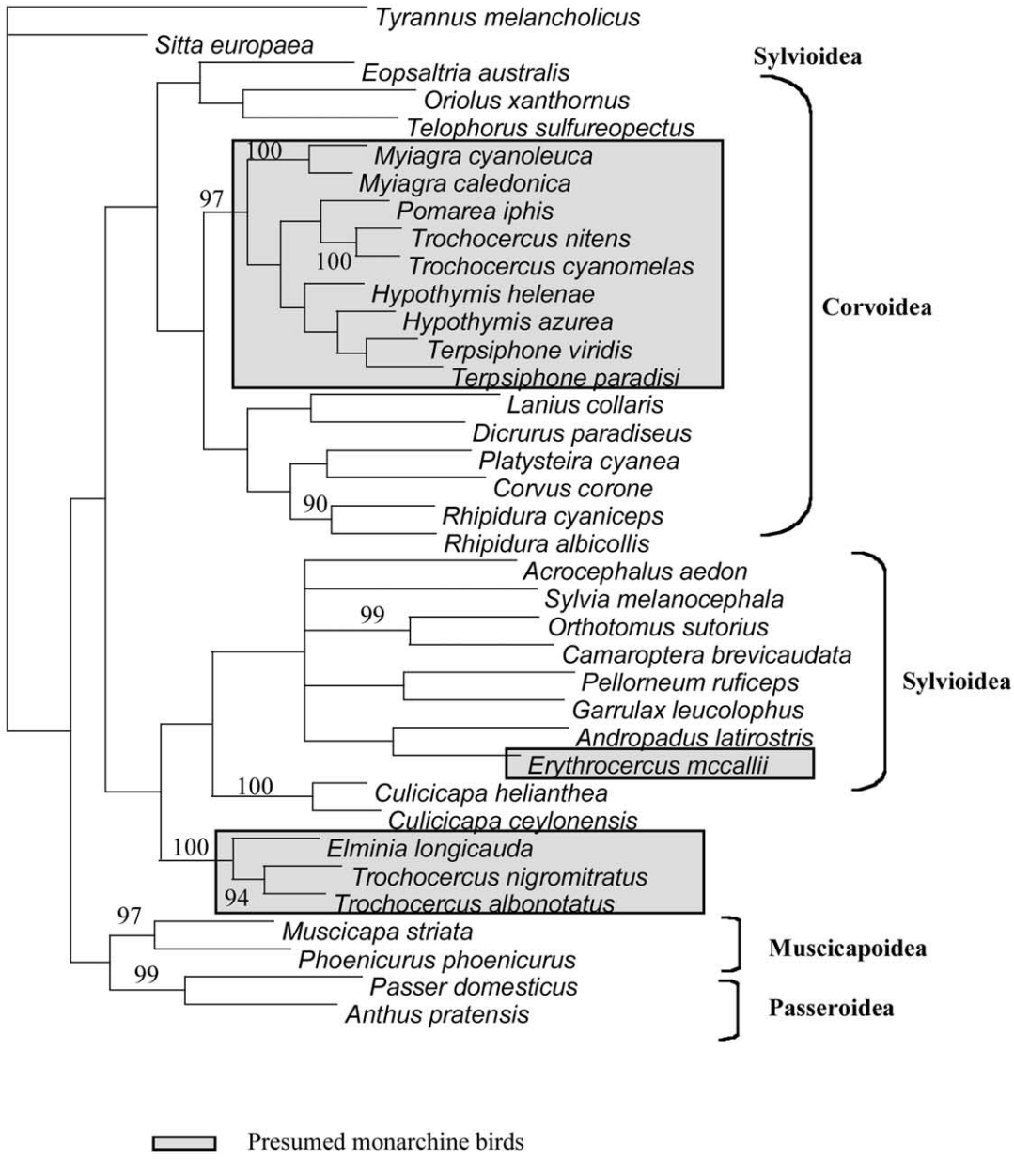

Fig. 2. Strict consensus of 4 MP trees obtained from combined datasets (cytb and 16S). Bootstrap values shown are those higher than 90%.

Other reasonably supported associations between different genera confirm previous studies and will not be further discussed: a) *Anthus* and *Passer* [see 1, 41], b) *Pellorneum* and *Garrulax* (see [26]), c) *Orthotomus* and *Camaroptera* (see [26, 42–44]), and d) *Muscicapa* and *Phoenicurus* (see [1]).

