

# Immigration, species radiation and extinction in a highly diverse songbird lineage: white-eyes on Indian Ocean islands

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## Abstract

Molecular phylogenetic hypotheses of species-rich lineages in regions where geological history can be reliably inferred may provide insights into the scale of processes driving diversification. Here we sample all extant or recently extinct white-eye (*Zosterops*) taxa of the southwest Indian Ocean, combined with samples from all principal continental lineages. Results support a high dispersal capability, with at least two independent continental sources for white-eyes of the region. An early (within 1.8 million years ago) expansion into the Indian Ocean may have originated either from Asia or Africa; the three resulting lineages show a disparate distribution consistent with considerable extinction following their arrival. Africa is supported as the origin of a later expansion into the region (within 1.2 million years ago). On two islands, a pair of *Zosterops* species derived from independent immigrations into the Indian Ocean co-occur or may have formerly co-occurred, providing strong support for their origin by double-island colonization rather than within-island (sympatric or micro-allopatric) speciation. On Mauritius and La Réunion, phylogenetic placement of sympatric white-eyes allow us to rule out a scenario in which independent within-island speciation occurred on *both* islands; one of the species pairs must have arisen by double colonization, while the other pair is likely to have arisen by the same mechanism. Long-distance immigration therefore appears to be responsible for much of the region's white-eye diversity. Independent immigrations into the region have resulted in lineages with mutually exclusive distributions and it seems likely that competition with congeneric species, rather than arrival frequency, may limit present-day diversity.

*Keywords:* diversification, extinction, immigration, Indian Ocean islands, molecular phylogeny, *Zosterops*

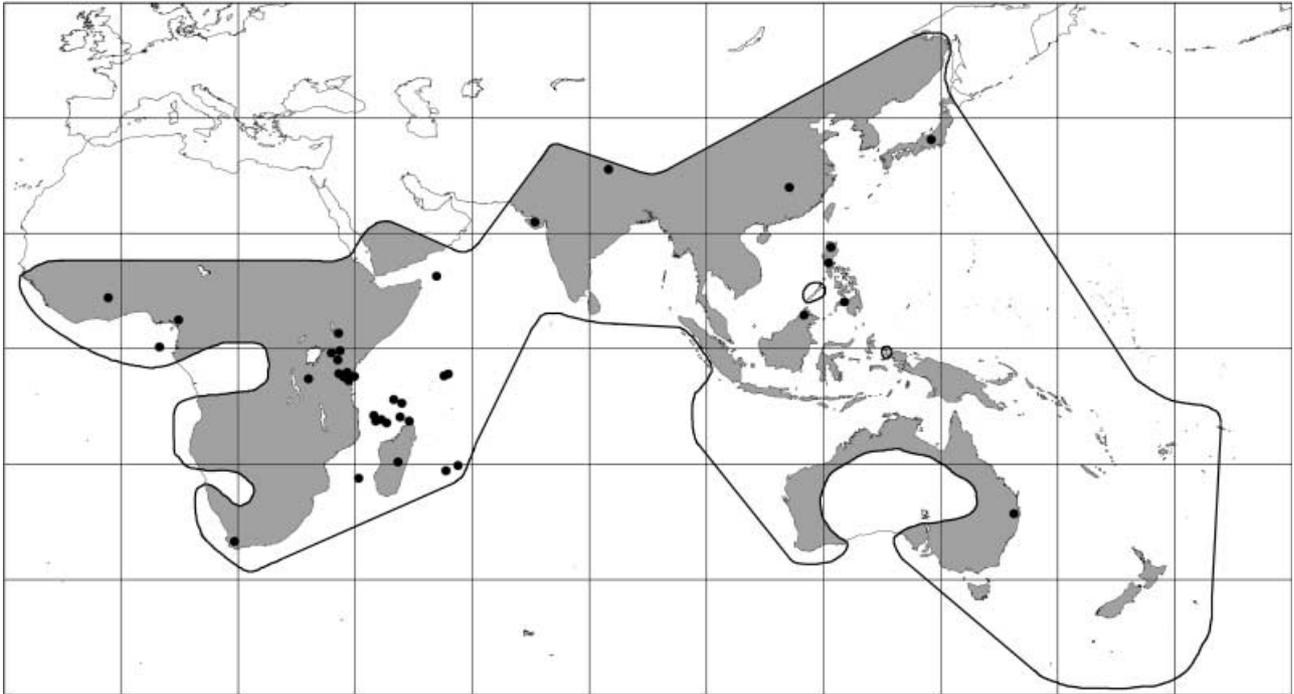
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## Introduction

Why do some geographical regions contain many more species than others? Increasingly, ecologists have recognized the need to consider both *in situ* processes and regional processes, especially long-distance immigration, in understanding

local species richness (He *et al.* 2005 and references therein). However, for any particular group of organisms, the relative contribution of these two classes of processes may not be intuitively apparent, even with a good understanding of their biology. For example, two conflicting effects of dispersal ability on lineage diversification seem likely. First, higher dispersal abilities may result in wider distributions in which more geographical opportunities for speciation are afforded, and buffering from local environmental disturbances reduces extinction risk (Rosenzweig 1995). Second, higher dispersal ability may dampen speciation

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**Fig. 1** The global distribution of white-eyes (*Zosteropidae*) indicated by the thick dark line, and shaded grey on land. Based on Mees (1957) and Moreau (1957) with modifications to the Arabian distribution from Gallagher & Woodcock (1980). White-eyes have also been sighted in the mangroves of southern Iran and eastern Oman (Reynolds 1978; Eriksen *et al.* 2001), but their distribution in these areas is unknown. Black dots indicate sampling localities. See Fig. 2 for an enlarged map of the southwest Indian Ocean with distributions and sampling localities.

rates by reducing the impact of barriers in interrupting gene flow (Jablonski & Roy 2003). As a result, at any particular spatial scale, it may be difficult to decipher the contributions of species immigration into a region vs. localized speciation and extinction history in determining community diversity. We may likely obtain a more accurate picture by investigating geographical histories of component lineages than by making broad inferences based on their ecology. Therefore, to understand the production of current biogeographic patterns of species diversity, we must first test geographic hypotheses regarding the spatial scale of diversification events.

White-eyes (*Zosterops*) of the southwest Indian Ocean are a useful group in which to evaluate the likely importance of long-distance dispersal vs. *in situ* processes in driving species diversification. On the one hand, white-eyes appear to have among the highest dispersal capabilities of birds, with an exceptionally wide distribution and high colonizing potential; they have colonized more islands globally than any other passerine group (Moreau 1964). On the other hand, distance appears to pose an important barrier to gene flow in *Zosterops*, since 46% of the world's forms are single-island endemic species or subspecies, making it the second most species-rich bird genus in the world (*Nectarinia* is the first). In the southwest Indian Ocean, almost all islands have been

colonized by white-eyes. Most taxa have a very localized distribution, 87% of them being single-island endemic species or subspecies. Since *Zosterops* are found throughout the old world tropics (Fig. 1), both Asia and Africa are potential sources of Indian Ocean diversifications. However, range splitting (vicariance) can reasonably be rejected as a mechanism of diversification; none of the archipelagos in the western Indian Ocean (the Mascarenes, Comoros, Seychelles and Aldabras) show any evidence of having been joined to each other or to Madagascar, Africa or India in the last 50 million years (Myr). With the exception of small islands within the granitic Seychelles, all Indian Ocean islands have been isolated since their origin and white-eyes can only have reached them by dispersal. Despite the antiquity of continental isolation in the region, barriers to dispersal have varied significantly over time since archipelagos of the region are extremely diverse in geological origin and age (0.015–64 Myr; Warren *et al.* 2003, 2005), and sea levels have fluctuated considerably (Colonna *et al.* 1996; Camoin *et al.* 2004).

Here we use mitochondrial sequence data from all known southwest Indian Ocean *Zosterops* taxa, along with a selection of the principal white-eye lineages from Africa, Asia and Australia, to test three hypotheses regarding the origin and diversification of the genus within the southwest

Indian Ocean. First, to understand whether there was a single immigration event into the region, or multiple events, we test the hypothesis that southwest Indian Ocean white-eyes are a monophyletic group. This hypothesis would imply that they have radiated following a single colonization of the region, and therefore that speciation within the region has predominated in determining current diversity. Second, based on island biogeographic theory, we would expect colonists to immigrate into the region from their closest mainland source. In line with this idea, we test the hypothesis that southwest Indian Ocean and African white-eyes form a monophyletic group excluding Asian white-eyes. Third, given the presence of two *Zosterops* species on several Indian Ocean islands, we test a null hypothesis that species occupying the same island are monophyletic. This placement would be consistent with within-island speciation (sympatric or microallopatriic), rather than multiple immigration events, in the accumulation of diversity at a local scale.

## Materials and methods

### *Indian Ocean white-eye taxonomy*

White-eyes are notorious for the difficulty they present to taxonomists in resolving relationships and species boundaries; plumage appears to change very readily, while morphological differences among most of the world's white-eyes are slight. The taxonomic literature shows little consensus over the relationships and species status of southwest Indian Ocean white-eyes (Rand 1936; Moreau 1957; Benson 1960, 1984; Benson *et al.* 1977; Louette 1988a, 1988b). One exception is *Zosterops mouroiensis*, restricted to upper parts of the Grande Comore volcano, which has been consistently placed in a separate species from neighbouring forms (Benson 1960; Louette 1988a, 1988b). In addition, the islands of Mauritius and La Réunion in the Mascarenes are each inhabited by two unusual sympatric white-eye species, and these four taxa have been universally divided into two groups: the Mascarene grey white-eyes and olive white-eyes (Gill 1971). However, proposed relationships between the Mascarene grey white-eyes, the Mascarene olive white-eyes, *Z. mouroiensis*, and the other white-eyes of the southwest Indian Ocean have been largely speculative. Further, the relationship between Indian Ocean white-eyes and those of neighbouring continents has remained elusive. While Mees (1957, 1961, 1969) could specify relationships and species boundaries in the oriental white-eyes relatively precisely, the gradation and geographical complexity of morphological and plumage variation in the white-eyes of Africa resulted in much uncertainty in Moreau's (1957) thorough attempt at classifying the Zosteropidae of Africa and the Indian Ocean. While Benson (1960) presumed an African origin for several Indian Ocean species, very little supporting evidence was presented.

