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Evolutionary history and biogeography of the drongos (Dicruridae), a tropical Old World clade of corvoid passerines

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Abstract

We address the phylogenetic relationships of the drongos (Dicruridae) at the species-level using sequences from two nuclear (myoglobin intron-2 and c-mos) and two mitochondrial (ND2 and cytochrome *b*) loci. The resulting phylogenetic tree shows that the most basal species is *D. aeneus*, followed in the tree by a trichotomy including (1) the Asian *D. remifer*, (2) a clade of all African and Indian Ocean islands species as well as two Asian species (*D. macrocercus* and *D. leucophaeus*) and (3) a clade that includes all other Asian species as well as two Australasian species (*D. megarhynchus* and *D. bracteatus*). Our phylogenetic hypotheses are compared to [Mayr, E., Vaurie, C., 1948. Evolution of the family Dicruridae (Birds). Evolution 2, 238–265.] hypothetical family "tree" based on traditional phenotypic analysis and biogeography. We point out a general discrepancy between the so-called "primitive" or "unspecialized" species and their position in the phylogenetic tree, although our results for other species are congruent with previous hypotheses. We conduct dating analyses using a relaxed-clock method, and propose a chronology of clades formation. A particular attention is given to the drongo radiation in Indian Ocean islands and to the extinction–invasion processes involved. The first large diversification of the family took place both in Asia and Africa at 11.9 and 13.3 Myr, respectively, followed by a dispersal event from Africa to Asia at ca 10.6 Myr; dispersal over Wallace line occurred later at ca 6 Myr. At 5 Myr, Principe and Indian Ocean Islands have been colonized from an African ancestor; the most recent colonization event concerned Anjouan by an immigrating population from Madagascar.

Keywords: Dicruridae; Phylogeny; Biogeography; Molecular dating; Myoglobin intron-2; Cmos; ND2; Cytochrome *b*

1. Introduction

The family Dicruridae is one of the very few families of passerines that are morphologically highly homogeneous, like the Paridae, Sittidae or Certhiidae. Excluding *Chaetorhynchus papuensis*, which is now believed to belong to Rhipiduridae (Barker et al., 2004), the Dicruridae includes only one genus and 21 insectivorous species (Dickinson, 2003). All drongos are of moderate size (wing length from 107 to 184 mm) and present a glossy black coloration (some forms show reduction in pigmentation or gloss), with 10 feathers on a more or less forked tail and no sexual dimorphism (Vaurie, 1949). Several species show highly modified feathers like crest, hackles or spangles on head, or outermost tail feathers elongated in three species (Vaurie, 1949). The family is distributed throughout the Old World, mostly in tropical areas: Africa (including the

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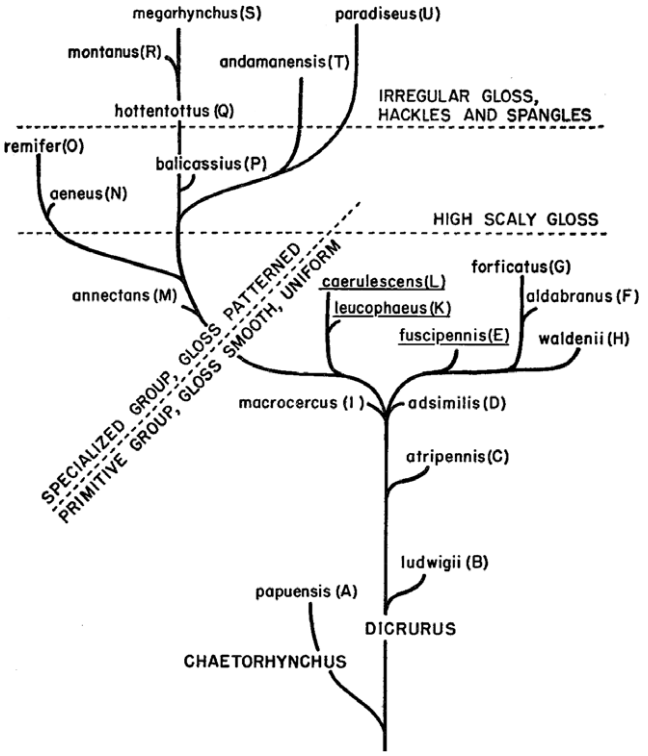

Fig. 1. Diagrammatic presentation of the relationships of the 20 species of drongos (Dicruridae) proposed by Mayr and Vaurie (1948).

island of Principe), the Indian Ocean archipelagos, Central Asia, India, East Asia North to Manchuria and South to Indonesia, Philippines, Sulawesi and Australasia.

The family Dicruridae has been the subject of a major taxonomic revision by Vaurie (1949), synthesized by Mayr and Vaurie (1948) who proposed an hypothetical family "tree" (Fig. 1), mainly based on geographical distributions, overall size and external morphology (including bill size, the amount of pigmentation or gloss, and the presence of modified feathers). Mayr and Vaurie (1948) proposed a split between primitive and specialized taxa, and placed them in their tree, in basal and terminal positions respectively. Since this pioneering study, there has been no attempt to test or clarify phylogenetic relationships within the Dicruridae. We used sequences data from two mitochondrial genes (ND2 and cytochrome *b*) and two nuclear loci (myoglobin intron-2 and cmos) obtained from 18 out of the 21 recognized *Dicrurus* species to reconstruct the phylogenetic relationships of the drongos and discuss biogeographical, systematic and evolutionary implications of this phylogeny with particular emphasis on the Malagasy region that hosts five island taxa.

2. Material and methods

2.1. Taxonomic sampling

We obtained samples (liver, blood, feathers, toe pads) from 18 out of 21 recognized drongos species (Dickinson, 2003), and when possible, included two individuals per species (Table 1). Only samples from *D. caerulescens* (India), *D. montanus* (Sulawesi) and *D. andamanensis* (Andaman Islands) were lacking. We also included representatives of the major crown corvoid lineages, encompassing the putative closest relatives of the drongos (Pasquet et al., 2002; Barker et al., 2004; Fuchs et al., 2004, 2006; Jønsson and Fjeldså, 2006). Phylogenies were rooted using sequences from the House Sparrow *Passer domesticus*.