4. Discussion

Our results clearly illustrate the polyphyly of the African monarchine birds and that none of them is closely related to Muscicapoidea and Passeroidea. The genus *Trochocercus* in particular appears polyphyletic and, on the basis of body shape, proportions, skull characters and etho-ecological arguments [e.g., 18–22, 45], was already divided previously into two groups: *Trochocercus-1* (*cyanomelas* and *nitens*) and *Trochocercus-2* (*albonotatus*, *nigromitratus* and *albiventris*). Our molecular results confirm this division and that *Hypothymis*, *Terpsiphone* and *Trochocercus-1* are monarchine birds (in Corvoidea), as proposed by Sibley and Ahlquist [1], who based their study only on

Terpsiphone paradisi, *Hypothymis azurea*, *Trochocercus cyanomelas*, but with a larger Australasian taxonomic sampling. These species belong to a clade including typical monarchine taxa like *Myiagra* or *Pomarea*. The generic name *Trochocercus* given by Cabanis (1850) to *Muscicapa cyanomelas* Vieillot, 1818 (type species by monotypy), but used subsequently for the five species *cyanomelas, nitens, nigromitratus, albiventris* and *albonotatus*, must now be restricted to the species *cyanomelas* and *nitens* (*i.e. Trochocercus-1*). These latter *Trochocercus* species were already believed to be close to *Terpsiphone* on the basis of similar skull osteology, and it was even suggested that both genera could be merged [21, 22]. But most taxonomists [see particularly 46] maintained *Trochocercus* as a distinct genus, and Erard et al. [47] eventually adopted this conservative position. General habits of *Hypothymis*, as well as calls and songs (C. Chappuis, pers. comm.), are also quite similar to those of *Terpsiphone*, suggesting that these three genera are closely related. Our molecular results show that *Terpsiphone* is closer to *Hypothymis* than to *Trochocercus-1* and that, unless all three genera are merged, *Trochocercus* should be maintained as a distinct genus.

Species from *Trochocercus-2* were believed to be related to *Elminia* [20, 21, 45], and this is confirmed by our study. General behaviour, breeding habits, and vocalizations supported this close relationship [see 21, 47 for details]. However, differences exist in plumage patterns and body proportions, so one may wonder whether maintaining two separate genera would be appropriate, particularly in the light of the clear molecular divergence, but unclear relationship, existing between *Elminia longicauda* and the pair *albonotatus*/*nigromitratus*. We propose here to merge *nigromitratus, albiventris* and *albonotatus* into *Elminia* (s.l.), as it was done by Howard and Moore [12], but not by Sibley and Monroe [11].

Because of our large non-monarchine taxonomic sampling, the values of the tests and the general pattern of supported nodes, we can also conclude that *Trochocercus-2* and *Elminia*, as well as *Erythocercus*, are not monarchine. *Elminia* (s.s.) and *Erythrocercus* have previously always been considered to be monarchine. Species now included in the genus *Elminia* (s.l.) are known to share a characteristic behaviour with *Rhipidura*; continuously they fan their tail, droop their partly open wings, and progress with jerky pivoting movements. Because of these striking similarities in behaviour and also some similarity in external morphology and breeding habits, including nest characteristics, some authors (e.g., [20, 47, 48]) have speculated about a possible relationship between them. Present molecular data do not provide support for such a close relationship. Likewise they do not support the very close relationship between *Rhipidura* species and monarchs suggested by some authors, who lumped them in the same family. Ames [5] and Olson [22] have stressed the fact that *Elminia* (s.l.) and *Erythrocercus* lack the corvine configuration of the humerus (unlike corvids, they have a non-pneumatic humerus, with a double tricipita fossa) and the amphirhinal condition of ossification of the nostril, characters present in true monarchs. *Elminia* (s.l.) and *Erythrocercus* are thus neither monarchine, nor rhipidurine, nor muscicapid birds (they also lack the 'turdine thumb' pattern of the syrinx [5], and spotted juvenile plumage). They do not belong to the Corvoidea, but seem close to the Sylvioidea.

Traylor [16] suggested a close relationship between *Erythrocercus* and *Culicicapa*, but Erard et al. [47] found the call and songs of *Erythrocercus* reminiscent of those of *Elminia* species. Molecular results obviously indicate that *Erythrocercus* is not a close relative of these birds. Moreover, all analyses place *Erythrocercus* within the Sylvioidea clade with clear though not strong support (clade #21, 82–84%, see Table 3).