### *Sampling*

We obtained mitochondrial DNA (mtDNA) sequence data from 41 individuals representing all the southwest Indian Ocean *Zosterops* taxa and including all island populations of *Zosterops maderaspatanus* with the exception of the Cosmoledo population (Fig. 2). In recognition of the high colonizing potential of white-eyes, and the accessibility of the Indian Ocean from Africa and Asia, we obtained further sequence data from 33 individuals distributed across Africa, including highland forms from the rift valley, and across Asia into Australasia, including species from the Indian Subcontinent, China, the Philippines, Borneo, and Australia (Fig. 1). These represent the principal *Zosterops* lineages identified in earlier taxonomic studies (Mees 1957, 1961, 1969; Moreau 1957). Given the uncertain relationship of white-eyes in the genus *Speirops* to *Zosterops* (Moreau 1957), we further sampled two taxa of *Speirops*, one from Mount Cameroon and one from the Gulf of Guinea. We also obtained sequences of *Stachyris whiteheadi* and *Sylvia atricapilla*, since the results of a previous molecular phylogenetic study (Slikas *et al.* 2000) showed that these genera make suitable outgroups to *Zosterops*. We sequenced two or three individuals per Indian Ocean *Zosterops* taxon and per island population, with the exception of *Zosterops vaughani*, *Z. maderaspatanus* in the Iles Glorieuses, and the extinct *Zosterops semiflavus*, for which only one individual each was sequenced from museum skin samples. Blood samples were obtained during collecting expeditions to Madagascar, the Comoros, the Mascarenes, the granitic Seychelles and Aldabra between July 1999 and February 2002, and were preserved in Queen's lysis buffer (Seutin *et al.* 1991, 1993). Since three southwest Indian Ocean *Zosterops* species are threatened, two having estimated population sizes of only 50–296 individuals (BirdLife International 2006), all blood samples were taken non-destructively from mist-netted individuals. Additional Indian Ocean sequences were obtained from museum skin samples from The Natural History Museum, Tring, UK (BMNH), and from tissue samples of *Z. maderaspatanus* from Madagascar granted to us by the Field Museum of Natural History (FMNH) (Appendix I). Blood, tissue and skin samples of all the non-Indian Ocean taxa were generously granted by a number of institutions and individuals (Appendix I). A total of 662 bp of sequence data was obtained for all individuals from the mitochondrial NADH dehydrogenase subunit 3 (ND3) and cytochrome *b* (*cyt b*) genes. To improve the resolution of phylogenetic relationships the two-gene (ND3 and *cyt b*) data were supplemented with full mitochondrial ATP synthase 6 (ATPase6), and ATP synthase 8 (ATPase8) sequences of one individual from each of the principal evolutionary lineages, providing a four-gene data set totalling 1514 bp.

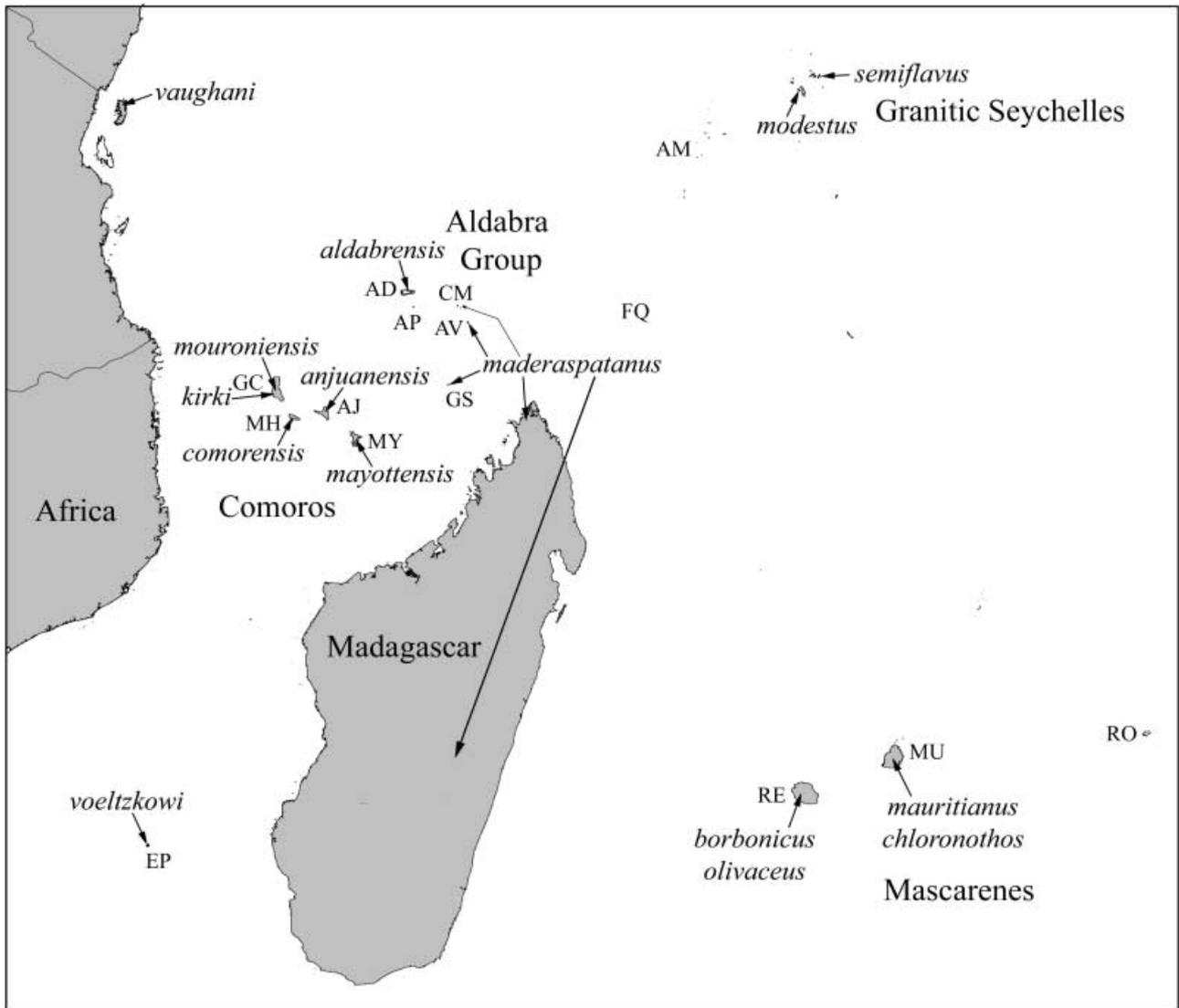


Fig. 2 Distribution of white-eye taxa of the southwest Indian Ocean. All island populations were sampled with the exception of the Cosmoledo population of *Zosterops maderaspatanus*, indicated with a thin arrow. Within the Madagascar population of *Z. maderaspatanus*, arrows indicate sampling localities. White-eye taxa of Africa are not indicated. GC, Grande Comore; MH, Moheli; AJ, Anjouan; MY, Mayotte; AD, Aldabra; AP, Assumption; CM, Cosmoledo; AV, Astove; FQ, Farquhars; AM, Amirantes; GS, Iles Glorieuses; EP, Europa; RE, La Réunion; MU, Mauritius; RO, Rodrigues.

#### DNA extraction, PCR and sequencing

DNA was extracted from blood and tissue samples following the phenol–chloroform protocol of Seutin *et al.* (1993), except that the final suspension was purified by dialysis instead of ethanol precipitation. Polymerase chain reaction (PCR) primers and conditions are detailed in Table 1. Strict procedures appropriate for ‘ancient DNA’ were used to obtain ND3 and *cyt b* sequences from museum skins, using the same primer sequences and PCR cycles used for the other *Zosterops* samples. These procedures have already been described elsewhere (Warren *et al.* 2005), as have the

steps following PCR (Warren *et al.* 2003). To guard against amplification of nuclear copies of mitochondrial genes, the absence of misplaced stop codons and frame shift mutations was checked for all sequences.

#### Phylogenetic analysis

*Two-gene data for the full sample set.* The congruence of the ND3 and *cyt b* data sets was checked using the partition homogeneity test (Farris *et al.* 1995) implemented in PAUP\*, and data from these two genes were combined for further analysis. An unweighted parsimony analysis was performed

**Table 1** Primers and experimental conditions used for white-eye PCR amplification and DNA sequencing

Gene region	Primer names	Source	PCR conditions			
			Denaturation	Annealing	Extension	Number of cycles
ATPase 6&8	CO2GQL & CO3HMH	http://striweb.si.edu	94 °C for 45 s	55 °C for 45 s	72 °C for 1 min	30
ND3	L10755 & H11151	Chesser 1999	95 °C for 45 s	54 °C for 30 s	72 °C for 45 s	30
Cyt <i>b</i>	CB1 & CB2	Palumbi 1996	94 °C for 45 s	55 °C for 45 s	72 °C for 1 min	35

on the combined data set using the heuristic search algorithm, holding 10 trees at each step and branch swapping on all trees, using the steepest descent option. In addition, the combined data set excluding outgroups (*Sylvia* and *Stachyris*) was analysed in MODELTEST (Posada & Crandall 1998) to determine the substitution model which best describes the data.

