

# Molecular phylogeography reveals island colonization history and diversification of western Indian Ocean sunbirds (*Nectarinia*: Nectariniidae)

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## Abstract

We constructed a phylogenetic hypothesis for western Indian Ocean sunbirds (*Nectarinia*) and used this to investigate the geographic pattern of their diversification among the islands of the Indian Ocean. A total of 1309 bp of mitochondrial sequence data was collected from the island sunbird taxa of the western Indian Ocean region, combined with sequence data from a selection of continental (African and Asian) sunbirds. Topological and branch length information combined with estimated divergence times are used to present hypotheses for the direction and sequence of colonization events in relation to the geological history of the Indian Ocean region. Indian Ocean sunbirds fall into two well-supported clades, consistent with two independent colonizations from Africa within the last 3.9 million years. The first clade contains island populations representing the species *Nectarinia notata*, while the second includes *Nectarinia souimanga*, *Nectarinia humbloti*, *Nectarinia dussumieri*, and *Nectarinia coquereli*. With respect to the latter clade, application of Bremer's [Syst. Biol. 41 (1992) 436] ancestral areas method permits us to posit the Comoros archipelago as the point of initial colonization in the Indian Ocean. The subsequent expansion of the *souimanga* clade across its Indian Ocean range occurred rapidly, with descendants of this early expansion remaining on the Comoros and granitic Seychelles. The data suggest that a more recent expansion from Anjouan in the Comoros group led to the colonization of Madagascar by sunbirds representing the *souimanga* clade. In concordance with the very young geological age of the Aldabra group, the sunbirds of this archipelago have diverged little from the Madagascar population; this is attributed to colonization of the Aldabra archipelago in recent times, in one or possibly two or more waves originating from Madagascar. The overall pattern of sunbird radiation across Indian Ocean islands indicates that these birds disperse across ocean barriers with relative ease, but that their subsequent evolutionary success probably depends on a variety of factors including prior island occupation by competing species.

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## 1. Introduction

Islands in the western Indian Ocean around Madagascar (the Seychelles, Comoros, and Mascarenes) vary in geological age from 15,000 years (Aldabra Group) to 75 million years (granitic Seychelles) (Fig. 1), providing an attractive system for evolutionary studies relating estimated divergence times to island age. Recent years have seen a growing interest in evolutionary studies of

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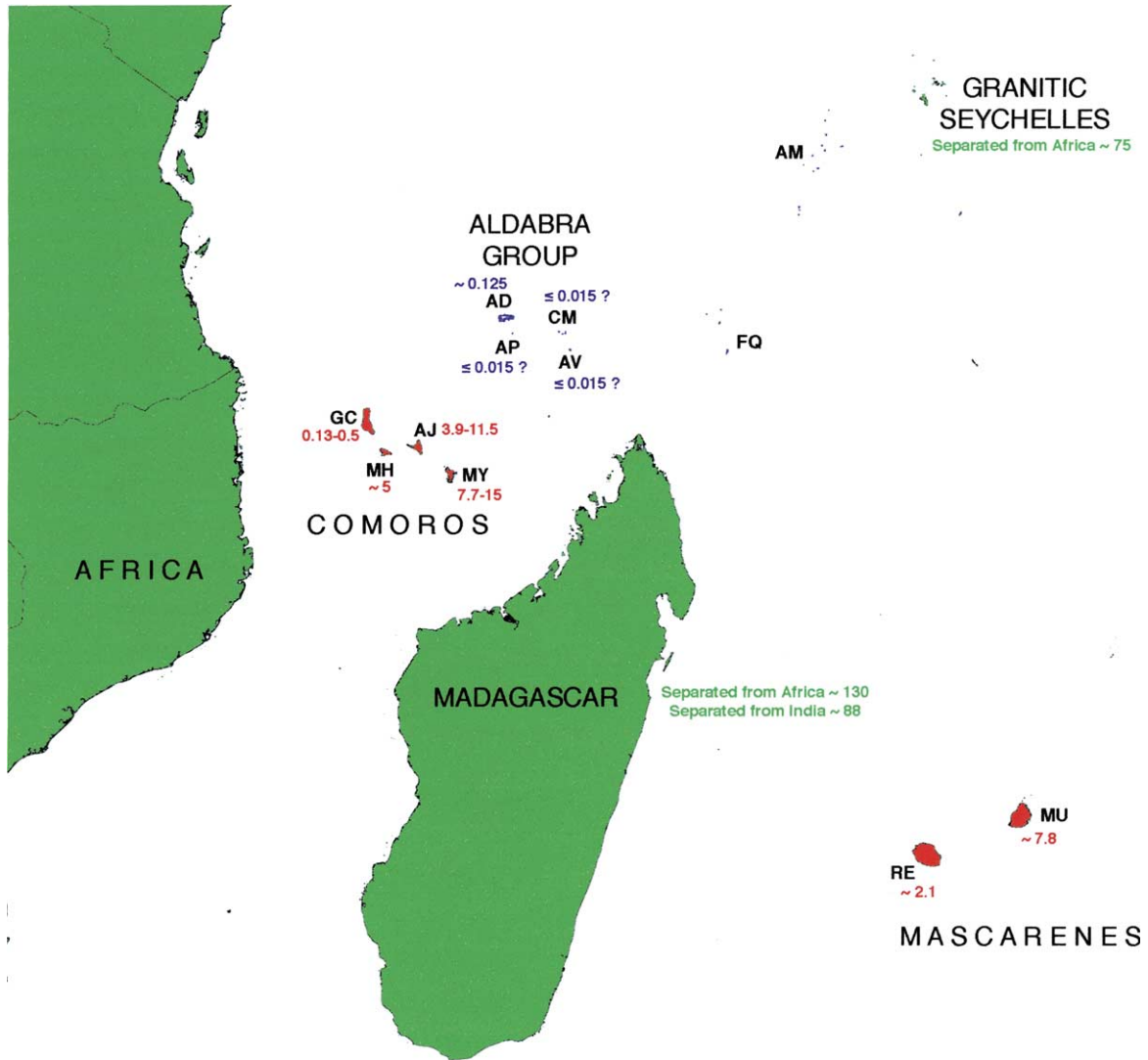


