ORIGINAL ARTICLE

Phylogeography of the magpie-robin species complex (Aves: Turdidae: *Copsychus*) reveals a Philippine species, an interesting isolating barrier and unusual dispersal patterns in the Indian Ocean and Southeast Asia

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ABSTRACT

Aim The oriental magpie-robin (*Copsychus saularis*) of South and Southeast Asia is a phenotypically variable species that appears to be closely related to two endemic species of the western Indian Ocean: the Madagascar magpie-robin (*Copsychus albospecularis*) and the Seychelles magpie-robin (*Copsychus sechellarum*). This unusual distribution led us to examine evolutionary relationships in magpie-robins, and also the taxonomic significance of their plumage variation, via a molecular phylogenetic and population genetic analysis of *C. saularis* and *C. albospecularis*.

Location Southern Asia from Nepal across Indochina to southern China, and the Indian Ocean from Madagascar to the Greater Sunda and Philippine islands.

Methods We sequenced 1695 nucleotides of mitochondrial DNA comprising the complete second subunit of the nicotinamide adenine dinucleotide dehydrogenase (ND2) gene and 654 bases of the cytochrome *c* oxidase subunit I (COI) region in 51 individuals of eight *C. saularis* subspecies, 10 individuals of *C. albospecularis* (one subspecies) and single individuals of two other *Copsychus* species as outgroups. The data were analysed phylogenetically, with maximum likelihood, Bayesian, relaxed clock and parsimony methods, and geographically for patterns of genetic diversity.

Results Phylogenetic analysis indicated that *C. albospecularis* lies within the nominal *C. saularis*, making *C. saularis* polyphyletic. Malagasy and non-Philippine Asian populations form a monophyletic group that is sister to a clade of Philippine populations. Within non-Philippine Asian populations, two groups are evident: black-bellied birds in the eastern Greater Sunda islands and white-bellied birds in the western Sundas and on mainland Asia.

Main conclusions The phylogeny of magpie-robins suggests a novel pattern of dispersal and differentiation in the Old World. Ancestral magpie-robins appear to have spread widely among islands of the Indian Ocean in the Pliocene, probably aided by their affinity for coastal habitats. Populations subsequently became isolated in island groups, notably the Philippines, Madagascar and the Greater Sundas, leading to speciation in all three areas. Isolation in the Philippines may have been aided by competitive exclusion of *C. saularis* from Palawan by a congener, the white-vented shama (*Copsychus niger*). In the Greater Sundas,

white-bellied populations appear to have invaded Borneo and Java recently, where they hybridize with resident black-bellied birds.

INTRODUCTION

The complex geography and dynamic geological history of Southeast Asia has fostered tremendous species diversity and endemism (Heaney, 1991; Hall, 2001), and consequently many opportunities for biogeographical investigation (Wallace, 1883). However, despite this potential, Southeast Asian taxa have received much less attention than groups in other tropical areas, particularly with respect to molecular phylogeography (e.g. Kolbe *et al.*, 2008; Ricklefs & Bermingham, 2008). This neglect is disturbing given that Southeast Asia has suffered some of the highest levels of natural habitat destruction in the world (Sodhi *et al.*, 2004) and opportunities for research in natural settings are diminishing. The few phylogeographical studies that already exist for the region have revealed a remarkable range of evolutionary patterns and insights, from trivial diversification within widespread species (Fuchs *et al.*, 2008), to unusually high levels of diversification (Moyle *et al.*, 2005; Zou *et al.*, 2007), to the existence of cryptic species largely unrecognizable by morphology alone (Moyle *et al.*, 2005, 2008; Bickford *et al.*, 2007). Continued biogeographical work in Southeast Asia will undoubtedly provide much more insight into patterns and mechanisms of evolution, as well as into taxa and sites critical for conservation.

The oriental magpie-robin, *Copsychus saularis* (Linnaeus, 1758), is a common bird of Southeast Asia found at lower elevations in gardens, plantations, cultivated fields, coastal woodlands and mangroves from India and Sri Lanka east across Indochina and southern China and south to the Philippines and Sundaland (the Malay Peninsula, Borneo, Sumatra, Java and smaller islands on the Sunda continental shelf). As an excellent singer, this magpie-robin is often captured in the wild and sold in markets. It resembles a typical thrush (Turdidae: Turdinae) in its ground-feeding habit, upright posture and large size, but its nearest relatives are the chatlike robins (Saxicolinae), including scrub-robins (*Cercotrichas*), alethes (*Alethe*) and other small species distributed across Africa and southern Asia (Cibois & Cracraft, 2004; Voelker & Spellman, 2004). Within the genus *Copsychus*, which consists of seven species (Collar, 2005), *C. saularis* appears to be most closely related to the Madagascar magpie-robin, *Copsychus albospecularis* (Eydoux & Gervais, 1836), and the Seychelles magpie-robin, *Copsychus sechellarum* Newton, 1865, with which it is sometimes united in a superspecies (Sibley & Monroe, 1990).

Copsychus saularis is a black and white bird that exhibits substantial geographical variation in plumage and size. The number of recognized subspecies varies from 8 (Collar, 2005) to 17 (Ripley, 1964). Over most of its range, *C. saularis* has the plumage of its 'nominate group' (Collar, 2005), which includes a white belly and white outer tail feathers. Members of the '*mindanensis* group' in the Philippines have white bellies but no white in the tail, and those of the '*amoenus* group' in eastern Java and eastern Borneo have black bellies and (usually) white in the tail. In some subspecies, such as those in the Philippines and the Mentawai Islands of western Sumatra, differences in size and plumage seem minor. Across mainland Asia, some subspecific characters appear to be clinal and do not define distinct taxonomic units. In other cases, however, differences among populations appear substantial (e.g. black vs. white bellies, or black vs. black-and-white tails). On Borneo and Java, where the white-bellied and black-bellied birds meet, the two plumage morphs appear to hybridize (Mees, 1986), suggesting allopatric isolation followed by recent secondary contact. In addition to these natural patterns of plumage variation, there is also the possibility that humans have influenced geographical variation by transporting captive birds within and among regions.

We compared mitochondrial DNA sequences of populations across the range of *C. saularis* and its close congener *C. albospecularis* to address the evolutionary significance of plumage differences among populations and the phylogenetic accuracy of current species and subspecies classification. These comparisons permit consideration of the biogeographical and anthropogenic forces that have shaped the distribution of variation in these species. Although comprehensive sampling of every subspecies was not logistically feasible, we were able to assemble a geographically extensive dataset by sampling across the Sino-Indian region, on Madagascar, and in the Greater Sunda and Philippine islands.