Bayesian analysis was performed on the combined data set using the program MRBAYES 2.01 (Huelsenbeck *et al.* 2001). Base frequencies were estimated from the data. Four Markov chains were run simultaneously for 1 million generations, and sampled every 10 generations. Variation in the maximum-likelihood (ML) scores in this sample was examined graphically. The trees generated prior to stationarity were discarded, and the consensus phylogeny and posterior probability of its nodes were determined from the last 80 000 trees in the chain. To check our results and guard against the possibility of multiple optima, we ran this analysis a second time for 3 million generations and used the last 250 000 trees to determine the consensus phylogeny and posterior probability of nodes.

For a few of the 'ancient DNA' museum skin samples, some of which date back to 1888, we were successful in obtaining the *cyt b* region but were unable to amplify ND3 despite numerous efforts, and vice versa (Appendix I). In addition to the above analyses, we therefore re-ran the *cyt b* and ND3 data sets in MODELTEST independently, including these additional samples. Bayesian analysis was run for 2 million generations on the ND3 and *cyt b* regions independently, and the last 150 000 trees were used to obtain the consensus phylogeny and posterior probability of nodes in both cases.

*Four-gene data for the restricted sample set.* In an attempt to improve resolution of nodes with low Bayesian and Bootstrap support, we selected one sample from each of the principal lineages of interest in the two-gene data set and supplemented the 662 bp of sequence data from ND3 and *cyt b* with an additional 852 bp of sequence from the mitochondrial ATPase6 and ATPase8 genes. Again, we used the partition homogeneity test (Farris *et al.* 1995) to determine if the four gene data sets could be combined for phylogenetic analysis. The combined data set (excluding the outgroup: *S. whiteheadi*) was analysed in MODELTEST.

The optimal model defined by MODELTEST was used to determine the ML distances for both a neighbour-joining (NJ) analysis in PAUP\*, and a ML analysis in PAUP\* with the heuristic search algorithm. In addition, an unweighted parsimony analysis was performed as for the two-gene data. Bayesian analysis followed the procedures for the two-gene data with the first analysis run for 1 million generations and the consensus phylogeny and posterior probability of its nodes determined from the last 80 000 trees, while the final analysis was run for 5 million generations and the last 450 000 trees were used to obtain the consensus.

*Hypothesis testing.* The method of Shimodaira & Hasegawa (1999) implemented in PAUP\* was used to test the monophyly of white-eye species and discriminate between alternative scenarios of island colonization as well as identify the most likely geographical modes of speciation. Since the construction of ML or Bayesian alternative topologies was computationally prohibitive, we used the Shimodaira-Hasegawa (SH) test to compare the optimal four-gene Bayesian topology with alternative topologies reconstructed using parsimony.

#### *Estimation of divergence times*

Rate heterogeneity was first tested using PAUP\* to determine whether branch lengths were consistent with a molecular clock. Ages were assigned to nodes in the tree based on geological estimates of island ages. Where closely related taxa are found on neighbouring islands we consider that the age of the younger island represents an approximate estimate for the maximum age of the split between these island lineages. Fleischer *et al.* (1998) and Warren *et al.* (2003) summarize eight assumptions made when dating nodes with this method.

#### *Estimation of direction and sequence of colonization*

To infer the distribution of the ancestors of two nested white-eye clades we applied the ancestral areas method of Bremer (1992) to the Bayesian tree for the four-gene data. This method is cladistic, makes no assumptions about the mechanism of speciation, and relies solely on topological

information from the tree. In our application of the method to the whole of the white-eye phylogeny, taxa were replaced by the geographical regions (Africa, Asia, Indian Ocean) in which they occur, while in our application of the method to the *maderaspatanus* clade of the Indian Ocean, taxa were replaced with the island archipelago in which they occur. Two parsimony analyses, which assume irreversibility of character states, were used to determine the number of gains and losses representing each area and calculate relative probabilities of belonging to the ancestral area for the group. Under this method, regions containing taxa that diverge from deep nodes are considered more likely to represent the ancestral area than are regions containing taxa derived from shallower nodes.

## Results

### *Two-gene data for the full sample set*

A partition homogeneity test (Farris *et al.* 1995) on the combined mtDNA data (two partitions; 662 bp) indicated that the ND3 and *cyt b* regions did not differ significantly ( $P = 0.23$ ). We therefore combined these two data sets for further analysis. An unweighted parsimony analysis resulted in 111 most parsimonious trees with length 597. A strict consensus was calculated and bootstrapping was on this consensus tree (stepwise-addition, 1000 replicates). MODELTEST identified the transition (TIM) model of DNA substitution (Rodríguez *et al.* 1990) with invariable sites and gamma shape parameter (TIM+I+G) as best describing the data under the Akaike information criterion (see Appendix II for parameters). For Bayesian analysis we used a general time reversible (GTR) with invariant sites and gamma distribution shape parameter (GTR+I+G) model of DNA substitution, given that it is the model available in MRBAYES that best matches the TIM+I+G model.

### *Four-gene data for the restricted sample set*

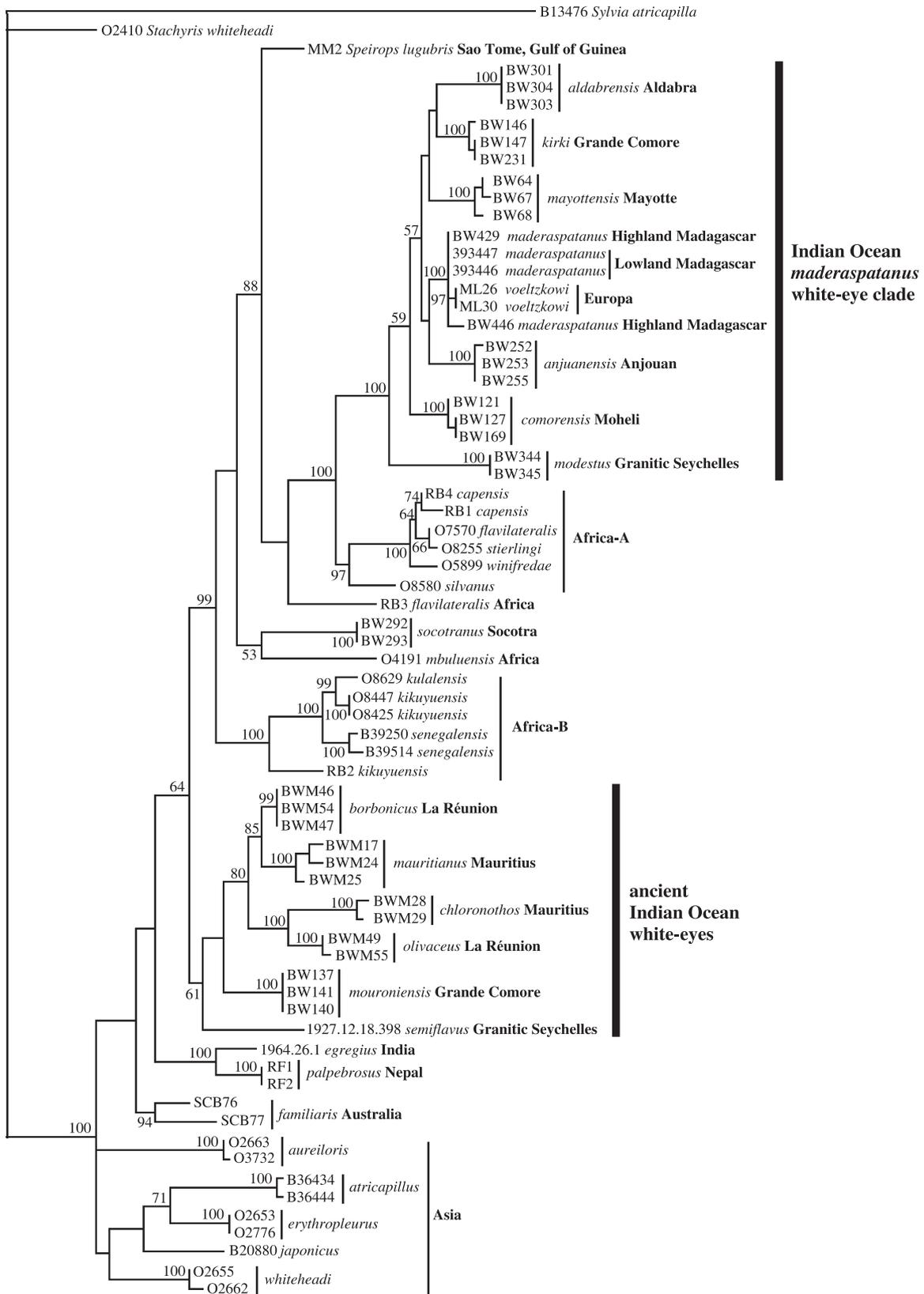
The partition homogeneity test (Farris *et al.* 1995) on the combined mtDNA data (four partitions; 1514 bp) indicated that the ATPase6, ATPase8, ND3 and *cyt b* regions did not differ significantly ( $P = 0.57$ ). We therefore combined these four data sets for further analysis. An unweighted parsimony analysis resulted in a single most parsimonious tree with length 977 (CI 0.531, RI 0.595, RC 0.316, HI 0.469). MODELTEST again identified the TIM+I+G model of DNA substitution (Rodríguez *et al.* 1990) as best describing the data under the Akaike information criterion (see Appendix II for parameters). We used parameters and nucleotide frequencies from this model in the NJ and ML analyses. For Bayesian analysis we again used a GTR+I+G model of DNA substitution, given that it is the model available in MRBAYES that best matches the TIM+I+G model.