Fig. 1. The geology of the western Indian Ocean. Continental land masses are indicated in green, volcanic islands in red, coralline, and sand islands in blue. Dates correspond to island age in Ma. GC, Grande Comore; MH, Moheli; AJ, Anjouan; MY, Mayotte; AD, Aldabra; AP, Assumption; CM, Cosmoledo; AV, Astove; FQ, Farquahars; AM, Amirantes; RE, La Reunion; and MU, Mauritius.

island archipelagos such as the Hawaiian Islands, Canary Islands, the Galapagos, and the Caribbean, for a number of reasons. The presence or absence of a terrestrial species on any particular island often allows both its current distribution and inferred ancestral distributions and colonizations to be more easily defined than they could be on a continent. Conversely, expanses of open ocean are easy to identify as barriers to gene flow. Species-level phylogenies in island settings can potentially provide useful insights into the rate of species diversification or lineage accumulation with time (Barracough and Nee, 2001; Lovette and Bermingham, 1999; Ricklefs and Bermingham, 2001), and into the mode of speciation (Coyne and Price, 2000; Turelli et al., 2001; Via, 2001), in particular the role of geography (Barracough and Vogler, 2000; Hewitt, 2001). Potentially they might also indicate the vulner-

ability of species to extinction (Ricklefs and Bermingham, 1999) and provide information on rates of population turnover in island communities. Archipelagos also provide the opportunity to trace the evolutionary history of multiple evolutionary lineages in the same geographical setting. This can provide 'replicates' for the testing of evolutionary hypotheses, and improve our understanding of the relative contribution of local, regional, and historical processes, as well as unique events and circumstances, to community diversity (Arnold, 2000; Bermingham and Moritz, 1998; Juan et al., 2000; Ricklefs, 1987; Ricklefs and Bermingham, 2001).

The western Indian Ocean setting provides an opportunity to examine the extent to which avian colonization patterns are influenced by geological events, which show greater variation in time-scale than in other

island regions. Owing to a widespread distribution across many Indian Ocean islands and a high level of endemism, sunbirds in the genus *Nectarinia* provide a good model for an initial assessment of avian colonization patterns in this region. Here we use 1309 bp of mitochondrial sequence data to address the origin and diversification of the sunbirds of the western Indian Ocean. Using topological and branch-length information, combined with estimates of divergence times and geological data, we present hypotheses for the direction and sequence of colonization events among islands of the western Indian Ocean.

Although much of the Madagascar and Indian Ocean avifauna shows a high level of provincialism, *Nectarinia* is distributed across Afrotropical and Oriental regions into Australasia and may be the most speciose avian genus in the world with 80 species. For the purposes of this study, we retain the genus name *Nectarinia* instead of *Cinnyris*, which has recently been proposed for many of the sunbirds (Cheke et al., 2001; Fry, 2000; Irwin, 1999), because results from a higher level systematics study strongly suggest that *Cinnyris* is polyphyletic under Irwin's (1999) or Cheke et al.'s (2001) classification scheme (Bowie, unpublished). The taxonomic placement