MATERIALS AND METHODS

We compared 51 individuals of *C. saularis* representing eight subspecies (Fig. 1 and Appendix 1). Missing were subspecies from Sri Lanka, the Andamans, the west Sumatran islands and Java. In preliminary comparisons (F. Zou *et al.*, work in preparation), we examined the phylogeny of all species of *Copsychus* and determined two appropriate outgroups: white-rumped shama, *Copsychus malabaricus* (Scopoli, 1788), and white-browed shama, *Copsychus luzoniensis* (Kittlitz, 1832). Because the preliminary phylogeny indicated that *C. saularis* might be polyphyletic by including *C. albospecularis*, we included individuals of *C. albospecularis* in the current study.
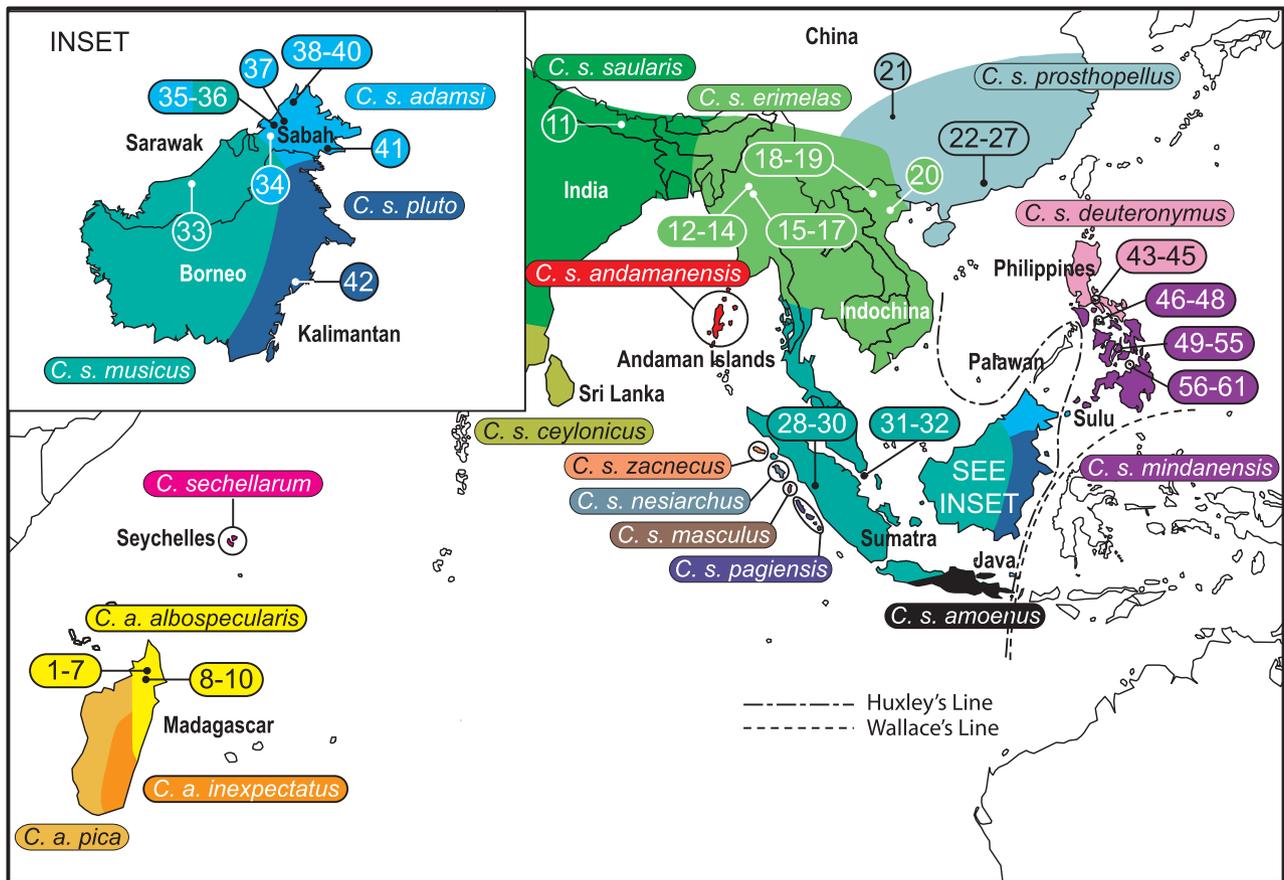
Figure 1 Distribution map of *Copsychus saularis*, *Copsychus albospecularis* and *Copsychus sechellarum*. Colours indicate ranges of subspecies from Dickinson (2003). Numbers indicate specimen localities (see Appendix 1). Distributions on Java and Borneo are from Mees (1986).

Voucher specimens were collected at all localities where wild birds were sampled, except one site in eastern Kalimantan (sample 42). Some non-vouchered captive birds are included in the study (Appendix 1) but are of little biogeographical value because their collecting localities are unknown and their skins unavailable for subspecies determination.

Total genomic DNA was extracted from muscle tissue or blood using proteinase K digestion following the manufacturer's protocol (DNeasy; Qiagen, http://www.qiagen.com/). Two mitochondrial genes were sequenced: the entire second subunit of nicotinamide adenine dinucleotide dehydrogenase (ND2) and the barcoding region of cytochrome *c* oxidase subunit I (COI). Primers and protocols for amplification and sequencing of ND2 generally followed Zou *et al.* (2007), but the gene was amplified in two overlapping fragments in some samples using primers L5215 paired with CopsyND2-R1 (TCMCTCAACCCCACACTMCTAG) and CopsyND2-F2 (GTTTGTGTTTGGTTTAGGCC) paired with H6313. COI was amplified and sequenced with primers PasserF1 (CCAAC-CACAAAGACATCGGAACC) and PasserR1 (GTAAACTTC-TGGGTGACCAAAGAATC) using the protocol of Lohman *et al.* (2009). Sequences have been deposited in GenBank (Appendix 1).

Nucleotide site characteristics were determined with MEGA version 3 (Kumar *et al.*, 2004). A maximum likelihood (ML) model for phylogenetic estimation was determined using the Akaike information criterion in MrModeltest (Nylander, 2004). Maximum likelihood trees were constructed with PAUP* version 4.0b10 (Swofford, 2002), using random sequence additions with 10 replicates, branch swapping by tree bisection–reconnection and branch support assessment via 100 nonparametric bootstrap replicates (Felsenstein, 1985). Mixed model Bayesian phylogenetic analysis was conducted with MrBayes version 3.1.2 (Huelsenbeck & Ronquist, 2001). Both genes were partitioned by codon position and gene, and models for each partition were determined with MrModel-test. Two simultaneous runs of five million generations were conducted, each sampled every 100 generations, running four Metropolis coupling chains with the heating scheme set to default and discarding the first 25% of sampled trees as burn-in. Nodal support in the parsimony analysis was assessed with 10,000 symmetric resampling bootstrap replicates (Goloboff *et al.*, 2003) using a traditional search with 33% change probability performed with TNT 1.1 (Goloboff *et al.*, 2008). Jackknife support was assessed with 10,000 replicates using a traditional search with a 36% removal probability.