### *Phylogenetic relationships of southwest Indian Ocean white-eyes*

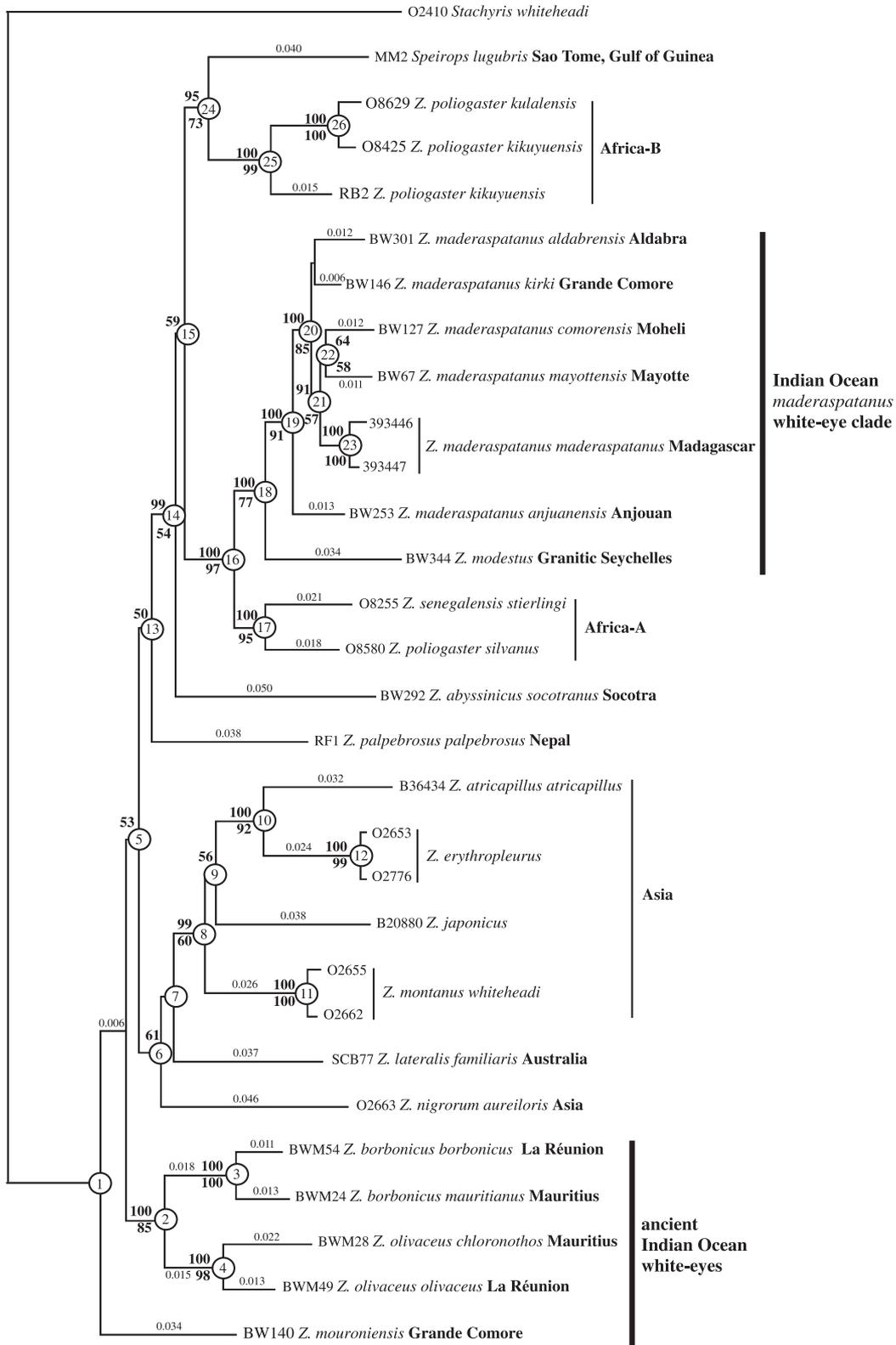
Bayesian branch support (BBS) values, being the proportion of the sampled trees containing a given clade, can be interpreted as the Bayesian posterior probability of the given clade. We therefore set a BBS value of 95%, below which we consider nodes not to be significantly supported. The topology of the Bayesian tree for the combined two-gene data alone (Fig. 3) is fully concordant with the topology of the Bayesian tree for the four-gene data (Fig. 4), with respect to nodes gaining  $\geq 95\%$  BBS in the two-gene tree. Furthermore, the additional sequence data in the four-gene data set increases the resolution of phylogenetic relationships in certain parts of the tree, since some nodes gaining  $< 95\%$  BBS in Fig. 3 gain  $\geq 95\%$  support in Fig. 4. In both the two-gene and four-gene data sets different methods of inferring molecular phylogeny [ML, maximum parsimony (MP), NJ and Bayesian analyses] yield identical tree topologies with respect to all nodes gaining  $\geq 95\%$  BBS, with the exception of the placement of *Zosterops abyssinicus socotranus* in the four-gene data set, which shows two different placements among the African forms under different methods. It is also noteworthy that in the MP and NJ trees, *Zosterops nigrorum* of the Philippines falls outside a monophyletic group containing all the other white-eyes (*Zosterops* and *Speirops*), gaining 77% and 62% bootstrap support, respectively. This relationship is not observed in the Bayesian (Fig. 4) or ML trees.

The SH test (Table 2) allows us to reject our first hypothesis that southwest Indian Ocean white-eyes form a monophyletic group. We can therefore infer that they have at least two independent origins. First, all named taxa within the species *Z. maderaspatanus* form a monophyletic group including *Zosterops modestus* of the granitic Seychelles with 100% BBS. This *maderaspatanus* clade pairs as the sister to a clade of African *Zosterops* (Africa-A) with 100% BBS. We further find 99% BBS for the monophyly of a clade containing all the African white-eyes (including *Speirops* from the Gulf of Guinea and *Z. a. socotranus* from Socotra) and the *maderaspatanus* clade. Second, the other white-eyes of the southwest Indian Ocean including the Mascarene white-eyes (*Zosterops borbonicus borbonicus*, *Zosterops borbonicus mauritianus*, *Zosterops olivaceus olivaceus* and *Zosterops olivaceus chloronothos*), the Mount Karthala white-eye of Grande Comore (*Z. mouroniensis*), and the extinct chestnut-flanked white-eye of the granitic Seychelles (*Z. semiflavus*) group near the base of the two-gene phylogeny (Fig. 3), although there is no BBS for their monophyly. We hereafter refer to this group of species as the ancient Indian Ocean white-eyes.

Non-significance of the SH test (Table 2) prevents us from rejecting our second hypothesis that southwest Indian Ocean and African white-eyes form a monophyletic



**Fig. 3** Bayesian analysis of the mitochondrial ND3 and *cyt b* data set for all white-eye samples. Consensus of the last 250 000 trees after 3 million generations based on the GTR+I+G model. Bayesian branch support values are indicated. All *Zosterops* taxa are labelled with their subspecific names only. Other genera are labelled with their full genus and species name. We follow the taxonomy of Mees (1957, 1961, 1969) for Asian forms, Fry (2000) for African forms, Moreau (1957) for the granitic Seychelles, Gill (1971) for the Mascarenes and Louette (1988a) for all other southwest Indian Ocean forms.



**Fig. 4** Bayesian analysis of the mitochondrial ATPase6, ATPase8, ND3 and cyt *b* data set for the principal white-eye lineages. Consensus of the last 450 000 trees after 5 million generations based on the GTR+I+G model. Bayesian branch support values are indicated above ML bootstrap values (stepwise-addition, 100 replicates). Node numbers correspond to divergence time estimates in Table 3. Trinomials follow the taxonomy of Fig. 3.

**Table 2** Results of SH tests of alternative topologies. Significant results (bold) denote rejection of the null hypotheses stated. All topologies were based on the four-gene data set except for Marianne where the two-gene data set was used because sequence data for all four genes was unavailable. For Marianne, the SH test was performed once using one of the 60 most parsimonious constrained topologies obtained. For all other hypotheses, SH tests were performed on all the most parsimonious constrained topologies and the range of *P* values are quoted

Null hypothesis	<i>P</i>
1. Monophyly of southwest Indian Ocean white-eyes	<b>0.003–0.004</b>
2. Monophyly of African and southwest Indian Ocean white-eyes	0.109–0.116
3. Monophyly of white-eyes occupying the same island	
Mauritius	< <b>0.001</b>
La Réunion	< <b>0.001</b>
Grande Comore	< <b>0.001</b>
Marianne (Granitic Seychelles)	<b>0.004</b>

group excluding Asian white-eyes, despite the absence of significant BBS for the relationship. A basal polytomy in the white-eye phylogeny separates the single clade containing all the African and *maderaspatanus* white-eyes from the Mascarene white-eyes, all other non-*maderaspatanus* southwest Indian Ocean white-eyes, and four lineages of Asian white-eyes.

On all three islands where two *Zosterops* species co-occur (Mauritius, La Réunion and Grande Comore), in addition to one archipelago where they are likely to have formerly co-occurred (granitic Seychelles), the SH test allows us to reject our third null hypothesis that species occupying the same island are monophyletic (Table 2). This result is consistent with double island colonization rather than within-island (sympatric or microallopatric) speciation.

Results from the four-gene data show that the Mascarene white-eyes form a monophyletic group (100% BBS: Fig. 4) consisting of two monophyletic subgroups – the Mascarene olive white-eyes (*Z. o. olivaceus* and *Z. o. chloronothos*: 100% BBS) and the Mascarene grey white-eyes (*Z. b. borbonicus*, *Z. b. mauritianus*: 100% BBS) – each of which is represented by one species on La Réunion and the other on Mauritius.