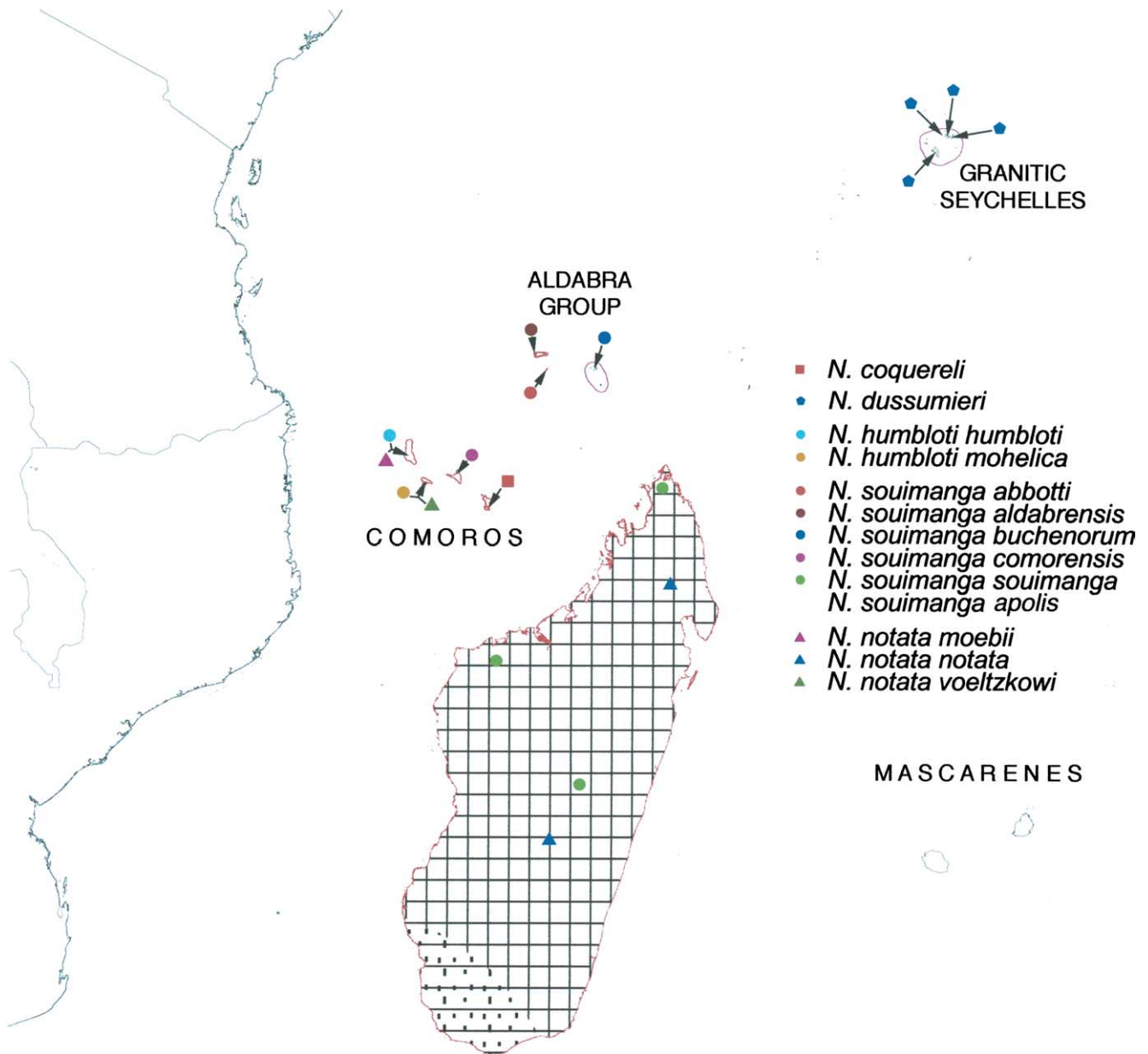


Fig. 2. Distribution of sunbirds of the western Indian Ocean. Symbols indicate sampling localities for each taxon. Coastlines representing the edge of a taxon's range are depicted in red. Purple lines show the distribution of taxa occupying a number of small islands. On Madagascar, the range of *N. notata notata* is shaded with horizontal lines, *N. souimanga souimanga* with vertical lines, and *N. souimanga apolis* with vertical broken lines.

of the Indian Ocean sunbirds is difficult to determine using purely morphological characters due to the structural and skeletal uniformity within the Nectariniidae (Farquhar et al., 1996) and plumage variation is also not strongly informative because female coloration is relatively uniform and male color pattern appears to be under strong sexual selection. Notwithstanding the uncertain relationships of the sunbirds, linear taxonomies generally recognize three or more independent origins of Indian Ocean *Nectarinia* (Benson, 1960; Sibley and Monroe, 1990). Five endemic sunbird species are generally recognized in the western Indian Ocean region: *Nectarinia notata*, *Nectarinia souimanga*, *Nectarinia humbloti*, *Nectarinia dussumieri*, and *Nectarinia coquereli* and these are represented by 13 subspecies (Fig. 2) (Irwin, 1999; Louette, 1988). Of these, *N. notata* is consistently represented as having a separate origin from the others, but the literature indicates little consensus regarding the relationships and origins of the other four species of Indian Ocean sunbirds (Benson, 1960, 1984; Louette, 1992; Sibley and Monroe, 1990).

### 1.1. Geological background for the islands of the western Indian Ocean

The islands of the western Indian Ocean can be broadly divided into three groups distinguished by both age and geology. Madagascar and the granitic Seychelles are remnants of continental blocks that were isolated around 75–130 Ma (megannum) (Coffin and Rabinowitz, 1987; Kingdon, 1990; Rabinowitz et al., 1983). The Comoros archipelago is of volcanic origin and its islands are intermediate in age (Emerick and Duncan, 1982; Nougier et al., 1986), whereas the raised coralline islands of the Aldabra group along with the Farquhars and Amirantes represent the youngest archipelagos in the region (Radtkey, 1996; Thomson and Walton, 1972).

While Madagascar is famous for its insular fauna and flora, sunbirds are one of a number of groups of organisms which are not highly diverged morphologically from neighboring island and continental forms. Therefore, we consider that the continental origins of Madagascar and the granitic Seychelles, and their isolation from other land masses, considerably predate the arrival of sunbirds in the Indian Ocean. Likewise, while seamounts in the Mozambique channel between Africa and Madagascar may have aided the arrival of some Madagascar endemics, including certain groups of birds (Fjeldså et al., 1999), they are unlikely to have been important to Indian Ocean sunbird colonization owing to their relatively old dates, approximately 45–26 Ma (Bassias, 1992; Leclaire et al., 1989; McCall, 1997).

The volcanic origins of the Comoro islands span a range of times likely to coincide with sunbird origins in the Indian Ocean. Potassium–Argon (K–Ar) dating of

the oldest exposed lavas on the islands of the Comoros archipelago are as follows (Emerick and Duncan, 1982; Nougier et al., 1986), with the estimated age of the volcanic origin for three of the islands provided parenthetically (Montaggioni and Nougier, 1981; Nougier et al., 1986; R. Duncan, personal communication): Mayotte,  $7.7 \pm 1.0$  (10–15 Ma); Anjouan,  $3.9 \pm 0.3$  (11.5 Ma); Moheli,  $5.0 \pm 0.4$ ; and Grande Comore,  $0.13 \pm 0.02$  (0.5 Ma).

The youngest archipelagos in the western Indian Ocean are the raised coralline islands of the Aldabra group (Aldabra, Assumption, Cosmoledo, and Astove), the Farquhars and the Amirantes. Thomson and Walton (1972) document a major inundation of Aldabra Atoll at the beginning of the last interglacial, ca. 0.125 Ma, which is suggested to have eliminated the contemporary terrestrial biota. Since the other islands in the Aldabra Group (Assumption, Cosmoledo, and Astove) probably only arose within the last 15,000 years (Radtkey, 1996), 0.125 Ma probably sets an upper bound on the age of the Aldabra Group. Although the Farquhar and the Amirante archipelagos lie between Aldabra and the granitic Seychelles, they are not occupied by sunbirds and thus are not considered further.

## 2. Materials and methods

### 2.1. Sampling

We obtained samples for genetic analyses from 35 individuals representing all but one of the Indian Ocean island sunbird taxa (Fig. 2). Our sample consisted of two or more individuals of each taxon, with the exception of *Nectarinia souimanga abbotti* and *Nectarinia balfouri* for which only one individual was sampled, and *N. s. apolis* (southern Madagascar) which we did not have the opportunity to collect. Blood samples were obtained during collecting expeditions to Madagascar, the Comoros, granitic Seychelles, Aldabra, Assumption, and Socotra between November 1999 and February 2002, and were preserved in Queen's lysis buffer (Seutin et al., 1991, 1993). Feather samples of *N. s. buchenorum* (Cosmoledo) were obtained in June 1999. All blood and feather samples were taken non-destructively from mist-netted individuals. The Field Museum of Natural History (FMNH) loaned us tissue samples of *N. notata* and *N. souimanga* from Madagascar. A total of 1309 bp of sequence data was obtained from the mitochondrial ATP synthase 6 (ATPase6), NADH dehydrogenase subunit 3 (ND3), and cytochrome *b* (*cytb*) genes. These data set were combined with 989 bp of ATPase 6 and ND3 from 16 continental African and Asian taxa, representing all the major sunbird lineages. The phylogenetic results from a family level study of sunbirds support an Asian origin for the Nectariniidae (Bowie, unpublished).