Adherence of the *Copsychus* tree to a molecular clock was tested with the likelihood ratio test (Huelsenbeck & Rannala, 1997) implemented in paup*. Confidence limits on divergence dates were calculated using a relaxed clock, uncorrelated lognormal model in the program beast version 1.4.7 (Drummond & Rambaut, 2007). The uncorrelated lognormal model performs well when data are roughly clock-like, as ours were (Drummond *et al.*, 2006). In the absence of relevant calibration points to date nodes, we assumed a rate of 2% Myr^{-1} (Weir & Schluter, 2008). Ancestral polymorphism can cause an overestimate of the age of recent divergence dates (Ho, 2007), but this is a minor problem for most of the important *Copsychus* nodes because of their relatively old ages (see Results). Based on the average effective population size, Edwards & Beerli (2000) and Weir & Schluter (2008) recommended corrections of 175,000 and 185,000 years, respectively.

Parsimony haplotype networks were constructed using tcs version 1.21 (Clement *et al.*, 2000) on concatenated ND2 + COI sequences (the 52 nucleotide sites that had missing data in any sample were excluded from the analysis). Indices of molecular diversity were calculated using DnaSP version 4.5 (Rozas *et al.*, 2003) for samples grouped by geographically defined populations or subspecies (Table 1). To determine whether individual populations evolved according to the Wright–Fisher model and whether substitutions were neutral, we calculated Fu's F_s statistic (Fu, 1997) and Tajima's D statistic (Tajima, 1989), and also performed the McDonald–Kreitman test (McDonald & Kreitman, 1991) using DnaSP. The significance of the F_s statistic was tested via Markov chain Monte Carlo simulations, and Tajima's D was tested against the null assumption of a beta distribution. We also investigated population structure under the framework of nested analysis of molecular variance (AMOVA), computing the fraction of total genetic variation distributed within and among clades and subspecies using Tamura–Nei (TrN) distances (Tamura & Nei, 1993). The significance of this partitioning against a null model of no significant difference was tested with a permutation procedure (Excoffier *et al.*, 1992). AMOVAs and related statistics were calculated with arlequin version 3.1 (Excoffier *et al.*, 2005). Tabulation of fixed nucleotide differences was accomplished with DnaSP.

RESULTS

We obtained *c.* 1695 nucleotides of sequence for each individual. These data consisted of the entire ND2 gene (1041 bases) and 654 bases of COI corresponding to chicken sites 6699–7349 (Desjardins & Morais, 1990). Data for 52 of the 1695 sites (including four parsimoniously informative sites) were missing in some taxa. The ND2 and COI sequences appeared to be typical mitochondrial coding genes, with appropriate rates of codon-site substitution and no stop codons, insertions, or deletions. Among all samples, 5.0% of ND2 nucleotide sites varied, whereas 2.0% of COI sites varied. Within all but one geographical region, Madagascar, the

percentage of transitions, transversions and total polymorphic sites was higher in ND2 (Table 1). Sequences of *C. albospecularis* had the lowest haplotype diversity for ND2, and the highest haplotype diversity for COI (Table 1).

Model testing for ML analysis indicated GTR + Γ as the appropriate model with the following parameters: base frequencies, A = 30%, C = 35%, G = 12% and T = 23%; substitution rates, A → C 1.81, A → G 47.92, A → T 0.98, C → G 1.80, G → T 22.11; shape parameter of the Γ distribution (α) = 0.15. For Bayesian tree construction, the six codon partitions were treated with the following models (partitions 1, 2 and 3 represent ND2 codon positions 1, 2 and 3; partitions 4, 5 and 6 represent COI positions 1, 2 and 3): partitions 1 and 2, HKY85 + Γ + I (Hasegawa *et al.*, 1985); 3 and 6, GTR + Γ; 4, K80 (Kimura, 1980); and 5, F81 (Felsenstein, 1981). All phylogenetic analyses (Fig. 2) indicated two distinct clades of nominal *C. saularis*: Philippine populations vs. all other populations, including *C. albospecularis*. Within the Philippine clade, we detected no genetic differences between nominal subspecies or among sampling localities. The Philippine clade was sister to a clade consisting of two well-supported, basally bifurcating lineages: *C. albospecularis* from Madagascar and non-Philippine *C. saularis*. The latter showed substantial geographical structure. Populations of the black-bellied subspecies *adamsi* and *pluto* in eastern Borneo formed the sister of the white-bellied subspecies *musicus* (from western Borneo, Sumatra, Singapore) and *saularis*, *erimelas* and *prosthopellus* (from the Sino-Indian region). The phylogenetic position of the Bornean members of *musicus* (nos 33 and 36 in Figs 2 and 3) was unresolved, but they were marginally closer to Sumatran and Singaporean populations than to mainland populations (Table 2). Among the Sino-Indian populations, no relationship was evident between genetic variation and geographical location or subspecies.

The likelihood ratio test of evolutionary rates revealed no significant differences among clades. beast analysis indicated that Philippine populations were separated from others about 3.7 Ma, with a 95% confidence interval ranging from 2.2 to 5.2 Ma. If the rate is doubled to 4%, Philippine populations were isolated 1.1 Ma at the earliest. Under the 2% rate, the Malagasy population diverged from non-Philippine Asian populations about 2.0 (1.2–2.8) Ma, eastern Bornean populations diverged from mainland populations about 1.2 (0.8–1.7) Ma, and western Bornean *musicus* diverged from other *musicus* populations 468,000 (256,200–708,000) years ago.

Four separate parsimony haplotype networks were formed with a 99% connection limit. None of these networks could be joined to another within a connection limit of 90%, the lowest limit allowed by tcs 1.21 (Fig. 3). About one-half (31/61) of the specimens shared their ND2 + COI haplotype with at least one other specimen. The network spanning the greatest divergence comprised mainland Asia and western Sunda populations (Fig. 3d); at least 11 substitutions separated mainland Asia from Sumatra, Singapore and western Borneo. Convergence of mutations at identical nucleotide sites in several *C. albospecularis* specimens from Madagascar led to a

Table 1 Molecular diversity and tests of neutral evolution of *Copsychus saularis* and *Copsychus albospecularis* populations grouped by geographical region and clades (see Figs 2 and 3).