Based on the two-gene data we find no differentiation between *Zosterops maderaspatanus maderaspatanus* at lowland and highland localities, despite the fact that the form sampled from Mount Ankaratra in highland Madagascar is noticeably larger than the lowland population, and was formerly classified as a separate subspecies of *Z. maderaspatanus*. The white-eyes of Europa island (*Zosterops maderaspatanus voeltzkowi*) and Astove (*Z. m. maderaspatanus*) form part of this monophyletic group of Madagascar white-eye haplotypes (with *Z. m. maderaspatanus* of Madagascar),

**Table 3** White-eye divergence times estimated from molecular clock methods. Calibration using geologically determined island ages. Dating based on fixing node 20 at 0.5 Ma. All dates are in millions of years before present. Nodes 1–26 have been labelled in Fig. 4

Node number	Genetic distance from node to tip	Divergence time estimates
1	0.0430	1.84
2	0.0284	1.22
3	0.0100	0.43
4	0.0149	0.64
5	0.0421	1.80
6	0.0379	1.62
7	0.0368	1.58
8	0.0307	1.31
9	0.0299	1.28
10	0.0215	0.92
11	0.0018	0.08
12	0.0007	0.03
13	0.0405	1.73
14	0.0381	1.63
15	0.0371	1.59
16	0.0281	1.20
17	0.0209	0.90
18	0.0238	1.02
19	0.0157	0.67
20	0.0116	0.50
21	0.0103	0.44
22	0.0099	0.43
23	0.0022	0.09
24	0.0314	1.35
25	0.0159	0.68
26	0.0034	0.14

but are both slightly diverged from the Madagascar haplotypes (97% BBS in both cases). The single sample from the Iles Glorieuses is undiverged from the Madagascar haplotypes. *Z. m. maderaspatanus* sequences from Iles Glorieuses and Astove are not included in Fig. 3 since ND3 would not amplify for these museum skin samples, and therefore their relationships are determined from the *cyt b* data alone.

#### Estimation of divergence times

A log-likelihood test with outgroup (*Stachyris whiteheadi*) excluded failed to reject the null hypothesis of rate constancy ( $-\ln L$  clock-enforced tree = 6378.24429,  $-\ln L$  unconstrained tree 6363.89259,  $X^2 = 28.70$ ,  $DF = 27$ ,  $P > 0.25$ ). We therefore used the ML tree constructed under the assumption of a molecular clock for estimating divergence times (Table 3). Node 20 (Fig. 4) in the ML tree can be used as a calibration point, since the maximum age of divergence can be inferred. This node separates the lowland Grande Comore white-eye (*Zosterops maderaspatanus kirki*) from other white-eyes in the *maderaspatanus* clade and is taken as the earliest

**Table 4** Application of Bremer's (1992) ancestral areas method to the topology of Fig. 4 with nodes gaining less than 95% Bayesian branch support collapsed. A, ancestral area analysis of the *maderaspatana* clade only; B, ancestral area analysis for the whole white-eye phylogeny, grouping the islands of Socotra and the Gulf of Guinea with Africa; C, ancestral area analysis for the whole white-eye phylogeny, with the islands of Socotra and the Gulf of Guinea as separate groupings

Area	Gains	Losses	Gains/Losses	Ancestral area probability
<b>A</b>				
Comoros	4	3	1.33	1.00
Madagascar	1	6	0.17	0.13
Aldabra Group	2	5	0.40	0.30
Seychelles	1	1	1.00	0.75
<b>B</b>				
Indian Ocean	4	7	0.57	0.57
Africa	3	8	0.38	0.38
Asia	4	4	1.00	1.00
<b>C</b>				
Indian Ocean	4	7	0.57	0.57
Africa	2	10	0.20	0.20
Asia	4	4	1.00	1.00
Gulf of Guinea	1	10	0.10	0.10
Socotra	1	9	0.11	0.11

possible colonization of Grande Comore since the estimated volcanic origin of the island [0.5 megannum (Ma) (i.e. million years ago)]. Since nodes separating *Z. m. kirki*, *Z. m. maderaspatanus*, *Zosterops maderaspatanus comorensis*, *Zosterops maderaspatanus mayottensis* and *Zosterops maderaspatanus aldabrensis* show less than 95% BBS, for dating purposes we collapse these nodes to form a polytomy and attach the 0.5 Ma dating to node 20. Based on this method of calibration, the average white-eye pairwise mtDNA divergence rate for the combined four-gene data of 4.66% per Myr is higher than the 2% tick rate of the commonly used passerine mtDNA *cyt b* clock (Fleischer *et al.* 1998). However, it is not as high as the 8.29% per Myr tick rate of the sunbird mtDNA clock, which also included ATPase6 and ND3 genes (Warren *et al.* 2003; and see García-Moreno 2004).

#### *Estimation of direction and sequence of white-eye colonization*

In our application of Bremer's (1992) ancestral areas method to the full white-eye clade and to the *maderaspatanus* white-eye clade we collapsed nodes gaining less than 95% BBS. In the analysis of the full white-eye clade we experimented first with the grouping of all areas into Africa, Asia and southwest Indian Ocean, with the islands of Socotra (Gulf of Aden) and Sao Tome (Gulf of Guinea) as part of our

African grouping, and second with the islands of Socotra and Sao Tome as separate groupings. In both cases, Asia gains the highest probability of being the ancestral area for white-eyes (*Zosterops* and *Speirops*) (Table 4). In our analysis of the *maderaspatanus* clade, the Comoros gains the highest probability of being the ancestral area (Table 4).

## Discussion

### *Immigration events*

That we are able to reject our first hypothesis of the monophyly of southwest Indian Ocean white-eyes provides strong support for two or more independent white-eye colonizations of the southwest Indian Ocean. The phylogenetic placement of the *maderaspatanus* white-eyes makes an African origin for the clade seem much more likely than an Asian origin. A direct Asian origin for the *maderaspatanus* clade would require three independent colonizations of Africa and its nearby islands from Asia, in addition to the colonization of the southwest Indian Ocean from Asia (regardless of whether white-eyes as a whole originated in Asia or Africa). By contrast, an African origin for the clade requires only a single colonization of Africa from Asia, followed by a single colonization of the southwest Indian Ocean from Africa. However, for the ancient Indian Ocean white-eyes, topological uncertainty prevents us from rejecting either an African or Asian origin.

### *Likely speciation mechanisms for congeneric species pairs*

An important line of empirical evidence for sympatric speciation is the monophyly of sympatric taxa from small isolated environments (Schliewen *et al.* 1994; Wilson *et al.* 2000). Southwest Indian Ocean white-eyes show three cases of extant endemic *Zosterops* taxa occupying the same island. Both Mascarene islands support a sympatric white-eye species pair (*Z. b. borbonicus* and *Z. o. olivaceus* on La Réunion, and *Z. b. mauritanus* and *Z. o. chloronothos* on Mauritius), and Grande Comore is occupied by both *Z. mouroi* and *Z. m. kirki*. The latter two species are altitudinally segregated, *Z. mouroi* being restricted to the summit and upper slopes of Mount Karthala while *Z. m. kirki* is confined to the lowlands of the island, with a narrow zone of overlap around 1700–1900 m. In addition, two white-eye species formerly occupied the granitic Seychelles archipelago: *Z. semiflavus*, now extinct, and *Z. modestus*. The original distributions of this species pair are uncertain; while it has been speculated that they formerly divided the archipelago between them (Moreau 1957), there is evidence that both species used to occur on Marianne (Diamond & Feare 1980; Diamond 1984).

Contrary to our third null hypothesis, none of the four species pairs are monophyletic (Figs 3 and 4; Table 2), and

therefore the data are more consistent with double island colonization than with within-island speciation. In the Grande Comore and granitic Seychelles species pairs, one member of the pair belongs to the *maderaspatanus* clade, while the other member is one of the ancient Indian Ocean white-eyes. Such placement of species from the same island in different groups inferred to represent independent colonizations of the Indian Ocean is further clear evidence for double island colonization in explaining their occurrence. The Mascarene white-eyes form a monophyletic group consisting of two lineages, each of which is represented by one species on La Réunion and the other on Mauritius. Based on their phylogenetic placement, we cannot rule out the possibility that within-island speciation occurred on one of the two islands. However, we can rule out a scenario in which independent within-island speciation occurred on *both* islands; double island colonization must have given rise to at least one of the two species pairs, and we assess alternative geographical scenarios in our hypothesis section. Our results are therefore consistent with Coyne & Price's (2000) conclusion that there is little evidence for sympatric speciation in island birds.

#### *Diversification in southwest Indian Ocean white-eyes: a hypothesis*

*Island colonization.* That the ancestral areas method applied to the Bayesian tree supports an Asian origin for white-eyes, rather than an Indian Ocean or African origin, is in accordance with the basal divergence of *Z. nigrorum* of the Philippines under MP and NJ as well as with a recent molecular phylogenetic study of the higher-level taxonomy of the old world insectivorous passerines, in which *Zosterops japonicus* is placed among yuhinas (Cibois 2003). Since *Yuhina* is restricted to the Indo-Malayan region, with a large diversification in the Philippines, the close relationship between white-eyes and yuhinas also suggests an Asian origin for *Zosterops* (Cibois 2003).

Although absolute timings of divergence events are susceptible to error in calibration, it is the *relative* timing of these events which is important to our interpretation of geographic patterns of white-eye colonization history. Based on our phylogeny, the colonization of the Indian Ocean by the *maderaspatanus* clade of white-eyes (node 16, estimated 1.2 Ma) considerably postdates the Indian Ocean arrival of the ancestors of the ancient Indian Ocean white-eyes (node 1, estimated 1.8 Ma). Since the ancient Indian Ocean white-eyes all emanate directly from the basal *Zosterops* polytomy, we hypothesize that the ancestor or ancestors of these species colonized the Indian Ocean in an early wave of expansion from Asia. As already noted, an African origin for the *maderaspatanus* clade requires fewer intercontinental colonizations than an Asian one; we therefore hypothesize that arrival of the ancient Indian Ocean white-eyes was

followed by the white-eye colonization of Africa, either from the Indian Ocean or directly from Asia, and secondary colonization of the Indian Ocean from Africa to form the *maderaspatanus* clade.