We thus included *Aethopyga* and representatives of both African and Asian species of the broadly distributed genera *Nectarinia* and *Anthreptes* in our analyses.

## 2.2. DNA extraction, PCR, and sequencing

DNA was extracted 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) amplification of two regions of the mitochondrial genome was performed for all individuals. The primer pair A8PWL and CO3HMH (<http://nmg.si.edu/bermlab.htm>) were used to amplify 609 base pairs of the ATPase6 gene, and L10755 and H11151 (Chesser, 1999) to amplify a 380 base pair region including the entire ND3 gene, 11 base pairs in the upstream region and 18 base pairs in the downstream region. In addition, CB1 and CB2 (Palumbi, 1996) were used to amplify a 320 base pair portion of *cytb* for all individuals of *N. souimanga*, *N. humbloti*, *N. dussumieri*, and *N. coquereli*. PCR conditions are detailed in Table 1.

PCR products were run electrophoretically on low melting point agarose gels. The amplification product was cut and the agarose digested using 1 µl GELase (Epicenter Technologies, Madison WI) at 70 °C for 5 min, followed by 4 h at 45 °C. Sequencing reactions were performed using the Taq-DyeDeoxy Terminator Cycle Sequencing kit (Applied Biosystems, Forest City, CA). After cycle-sequencing, products were cleaned by centrifugal passage through Sephadex columns (3600 rpm for 3 min) and run on an Applied Biosystems model 377 automated DNA sequencer. All sequences were imported into Sequencher 3.1 and complementary strands and consensus sequences were aligned using the automatic assembly function (GenBank Accession Nos. AY233986 to AY234017, and AY235475 to AY235569).

## 2.3. Phylogenetic analysis

### 2.3.1. All taxa

To examine the relationships between Indian Ocean taxa and their continental congeners, our first set of analyses made use of the complete ATPase6 and ND3 data sets, which included all available sunbird sequences

from the Indian Ocean, Africa, and Asia. Initially these two gene data sets (excluding outgroups) were analyzed independently using MODELTEST (Posada and Crandall, 1998) to determine the substitution model which best describes each data set. For both ATPase6 and ND3 data sets, the optimal model defined by MODELTEST was used to determine the maximum likelihood distances for a preliminary neighbor-joining (NJ) analysis using PAUP\*. The method of Shimodaira and Hasegawa (1999) implemented in PAUP\* was used to test whether the tree topologies based on the two data sets fall within the same confidence limits. As a further check of the congruence of the two data sets we used the partition homogeneity test (Farris et al., 1995) implemented in PAUP\*.

Multiple haplotypes of the same taxon were pruned from the data sets to reduce computation time for a maximum-likelihood (ML) analysis. The combined ATPase6–ND3 data set was re-run through MODELTEST, and the optimal model defined by this program was then used for a ML analysis in PAUP\* with the heuristic search algorithm. In addition, an unweighted parsimony analysis was performed using the heuristic search algorithm, holding 10 trees at each step and branch swapping on all trees, using the steepest descent option.

Further to these analyses we performed Bayesian analysis on the full data set using the program MrBayes 2.01 (<http://morphbank.ebc.uu.se/mrbayes/>) (Huelsenbeck et al., 2001). This program implements the Markov Chain Monte Carlo (MCMC) algorithm to approximate the posterior probability distribution of a large number of trees. This algorithm proposes a new tree by stochastically perturbing a previous tree. The new tree can either be accepted or rejected based on its probability. If it is accepted it is subject to further perturbation. The proportion of the time that any tree is visited in the chain is taken as an approximation of the posterior probability of that tree. Further, the posterior probability of any clade is the sum of the posterior probabilities of all the trees that contain that clade (Huelsenbeck et al., 2001). Base frequencies were estimated from the data. Four Markov chains were run simultaneously for 500,000 generations starting from random initial trees, and sampled every 10 generations. Variation in the ML

Table 1  
Primers and experimental conditions used for sunbird PCR amplification and DNA sequencing

Gene region	Primer names	Source	PCR conditions			
			Denaturation	Annealing	Extension	Number of cycles
ATPase 6	A8PWL and CO3HMH	<a href="http://nmg.si.edu/bermlab.htm">http://nmg.si.edu/bermlab.htm</a>	95 °C for 45 s	52 °C for 30 s	72 °C for 45 s	30
ND3	L10755 and H11151	Chesser (1999)	95 °C for 45 s	54 °C for 30 s	72 °C for 45 s	30
Cytb	CB1 <sup>a</sup> and CB2	Palumbi (1996)	94 °C for 45 s	55 °C for 45 s	72 °C for 1 min	35

<sup>a</sup> For *N. humbloti* CB1 was substituted for CBVL14828 (5'CCACCCTCCACTCAGGCCTAATCAA3').



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 45,000 trees in the chain. To check our results and guard against the possibility of multiple optima we repeated this process four times. As a further check for multiple optima we ran this analysis a fifth time with four million generations, and used the last 350,000 trees to determine the consensus phylogeny and posterior probability of nodes.

### 2.3.2. The *souimanga* clade of the Indian Ocean

In an attempt to improve resolution in the *souimanga* clade by enlarging our data set we collected 320 bp of *cytb* data in addition to the ATPase6 and ND3 data collected for all taxa. A data set including only *N. souimanga*, *N. humbloti*, *N. dussumieri*, and *N. coquereli* for both ATPase6–ND3 and for *cytb* was analyzed independently in MODELTEST to determine the substitution model which best describes each data set as in Section 2.3.1. These models provided maximum likelihood distances for two preliminary neighbor-joining (NJ) analyses using PAUP\*. Again we used the Shimodaira and Hasegawa (1999) test along with the partition homogeneity test (Farris et al., 1995) to determine if the two data sets could be combined for phylogenetic analysis. The combined data set was re-analyzed in MODELTEST. The optimal model defined by this program was then used for a ML analysis in PAUP\* with the heuristic search algorithm.