2.2. Laboratory procedure

Total DNA was extracted using a CTAB-based protocol (Winnepenninckx et al., 1993) with an overnight Proteinase K (0.1 mg ml^{-1}) digestion. The myoglobin intron-2 was amplified with primers Myo2 and Myo3F (Slade et al., 1993; Heslewood et al., 1998). Internal primers Myo2int, Myo3int, Myo-Ma169R, Myo-Ma183F, Myo-Ma280F, Myo-Ma329R, Myo-Ma405R, and Myo-Ma440F were used to amplify myoglobin intron-2 from the skins samples (Fuchs et al., 2004, 2005). A fragment of the cmos locus was amplified with primers 944 and 1550 (Cooper and Penny, 1997) as well as with internal primers (cmosintF 5′-TGCACCATGTAATTTATKGCAC-3′ and cmosintR: 5′-AGCCACACARTCTCYTCCATGCTC-3′) (Fuchs et al., in press). ND2 was amplified using primers L5219 and H6313 (Sorenson et al., 1999) as well as with internal primers specifically designed for this study (L-ND2Di360F 5′-GRT TYCCAGARGTNCTTCAAGG-3′, L-ND2Di250F, 5′-ACCGGYCARTGRGAYATTAC-3′, H5736 5′-TGRTT GARKCCYATTCATCC-3′, H-ND2Di670R 5′-GARGT YATYAGYGTRGARAGTT-3′). A 441 bp portion of the cytochrome *b* was amplified with the primer pairs L14990 (Kocher et al., 1989) and H15509 5′-CATCCTGT TTCGTGTAGGAATGT-3′ (designed for this study). DNA from the museum skins was amplified using two pairs of primers specifically designed for the present study: CYTB8DIC 5′-GGCATYTGCYTAATRACACAAATC-3′ with CYTB10DIC 5′-GATCCTGTTTCGTGKAGGAA 3′ and CYTBFDIC 5′-CAAAGARACYTGAAAYATYG GAGT-3′ with CYTBRDIC 5′-CCTCAGAAKGATAT TTGTCCTC-3′. The amplification protocol was: 2 min at 94 °C, followed by 36 cycles of 94 °C for 40 s, 52–56 °C for 45 s, 72 °C for 40–50 s, with a final extension at 72 °C for 5 min. Cycle-sequencing reactions were performed using the CEQ Dye Terminator Cycle Sequencing kit (Beckman Coulter, Inc., Fullerton, CA, USA) or the Big Dye (Applied Biosystems Inc. Foster City, CA, USA) terminator chemistries kit using the same primers as for PCR amplifications. Both DNA strands were sequenced for all taxa on automated sequencers (CEQ2000 DNA Analysis System or ABI3100). Sequences were edited, assembled, and aligned using Sequencher 4.1 (Gene Codes Corporation, Ann Arbor, MI, USA).

2.3. Phylogenetic analyses

Molecular phylogenies were estimated using model-based approaches (maximum likelihood, ML, and Bayesian

Table 1
List of taxa, origin, samples and Genbank accession numbers

Species	Origin	Sample n°	Cytochrome *b*	ND2	Myoglobin	Cmos
Dicrurus adsimilis	Kenya	ZMUC 116137	EF449740	EF449644	EF449724	EF449681
Dicrurus aeneus	Thailand	MNHN 4-3A	EF449759	EF449663	EF449726	EF449687
Dicrurus aeneus	Thailand	MNHN 4-2J	EF449744	EF449649	EF449721	EF449702
Dicrurus aldabranus	Aldabra	MNHN 13-12	EF449760	EF449665	EF449728	EF449677
Dicrurus aldabranus	Aldabra	MNHN 13-19	EF449761	EF449666	EF449714	EF449695
Dicrurus annectans (1)	Borneo	LSUMZ B-47128	EF449767	EF449672	EF449718	EF449700
Dicrurus atripennis	Ghana	LSUMZ B-45252	EF449765	EF449670	EF449719	EF449697
Dicrurus atripennis	Ghana	LSUMZ B-27116	EF449766	EF449671	EF449713	EF449698
Dicrurus balicassius	Philippines	ZMUC 116140	EF449741[a](207)	EF449645[a](825)	EF449725	EF449680
Dicrurus balicassius	Philippines	ZMUC 116141	NS	EF449646[a](893)	EF449722	EF449690
Dicrurus balicassius	Philippines	USNM B-3611	EF449768	EF449674	EF449716	EF449701
Dicrurus bracteatus	New Guinea	UWBM 68045	EF449756	EF052784	EF2052839	EF052735
Dicrurus forficatus forficatus	Madagascar	FMNH 346029	EF449745	EF449650	EF449720	EF449683
Dicrurus forficatus forficatus	Madagascar	FMNH 353035	EF449746	EF449651	EF449732	EF449684
Dicrurus forficatus potior	Anjouan (Comoro)	MNHN E52	EF449763	EF449668[a](604)	EF449736	EF449676
Dicrurus fuscipennis	Grand Comoro (Comoro)	MNHN CG 1959-480 (s)	EF449752	EF449657[a](688)	EF449737	EF449707
Dicrurus fuscipennis	Grand Comoro (Comoro)	MNHN CG 1964-1017 (s)	EF449751	EF449656[a](688)	EF449738	EF449706
Dicrurus hottentotus	Cambodia	MNHN 35-1B	EF449758	EF449662	EF449723	EF449688
Dicrurus hottentotus	Thailand	MNHN CG 1989-35 (s)	EF449750	EF449655	EF449731	EF449703
Dicrurus leucophaeus	China	MNHN 14-30	EF449748	EF449653	EF449715	EF449682
Dicrurus leucophaeus	Laos	MNHN 33-8C	EF449755	EF449660	EF449727	EF449686
Dicrurus leucophaeus	Myanmar	USNM B-06197	EF449766	EF449675	EF449712	EF449699
Dicrurus ludwigii	Tanzania	ZMUC 122632	EF449743	EF449648	EF449730	EF449691
Dicrurus ludwigii	Tanzania	ZMUC 121877	EF449742	EF449647	EF449729	EF449679
Dicrurus macrocercus	Pakistan	FMNH 347969	EF449747	EF449652	EF449733	EF449685
Dicrurus megarhynchus	New Ireland	USNM B40070	EF449770	EF449673	EF449711	EF449696
Dicrurus modestus	Principe	M. Melo, uncatalogued	EF449753	EF449658	EF449734	EF449678
Dicrurus modestus	Principe	M. Melo, uncatalogued	EF449754	EF449659	EF449739	EF449693
Dicrurus paradiseus	Laos	MNHN 5-57	AF096473	AY529951	AY529916	EF052714
Dicrurus paradiseus	Cambodia	MNHN 34-6G	EF449757	EF449661	EF449735	EF449689
Dicrurus remifer	Thailand	MNHN CG 1989-32 (s)	EF449749	EF449654[a](938)	EF449708	EF449692
Dicrurus remifer	Myanmar	USNM B-2107	EF449764	EF449669	EF449717	EF449704
Dicrurus waldenii	Mayotte (France)	MNHN E13	EF449762	EF449667	EF449709	EF449694
Outgroup taxa						
Corvus corone	France	MNHN 13-16	AF094613	AY529949	AY529914	EF052706
Lanius collaris	Cameroon	MNHN 2-26	AF094614	AY529960	AY529925	EF052707
Rhipidura albicollis	Laos	MNHN 5-48	AF096462	AY529969	AY529934	EF052713
Terpsiphone viridis	Cameroon	MNHN 2-20	AF094616	DQ125996	AY529939	EF052708
Platysteira cyanea	Cameroon	MNHN 2-22	AF096452	AY529965	AY529930	EF052717
Chlorophoneus sulfureopectus	Malawi	MNHN CG 1998-823	AF096456	AY529947	AY529912	AF052701
Oriolus xanthornus	Thailand	MNHN 4-10D	AF094615	AY529964	AY529929	EF052715
Passer domesticus	France	MNHN 28-43	AF094639	EF449664	EF449710	EF449705