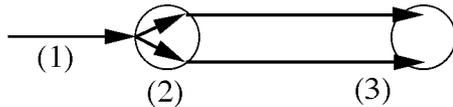
An Asian origin for some of the southwest Indian Ocean *Zosterops* might seem unlikely given the much greater distance of Madagascar from India (3800 km) than from Africa (420 km), and the present day expanse of open ocean (2750 km) between the Seychelles and India vs. the small distances involved in traversing the Mozambique Channel either directly from Mozambique to Madagascar by the shortest crossing (420 km), or using the Comoros as stepping-stones (the greatest single crossing being the 320 km between Mayotte and Madagascar). However, examination of the topography of the Indian Ocean floor supports this inference. Data on sea level changes within the Pliocene and Quaternary show sea level low-stands of 80–145 m below present sea level, some of which persisted for up to 50 000 years at a time, occurring at 2.4 Ma, 1.6 Ma and in six episodes within 0.5 Ma (Haq *et al.* 1987; Colonna *et al.* 1996; Rohling *et al.* 1998). Assuming relatively constant ocean floor topography, a drop in sea level of 80 m or more would result in much larger landmasses being exposed in the granitic Seychelles and at other sites along the Mascarene bank, with a chain of smaller islands appearing between these landmasses and India (for illustration, see Benson 1984). This chain of islands could have served as stepping-stones for a white-eye colonization of the Indian Ocean from Asia at around 1.6 Ma, greatly reducing the distance of open ocean to be crossed.

The relatively close relationship between *Z. semiflavus*, *Z. mouroniensis* and the Mascarene white-eyes is not only unexpected based on existing taxonomy, but is also biogeographically surprising. The islands occupied by these ancient Indian Ocean white-eyes — the granitic Seychelles, Grande Comore and Mascarenes — are disparate island groupings, while the intervening islands of the eastern Comoros (Moheli, Anjouan and Mayotte) as well as the much larger landmass of Madagascar are devoid of ancient Indian Ocean white-eyes, and are solely occupied by white-eyes of the *maderaspatanus* clade. The lack of branch support for relationships between *Z. semiflavus*, *Z. mouroniensis*, the Mascarene white-eyes and the five other basal white-eye lineages, as well as relatively long branch lengths in these species, suggest that these lineages result from an early and relatively rapid white-eye expansion at around 1.8 Ma followed by long periods of genetic isolation between lineages.

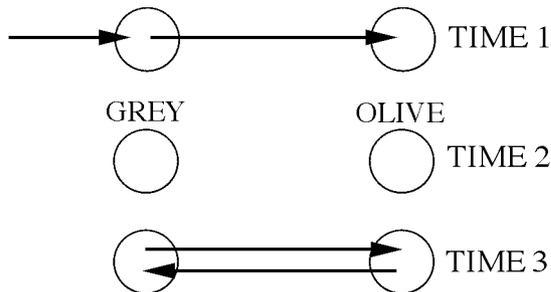
Although the rapid expansion early in the history of the ancient Indian Ocean white-eyes makes it difficult to interpret direction of island colonization, in the Mascarenes species relationships are clearly resolved. While single colonization of one of the Mascarene islands, followed by within-island speciation and colonization of the second island by both lineages is possible (Fig. 5, scenario 1), it would appear

**Fig. 5** Hypotheses for the origin of the Mascarene white-eyes on La Réunion and Mauritius.

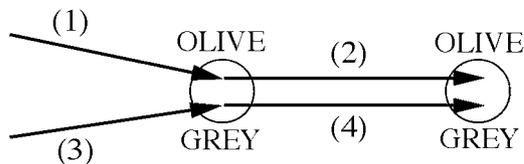
Scenario 1: Single archipelago colonization with within-island (sympatric or microallopatric) speciation. (1) Ancestral white-eye colonizes one Mascarene island. (2) Within-island speciation. (3) Colonization of the neighbouring island by both of the resulting lineages.



Scenario 2: Single archipelago colonization with species exchange between islands. TIME 1: Ancestral white-eye lineage colonizes both Mascarene islands. TIME 2: The two island populations speciate to form grey and olive lineages as a result of environmental differences between the two islands. TIME 3: Both new island-endemic species colonize their neighbouring island.



Scenario 3: Double archipelago colonization. (1) First colonization of the archipelago from an external source. (2) Inter-island colonization. (3) Second colonization of the archipelago from an external source. (4) Inter-island colonization.



unlikely on the basis of divergence times. The divergence of the grey and olive white-eyes is dated at 1.2 Ma, while the first divergence between La Réunion and Mauritius populations is dated at 0.64 Ma, 0.58 Myr later. The *Z. m. maderaspatanus* colonizations of Astove, Cosmoledo, Europa and the Iles Glorieuses demonstrate that white-eyes are relatively mobile and are able to colonize small distant islands within about 0.015 Ma. Therefore, the 0.58 Ma time delay to colonize one Mascarene island from the other seems long, and we favour either one of two alternative scenarios (2 and 3; Fig. 5) over this one. Distinguishing single archipelago colonization with species exchange between islands (Fig. 5, scenario 2) from double archipelago colonization (Fig. 5, scenario 3) would require a more detailed population

level study. Double archipelago colonization was favoured by Gill (1971). Given that the olive white-eye divergence (0.64 Ma) preceded that of the grey white-eyes (0.43 Ma), under scenario 3 we further hypothesize that the olive white-eye lineage colonized the Mascarenes before the grey white-eye lineage (Fig. 5, scenario 3). This hypothesis based on mtDNA evidence conforms to predictions made by Gill (1971) on morphological grounds.

In the *maderaspatanus* white-eyes, Bremer's (1992) ancestral areas method supports the Comoros as the centre of origin of the clade in the Indian Ocean. Such an origin is plausible given the geographical location of the Comoros as 'stepping-stones' equidistant from Africa and Madagascar. We estimate an age of 1.2 Ma for this initial colonization of the Indian Ocean from Africa (Node 16, Fig. 4). Since Grande Comore's estimated origin (0.5 Ma) postdates this colonization, we suggest that the ancestor of the *maderaspatanus* clade probably first colonized one of the older Comoro islands of Moheli, Anjouan or Mayotte.

Given the early divergence of the *Z. modestus* lineage from other lineages of the *maderaspatanus* clade (Fig. 4) we hypothesize that the granitic Seychelles were colonized from the Comoros early in the history of the *maderaspatanus* clade (1.0 Ma based on our dating of node 18, Fig. 6). The absence of the *Z. modestus* lineage from the intervening islands between these two points (the Aldabra Group, Farquhars and Amirantes) can be reasonably explained by the fact that these islands are all much younger than the 1.0 Ma expansion or have been completely inundated since this time causing the extirpation of any remnant descendants.

Divergence of the Anjouan white-eye (*Zosterops maderaspatanus anjuanensis*) from the rest of the *maderaspatanus* clade (0.67 Ma, node 19, Fig. 4) appears to have been followed by a rapid expansion of the Comoro lineage sister to the one occupying Anjouan across the islands of the Comoros, Aldabra and Madagascar at roughly 0.5 Ma (node 20, Fig. 4), giving rise to five other *maderaspatanus* lineages (*Z. m. comorensis*, *Z. m. kirki*, *Z. m. mayottensis*, *Z. m. maderaspatanus* and *Z. m. aldabrensis*, Fig. 6). Surprisingly, the colonization of Madagascar by the ancestor of *Z. m. maderaspatanus* at 0.5 Ma (node 20, Fig. 4) appears to postdate the arrival of the *maderaspatanus* clade ancestor in the Comoros (1.2 Ma) considerably (node 16, Fig. 4). We speculate that colonization of Madagascar may have been delayed owing to processes opposing community invasion, possibly competitive displacement by an ancient Indian Ocean white-eye on Madagascar, which later became extinct. Alternatively, earlier colonization by the *maderaspatanus* clade may itself have given rise to forms that are now extinct.