	No.	n	S (%)	No. ts (%)	No. tv (%)	k (var)	$h \pm$ SD	$\pi \pm$ SD	$\theta_{W} \pm$ SD	Tajima's D		Fu's F_s	
										D	P (D)	F_s	P (F_s)
COI													
A. Philippines	19	3	3 (0.46)	3 (0.46)	0 (0)	0.620 (0.260)	0.485 ± 0.104	0.00100 ± 0.00032	0.00139 ± 0.0009	−0.755	0.252	0.160	0.297
B. Madagascar (*C. albospecularis*)	10	6	5 (0.76)	4 (0.61)	1 (0.15)	1.311 (0.793)	0.844 ± 0.103	0.00205 ± 0.00047	0.00277 ± 0.0016	−1.035	0.181	−3.023	0.038
C. Borneo (*C. saularis adamsi* + *C. saularis pluto* only)	8	3	2 (0.31)	2 (0.31)	0 (0)	0.500 (0.222)	0.464 ± 0.200	0.00077 ± 0.00037	0.00119 ± 0.00092	−1.310	0.101	−0.999	0.207
D. Mainland + *C. saularis musicus*	24	8	15 (2.29)	13 (1.99)	2 (0.31)	3.634 (3.639)	0.757 ± 0.075	0.00570 ± 0.00083	0.00589 ± 0.00241	−0.266	0.443	0.212	0.192
E. Western Sundaland (*C. saularis musicus*)	7	5	8 (1.22)	7 (1.07)	1 (0.15)	3.33 (1.030)	0.857 ± 0.137	0.00514 ± 0.00131	0.00504 ± 0.00276	0.109	0.568	−0.495	0.259
F. Mainland (Sino-Indian) populations	17	4	5 (0.76)	4 (0.62)	1 (0.15)	1.382 (0.799)	0.551 ± 0.116	0.00217 ± 0.00054	0.00232 ± 0.0013	−0.211	0.443	0.562	0.265
ND2													
A. Philippines	19	12	17 (1.63)	14 (2.14)	3 (0.46)	3.953 (4.287)	0.947 ± 0.030	0.00383 ± 0.00040	0.00472 ± 0.0019	−0.709	0.261	−4.068	0.013
B. Madagascar (*C. albospecularis*)	10	2	2 (0.19)	2 (0.30)	0 (0)	0.711 (0.337)	0.356 ± 0.159	0.00068 ± 0.00031	0.00068 ± 0.0005	0.019	0.628	1.523	0.360
C. Borneo (*C. saularis adamsi* + *C. saularis pluto* only)	8	6	11 (1.06)	9 (0.86)	2 (0.19)	2.929 (2.934)	0.893 ± 0.111	0.00281 ± 0.00089	0.00408 ± 0.00207	−1.547	0.0466	−1.666	0.124
D. Mainland + *C. saularis musicus*	24	13	29 (2.79)	28 (2.69)	1 (0.10)	6.757 (10.889)	0.928 ± 0.030	0.00649 ± 0.00076	0.00746 ± 0.00273	−0.488	0.347	−1.396	0.100
E. Western Sundaland (*C. saularis musicus*)	7	4	13 (1.25)	13 (1.25)	0 (0)	5.333 (8.573)	0.714 ± 0.181	0.00512 ± 0.00131	0.00510 ± 0.00260	0.0281	0.525	1.874	0.265
F. Mainland (Sino-Indian) populations	17	9	14 (1.34)	13 (1.99)	1 (0.15)	3.676 (3.825)	0.897 ± 0.048	0.00353 ± 0.00052	0.00398 ± 0.0017	−0.429	0.364	−1.599	0.101

No., number of samples; n, number of haplotypes; S, number of segregating (polymorphic) sites; No. ts, number of transitions (group average in relation to all other sequences; also expressed as the total number of transitions/transversions as a percentage of nucleotide sites within each gene); No. tv, number of transversions (see No. ts); k, average number of nucleotide differences; h, haplotype diversity and its standard deviation (Nei, 1987); π, nucleotide diversity and its standard deviation (Tajima, 1983); θ_{W}, population parameter from segregation sites (Weir & Cockerham, 1984).
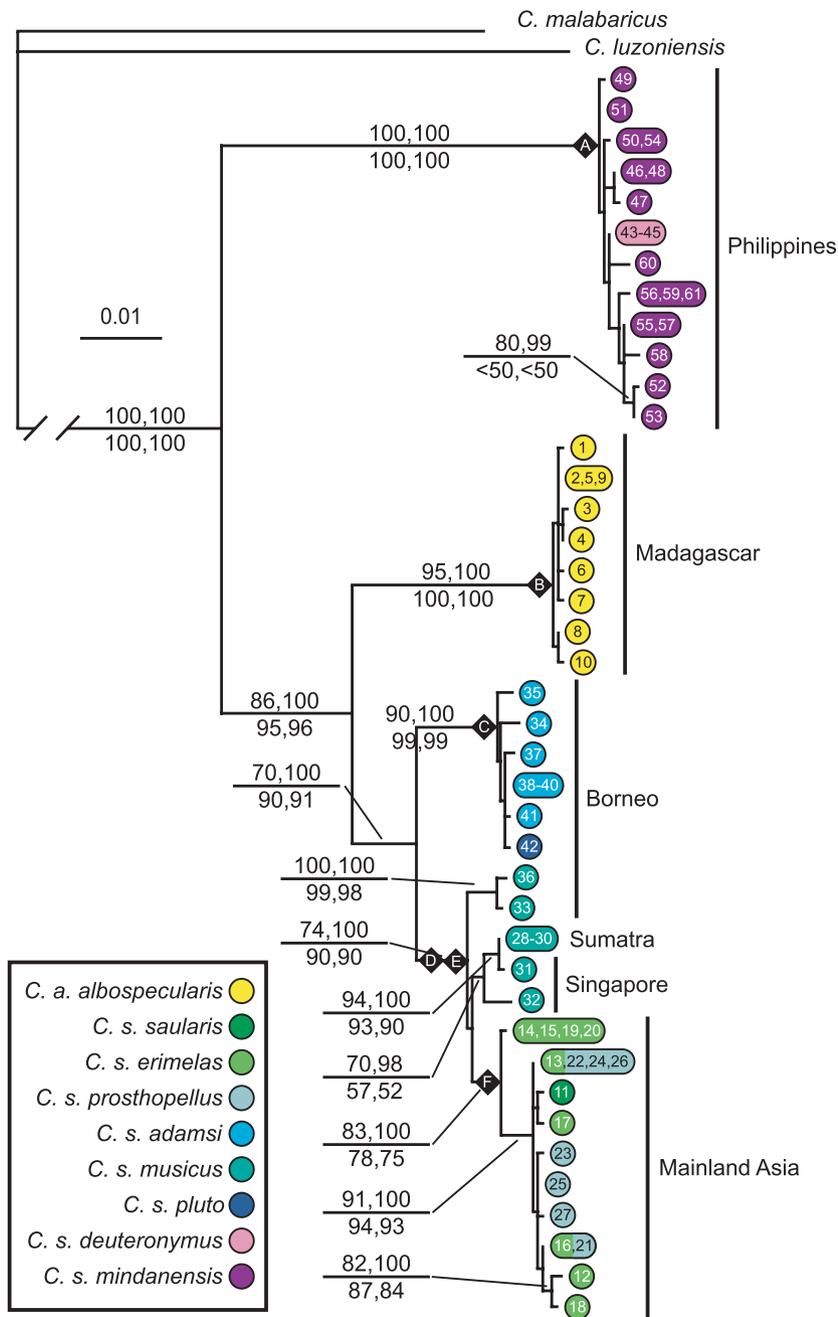
Figure 2 Maximum likelihood phylogenetic estimate of *Copsychus saularis* and *Copsychus albospecularis* mitochondrial haplotypes based on a maximum of 1695 bp of mtDNA sequence (ND2 and COI). Collection and locality information for each numbered sample is provided in Fig. 1 and Appendix 1. The colour of each sample corresponds to its nominal subspecies. Numbers above branches indicate maximum likelihood bootstrap support and Bayesian posterior probabilities, respectively, and support measures below branches indicate symmetric resampling and jackknife parsimony support, respectively. Letters denote clades used in population genetics analyses (Table 1). The letter 'E' denotes the *C. saularis* subspecies *musicus*. Because this clade does not appear on the tree, we have placed 'E' on the same branch as 'D', which denotes the combined western Sunda and mainland clade.