Acronyms refer to: FMNH, Field Museum of Natural History, Chicago; LSUMZ, Museum of Natural Science, Louisiana State University, Baton-Rouge; MNHN, Muséum National d'Histoire Naturelle, Paris; USNM United States National Museum, Washington; UWBM, University of Washington, Burke Museum, Seattle; ZMUC, Zoological Museum University of Copenhagen. (1) Identification of the voucher has been checked (D.L. Dittmann, pers. com.). (s) indicates the skin samples.

[a] Cases where only partial sequences were obtained, numbers in brackets indicate the length of the partial sequences. NS means no PCR product obtained.

inferences, BI), as implemented in PHYML v2.4 (Guindon and Gascuel, 2003) and MRBAYES 3.1 (Huelsenbeck and Ronquist, 2001, 2003; Ronquist and Huelsenbeck, 2003). Likelihood models were estimated with MRMODELTEST 2.0 (Nylander, 2004), using the Akaike Information Criterion, AIC (Akaike, 1973). Selected models and model parameters are provided in Table 2. Clade support for the ML analyses was assessed by non-parametric bootstrapping (100 replicates; Felsenstein, 1985).

The four gene regions we sequenced differ considerably in their properties and substitution dynamics, as shown by the parameters of the different selected models (Table 2). We therefore did not perform ML and BI analyses that assume a single model of evolution for the concatenated

Table 2
Length of sequenced loci, number of variable and informative sites, selected models, estimates of the model parameters and scores of the phylogenetic analyses

	Myoglobin intron-2	Cmos	ND2	Cytochrome *b*	Concatenated
Number of base pairs	677	608	1041	441	2767
Number of variable/informative sites	145/58	94/36	557/470	177/154	973/718
Model	K80 + G	GTR + G	GTR + G + I	GTR + G + I	NA
First codon position	NA	HKY + I	GTR + G	SYM + G	NA
Second codon position	NA	HKY	GTR + G + I	GTR + I	NA
Third codon position	NA	HKY + G	GTR + G + I	HKY + G + I	NA
ML score (−ln)	2028.38	1600.95	10497.02	3664.81	NA
Freq A	0.25	0.22509	0.32762	0.28896	NA
Freq C	0.25	0.24981	0.34897	0.28755	NA
Freq G	0.25	0.32907	0.09852	0.16075	NA
Freq T	0.25	0.19603	0.22489	0.26274	NA
$R_{A\text{-}C}$	NA	1.56016	0.83965	3.9914	NA
$R_{A\text{-}G}$	NA	6.42617	13.49195	27.7439	NA
$R_{A\text{-}T}$	NA	0.88933	1.00806	3.0392	NA
$R_{C\text{-}G}$	NA	1.29422	0.34274	1.986	NA
$R_{C\text{-}T}$	NA	13.70516	8.26004	61.337	NA
$R_{G\text{-}T}$	NA	1.0	1.0	1.0	NA
Ts/TV	4.317	NA	NA	NA	NA
Γ	0.795	0.183	0.912	1.811	NA
I	NA	NA	0.383	0.558	NA
BI score (−ln arithmetic/harmonic means)	2267.74	1753.95/1807.46	10521.70/10566.22	3671.19/3719.85	NA
BI score partitioned by codon position (−ln arithmetic/harmonic means)	NA	1646.70/1687.35	10072.62/10120.40	3422.88/3469.60	17305.84
Bayes Factor [a]	NA	240.2	891.6	500.5	NA

NA means not applicable. Estimates of the parameters were obtained using PHYML.
[a] A value greater than 10 for the Bayes Factor indicates strong support for the more complex model (i.e. partitioned by codon position).

data set. The relevance of partitioning the data set by gene and codon position was assessed with the Bayes Factor (Kass and Raferty, 1995; Nylander et al., 2004). We used the harmonic mean approximations of the marginal likelihood of the two compared models (M_0 and M_1) to calculate the Bayes Factors (BF), which is the ratio between the two compared models. A value greater than 10 for $2\ln BF_{10}$ was considered as strong evidence against the simpler model (M_0) (Kass and Raferty, 1995; Nylander et al., 2004).