The systematic position of *Culicicapa* has been much debated. For a long time, *Culicicapa* was believed to be either a member of Muscicapidae [2], or a close relative of *Rhipidura* [15], of *Erythrocercus* [16], or a member of the Petroicidae [11]. Unfortunately, Sibley and Ahlquist [1] provided no data. A relationship between *Elminia* (s.l.) and *Culicicapa* is only suggested by our molecular data and together they may constitute the sister-group of the Sylvioidea considered in the present study. However, *Culicicapa* and *Elminia* differ greatly in general postural and vocal behaviour, as well as in foraging behaviour [47, Pasquet pers. obs.]. *Culicicapa*, like *Elminia*, belongs neither to Muscicapidae nor to Petroicidae. Though one may be tempted to introduce new family names for them, the exact relationships of *Erythrocercus*, *Culicicapa* and *Elminia* need further detailed studies with more material for comparisons.

5. Conclusion

Though African *Terpsiphone* and part of *Trochocercus* (i.e. *Trochocercus-1*) are really monarchs and belong to the Monarchidae, it is sure that other *Trochocercus* (i.e. *Trochocercus-2*), *Elminia* and *Erythrocercus* are not. *Trochocercus-2* and *Elminia* belong to the same family, but this family will remain indeterminate as long as the exact relationships with *Culicicapa* and the various families of the Sylvioidea are unresolved. *Erythrocercus* belongs to another family, which

also requires further study before it is clearly identified. More material and wider taxonomic coverage will probably show that new family names are necessary for these birds.

Morphological (e.g. bill and tail shape, plumage texture and pattern, body proportions), ecological and behavioural (e.g. foraging location and behaviour, nesting habits) similarities that these birds share with monarchs reflect more convergent fine-tuned adaptations to habitat and resource exploitation than relationships.

Obviously oscine families require revisions and better definitions. More comparative phylogenetic studies, based on large samples and wide taxonomic coverage, are needed before it can be envisioned a reliable picture of the evolution of passerines and of the relationships among families in this order, which gathers more than half of extant bird species.

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References

[1] Sibley C.G., Ahlquist J.E., Phylogeny and classification of birds. A study in molecular evolution, Yale University Press, New Haven, 1990.

[2] Vaurie C., A generic revision of flycatchers of the tribe Muscicapini, Bull. Amer. Mus. Nat. Hist. 100 (1953) 459–538.

[3] Storr G.M., On the classification of the Old World flycatchers, Emu 58 (1958) 277–283.

[4] Sibley C.G., Comparative study of the egg white proteins of passerine birds, Bull. Peabody Mus. Nat. Hist. 32 (1970) 1–131.

[5] Ames P.L., The application of syringeal morphology to the classification of the Old World insect eaters (Muscicapidae), Bonn. zool. Beitr. 26 (1975) 107–134.

[6] Harris T., Arnott G., Shrikes of Southern Africa, Struik Winchester, Cape Town, 1988.

[7] Harrison C.J.O., Some aspects of adaptation and evolution in Australian Fantailed Flycatchers, Emu 76 (1976) 115–119.

[8] Boles W.E., The relationships of the Australo-Papuan flycatchers, Emu 79 (1979) 107–110.

[9] Boles W.E., The Robins and Flycatchers of Australia, Angus and Robertson Publishers, North Ryde, Australia, 1988.

[10] Mayr E., Greenway J.C., A sequence of passerine families, Breviora 58 (1956) 1–11.

[11] Sibley C.G., Monroe B.L. Jr, Distribution and taxonomy of birds of the world, Yale University Press, New Haven, 1990.

[12] Howard R., Moore A., A complete checklist of the birds of the world, 2nd Edition, Academic Press, London, 1991.

[13] Boles W.E., The subfamily name of the Monarch Flycatchers, Emu 81 (1981) 50.

[14] White C.M.N., A revised Check List of African Flycatchers, Tits, Tree Creepers, Sunbirds, White-eyes, Honey Eaters, Buntings, Finches, Weavers and Waxbills, Government Printer, Lusaka, 1960.

[15] Parker S.A., Taxonomic position of the genus *Culicicapa* Swinhoe (Muscicapidae), Bull. Brit. Orn. Cl. 84 (1964) 45–46.

[16] Traylor M.A., Notes on African Muscicapidae, Ibis 112 (1970) 395–397.