Table 2 Uncorrected pairwise distances among major clades of *Copsychus saularis* and *Copsychus albospecularis*.

	Copsychus luzoniensis	Philippines	Madagascar (*C. albospecularis*)	East Borneo (*C. saularis adamsi* + *C. saularis pluto*)	West Borneo (*C. saularis musicus*)	Sumatra and Singapore (*C. saularis musicus*)
Philippines	0.144					
Madagascar	0.131	0.063				
East Borneo	0.134	0.059	0.039			
West Borneo	0.136	0.062	0.035	0.021		
Sumatra + Singapore	0.134	0.062	0.037	0.019	0.008	
Mainland	0.132	0.057	0.039	0.020	0.011	0.009

Figure 3 Most parsimonious haplotype networks of *Copsychus saularis* and *Copsychus albospecularis* based on 1695-bp sequences of mtDNA (ND2 and COI) calculated with a connection limit of 99%. Collection and locality information for each numbered sample is provided in Fig. 1 and Appendix 1. The colour of each sample corresponds to its nominal subspecies. Letters denote clades used in population genetics analysis (Table 1).
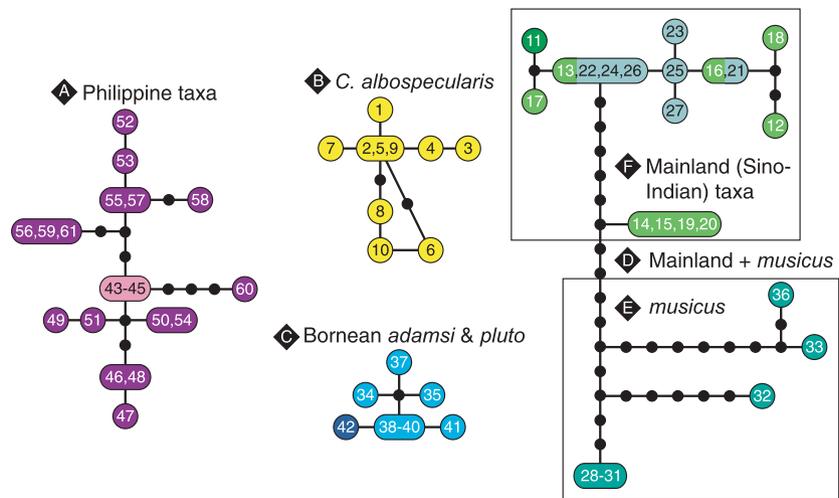
single loop (Fig. 3b). When the 52 sites with missing data were included, changes to the resulting haplotype trees were minor and did not change the conclusions (see below). AMOVA determined that more than 90% of molecular variance was partitioned among rather than within the four main haplotype groups (Table 3). Genetic differences among these groups were significant under the null hypothesis of no difference. In summary, haplotypes separate into four distinct clusters and genetic variation is generally geographically structured, but subspecies do not tend to be genetically cohesive entities divergent from other subspecies located in the same region. The only exception is *musicus* in western Borneo vs. *adamsi* and *pluto* in eastern Borneo.

Our molecular diversity and allelic distribution data suggest no strong departures from the neutral Fisher–Wright model of mutation in any population (Table 1). None of the groups had values significantly different from zero for both Tajima's D and Fu's F_s at the same time. Pairwise McDonald–Kreitman tests of amino acid selection between clades were not significant. For both genes, the group consisting of *musicus* and Sino-Indian taxa had the greatest amount of molecular diversity. This is

probably a function of its wide geographical distribution and inclusion of several modestly distinct clades. Sino-Indian populations had a low level of diversity (as indicated by π and θ_W), whereas *musicus* was unusually diverse because of the west Bornean population (Fig. 3). Genetic diversity within the Philippines might have been increased by divergence among the multiple islands we sampled. In general, these estimators of genetic diversity should be regarded as suggestive and tentative, as logistical considerations prevented intensive sampling in many areas and because isolation as well as genetic drift in small island populations may have influenced some more than others.

DISCUSSION

Biogeography

The inclusion of *C. albospecularis* (and almost certainly *C. sechellarum*) within *C. saularis* results in a species complex encompassing three faunal regions: the oceanic islands of the Philippines, the mainland and continental islands of Southeast Asia, and the isolated islands of the western Indian Ocean (Madagascar and the Seychelles). The ancestor of this complex appears to have been distributed widely in the Indian Ocean and South China Sea in the Pliocene. Philippine populations became isolated, probably in the mid to late Pliocene (assuming the 2% clock), followed by the Malagasy and non-Philippine Asian populations. In Southeast Asia, black-bellied birds on Borneo (and probably Java) were separated from white-bellied populations in the western Sundas and mainland Asia in the early Pleistocene. Most recently, white-bellied birds appear to have invaded the interior of South Asia and the islands of Borneo and Java, where they are hybridizing with black-bellied birds. This scenario suggests an impressive initial dispersal of ancestral magpie-robins across the Indian Ocean. It also suggests invasion of the interior of mainland Asia from Sundaland, which is opposite to the traditional Southeast Asian model of colonization from the mainland to islands (Heaney, 1986; Inger & Voris, 2001). Such unanticipated

Table 3 AMOVA of *Copsychus saularis* and *Copsychus albospecularis* genetic variation by four major clades (A–D, Fig. 2) and by subspecies.

Source	d.f.	SS	Variance	%
Clades				
Among clades	3	1752.844	40.57994	92.48
Within clades	57	188.150	3.30089	7.52
Total	60	1940.995	43.88083	
			$P = 0.0000$	
Subspecies				
Among subspecies	8	1784.475	34.44653	92.07
Within subspecies	52	154.37	2.96866	7.93
Total	60	1938.846	37.41519	
			$P = 0.0000$	

SS, sum of squares.

dispersal patterns, however, are not unreasonable given the ecological characteristics of magpie-robins, the geography of the three regions they inhabit, and growing evidence of invasion from islands to mainland areas (Bellemain & Ricklefs, 2008).