Ten partitions were distinguished for the Bayesian analyses according to the functional properties of the markers. Bayesian analyses for the concatenated data set were performed allowing base frequencies, branch lengths, rate matrix, shape parameter and proportion of invariable sites to vary between the partitions (using the *unlink* and *prset ratepr = variable* commands). Four Metropolis-coupled Markov Chain Monte Carlo (MCMC) chains (three hot and one cold) were run for five million generations with trees sampled every 100 generations. The first 500,000 generations (5000 trees) were discarded ('burn-in' period) and the posterior probabilities were estimated for the remaining sampled generations. Two independent Bayesian runs initiated from random starting trees were also performed for each locus, and the log-likelihood values and posterior probabilities were checked to ascertain that the chains had reached stationarity. We also checked that the Potential Scale Reduction Factor (PSRF) approached 1.0 for all parameters and that the average standard deviation of split frequencies converged towards zero. We searched for significant incongruence between the individual loci trees by comparing the topologies and nodal support obtained under the two different analytical methods (ML, BI) to check for possible errors in amplifying and sequencing procedures and to detect eventual conflicts between individual loci trees. Criteria for incongruence were set at 70% for the bootstrap values (Hillis and Bull, 1993), and at 0.95 for posterior probabilities (Huelsenbeck and Ronquist, 2001). Trees were edited with the assistance of MRENT 1.2 (Zuccon and Zuccon, 2006).

2.4. Molecular dating analyses

Bayesian approaches for the divergence time estimates were performed using a relaxed clock method as implemented in the MULTIDIVTIME package (Thorne et al., 1998; Thorne and Kishino, 2002). We estimated different branch length variance–covariance matrices for our ten partitions (myoglobin intron-2 and three codon positions for cmos, ND2 and cytochrome *b*) using ESTBRANCHES. Outputs were then analyzed simultaneously by MULTIDIVTIME. The MCMC analyses followed the default settings of the software, and as priors we set the distance between the tip and the root to 50 ± 25 Myr and the substitution rate per site per million years at the root node to 0.0052 ± 0.0052 (estimated using the procedure described in the multidivtime.readme file). The topology obtained with the

concatenated data set partitioned by gene and codon position was used for the dating analyses.

Three calibration points were used, independently or in combination, to estimate the divergence times among drongo lineages. These calibration points are:

(1) The split between *Platysteira cyanea* and *Chlorophoneus sulfureopectus* at 33.9 ± 4.4 Myr BP (Fuchs et al., 2006). Instead of using a fixed date, we used upper (38.3 Myr BP) and lower (29.5 Myr BP) bounds that incorporate the standard deviations. These estimates are derived from the Suboscines–Oscines split, believed to have occurred between 71 and 77 Myr BP (Van Tuinen and Hedges, 2001; Ericson et al., 2002; Barker et al., 2004), and are based on a Gondwanan time frame for the passerine evolution.
(2) The split between *Dicrurus fuscipennis* (endemic to the volcanic island of Grand Comoro) and its closest relatives, which cannot be more than 0.5 Myr BP (Nougier et al., 1986). This date corresponds to the oldest age that would allow the colonization of Grand Comoro by the *D. fuscipennis* lineage.
(3) The split between *Dicrurus aldabranus* and its sister-group dating back to no more than 0.125 Myr BP. This date corresponds to the oldest age that would allow the colonization of the Aldabra atoll by the *D. aldabranus* lineage. The Aldabra Atoll was inundated at ca 0.125 Myr (Thomson and Walton, 1972), an event that may have eliminated all terrestrial biota. All other islands from the Aldabra group (Assumption, Cosmoledo, and Astove) probably only arose within the last 15,000 years (Radtkey, 1996).

2.5. Biogeographic analyses

The ancestral areas at nodes were inferred from distributional data using dispersal-vicariance analysis with the assistance of DIVA 1.2 (Ronquist, 1996, 1997). The following areas were specified for the analyses: Africa (Af), Indo-Malaya (IM) and Australasia (Au). The Indian Ocean islands (Comoros and Madagascar) were considered as part of the African realm and we used the Wallace line to delimit the Indo-Malayan and Australasian biomes (see Newton, 2003). The dispersal-vicariance analyses were only performed with the members of the Dicruridae in the input tree to avoid any biases associated with the fact that we did not sample representatives of all corvoid lineages. We used the topology resulting from the Bayesian analyses of the concatenated data set for the biogeographic analyses.

3. Results

Length of the sequenced loci, numbers of variable and parsimony informative sites, selected models and likelihood scores of the phylogenetic analyses are given in Table 2. The length of the concatenated dataset was 2767 bp, among which 973 and 718 sites were variable and parsimony informative, respectively. ML or BI results were very similar for each gene and no significant conflicts were detected between the individual locus trees. The trees obtained with the various loci presented uneven resolution (see Supplementary material). Monophyly of the Dicruridae was recovered with significant posterior probabilities in most analyses of the individual loci. In fact, only the comparatively short cytochrome *b* segment could not unambiguously support this monophyly. Apart from the well supported nodes linking individuals pertaining to the same species, the number of strongly supported nodes within the Dicruridae varied from eight (ND2) to six (Myoglobin intron-2 and cytochrome *b*) and three (cmos). The Bayes factor strongly suggested that the best partitioning strategy is by codon position for all three protein-coding genes (cmos, cytochrome *b*, ND2) (Table 2).