[17] Greig-Smith P.W., Convergence between *Terpsiphone corvina, T. atrochalybea* and *Piezorhynchus alecto*, Bull. Brit. Orn. Cl. 98 (1978) 41–43.

[18] Hall B.P., Moreau R.E., An atlas of speciation in African passerine birds, British Museum (Natural History), London, 1970.

[19] Dowsett R.J., Stjernstedt R., The birds of the Mafinga Mountains, Puku 7 (1973) 107–123.

[20] Dowsett R.J., Dowsett-Lemaire F., The systematic status of some Zambian birds, Gerfaut 70 (1980) 151–199.

[21] Érard C., Écologie et comportement des gobe-mouches (Aves: Muscicapinae, Platysteirinae, Monarchinae) du Nord-Est du Gabon, Vol 1, Mém. Mus. Nat. Hist. Nat., Série A, Zoologie 138 (1987) 1–256.

[22] Olson S.L., Preliminary systematic notes on some Old World passerines, Riv. Ital. Orn. 59 (1989) 183–195.

[23] Moore W.S., DeFilippis V.R., The window of taxonomic resolution for phylogenies based on mitochondrial cytochrome *b*, in: Mindell D.P. (Ed.), Avian molecular evolution and systematics, Academic Press, San Diego, 1997, pp. 83–115.

[24] Miyaki C.Y., Matioli S.R., Burke T., Wajntal A., Parrot evolution and paleogeographic events: mitochondrial DNA evidence, Mol. Biol. Evol. 15 (1998) 544–551.

[25] Sturmbauer C., Berger B., Dallinger R., Foeger M., Mitochondrial phylogeny of the genus *Regulus* and implications on the evolution of breeding behaviour in Sylvioid songbirds, Mol. Phyl. Evol. 10 (1998) 144–149.

[26] Cibois A., Pasquet É., Schulenberg T.S., Molecular systematics of the Malagasy babblers (Passeriformes: Timaliidae) and warblers (Passeriformes: Sylviidae), based on cytochrome *b* and 16S rRNA sequences, Mol. Phyl. Evol. 13 (1999) 581–595.

[27] Bock W.J., History and nomenclature of avian family-group names, Bull. Am. Mus. Nat. Hist. 222 (1994) 1–281.

[28] Prum R.O., Syringeal morphology, phylogeny, and evolution of the neotropical manakins (Aves: Pipridae), Am. Mus. Novit. 3043 (1992) 1–65.

[29] Doyle J.J., Doyle J.L., A rapid DNA isolation procedure for small amounts of fresh leaf tissue, Phytochem. Bull. 19 (1987) 11–15.

[30] Winnepenninckx B., Backeljau T., De Wachter R., Extraction of high molecular weight DNA from molluscs, Trends in Genetics 9 (1993) 407.

[31] Desjardins P., Morais R., Sequence and gene organisation of the chicken mitochondrial genome, J. Mol. Biol. 212 (1990) 599–634.

[32] Philippe H., MUST. A computer package of management utilities for sequences and trees, Nucleic Acids Res. 21 (1993) 5264–5272.

[33] Wheeler W., Gladstein D., MALIGN: a multiple sequence alignment program, J. Hered. 85 (1994) 417.

[34] Bioinformatique, BARCOD, Université Pierre-et-Marie-Curie, Paris, 1988.

[35] Farris J.S., Källersjö M., Kluge A.G., Bult C., Testing significance of incongruence, Cladistics 10 (1995) 315–319.

[36] Swofford D.L., PAUP*. Phylogenetic analysis using parsimony (* and other Method), Version 4, Sinauer Associates, Sunderland, Massachusetts, 1999.

[37] Irwin D.M., Kocher T.D., Wilson A.C., Evolution of the cytochrome *b* gene of mammals, J. Mol. Evol. 32 (1991) 128–144.

[38] Felsenstein J., Confidence limits on phylogenies: an approach using bootstrap, Evolution 39 (1985) 783–791.

[39] Templeton A.R., Phylogenetic inference from restriction endonuclease cleavage site maps with particular reference to the evolution of humans and apes, Evolution 37 (1983) 221–244.

[40] Kishino H., Hasegawa M., Evaluation of the maximum-likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea, J. Mol. Evol. 29 (1989) 170–179.