### 2.4. Parametric bootstrap

To test the null hypothesis that the Indian Ocean archipelagos under study had been colonized only once and that Indian Ocean taxa under study are monophyletic (derived from a single colonization event), we implemented the parametric bootstrap test proposed by Huelsenbeck et al. (1996a). Testing for monophyly requires score estimates for trees obtained both with and without the constraint of monophyly, and in turn comparing the difference in scores with the distribution of differences generated through simulation. While Huelsenbeck et al. (1996b) propose a ML approach for obtaining score estimates, we have followed the MP approach adopted by Ruedi et al. (1998) owing to prohibitive computation time under the ML criterion.

DNA sequences were simulated using Seq-Gen 1.1 (Rambaut and Grassly, 1997), which simulates the evolution of sequences along a defined phylogeny using a defined model of DNA substitution. Since this program cannot simulate incomplete sequences, the combined ATPase6–ND3 data set was trimmed to remove any slight differences in length between sequences, and the 949-bp data set was re-analyzed using MODELTEST before simulation. For the hypothesis of mono-

phyly the constrained NJ tree was constructed with PAUP\* using ML distances and parameter estimates derived from MODELTEST analysis. Sequences were then simulated using the same model of sequence evolution and parameter estimates. One hundred new data sets were simulated for the constrained topology, and heuristic searches were carried out on each of these, first with and then without the constraint. The difference between the empirical constrained and non-constrained trees was then compared to the distribution of simulated tree length differences.

### 2.5. Estimation of divergence times

Rate heterogeneity was first tested using PAUP\* to determine whether branch lengths were consistent with a molecular clock. We compared divergence times obtained under the assumption of a molecular clock with those obtained following the Penalized Likelihood method of Sanderson (2002). This method combines a parametric model having a different substitution rate on every branch with a nonparametric roughness penalty, and introduces a cost if rates change too quickly from branch to branch. The relative contribution of these two components to the model is determined by a smoothing parameter. We used the program r8s 1.06 (<http://ginger.ucdavis.edu/r8s/>) which allows one or more nodes in the tree to be assigned ages. The relative age of other nodes is then calculated. In our final analysis the solution was perturbed 10 times using a factor of 0.05.

Ages were assigned to nodes in the tree based on geological estimates of island ages. Where sister taxa are found on neighboring islands we assume that age of the younger island represents an approximate estimate for the maximum age of the split between the ‘offspring’ population on the younger island and the ‘parental’ population on the older island. Fleischer et al. (1998) summarize seven assumptions made when dating nodes with this method. A further assumption not listed by Fleischer et al. (1998) applying to volcanic islands is that the subaerial lavas representing the first emergence of the island above sea level are accessible to geologists, and have not been deeply buried by later strata.

### 2.6. Estimation of direction and sequence of colonization in the *souimanga* clade

Based on the ML tree, we employed two different methods to infer the direction and sequence of colonization of the *souimanga* clade: (i) To infer the ancestral distribution of this clade we used the ancestral areas method of Bremer (1992). This method is cladistic, makes no assumptions about the mechanism of speciation, and relies solely on topological information from the ML tree. (ii) We also used the Topology Plus Branch Length method (Thorpe et al., 1994), which

makes use of ML tree topology and branch lengths to infer sequence and direction of inter-island colonization under the assumption of speciation following dispersal.

In our application of the ancestral areas method to Indian Ocean sunbirds, the taxa in the *souimanga* clade were replaced by the archipelagos on which they occur. Two parsimony analyses, which assume irreversibility of character states, were used to determine the number of gains and losses representing each archipelago. For each archipelago, the number of gains was divided by the number of losses, and the area with the highest gain/loss ratio was set to a value of one. Values for the other archipelagos were re-scaled to provide the relative probabilities of belonging to the ancestral area for the group. Under this method, regions containing basal taxa are considered more likely to represent the ancestral area than are regions containing terminal taxa.

The Topology Plus Branch Length method has a number of underlying assumptions (Thorpe et al., 1994), but basically argues that when an ancestral population (a) on island A colonizes island B, the resulting populations (a') on island A, and (b) on island B will not be equally divergent from the ancestral population (a). Population (b) will be more divergent than (a') due to founder effects. In the Topology Plus Branch Length method applied to Indian Ocean sunbirds, each ancestral node was assigned the distribution occupied by the anagenetically closest descendant.

### 3. Results

#### 3.1. Phylogenetic analysis

##### 3.1.1. All taxa

We initially conducted independent NJ analyses of the ATPase6 and ND3 data sets using the GTR + I + G and TIM + I + G models of nucleotide substitution identified, respectively, by MODELTEST. This yielded two trees that differed in the placement of the basal nodes in the *souimanga* clade. Bootstrap support for the relationships among lineages early in the diversification of the *souimanga* clade in both the ATPase6 and ND3 trees was less than 60% based on 1000 replicates of stepwise-addition searches. The method of Shimodaira and Hasegawa (1999) showed that the topology of the ATPase6 tree fell within the confidence interval of the ND3 tree, and vice versa (1000 bootstrap replicates,  $P < 0.01$  in both cases). In addition, a partition homogeneity test (Farris et al., 1995) on the combined mtDNA data (2 partitions; 989 bp) indicated that the gene regions did not differ significantly ( $P = 0.68$ ). We therefore combined these two gene data sets for the analysis. MODELTEST identified the TIM model of DNA substitution (Rodríguez et al., 1990) with invariant sites and  $\gamma$ -shape parameter (TIM + I + G) under the

Akaike information criterion. Estimates of substitution rates under this model are A–C, 1; A–G, 24.3423; A–T, 0.3701; C–G, 0.3701; C–T, 9.6863; and G–T, 1. The proportion of invariant sites and  $\gamma$  distribution shape parameter are estimated as 0.521 and 1.4145, respectively. We used these parameters and nucleotide frequencies in the ML analysis.