Results of the concatenated analysis (for which only the partitioned BI was performed, see Section 2) yielded a 50% majority-rule consensus tree where 22 out the 29 nodes within the Dicruridae received posterior probabilities equal or greater than 0.95 (Fig. 2). The Asian species *Dicrurus aeneus* is the first taxon to branch-off in the Dicruridae tree. More inclusively, the tree shows a trichotomy including the Asian *D. remifer* and two well supported clades G and D. The clade G contains all species from Africa (*atripennis*, *ludwigii*, *adsimilis*, *modestus*), from Indian Ocean islands (*fuscipennis*, *aldabranus*, *forficatus*, *waldenii*) as well as two Asian species (*macrocercus* and *leucophaeus*). The structure of this clade therefore does not reflect any clear geographical pattern of species distribution. *D. atripennis* and *ludwigii*, two African species, are basally positioned and their sister-group relationship does not receive significant support whereas other African species (*adsimilis, modestus*), all Indian Ocean species and the two Asian species are gathered in a strongly supported monophyletic group (clade I). Within this clade, *D. leucophaeus* is the first emerging taxon and the final crown (clade J) groups all the species from Indian Ocean, the Asian *macrocercus* and the African *adsimilis* and *modestus*. Inside this clade J, only two relationships are well supported, (1) between *aldabranus* and *forficatus* (including the Anjouan subspecies *forficatus potior*), and (2) between *adsimilis* and *macrocercus*, which are sister species expanded very recently, respectively, in Africa and in Asia. Although the placement of *D. fuscipennis* from Grand Comoro is not firmly established, this taxon is not closely related to the other insular species from the Indian Ocean. As a consequence, Indian Ocean insular species do not form a monophyletic assemblage in our phylogenetic tree.

The clade D includes all other Asian species which are divided in two strongly supported groups, (1) *annectans* and *paradiseus* which are very closely related, and (2) the four species *megarhynchus*, *bracteatus*, *balicassius* and

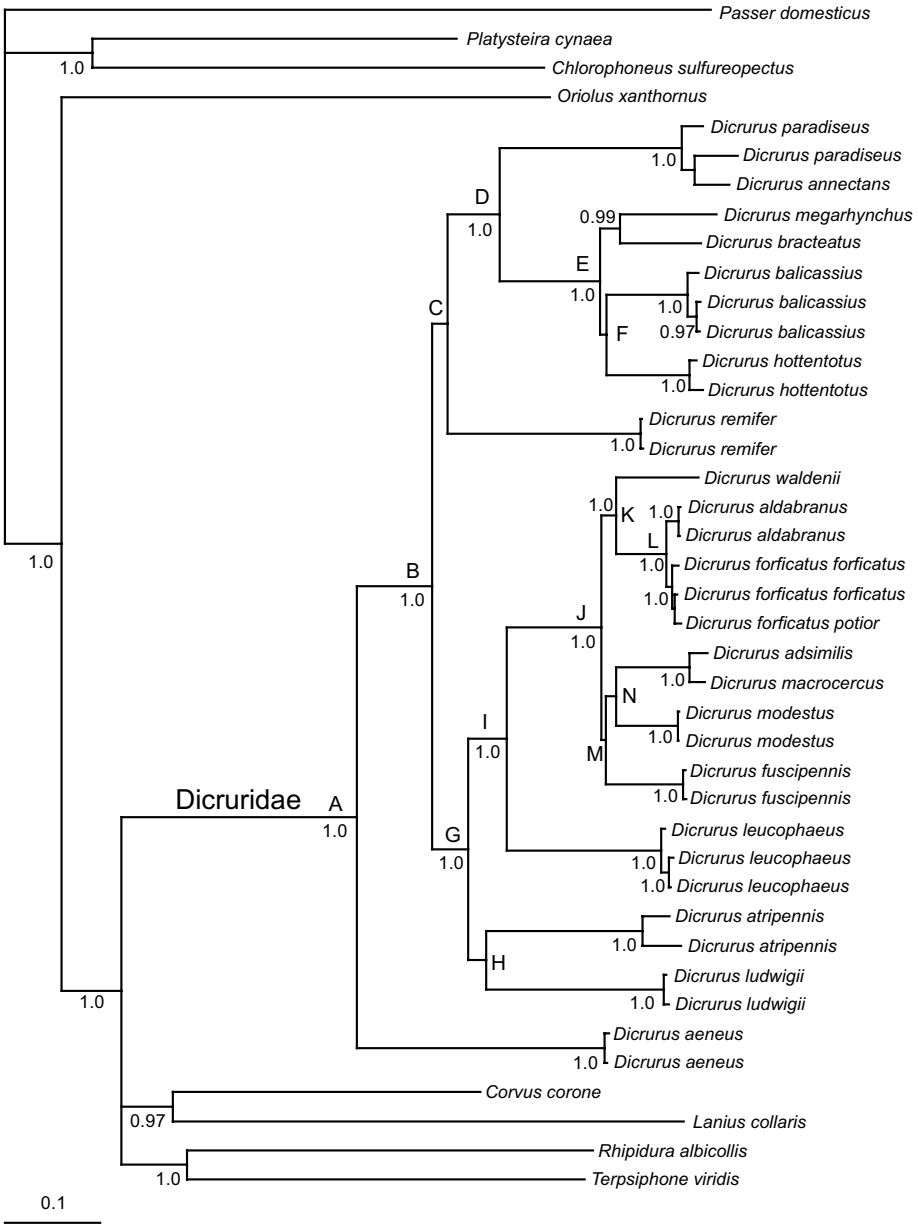

Fig. 2. Fifty percent majority-rule consensus tree (arithmetic mean $-\ln = 17305.84$) obtained with the concatenated dataset (myoglobin intron-2, cmos, ND2 and cytochrome *b*) under a mixed-model strategy with independent partitions corresponding to loci and codon positions (10 partitions). Values at node are BI posterior probability if greater or equal to 0.95. Letters close to nodes refer to those discussed in the text.

hottentotus, for which a supported relationship appears only between *megarhynchus* and *bracteatus*.