[41] Mindell D.P., Sorenson M.D., Huddleston C.J., Miranda H.C. Jr, Knight A., Sawchuk S.J., Yuri T., Phylogenetic relationships among and within select avian orders based on mitochondrial DNA, in: Mindell D.P. (Ed.), Avian molecular evolution and systematics, Academic Press, San Diego, 1997, pp. 213–247.

[42] Fry C.H., On the systematics of African and Asian tailor-birds (Sylviinae), Arnoldia (Rhodesia) 8 (1976) 1–15.

[43] Watson G.E., Traylor M.A. Jr., Mayr E., Families Sylviidae, Muscicapidae, and Monarchidae, in: Mayr E., Cottrell G.W. (Eds.), Check-list of birds of the world, Vol. XI, Museum of Comparative Zoology, Cambridge, Massachusetts, 1986, pp. 464–556.

[44] Urban E.K., Fry C.H., Keith S. (Eds.), The birds of Africa, Vol. V, Academic Press, London, 1997.

[45] Dowsett R.J., Dowsett-Lemaire F., Comments on the taxonomy of some Afrotropical bird species, Tauraco Res. Rep. 5 (1993) 323–389.

[46] Chapin J.-P., The birds of the Belgian Congo, Vol. 3, Bull. Amer. Mus. Nat. Hist. 75A (1953) 1–821.

[47] Erard C., Keith S., Naurois R. de, Monarchidae, paradise-flycatchers and monarchs, in: Urban E.K., Fry C.H., Keith S. (Eds.), The birds of Africa, Vol. 5, Academic Press, San Diego, London, 1997, pp. 508–547.

[48] Mayr E., Moynihan M., Evolution in the *Rhipidura rufifrons* group, Amer. Mus. Novit. 1321 (1946) 1–21.

[49] Helm-Bychowski K., Cracraft J., Recovering phylogenetic signal from DNA sequences: Rrelationships within the Corvine Assemblage (Class Aves) as inferred from complete sequences of the mitochondrial DNA cytochrome-*b* gene, Mol. Biol. Evol. 10 (1993) 1196–1214.

[50] Kocher T.D., Thomas W.K., Meyer A., Edwards S.V., Pääbo S., Villablanca F.X., Wilson A.C., Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers, Proc. Natl Acad. Sci. USA 86 (1989) 6196–6200.

[51] Pasquet É., Phylogeny of the nuthatches of the *Sitta canadensis* group and its evolutionary and biogeographic implications, Ibis 140 (1998) 150–156.

[52] Edwards E.V., Arctander P., Wilson A.C., Mitochondrial resolution of a deep branch in the genealogical tree for perching birds, Proc. Roy. Soc. London, Ser. B 243 (1991) 99–107.

[53] Hedges S.B., Molecular evidence for the origin of birds, Proc. Natl Acad. Sci. USA 91 (1994) 2621–2624.

[54] Hedges S.B., Sibley C.G., Molecules vs. morphology in avian evolution: the case of the ‘pelecaniform’ birds, Proc. Natl Acad. Sci. USA 91 (1994) 9861–9865.

Appendix. Manual alignment of 16S sequences corresponding to amplified segment; parts with insertions (noted *).