The Philippine Islands are an important area of diversification for the genus *Copsychus*, as they host four of the seven species, including three endemics: white-browed shama (*C. luzoniensis*), white-vented shama (*Copsychus niger*) and black shama (*Copsychus cebuensis*). This diversity is probably a function of isolation caused by the Philippines' archipelagic geography. Many of the islands have never been connected to the Asian mainland and some have never been connected to another island (Sathiamurthy & Voris, 2006). The only exception is Palawan, which lies on the Sunda shelf and forms a potential conduit between the Philippines and the rest of Sundaland via Borneo. Palawan was most recently connected to Borneo 160,000 years ago during a period of especially low sea level (Heaney & Rickart, 1990). The Sulu Archipelago, which approaches the eastern coast of Sabah in north Borneo, is another potential conduit, although it has never been connected to Borneo.

Given the endemicity of *Copsychus* in the Philippines, it is not unreasonable to suggest that ancestral magpie-robins arose in the Philippines and spread west. Although this may be true, molecular dating indicates that the Philippine magpie-robins have been separated from other populations since the Pliocene, despite lowered sea levels in the Pleistocene that would have facilitated dispersal to Borneo via Palawan. Their long isolation in the Philippines may be explained by the fact that magpie-robins do not occur on Palawan. They are replaced on that island by *C. niger*, which is a remarkably similar but not particularly closely related species (F. Zou *et al.*, work in preparation). This congener may have excluded magpie-robins from Palawan by competition and thus prevented gene flow between the northern Philippines and Borneo. The possibility of such isolation by competitive exclusion is an interesting addition to the usual list of extrinsic barriers to gene flow (Mayr, 1964).

Dispersal of magpie-robins between Borneo and the Sulu Archipelago may have been restricted by a persistent, deep water barrier corresponding to Wallace's line. However, we have not examined the genetics of magpie-robins in the Philippines south of Camiguin Sur, which is north of Mindanao. Given the close proximity of the Sulu Archipelago to eastern Sabah, it is possible that genetic exchange has occurred between these two areas, either by natural causes or human intervention. Such exchange would explain the Filipino-like black tails of *C. saularis adamsi* in Sabah. It would also imply that magpie-robins in the Sulu region are substantially different genetically from magpie-robins in the northern Philippines.

In the western Indian Ocean, biogeographical hypotheses about the spread of magpie-robins also need to be placed in a larger temporal context. Madagascar has been separated from Africa for over 160 Myr and fully isolated in the Indian Ocean for more than 90 Myr, after the separation of Indo-Madagas-

car (de Wit, 2003). As the Indian plate moved northwards, several massive portions broke off about 75 Ma forming the granitic Seychelles Islands, which comprise the known extant distribution of *C. sechellarum* (Skerrett *et al.*, 2001). Subsequently, neither Madagascar nor the granitic islands of the Seychelles had land connections. These events took place well before the divergence of *Copsychus* from its most recent common ancestor. More recently, the original human colonizers of Madagascar came from southern Borneo rather than Africa, as evidenced by linguistic, archaeological and genetic comparisons (Adelaar, 1995; Hurles *et al.*, 2005). This westward invasion raises the possibility that humans brought *Copsychus* to the island from Southeast Asia. However, human colonization of Madagascar occurred approximately 2300 years ago (Burney *et al.*, 2004; Perez *et al.*, 2005), which is too recent to account for the development of substantial plumage variation in *C. albospecularis* on Madagascar and the 3.5–3.9% sequence divergence between Malagasy and Southeast Asian members of the magpie-robin complex (Table 2).

This leaves the question of how the progenitor of *C. albospecularis,* a 25-g bird, could traverse 5000 km of ocean from Southeast Asia to Madagascar. Although a significant proportion of the modern land vertebrates of Madagascar dispersed from Africa, there are examples of Malagasy animals in taxa of Asiatic origin, both non-flying (Kosuch *et al.*, 2001) and flying (Griveaud, 1967). Most pertinent for this discussion are bulbuls in the genus *Hypsipetes* that appear to have colonized islands of the western Indian Ocean from Asia in a single wave within the last 2.6 Myr (Warren *et al.*, 2005). Another example is the bat genus *Emballonura*, which is particularly diverse in Southeast Asia. The westernmost Asiatic species is *Emballonura monticola*, found in southern Myanmar (Burma) and Sundaland (Simmons, 2005). Two endemic *Emballonura* species occur in Madagascar, but the genus is unknown in the intervening areas of South Asia and Africa (Goodman *et al.*, 2006). The ability of Asian animals to disperse to Madagascar over thousands of kilometres of Indian Ocean must have depended on fortuitous circumstances. These may have included strong easterly winds, such as those that enabled early human colonizers to sail vessels to Madagascar from the Sunda Islands, as well as lowered sea levels that exposed substantial island stepping stones 1.6 and 2.4 Ma (Haq *et al.*, 1987; Warren *et al.*, 2005). The ability of birds to colonize Indian Ocean islands would also have depended on a variety of ecological variables, such as habitat tolerance and reduced predation and competition. Isolated islands typically have few predators and competitors. They also feature landscapes attractive to *C. saularis*, most notably mangroves, beach strand and other coastal habitats (Collar, 2005).

In Southeast Asia, organisms are generally viewed as having spread from the mainland to the Greater Sunda Islands (Cracraft, 1988; Inger & Voris, 2001). However, as Reddy (2008) and others have discovered, the pattern of movement in Southeast Asia is often unexpected and complex. In many other parts of the world, invasion of the mainland from islands is well documented (Bellemain & Ricklefs, 2008). Thus, there is

no reason to believe such invasion has not occurred commonly in Southeast Asia as well. *Copsychus saularis* apparently spread first among islands and coastal areas before invading the interior of mainland Asia. Recent movement of the species away from the coast is suggested by the lack of genetic structure among the Sino-Indian populations. Inland dispersal may have been aided by the opening of forested lands by humans and the consequent creation of upland habitat appropriate for *C. saularis*. It might also have been helped by the transportation of caged birds.

An immediate effect of human influence on *C. saularis* is evident in our samples from Singapore. The once common *C. saularis* has become extremely rare in Singapore. To prevent its extirpation, wildlife managers have begun supplementing the Singapore population with individuals collected elsewhere (Wang & Hails, 2007). The substantial divergence between our two Singaporean individuals (nos. 31 and 32), both of which were salvaged after being found dead, suggests that one or the other or both were imported: 31 probably from Sumatra and 32 from Malaya or Java, based on genotype comparisons with Sumatran and Bornean individuals.

In Sundaland, distinct black-bellied and white-bellied populations of *C. saularis* appear to have evolved in isolation in the east and west, respectively, after their ancestors were separated in the early Pleistocene (*c.* 1 Ma). The two morphs have come into contact in Borneo and Java fairly recently, apparently as a result of invasion by white-bellied birds from the west (Mees, 1986). This scenario concurs with a general pattern that is emerging from genetic studies of Bornean groups: populations (or taxa) on the western side of Borneo are closer genetically to Malayan and Sumatran populations, whereas those on the eastern side are closer to Javan populations (Han, 2000; Moyle *et al.*, 2005, 2008). Early isolation on Borneo followed by recent invasion from the west explains why distinct subspecies of birds are found in Sabah and eastern Kalimantan when no current barriers exist to separate them. Past barriers to dispersal, apart from sea water, may have included extensive Pleistocene savanna between Borneo and the western Sundas during periods of lowered sea level (Bird *et al.*, 2005). However, it is difficult to relate the occurrence of this habitat to movements of *C. saularis* because of the uncertainty of timing (prior to the most recent glacial maximum 20,000 years ago) and the possibility that magpie-robins thrived in woodlands associated with savanna.