Estimates of divergence times within the Dicruridae, as obtained using either divergence between *Platysteira*/*Chlorophoneus* (29.5–38.3 Myr BP) or colonization of Aldabra (i.e., at or after 0.125 Myr BP), are very similar except for the two terminal nodes M and N (see Table 3 and Fig. 3). Analyses performed with calibration point 1 recover the *D. aldabranus*/*D. forficatus* split at 1.1 Myr (95% CI 0.4–2.3) instead of 0.125 Myr (Calibration point 3). Discrepancy between these two estimates can be attributed to the very recent age of the calibration point 3. Indeed, at such time scale, other factors than lack of gene flow, such as genetic drift, ancestral polymorphism or faster rate of molecular evolution in the 0–2 Myr windows may influence divergence time estimates (Ho et al., 2005; Ho and Larson, 2006). Analyses performed with calibration point 2 (colonization of Grand Comoro at or after 0.5 Myr BP) provided more recent values than those obtained with the two other calibration points (Table 3 and Fig. 3). As the analyses performed with calibration points 1 and 3 yield similar divergence times estimates for most nodes, we may consider these calibration points as congruent and combined them in order to obtain as accurate divergence time estimates as possible (Fig. 4).

Table 3
Estimates of divergence times (in Myr BP) for each node with its associated 95% confidence interval (in brackets), depending on the calibration point(s) used

Clade	1	2	3	2 + 3	1 + 3	Biogeographic event
Clade A	21.7 (15.5–28.9)	15.1 (7.5–25.1)	22.1 (15.6–29.6)	10.1 (5.9–17.6)	19.4 (13.4–26.3)	
Clade B	17.6 (12.1–24.3)	10.9 (4.8–19.9)	17.7 (11.2–24.5)	6.5 (3.7–11.9)	15.2 (10.3–21.2)	Colonisation of Africa
Clade C	16.9 (11.6–23.4)	10.5 (4.6–19.1)	16.9 (11.4–23.5)	6.2 (3.5–11.4)	14.6 (9.8–20.4)	(Node not supported)
Clade D	13.8 (9.0–19.6)	8.7 (3.7–16.0)	13.9 (9.0–20.1)	5.2 (2.9–9.6)	11.9 (7.7–17.1)	
Clade E	7.2 (4.4–11.3)	4.6 (1.9–8.6)	7.2 (4.3–11.0)	2.8 (1.4–5.2)	6.2 (3.7–9.5)	Dispersal over the Wallace line
Clade F	6.5 (3.9–10.1)	4.2 (1.7–7.7)	6.5 (3.9–10.6)	2.5 (1.3–4.8)	5.6 (3.3–8.6)	(Node not supported)
Clade G	15.6 (10.5–21.7)	9.1 (3.9–17.1)	15.4 (10.3–21.7)	5.2 (3.0–9.7)	13.3 (8.7–18.8)	
Clade H	14.5 (9.6–20.3)	8.5 (3.9–15.9)	14.4 (9.5–20.4)	4.8 (2.8–9.1)	12.4 (8–17.9)	(Node not supported)
Clade I	12.8 (8.4–18.9)	6.7 (2.7–13.5)	12.3 (7.8–17.8)	3.6 (2.0–6.9)	10.6 (6.6–15.6)	
Clade J	6.2 (3.7–9.7)	2.0 (0.7–4.6)	5.5 (3.2–8.5)	0.9 (0.5–1.8)	4.7 (2.7–7.4)	Expansion in Africa and on neighbouring islands
Clade K	5.6 (3.3–8.8)	1.8 (0.6–4.3)	4.9 (2.8–7.6)	0.8 (0.5–1.7)	4.2 (2.4–6.6)	Divergence Mayotte/Madagascar
Clade L	1.1 (0.4–2.3)	0.4 (0.1–1.2)	0.1 (0.04–0.12)	0.1 (0.04–0.12)	0.1 (0.05–0.12)	Colonisation of Aldabra
Clade M	5.6 (3.3–8.9)	0.5 (0.4–0.5)	5.0 (2.8–7.8)	0.5 (0.4–0.5)	4.3 (2.4–6.8)	Colonisation of Grand Comoro (node not supported)
Clade N	5.1 (2.9–8.0)	0.4 (0.2–0.5)	4.5 (2.5–7.1)	4.1 (2.9–4.9)	3.9 (2.2–6.2)	Colonisation of Principe (node not supported)
P. cyanea/*C. sulfureopectus*	36.2 (31.6–38.2)	37.1 (22.7–49.2)	43.4 (34.7–51.5)	29.7 (19.4–44.2)	36.0 (31.2–38.2)	

Codes are: 1, Calibration point *Platysteira*/*Chlorophoneus*; 2, Calibration point *D. fuscipennis*/sister-group; 3, Calibration point *D. aldabranus*/*D. forficatus*; 2 + 3, Combination of calibration points 2 and 3; 1 + 3, combination of calibration points 1 and 3. See Section 2 for details.

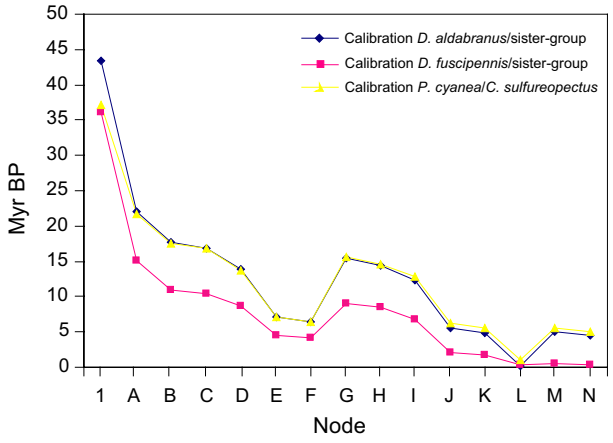

Fig. 3. Comparison of divergence date estimates for each clade using the three individual calibration points. Letters A–N refer to clades reported on Fig. 2, 1 refers to the calibration point *Platysteira*/*Chlorophoneus*. The figure highlights that analyses performed with calibration points *Platysteira*/*Chlorophoneus* and *Dicrurus aldabranus*/sister-group gave highly congruent estimates of divergence times. In contrast, dating analyses performed with the divergence *D. fuscipennis*/sister-group as a calibration point yielded estimates that are younger.