Positions	1 20 221 270
Tyrannus melancholicus	CAGTGACAA*TATGTTCAAC///CCCCTCCTACCCA*TGGAATATGCCTCT**GGACTTACTGGTCTGTA*TT
Oriolus xanthornus	-T-----CC*AG----T---///T-A-A--C--T***G--TT--C--ACT-*GCA-ACCG-----AC-C-*--
Myiagra cyanoleuca	-G-----CT*AG----A---///T-AA---C--T***G--TTC-C--A-A-TATAGG-A-T---C---C-*--
Myiagra caledonica	-G-----CT*AG----A---///T-AA---C--T***G--TTC-C--A-A-TATAGG-A-T---C---C-*--
Pomarea iphis	-G----TCC*AG-A--A---///T-AA---C--T***G--TTC-C--A-T-*ATAGG---T---C---C-*--
Trochocercus nitens	-------CT*AG----A---///--AA---C--T***G--TTC-C--A-T-*ATAGG-A-T---C---C-T--
Trochocercus cyanomelas	-------CT*AG----A---///--AA---C--T***G--TTC-C--A-T-*ATAGG-A-T---C---C-*--
Hypothymis helenae	--CT*AG----A---///T-AA---C--T***G--TTC-C--A-TATATAGG---T---C---C-A--
Terpsiphone viridis	-G-----TT*AG----A---///T-AA---C--T***G--TTC-C--A-T-*ATAGG-C-T---C---C-*--
Terpsiphone paradisi	-G-----TT*AG----A---///T-AA---C--T***G--TTC-C--A-T-*ATAGGC--T---C---C-*--
Hypothymis azurea	-------TT*A-----A---///T-AA---C--T***G--TTC-C--A-TA*ATAGG---T---C---C-*--
Lanius collaris	-------CC*AG----T---///T-A-A--C--T***G--TT--C--AC--*AAA-GGG-----C---C-*--
Dicrurus paradiseus	-G-----CT*AG----A---///T-A-A--C--T***G--TTC-C--A-A-**AA-G-CT----CA--C-*--
Platysteira cyanea	-G-----TC*A-C-------///TAA-A--C---***G--TTC-C-AACAC*ATA-AG-G----C---C-*--
Rhipidura cyaniceps	-G-----CT*AG----A---///T-A-A--C--T***G--TTC-C--ACAC*ATAGGGA---------C-*--
Rhipidura albicollis	-G-----CT*AG--------///T-A-A--C--T***G--TTC-C--ACA-*ATAGGGA---------C-*--
Corvus corone	-T-----TT*AG----T---///T-A-A--C--T***G--T-C-C--TCA-*ACAGGGA-----C---C-*--
Eopsaltria australis	-------CTTAG----T---///TTA-A--C--T***G--CC--CT-A-T-ACCA-GGCG----C---C-*--
Sitta europaea	-G-----CC*G-----T---///A-A-A--C--T***G--TTC-CA---A-**CA-AGC-----C---C-T--
Muscicapa striata	-G-----CT*G-G---T---///AAAAC--C-T-***G--CTC-CTAACAC*ATA-GCC-----C---CG*--
Phoenicurus phoenicurus	-G-----CT*G-----T---///A-A-A--C---***G--CTC-CTAACAC*ACA-GCC-----C---C-T--
Passer domesticus	-G-N---TC**G----T---///AT--C--C--T***G--CTC-CTG-CAA*ATA-G-CG--------CG*--
Anthus pratensis	-G-----TC**-C---A---///A-A-A--C---***G--CCC-CTGA---ACCA-GC-C-----TC-CG*--
Orthotomus sutorius	-G-----T-*G---------///TTATC--CCA--TCG--CTC-CTTAC-AATT-GGACGA-------C-T--
Camaroptera brevicaudata	-G-----TT*A-----A---///T-A-C--C----C*G--CTC-CTTA---ATT-GGACGA-------C-T--
Acrocephalus aedon	-G------T*GGC---T---///A-A-A--C-A-AC*G--CCC-CTTAC-C*ACAGGCC------TC-C-T--
Sylvia melanocephala	-G------T**G--------///T-A-A--C----C*G--CTC-CTTA--C*AA-GG-------CTA-C-T--
Pellorneum ruficeps	-G-----T***GA---T---///A-AAA--C---T**G--TTC-CT-AC-CGAC-GACC-----CTGAC-*--
Garrulax leucolophus	-G------***TA---T---///A--AA--C-T--**G--CTC-CTTA-TCTAC-GGC------CT--C-C--
Andropadus latirostris	-G-----C**G-----A---///T-A-A--C-T-TC*G--CGC-CTATC-A***CGGGCG-----TC-C-T--
Erythrocercus mccallii	-G-----TC*G-----A---///--A-A--C---TC*G--TTC-CTTAC--*AAAGGGAC----CGC-C-T--
Trochocercus nigromitratus	-G-----CT*G---------///A-A-A--C---***G--TTC-C--AC-C*ATA-AGCT----CTC-C-A--
Trochocercus albonotatus	-G-----CT*G-C-------///A-A-A--C---***G--TTC-C--A---*ATA-AGCT----CTC-C-A--
Elminia longicauda	-G-----CT*G-C---T---///A-A-A--C---***G--T-C-C--AC--*GCA-AG-T----CTC-C-A--
Culicicapa helianthea	-G-----CC*G-----A---///A-A-C--C--T***G--CTC-CTTA--C*ACA-ACCG-----AC-C-*--
Culicicapa ceylonensis	-G-----C**G-----A---///--A-C--C--T***G--CTC-CTTA--C*TCA-ACCG-----AC-C-*--
Telophorus sulfureopectus	-------TT*CG--------///T-AAA--C-T-***G--T-C-C-AACT-*ACA-GGAGA---C-----*--