Phylogeny and taxonomy

Copsychus saularis needs to be revised because *C. albospecularis* of Madagascar lies between Philippine and other populations of *C. saularis*, making nominal *C. saularis* polyphyletic. This problem cannot be solved by merging *C. albospecularis* into *C. saularis* because *C. albospecularis* is well diverged from other magpie-robins (3.5–3.9%) and is phenotypically distinct; it is smaller than its Asian counterparts and the females differ from all other magpie-robins in having pale grey and rufous brown backs. The Seychelles

magpie-robin, *C. sechellarum,* is also distinct from *C. saularis*. Although we were not able to compare this critically endangered species genetically, it is relatively large and all black (except for the white shoulder patch) with an elongate head and bill, and it is the only magpie-robin in which the males and females have similar plumage (Collar, 2005). The Philippine *C. saularis* merits distinction as a species, first because it is not monophyletic with other populations of *C. saularis* and second because it is morphologically diagnosable (white bellies and black tails) and has substantially diverged genetically (i.e. by 6%). The appropriate name for this taxon is the Philippine magpie-robin, *Copsychus mindanensis* (Boddaert, 1783). The two subspecies of *C. mindanensis* (*mindanensis* and *deuteronymus*) do not form distinct genetic or geographical groups and are morphologically distinguished from one another only by a minor plumage character: the underparts of females of *C. mindanensis deuteronymus* have a buff wash (Parkes, 1962). We recommend that *deuteronymus* no longer be recognized, rendering *C. mindanensis* monotypic.

In the newly constituted *C. saularis*, three major phylogenetic groups of subspecies are supported (Figs 2 and 3): (1) Sino-Indian *saularis*, *erimelas* and *prosthopellus*; (2) western Sundaic *musicus*; and (3) eastern Sundaic *adamsi*, *pluto* and *amoenus*. We were not able to obtain genetic samples of subspecies from Sri Lanka, the Andamans or the western Sumatran Mentawai islands, but assume based on plumage that these are part of *C. saularis*. The Sino-Indian taxa exhibited no geographical structure and can be lumped into the single subspecies *saularis* based on plumage (Collar, 2005). Genetic differences between the Sino-Indian birds and subspecies *musicus* are small (about 1%). Without sampling from the Thai-Malay Peninsula, it is not possible to determine whether the difference between these clusters is clinal or due to an abrupt genetic discontinuity, as might be expected at the Isthmus of Kra (Hughes *et al.*, 2003). Another uncertainty concerns the phylogenetic position of the Bornean population of subspecies *musicus*. Plumage similarity suggests it comprises a clade that is sister to Sumatran and Singaporean populations of subspecies *musicus* (Mees, 1986), but genetically it is distinct (Fig. 3d). Pending further investigation, it seems reasonable to keep the western Bornean population in subspecies *musicus* and to use that name as a convenient label to distinguish birds in western Sundaland from those in the Sino-Indian region and in eastern Sundaland.

The only substantially distinct genetic group in the newly constituted *C. saularis* consists of the black-bellied subspecies in eastern Borneo, *adamsi* and *pluto*, and presumably *amoenus* in eastern Java. The black-bellied taxa hybridize with white-bellied individuals of *C. saularis musicus* on Borneo and Java (Fig. 1), and individuals with introgressed plumage occur widely on both sides of the contact zones (Mees, 1986). For example, we collected specimens with *C. saularis musicus* and *C. saularis adamsi* genotypes at Klias Forest Reserve, Sabah (nos 35 and 36, respectively). The *musicus* specimen had plumage typical of the subspecies, but the genetic *adamsi*

individual, which should have been entirely black, had some white in its tail feathers. Although our genetic comparisons did not include the Javan subspecies *amoenus*, morphological similarity between subspecies *pluto* and *amoenus* is substantial – they can only be distinguished by size (Mees, 1986). This similarity, combined with the genetic cohesiveness and plumage variation of *adamsi* and *pluto* (Figs 2 and 3c), suggests that the three taxa may be lumped into a single subspecies, *amoenus*.

Predictions and future work

From our interpretation of magpie-robin biogeography, we can predict patterns that will be produced by better sampling in future studies. If the ancestors of *C. albospecularis* moved rapidly to Madagascar by island hopping, we would expect isolated Indian Ocean populations, such as those on the Seychelles, Sri Lanka and the Andamans, to be approximately the same age as *C. albospecularis* and exhibit similar relationships to Asian taxa. If the population on Sri Lanka is a relict of this early invasion, this would explain the existence of two distinct forms of *C. saularis* in south India; subspecies *saularis* would have invaded deeply into the subcontinent from the coast, whereas subspecies *ceylonensis* would have colonized the tip of the subcontinent recently from Sri Lanka. The occurrence of early invaders on oceanic islands would also explain the plethora of subspecies described for the western Sumatran islands, particularly the oceanic islands of Simeuluë, Nias and Mentawi, where prolonged isolation would be expected to produce distinct forms. With regard to the eastern Greater Sundas, samples from Java would verify the assumed close relationship of the Bornean and Javan populations. Samples from the southern Philippines would determine the extent of genetic exchange between Borneo and the Sulu Archipelago and between the north and south Philippines.

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APPENDIX 1

Name, tissue source, collection locality and GenBank number of specimens compared in this study.