For Bayesian analysis we used a GTR model with invariant sites and  $\gamma$  shape parameter model of DNA substitution, given that it is the model available in MrBayes that best matches the TIM model. Owing to prohibitive computation time of bootstrapping the ML tree, a NJ analysis was conducted using maximum likelihood distances based on TIM + I + G, and the bootstrap values from this tree (stepwise-addition, 1000 replicates) are labeled on the Bayesian tree (Fig. 3) along with the Bayesian support values. An unweighted parsimony analysis resulted in 16 most parsimonious trees with length 1493 (CI, 0.415; RI, 0.515; RC, 0.214; and HI, 0.585). Bootstrap values for the consensus tree (stepwise-addition, 1000 replicates) are concordant with the ML and Bayesian trees. With the exception of nodes with low bootstrap (<50%) or Bayesian support ( $\leq 58\%$ ), the ML, MP, and the Bayesian trees had identical topologies for the two Indian Ocean clades and their closest continental relatives.

The maximum observed divergence among the sunbirds analyzed here (excluding the basal Asian species *Anthreptes malacensis* and *Aethopyga boltoni*) was 16.2% (absolute distance), whereas the minimum divergence between the two Indian Ocean clades (*notata* and *souimanga*) was 10.9% (absolute distance). The Bayesian tree (Fig. 3) confirmed that the Indian Ocean sunbirds (excluding *Nectarinia balfourii* of Socotra) fall into two clades, which are not closely related. The *notata* clade has strong (100%) Bayesian branch support, and comprises phylogeographically differentiated populations of *N. notata* on Madagascar, Grande Comore, and Moheli. Although the pairing of the African *Nectarinia senegalensis* as sister to the *notata* clade has only weak Bayesian (64%) and bootstrap (<50%) support, the association of these two lineages as sister to African *Nectarinia adelberti* was well supported (100%) in the Bayesian tree. The *souimanga* clade comprises *N. souimanga*, *N. humbloti*, *N. coquereli*, and *N. dussumieri*, and also has strong (100%) Bayesian branch support. The African clade of *Nectarinia bowieri*, *Nectarinia venusta*, and *Nectarinia talatala* pairs as the sister to the *souimanga* clade, although with weaker Bayesian support (87%).

##### 3.1.2. The *souimanga* clade of the Indian Ocean

We initially conducted independent NJ analyses of the *cytb*, ATPase6, and ND3 data sets using maximum likelihood distances defined by MODELTEST. The topology of the ATPase6–ND3 trees fell within the

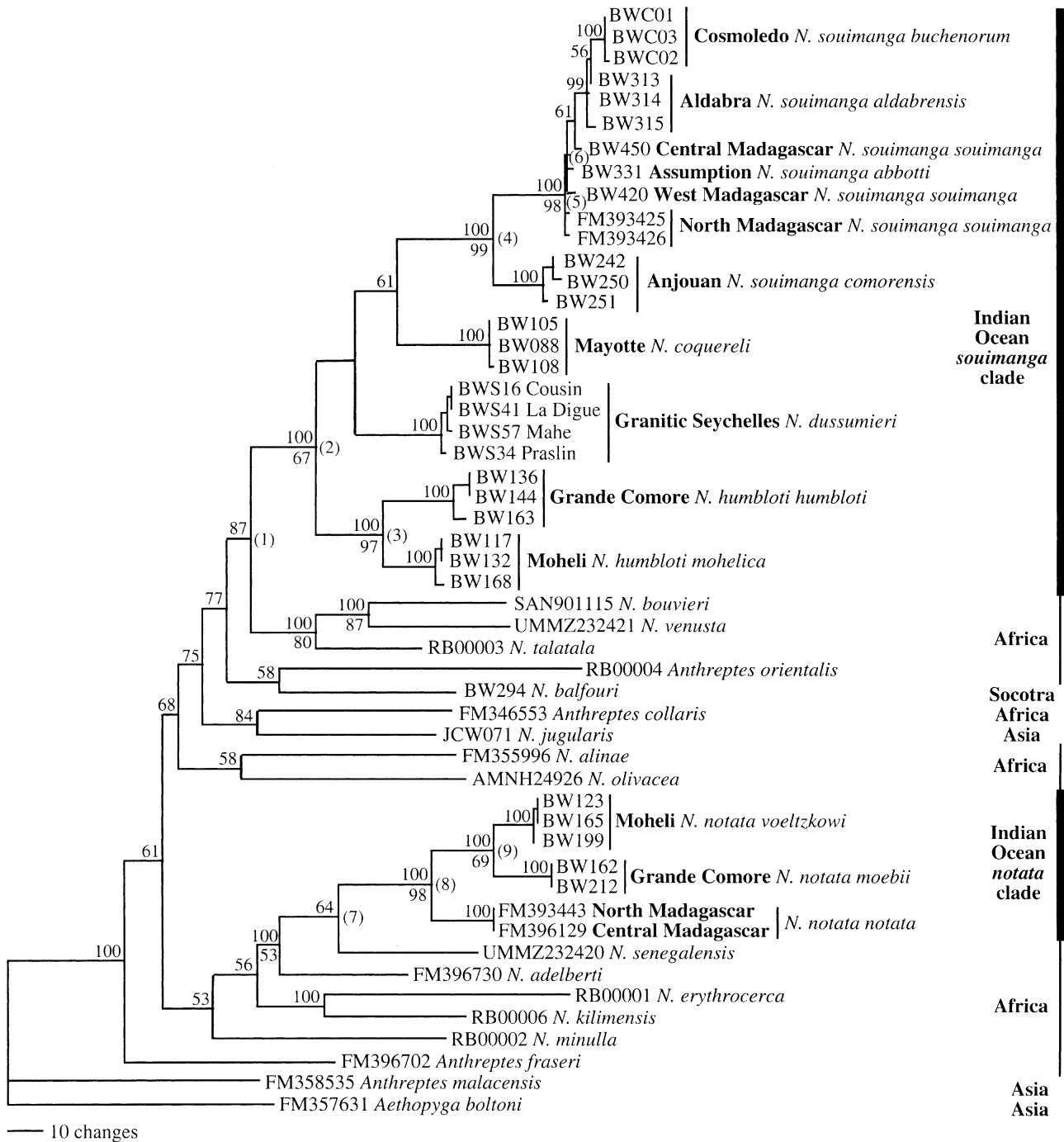


Fig. 3. Bayesian analysis of the mitochondrial ATPase6 and ND3 data set for Indian Ocean and continental sunbird taxa. Consensus of the last 350,000 trees after 4 million generations based on the GTR + I + G model. Bayesian branch support values are indicated above bootstrap values (stepwise-addition, 1000 replicates) from a NJ tree constructed using the TIM + I + G model.