The dispersal-vicariance analyses resulted in an exact solution where four dispersals events are required to explain the current distribution of drongos at the biome levels (Fig. 4). At least one further dispersal event is required to explain their occurrence on the Indian Ocean Islands. The ancestral area of the Dicruridae was inferred to be either Indo-Malaya or Indo-Malaya and Africa.

4. Discussion

4.1. Molecular analysis in comparison to previous systematics

The comparison of our molecular phylogeny with the traditional taxonomy based on morphological characters given by Mayr and Vaurie (1948) highlights obvious discrepancies but also many agreements in the species arrangement. The main difference is that our phylogeny is not rooted with the "primitive" or "unspecialized" species, as defined by Mayr and Vaurie (1948), like *ludwigii* and *atripennis*, but with *aeneus*, a middle sized species (wing length 111–132) bearing high metallic gloss. Furthermore, *D. remifer* is not directly related to *D. aeneus* but occupies a basal position within the Asian species group (clade C, Fig. 2).

Nevertheless, if we root the tree proposed by Mayr and Vaurie (1948) with *D. aeneus* (instead of *D. ludwigii*), the same corresponding two groups now emerge. The clade G (note that *caerulescens* has not been sampled in our study) corresponds to their Section 1, "the primitive group" which consists of the relatively small species (mean species wing length from 107 to 152 mm), having some reduction in black coloration, often with brown wings and loss of gloss. The clade G includes all the species distributed in Africa and Indian Ocean islands as well as the Asian *D. leucophaeus*. Vaurie (1949) considered that the Indian Ocean Islands taxa (*aldabranus*, *forficatus*, *fuscipennis* and *waldenii*) and the continental *adsimilis* (including *modestus*) and *macrocercus* form a superspecies. This assemblage was recovered by our analyses with strong

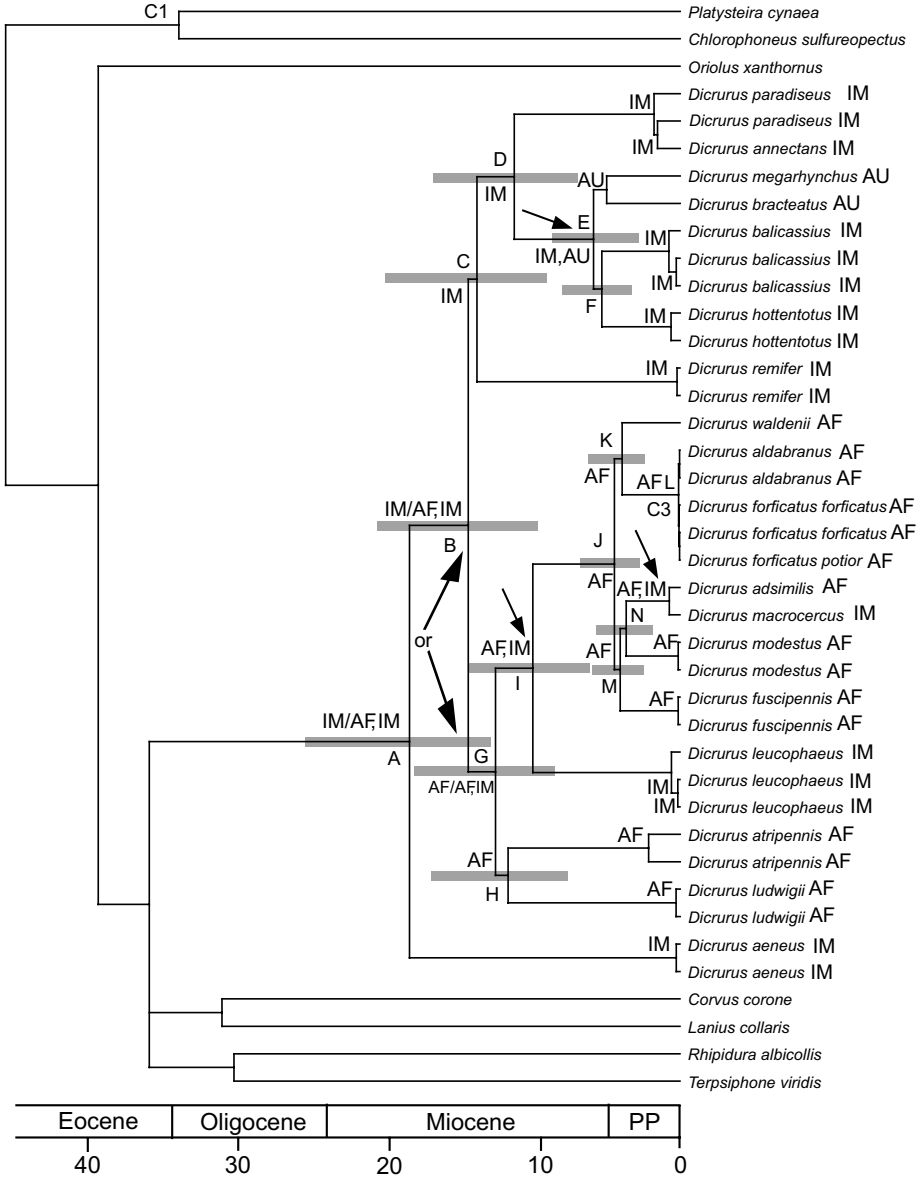

Fig. 4. Chronogram resulting from the dating analyses using the software MULTIDIVTIME and the topology obtained from the Bayesian analyses of the concatenated data set. Gray bars represents standard deviations. C1 and C3 indicate the location of the calibration points used for the dating analyses. The letters A–N refer to the clades discussed in the main text. The abbreviations AF (Africa including Indian Ocean Islands), AU (Australasia) and IM (Indo-Malaya) close to the nodes represent the ancestral area inferred from the dispersal-vicariance analyses performed with DIVA. All taxa but the Dicruridae were pruned from the data set for the biogeographic analyses. The arrows indicate the nodes where dispersal events between main biogeographic realms are involved. Coding states for the biogeographical analyses are indicated close to the taxon name. Note that at least one supplementary dispersal event is required to explain the occurrence of drongos on the Indian Ocean Islands (*D. forficatus*, *D. waldenii*, *D. aldabranus* and *D. fuscipennis*).

support (clade J). Yet, the high genetic distances among all these allopatric taxa suggest that the taxonomic level *superspecies* may not be adequate. As noted by Mayr and Vaurie (1948), the crested head has no phylogenetic value as it is present in both clades C (*hottentotus* and *paradiseus*) and K (*forficatus*); actually, this character appears highly variable even within species (e.g. *D. paradiseus*, Vaurie, 1949).