No	Name†	Source‡	Number§	Locality (notes)	COI	ND2
1	C. albospecularis albospecularis	FMNH	393316*	Antsiranana, Madagascar	FJ473000	FJ473250
2	C. albospecularis albospecularis	FMNH	393317*	Antsiranana, Madagascar	FJ473001	FJ473251
3	C. albospecularis albospecularis	FMNH	393318*	Antsiranana, Madagascar	FJ473002	FJ473252
4	C. albospecularis albospecularis	FMNH	393319*	Antsiranana, Madagascar	FJ473003	FJ473253
5	C. albospecularis albospecularis	FMNH	393320*	Antsiranana, Madagascar	FJ473004	FJ473254
6	C. albospecularis albospecularis	FMNH	393321*	Antsiranana, Madagascar	FJ473005	FJ473255
7	C. albospecularis albospecularis	FMNH	393322*	Antsiranana, Madagascar	FJ473006	FJ473256
8	C. albospecularis albospecularis	FMNH	393323*	Mahajanga, Madagascar	FJ473007	FJ473257
9	C. albospecularis albospecularis	FMNH	393324*	Mahajanga, Madagascar	FJ473008	FJ473258
10	C. albospecularis albospecularis	FMNH	393325*	Mahajanga, Madagascar	FJ473009	FJ473259
11	C. saularis saularis	AMNH	DOT5740*	Betrabati, Nepal	FJ473010	FJ473260
12	C. saularis erimelas	USNM	B2486*	Sagaing, Myanmar	FJ473047	FJ473298
13	C. saularis erimelas	USNM	B5612*	Sagaing, Myanmar	FJ473048	FJ473299
14	C. saularis erimelas	USNM	B5629*	Sagaing, Myanmar	FJ473049	FJ473300
15	C. saularis erimelas	USNM	B5735*	Sagaing, Myanmar	FJ473050	FJ473301
16	C. saularis erimelas	USNM	B6155*	Sagaing, Myanmar	FJ473051	FJ473302
17	C. saularis erimelas	USNM	B6160*	Sagaing, Myanmar	FJ473052	FJ473303
18	C. saularis erimelas	AMNH	DOT2661*	Ha Giang, Vietnam	FJ472996	FJ473246
19	C. saularis erimelas	AMNH	DOT6510*	Ha Giang, Vietnam	FJ473019	FJ473269
20	C. saularis erimelas	SNHM	NRM 20026683	Hanoi,Vietnam (captive)	FJ473035	FJ473286
21	C. saularis prosthopellus	KUNHM	11116*	Guizhou, China	FJ473012	FJ473262
22	C. saularis prosthopellus	LSUMNS	B51252	Guangdong, China (captive)	FJ473028	FJ473279
23	C. saularis prosthopellus	LSUMNS	B51265	Guangdong, China (captive)	FJ473029	FJ473280
24	C. saularis prosthopellus	LSUMNS	B51266	Guangdong, China (captive)	FJ473030	FJ473281
25	C. saularis prosthopellus	LSUMNS	B51267	Guangdong, China (captive)	FJ473031	FJ473282
26	C. saularis prosthopellus	LSUMNS	B51268	Guangdong, China (captive)	FJ473032	FJ473283
27	C. saularis prosthopellus	LSUMNS	B51270	Guangdong, China (captive)	FJ473033	FJ473284
28	C. saularis musicus	MZB	SPS32	W Sumatra, Indonesia	FJ473037	FJ473288
29	C. saularis musicus	MZB	SPS33	W Sumatra, Indonesia	FJ473038	FJ473289
30	C. saularis musicus	MZB	30925*	W Sumatra, Indonesia	FJ473039	FJ473290
31	C. saularis musicus	UAM	17941*	Singapore (salvage)	FJ473011	FJ473261
32	C. saularis musicus	RMBR	WLK 639*	Singapore (salvage)	FJ473036	FJ473287
33	C. saularis musicus	LSUMNS	B57071*	Bintulu, Sarawak	FJ473034	FJ473285
34	C. saularis adamsi	LSUMNS	B51004	Mendolong, Sabah	FJ473026	FJ473277
35	C. saularis adamsi	LSUMNS	B47215*	Klias, Sabah	FJ473025	FJ473276
36	C. saularis musicus	LSUMNS	B47166*	Klias, Sabah	FJ473024	FJ473275
37	C. saularis adamsi	LSUMNS	B36327*	Keningau, Sabah	EU541454	FJ473270

Appendix 1 (Continued).

No	Name†	Source‡	Number§	Locality (notes)	COI	ND2
38	C. saularis adamsi	LSUMNS	B46962*	Serinsim, Sabah	FJ473020	FJ473271
39	C. saularis adamsi	LSUMNS	B46966*	Serinsim, Sabah	FJ473021	FJ473272
40	C. saularis adamsi	LSUMNS	B46967*	Serinsim, Sabah	FJ473022	FJ473273
41	C. saularis adamsi	LSUMNS	B51045	Tawau Hills, Sabah	FJ473027	FJ473278
42	C. saularis pluto	MZB	BS06	East Kalimantan, Indonesia	FJ472995	FJ473245
43	C. saularis deuteronymus	PNM	19891*	Luzon, Philippines	FJ472992	FJ473242
44	C. saularis deuteronymus	PNM	102895	Luzon, Philippines	FJ472993	FJ473243
45	C. saularis deuteronymus	PNM	19896*	Luzon, Philippines	FJ472994	FJ473244
46	C. saularis mindanensis	FMNH	344996*	Sibuyan, Philippines	FJ472997	FJ473247
47	C. saularis mindanensis	FMNH	344997*	Sibuyan, Philippines	FJ472998	FJ473248
48	C. saularis mindanensis	FMNH	344998*	Sibuyan, Philippines	FJ472999	FJ473249
49	C. saularis mindanensis	UAM	21773*	Cebu, Philippines	FJ473040	FJ473291
50	C. saularis mindanensis	UAM	21776*	Cebu, Philippines	FJ473041	FJ473292
51	C. saularis mindanensis	UAM	21771*	Cebu, Philippines	FJ473042	FJ473293
52	C. saularis mindanensis	UAM	21767*	Cebu, Philippines	FJ473043	FJ473294
53	C. saularis mindanensis	UAM	21775*	Cebu, Philippines	FJ473044	FJ473295
54	C. saularis mindanensis	UAM	21774*	Cebu, Philippines	FJ473045	FJ473296
55	C. saularis mindanensis	UAM	21768*	Cebu, Philippines	FJ473046	FJ473297
56	C. saularis mindanensis	KUNHM	13925*	Camiguin Sur, Philippines	FJ473013	FJ473263
57	C. saularis mindanensis	KUNHM	13926*	Camiguin Sur, Philippines	FJ473014	FJ473264
58	C. saularis mindanensis	KUNHM	13934*	Camiguin Sur, Philippines	FJ473015	FJ473265
59	C. saularis mindanensis	KUNHM	13945*	Camiguin Sur, Philippines	FJ473016	FJ473266
60	C. saularis mindanensis	KUNHM	14365*	Camiguin Sur, Philippines	FJ473017	FJ473260
61	C. saularis mindanensis	KUNHM	14366*	Camiguin Sur, Philippines	FJ473018	FJ473268
	C. malabaricus	UWBM	73852*	Singapore (captive)	FJ473054	FJ473305
	C. luzoniensis	USNM	B3698*	Luzon, Philippines	FJ473053	FJ473304

†Classification according to Dickinson (2003). Subspecies names assigned by locality, except in Sabah.

‡FMNH, The Field Museum of Natural History; KUNHM, University of Kansas Natural History Museum; MZB, Museum Zoologicum Bogoriense, Indonesian Institute of Sciences-LIPI; LSUMNS, Louisiana State University Museum of Natural Science; PNM, National Museum of the Philippines; RMBR, Raffles Museum of Biodiversity Research; SNHM, Swedish Natural History Museum; UAM, University of Alaska Museum; USNM, National Museum of Natural History; UWBM, University of Washington Burke Museum.

§Asterisks indicate vouchered specimens. Among wild-caught birds, only one locality had no voucher (*C. saularis pluto* from East Kalimantan).