confidence interval of the *cytb* tree, and vice-versa (1000 bootstrap replicates,  $P < 0.05$  in both cases). A partition homogeneity test (Farris et al., 1995) of the combined mtDNA data (3 partitions; 1309 bp) indicated that the gene regions did not differ significantly ( $P \geq 0.05$ ). We therefore combined these three gene data sets for subsequent phylogenetic analysis of the *souimanga* clade.

Based on the Akaike information criterion, MODEL-TEST indicated that TIM (Rodríguez et al., 1990) with a  $\gamma$ -shape parameter (TIM + G) best modeled the nucleotide substitution pattern of the *souimanga* clade. Estimated substitution rates under this model are: A–C, 1; A–G, 21.6143; A–T, 0.3968; C–G, 0.3968; C–T, 9.1471; and G–T, 1. The  $\gamma$ -shape parameter was estimated to be



0.1975. The greatest observed divergence within the *souimanga* clade was 8.6% (absolute distance). Bootstrap values based on 100 ML analyses are presented on the tree pictured in Fig. 4. Both this ML tree and Fig. 3

yield essentially the same topology within the *souimanga* clade, comprising four main lineages: (i) *N. coquereli* of Mayotte in the Comoros; (ii) *N. humbloti* of the Comoros, with one mtDNA lineage found on Grande

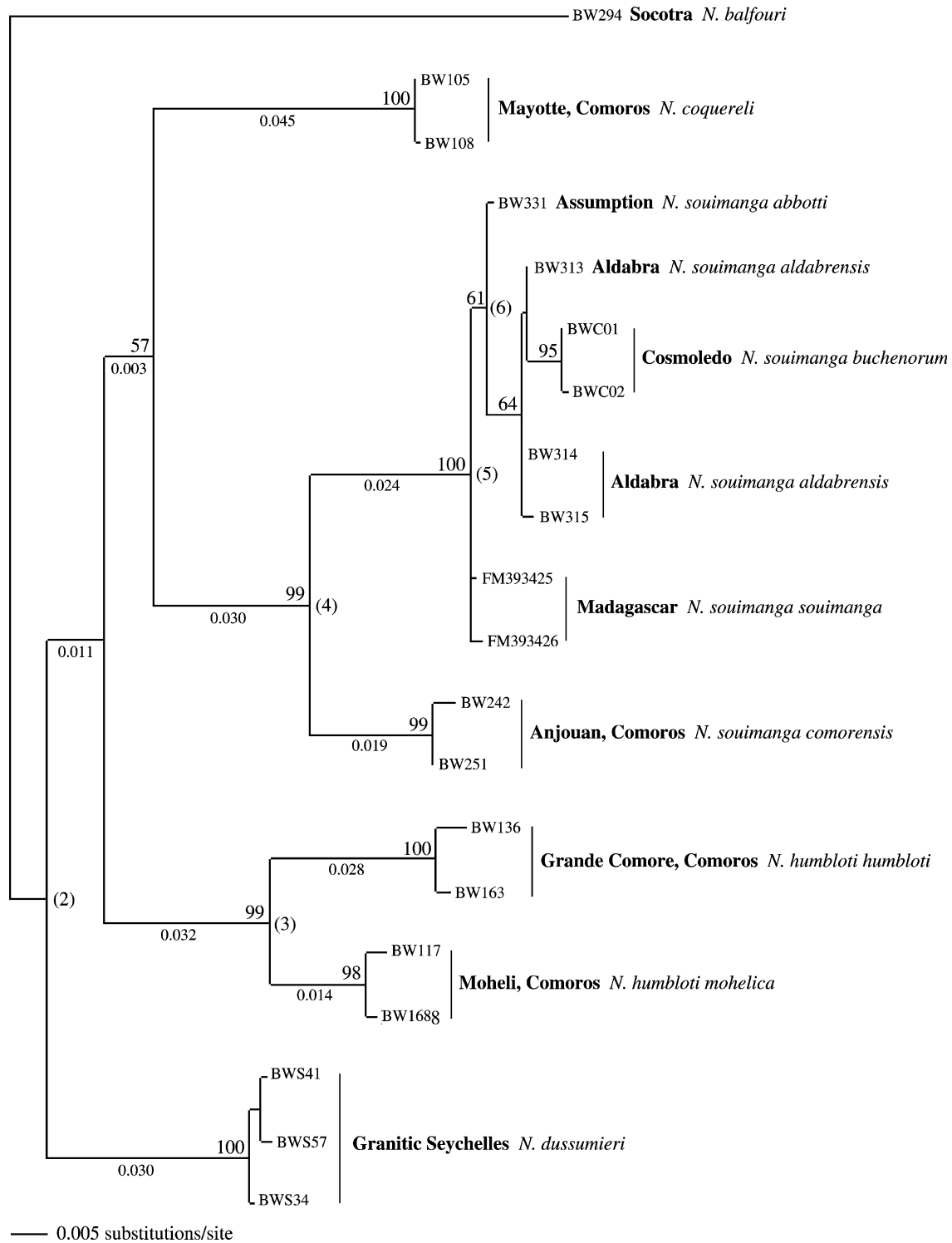


Fig. 4. Maximum likelihood tree based on the TIM + G model and the mitochondrial ATPase6, ND3, and cytb data for Indian Ocean sunbird taxa. Bootstrap values (stepwise-addition, 100 replicates) are indicated above TIM + G genetic distances.