The distribution and diversity of populations of *Z. m. maderaspatanus* and *Z. m. voeltzkowi* appear to reflect recent dispersal events, with some taxa still in the early stages of differentiation. Low levels of sequence divergence between the Madagascar population and populations on Astove

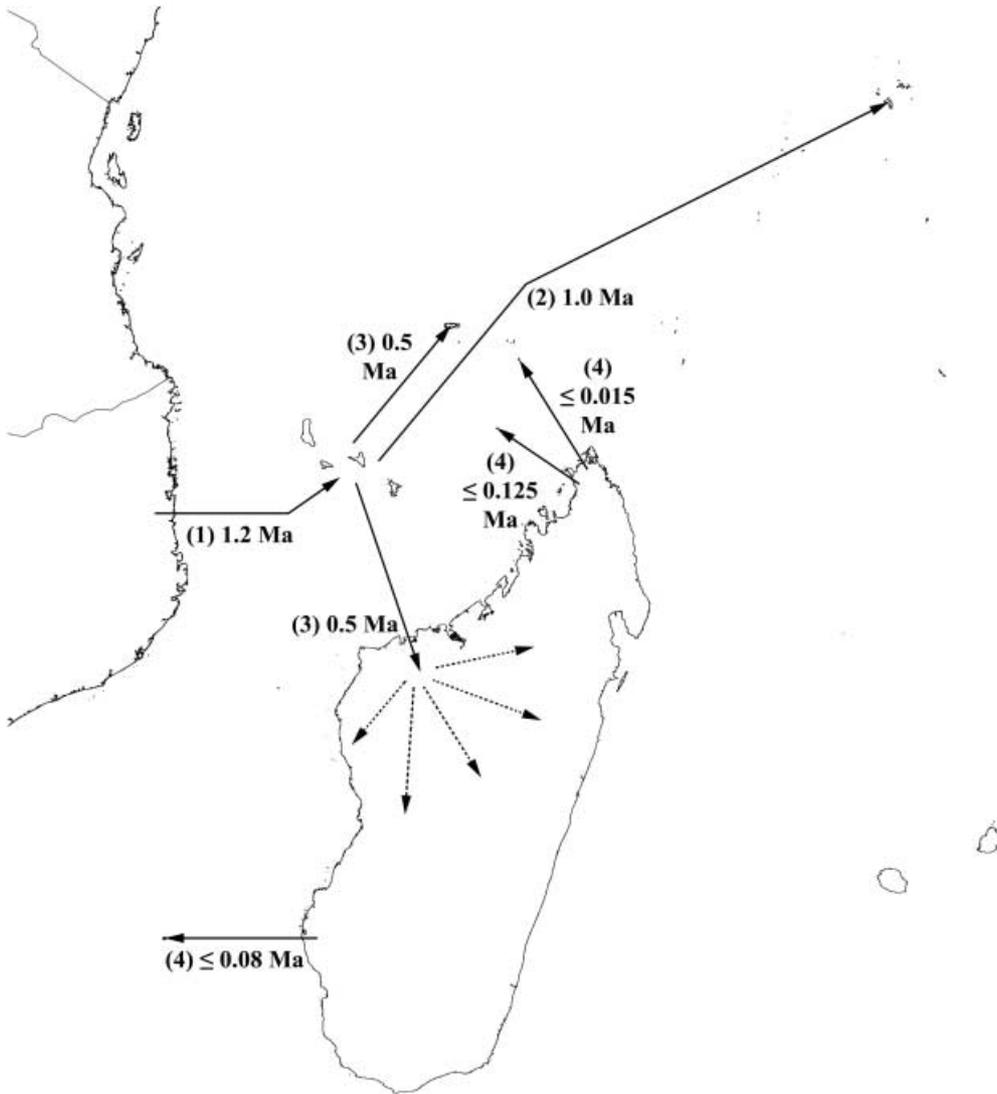


Fig. 6 Hypothesized pattern of colonization of the *maderaspatanus* white-eye clade.

(0.32% absolute divergence) and Europa (0.15% absolute divergence) support recent colonizations of the latter two islands from Madagascar. Finally, the *Z. m. maderaspatanus* population of the Iles Glorieuses has not diverged from the Madagascar population based on the available *cyt b* data. We therefore suggest that it represents a very recent colonization from Madagascar, or that there is ongoing gene flow from the Madagascar population.

*Speciation and extinction in relation to white-eye species diversity.*

The biogeographic disjunction between *Z. semiflavus*, *Z. mouroniensis* and the Mascarene white-eyes, coupled with the lack of branch support for relationships and high mtDNA divergences, suggest that related forms may have once existed on intervening landmasses which have now become extinct. Whether the lineage originated in Asia or

Africa, it is hard to imagine how white-eyes could have rapidly dispersed to Grande Comore, the Mascarenes and granitic Seychelles without colonizing Madagascar en route. Since the divergence of *maderaspatanus* white-eyes from an African ancestor did not occur until after ancient Indian Ocean white-eye arrival, there is no basis to predict competitive exclusion of ancient Indian Ocean white-eyes from Madagascar by any other white-eye lineage. In addition, a 1.8 Ma origin for *Z. mouroniensis* (based on the dating of the basal white-eye polytomy from which it is derived) considerably predates the volcanic origin of Grande Comore (0.5 Ma), the only island occupied by this species. The most likely explanation is that the divergence of the *Z. mouroniensis* lineage from other white-eye lineages does not represent a colonization of Grande Comore, but rather a colonization of Madagascar or one of the eastern Comoro

islands by a close relative to the extant *Z. mouroniensis* that is now extinct. That an undiscovered ancient Indian Ocean white-eye species persists in Madagascar or the eastern Comoros seems extremely unlikely. While the high-altitude form of Madagascar white-eye is larger than the lowland forms, our sampling of this population shows that it is undifferentiated from lowland *Z. m. maderaspatanus* based on ND3 and cyt *b* data (Fig. 3).

The combined evidence from distributions, geology and mtDNA divergence is therefore consistent with the view that extinction has played a role in determining the current distribution of the ancient Indian Ocean white-eyes.

## Conclusions

The early Indian Ocean white-eye expansion appears to have been followed by a long period of evolutionary independence, with a lack of gene flow between islands in which the ancestral form speciated, and many lineages may have subsequently become extinct. A second wave of immigration into the Indian Ocean from Africa by the ancestor of the *maderaspatanus* clade appears also to have been rapid, and was followed by evolutionary independence of the main lineages and a further secondary expansion of *Z. m. maderaspatanus* from Madagascar to the youngest islands. Where two *Zosterops* species occupy or may have formerly occupied the same Indian Ocean island, the fact that in two cases (Grande Comore and Marianne) the pair are not only nonmonophyletic but also derived from independent Indian Ocean colonizations makes a compelling case for double-island colonization rather than within-island speciation. Further, the phylogenetic placement of the Mascarene white-eyes allows us to rule out independent within-island speciation on one of the archipelago's two islands. Clearly, the distinction between *in situ* diversification events vs. long-distance immigration events varies depending on the geographical scale under consideration. Our results demonstrate that in white-eyes, long-distance immigration is an important component explaining diversity at the scale of the southwest Indian Ocean and below. At the scale of any individual southwest Indian Ocean island, accumulated white-eye diversity reflects immigration alone.

Although almost all southwest Indian Ocean islands are occupied by white-eyes as a result of two waves of immigration from the continents, our results reveal that the distribution of *maderaspatanus* white-eyes complements the distribution of the ancient Indian Ocean white-eyes, and the only locations where the two meet are on Grande Comore, where they are altitudinally segregated, and possibly in the granitic Seychelles prior to recent human arrival. It appears that in *Zosterops* (like in *Nectarinia*: Warren *et al.* 2003), one of two possible factors may be important in limiting the 'invasibility' (*sensu* Bruun & Ejrnæs 2006) of Indian Ocean island communities; the scarcity of geograph-

ical overlap in Indian Ocean white-eyes despite their high colonizing potential could be an indication that new island colonists hybridize with existing residents. Alternatively, it may be that species limit the distribution of their congeners as a result of low survival of small colonizing populations in competition with a larger resident population in the absence of sufficient niche partitioning. Further, the existence of more than one continental source of southwest Indian Ocean white-eyes in combination with their rapid expansion and wide distribution across the region suggests that chance dispersal events between islands are frequent in an evolutionary timeframe. Therefore, notwithstanding the high dispersal ability of white-eyes, it seems likely that the invasibility of island communities, rather than the incidence of island arrival events alone, may be the key in determining their present-day white-eye diversity.

## Dedication

We dedicate this paper to the memory of Aurélien Amjaud, a talented young researcher, who died aged 27, in a tragic accident on the island of La Réunion on July 5 2006. Aurélien had recently begun his PhD research into the causes of intra-island variation in the Mascarene White-eye *Zosterops borbonicus*.

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This work formed part of the PhD of Ben Warren who is currently involved in postdoctoral research into processes giving rise to the high species richness of Africa's Cape Flora. Christophe Thébaud is a Professor of evolutionary biology at Paul Sabatier University. He investigates the mechanisms driving evolutionary and ecological diversification, with special attention to Mascarene birds and plants. Dr Prys-Jones is head of the bird group of the Natural History Museum, UK. He has had a long-standing interest in the birds of the Indian Ocean, especially Aldabra. Dr Bermingham is a staff scientist at STRI in Panama with experience in the evolutionary genetics of Caribbean island birds, providing a parallel platform for Indian Ocean studies.

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## Appendix I

List of taxa included in this study and sampling localities. Gene regions sequenced for each sample are indicated in the right-hand three columns