Our clade D corresponds to the upper part of their Section 2 (see Fig. 1)—"specialized group with irregular gloss and hackles and spangles" which only includes Asian and Australasian large species (mean species wing length from 137 to 184 mm), with irregular gloss and modified head feathers, as well as rackets-like modified tail feathers for *D. remifer* and *D. paradiseus*, modifications of the outer-tail feathers having not been achieved by the same way in each species (Mayr and Vaurie, 1948). *D. remifer* is actually closer to clade D (albeit in a non supported relationship) and less closely related to *D. aeneus* than previously thought (Mayr and Vaurie, 1948), the latter species occupying a basal position in our phylogenetic tree.

Within the clade D, *D. balicassius* belongs to the clade E together with the members of the superspecies *hottentotus*

of Vaurie (1949), *D. hottentotus*, *D. megarhynchus* and *D. bracteatus*. More samples are however needed to explore the relationships inside the clade D and particularly within this superspecies *hottentotus*, where several particular forms and subspecies, presenting aberrant and highly modified tails, occur on islands from Asia to New Guinea (e.g. *D. hottentotus densus* from Timor, *D. hottentotus menagei* from Tablas Islands). *D. annectans* was previously considered as a link between the primitive and specialized groups (Mayr and Vaurie, 1948). Unexpectedly, our analyses place it close to, or within our *paradiseus* samples with significant posterior probabilities. The relative high level of molecular divergence between our two *paradiseus* samples highlights the need of further phylogeographic studies within this complex (Vaurie, 1949).

4.2. Evolutionary history of the Dicruridae with particular reference to Indian Ocean islands

Presumably of Asian origin, as it can be inferred from our biogeographic analyses, the drongos colonized Africa at around 15 Myr (node B). The first large diversification of the family took place in both continents, respectively, at 13.3 and 11.9 Myr (clades G and D), followed by a dispersal event from Africa to Asia that led to the *leucophaeus* lineage (clade I, 10.6 Myr). Dispersal over Wallace line (clade E) occurred later, at ca. 6 Myr and led to the formation of *D. megarhynchus* and *D. bractaetus*. More or less contemporaneously, the ancestor of the *adsimilis* superspecies expanded into sub-Saharan Africa, Asia (Near East and Middle East countries have probably lost their populations more recently), and colonized Principe and the Indian Ocean islands (clade J, 5 Myr).

The endemic *D. modestus* from Principe has traditionally been considered as a subspecies of *adsimilis* (Vaurie, 1949). Actually, our analysis revealed that these two taxa are not so closely related, *modestus* having diverged early within the *adsimilis* group, while *adsimilis* and *macrocercus* were sister species. The topology of our phylogenetic tree and the high molecular divergence favored the status of *modestus* as a distinct species. This was proposed by some authors (Pearson, 2000; Dickinson, 2003), although it was still considered as a subspecies of *adsimilis* by others (e.g. BirdLife International, 2006). More samples from continental *adsimilis* subspecies, especially from those of Western Africa (e.g. *D. a. coracinus*), from which Principe was likely colonized, are needed before drawing definitive conclusions on the range of the *modestus* lineage.

Three of the four Comoro islands (namely Mayotte, Anjouan, Moheli) are older than 5 Myr (Nougier, 1986). However, only Mayotte hosts an endemic lineage, *D. waldenii* that is as ancient as the island. In contrast, Anjouan was very recently colonized by an immigrated population from Madagascar (and led to *D. forficatus potior*), while Moheli has no drongo at all. Grand Comoro, a volcanic island erected very recently (0.5 Myr), also hosts an endemic species, *D. fuscipennis*, which is slightly older than the other Indian Ocean forms (*D. waldenii*, *aldabranus*, *forficatus*). This clearly rules out the native origin of the Grand Comoro species (*fuscipennis*) and emphazises the need for another island that served as a stepping stone in the speciation process. Moheli is the closest island of Grand Comoro, and could thus have acted as a reservoir for a *Dicrurus* population that later disappeared from Moheli. It should be noted that both islands share three exclusive endemic species: Comoro Vanga *Cyanolanius comorensis* (possibly now extinct on Grand Comoro, Sinclair and Langrand, 1998), the bulbul *Hypsipetes parvirostris* and the sunbird *Nectarinia humbloti* (Sinclair and Langrand, 1998). Warren et al. (2003, 2005) investigated the phylogeography of these latter two taxa, and found actually dissimilar patterns. For *Nectarinia*, divergence between Moheli and Grand Comoro subspecies may not be older than 0.5 Myr (Warren et al., 2003), thus in accord with the age of Grand Comoro. Conversely, for *Hypsipetes*, the Moheli and Grand Comoro subspecies are not closely related, and may have diverged as long as 2.6 Myr ago (Warren et al., 2005), well before the formation of Grand Comoro. This latter scenario may apply for the *Dicrurus* case, and highlights that the recent volcanic islands of the Indian Ocean may have been subject to high extinction rates and turnover of species due to demographic factors or colonization of competing lineages. In any cases, these results suggest complicated biogeographic patterns for which we have only *ad hoc* explanations.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ympev.2007.03.010.

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