Southwest Indian Ocean localities	Taxon	Number	Sample type	Collection	ATPase 6&8	ND3	Cyt b
La Réunion, Mascarenes	<i>Z. borbonicus borbonicus</i>	BWM46	Blood	B. Warren		X	X
La Réunion, Mascarenes	<i>Z. borbonicus borbonicus</i>	BWM47	Blood	B. Warren		X	X
La Réunion, Mascarenes	<i>Z. borbonicus borbonicus</i>	BWM54	Blood	B. Warren	X	X	X
Mauritius, Mascarenes	<i>Z. borbonicus mauritianus</i>	BWM17	Blood	B. Warren		X	X
Mauritius, Mascarenes	<i>Z. borbonicus mauritianus</i>	BWM24	Blood	B. Warren	X	X	X
Mauritius, Mascarenes	<i>Z. borbonicus mauritianus</i>	BWM25	Blood	B. Warren		X	X
La Réunion, Mascarenes	<i>Z. olivaceus olivaceus</i>	BWM49	Blood	B. Warren	X	X	X
La Réunion, Mascarenes	<i>Z. olivaceus olivaceus</i>	BWM55	Blood	B. Warren		X	X
Mauritius, Mascarenes	<i>Z. olivaceus chloronothos</i>	BWM28	Blood	B. Warren	X	X	X
Mauritius, Mascarenes	<i>Z. olivaceus chloronothos</i>	BWM29	Blood	B. Warren		X	X
Conception, Granitic Seychelles	<i>Z. modestus</i>	BW344	Blood	B. Warren	X	X	X
Conception, Granitic Seychelles	<i>Z. modestus</i>	BW345	Blood	B. Warren		X	X
Mayotte, Comoros	<i>Z. maderaspatanus mayottensis</i>	BW64	Blood	B. Warren		X	X
Mayotte, Comoros	<i>Z. maderaspatanus mayottensis</i>	BW67	Blood	B. Warren	X	X	X
Mayotte, Comoros	<i>Z. maderaspatanus mayottensis</i>	BW68	Blood	B. Warren		X	X
Moheli, Comoros	<i>Z. maderaspatanus comorensis</i>	BW121	Blood	B. Warren		X	X
Moheli, Comoros	<i>Z. maderaspatanus comorensis</i>	BW127	Blood	B. Warren	X	X	X
Moheli, Comoros	<i>Z. maderaspatanus comorensis</i>	BW169	Blood	B. Warren		X	X
Grande Comore, Comoros	<i>Z. maderaspatanus kirki</i>	BW146	Blood	B. Warren	X	X	X
Grande Comore, Comoros	<i>Z. maderaspatanus kirki</i>	BW147	Blood	B. Warren		X	X
Grande Comore, Comoros	<i>Z. maderaspatanus kirki</i>	BW231	Blood	B. Warren		X	X
Grande Comore, Comoros	<i>Z. mouroiensis</i>	BW137	Blood	B. Warren	X	X	X
Grande Comore, Comoros	<i>Z. mouroiensis</i>	BW140	Blood	B. Warren		X	X
Grande Comore, Comoros	<i>Z. mouroiensis</i>	BW141	Blood	B. Warren		X	X
Anjouan, Comoros	<i>Z. maderaspatanus anjuanensis</i>	BW252	Blood	B. Warren		X	X
Anjouan, Comoros	<i>Z. maderaspatanus anjuanensis</i>	BW253	Blood	B. Warren	X	X	X
Anjouan, Comoros	<i>Z. maderaspatanus anjuanensis</i>	BW255	Blood	B. Warren		X	X
Aldabra, Aldabra Group, Seychelles	<i>Z. maderaspatanus aldabrensis</i>	BW301	Blood	B. Warren	X	X	X
Aldabra, Aldabra Group, Seychelles	<i>Z. maderaspatanus aldabrensis</i>	BW303	Blood	B. Warren		X	X
Aldabra, Aldabra Group, Seychelles	<i>Z. maderaspatanus aldabrensis</i>	BW304	Blood	B. Warren		X	X
Mt Ankaratra, Madagascar	<i>Z. maderaspatanus maderaspatanus</i>	BW429	Blood	B. Warren		X	X
Mt Ankaratra, Madagascar	<i>Z. maderaspatanus maderaspatanus</i>	BW446	Blood	B. Warren		X	X
Southern Antsiranana, Madagascar	<i>Z. maderaspatanus maderaspatanus</i>	393447	Tissue	FMNH	X	X	X
Southern Antsiranana, Madagascar	<i>Z. maderaspatanus maderaspatanus</i>	393446	Tissue	FMNH	X	X	X
Europa	<i>Z. maderaspatanus voeltzkowi</i>	ML26	Blood	M. Le Corre		X	X
Europa	<i>Z. maderaspatanus voeltzkowi</i>	ML30	Blood	M. Le Corre		X	X
Glorieuse Island	<i>Z. maderaspatanus maderaspatanus</i>	1906.12.21.413	Skin	BMNH			X
Astove, Aldabra Group, Seychelles	<i>Z. maderaspatanus maderaspatanus</i>	1969.1.1	Skin	BMNH			X
Astove, Aldabra Group, Seychelles	<i>Z. maderaspatanus maderaspatanus</i>	1969.1.2	Skin	BMNH			X
Mkaoni, Pemba	<i>Z. vaughani</i>	1947.5.53	Skin	BMNH			X
Marianne, Granitic Seychelles	<i>Z. semiflavus</i> (extinct)	1927.12.18.398	Skin	BMNH		X	X

**Appendix I** *Continued*

Localities outside of the southwest Indian Ocean	Taxon	Number	Sample type	Collection	ATPase 6&8	ND3	Cyt b
Mt Cameroon, Cameroon	<i>Speirops melanocephalus</i>	1966.16.3378	Skin	BMNH		X	
Sasan, Kathiawar, Gujarat, India	<i>Z. palpebrosus egregious</i>	1964.26.1	Skin	BMNH		X	X
Sasan, Kathiawar, Gujarat, India	<i>Z. palpebrosus egregious</i>	1964.26.2	Skin	BMNH			X
Socotra, Gulf of Aden	<i>Z. abyssinicus socotranus</i>	BW292	Blood	B. Warren	X	X	X
Socotra, Gulf of Aden	<i>Z. abyssinicus socotranus</i>	BW293	Blood	B. Warren		X	X
Mt Trusmadi, Sabah, Borneo	<i>Z. atricapillus atricapillus</i>	B36434	Tissue	LSUMZ	X	X	X
Mt Trusmadi, Sabah, Borneo	<i>Z. atricapillus atricapillus</i>	B36444	Tissue	LSUMZ		X	X
Northern Region, Ghana	<i>Z. senegalensis senegalensis</i>	B39250	Tissue	LSUMZ		X	X
Northern Region, Ghana	<i>Z. senegalensis senegalensis</i>	B39514	Tissue	LSUMZ		X	X
From captivity	<i>Z. japonicus</i>	B20880	Tissue	LSUMZ	X	X	X
Helgoland, Germany	<i>Sylvia atricapilla</i>	B13476	Tissue	LSUMZ		X	X
Sao Tome, Gulf of Guinea	<i>Speirops lugubris</i>	MM2	Blood	M. Melo	X	X	X
Nepal	<i>Z. palpebrosus palpebrosus</i>	RF1	Blood	R. Fleischer	X	X	X
Nepal	<i>Z. palpebrosus palpebrosus</i>	RF2	Blood	R. Fleischer		X	X
Cape Town, South Africa	<i>Z. pallidus capensis</i>	RB1	Blood	R. Bowie		X	X
Mt Kenya, Kenya	<i>Z. poliogaster kikuyuensis</i>	RB2	Blood	R. Bowie	X	X	X
Nairobi, Kenya	<i>Z. abyssinicus flavilateralis</i>	RB3	Blood	R. Bowie		X	X
Cape Town, South Africa	<i>Z. pallidus capensis</i>	RB4	Blood	R. Bowie		X	X
Brisbane, Australia	<i>Z. lateralis familiaris</i>	SCB76	Blood	S. Clegg		X	X
Brisbane, Australia	<i>Z. lateralis familiaris</i>	SCB77	Blood	S. Clegg	X	X	X
Mt Kulal, E Turkana, Kenya	<i>Z. poliogaster kulalensis</i>	O8629	Blood	ZMUC	X	X	X
From captivity	<i>Z. erythropleurus</i>	O2776	Blood	ZMUC	X	X	X
Baidaihe, Hebei, China	<i>Z. erythropleurus</i>	O2653	Blood	ZMUC	X	X	X
Mt. Pulog, Luzon, Philippines	<i>Z. montanus whiteheadi</i>	O2655	Blood	ZMUC	X	X	X
Mt. Pulog, Luzon, Philippines	<i>Z. montanus whiteheadi</i>	O2662	Blood	ZMUC	X	X	X
Cayapa, Luzon, Philippines	<i>Z. nigrorum aureiloris</i>	O3732	Blood	ZMUC		X	X
Dinapique, Isabella, Philippines	<i>Z. nigrorum aureiloris</i>	O2663	Blood	ZMUC	X	X	X
Karisia, N. Aberdares, Kenya	<i>Z. poliogaster kikuyuensis</i>	O8425	Blood	ZMUC	X	X	X
Mbizi Forest, Tabora, Tanzania	<i>Z. abyssinicus flavilateralis</i>	O7570	Blood	ZMUC		X	X
Ngofi Forest, N Pare, Tanzania	<i>Z. poliogaster mbuluensis</i>	O4191	Blood	ZMUC		X	X
Mbololo, Teita, Kenya	<i>Z. poliogaster silvanus</i>	O8580	Blood	ZMUC	X	X	X
Mount Shengena, S Pare, Tanzania	<i>Z. poliogaster winifredae</i>	O5899	Blood	ZMUC		X	X
Uhafiwa, Tanzania	<i>Z. senegalensis stierlingi</i>	O8255	Blood	ZMUC	X	X	X
Karisia, N. Aberdares, Kenya	<i>Z. poliogaster kikuyuensis</i>	O8447	Blood	ZMUC		X	X
Mt Pulong, Luzon, Philippines	<i>Stachyris whiteheadi</i>	O2410	Blood	ZMUC	X	X	X

## Appendix II

Models of DNA substitution identified by MODELTEST AND associated parameters

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*(i) Two-gene data for the full sample set*

MODELTEST identified the TIM model of DNA substitution (Rodríguez *et al.* 1990) with invariable sites and gamma shape parameter (TIM+I+G) as best describing the data under the Akaike information criterion. Estimates of substitution rates under this model are A-C, 1; A-G, 18.1288; A-T, 0.5881; C-G, 0.5881; C-T, 12.0050; G-T, 1. The proportion of invariable sites and gamma distribution shape parameter are estimated as 0.6131 and 1.1672 respectively.

*(ii) Four-gene data for the restricted sample set*

MODELTEST again identified the TIM+I+G model of DNA substitution (Rodríguez *et al.* 1990) as best describing the data under the Akaike information criterion. Estimates of substitution rates under this model are A-C, 1; A-G, 20.4649; A-T, 0.3983; C-G, 0.3983; C-T, 9.9287; G-T, 1. The proportion of invariable sites and gamma distribution shape parameter are estimated as 0.5848 and 1.0767 respectively.

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