Comore and another on Moheli; (iii) *N. souimanga*, with one lineage, *N. s. comorensis*, on Anjouan in the Comoros, and a second mtDNA lineage on Madagascar (*N. s. souimanga*) and all sampled islands in the Aldabra archipelago (*N. s. abbotti*, *N. s. aldabrensis*, and *N. s. buchenorum*), and (iv) *N. dussumieri* of the granitic Seychelles archipelago. Although *N. dussumieri* is found on a number of islands in the granitic Seychelles, there is very little mtDNA sequence divergence among individuals from the different islands. Likewise short branches and nodes with low bootstrap support separate the mtDNA lineages representing *N. souimanga* on Madagascar and in the Aldabra archipelago.

The four main lineages of the *souimanga* clade are separated by very short internodes that lack strong bootstrap support (all bootstrap confidence values <60%). Fig. 5 presents a nucleotide saturation analysis of the combined mtDNA data, and plots the absolute number of observed substitutions between individuals of the *souimanga* clade against corrected patristic distances based on the TIM + G model of nucleotide substitution. The slope of this relationship indicates that nucleotide substitutions are not saturated in these comparisons, and thus we infer that the basal diversification of the *souimanga* group was rapid relative to the rate of mtDNA substitution.

### 3.2. Parametric bootstrap

If we constrain the western Indian Ocean sunbirds—the *notata* and the *souimanga* clades—to be monophyletic, the resulting tree is 29 steps longer than the shortest unconstrained tree.

This difference in tree lengths can be evaluated against parametric bootstrap simulations of the null hypothesis of monophyly. The largest observed difference between the null and alternative hypotheses generated from the simulated data was nine steps. The probability of observing a difference of 29 steps ( $P < 0.01$ ) permits us to reject the null hypothesis of monophyly for Indian Ocean sunbirds.

### 3.3. Estimation of divergence times

There are three nodes (3, 5, and 9, Figs. 3 and 4) in the ML tree which can be used as calibration points, since the maximum age of divergence can be inferred. Firstly, since *N. humbloti* is only found on Grande Comore and Moheli, the divergence between the two island mtDNA lineages (node 3, Fig. 4) is not likely to be older than the younger of the two islands. Since Grande Comore is a younger island than Moheli, we estimate the maximum age for the node separating the two *N. humbloti* lineages

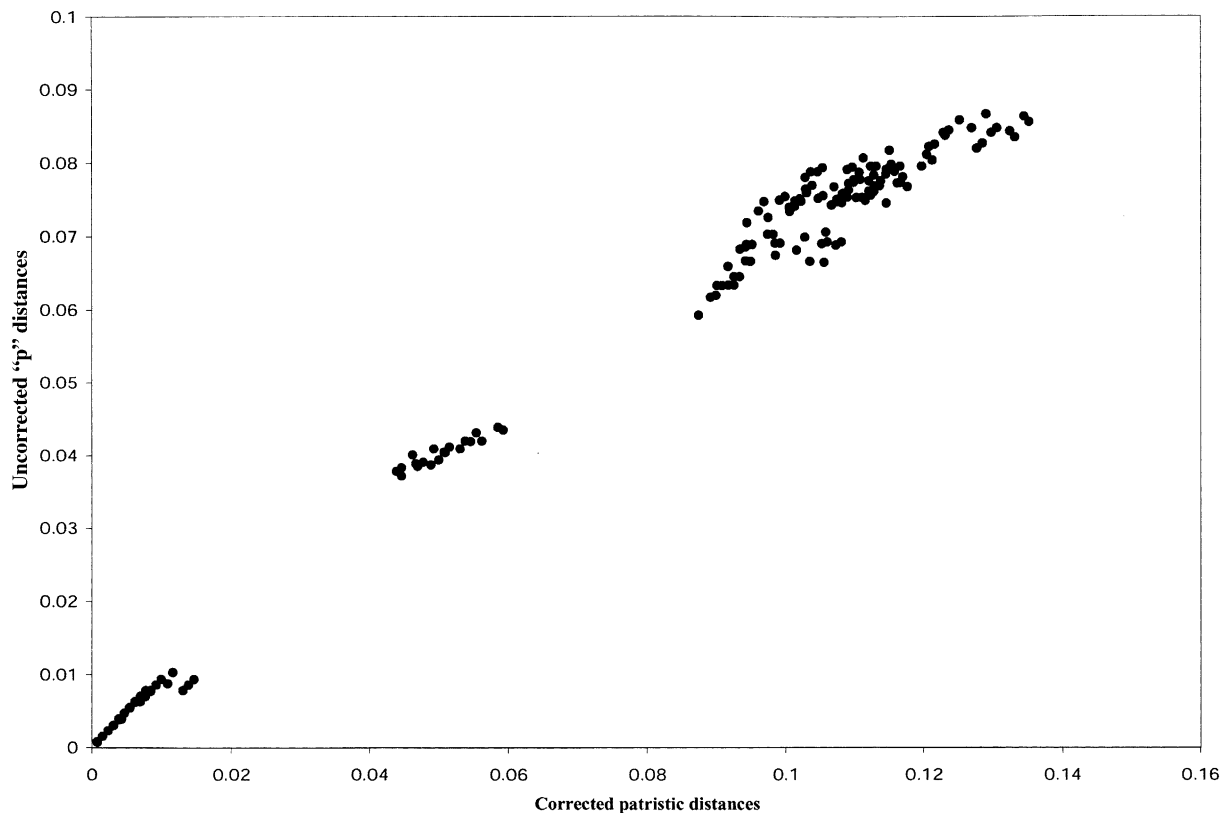


Fig. 5. Plot of uncorrected  $p$  distances against corrected patristic distances (using the TIM + G model) for the Fig. 4 sunbird mtDNA sequence data set with outgroup excluded.

to be 0.5 Ma. We reason that unless a single eruption covered the entirety of Grande Comore in lava, the current terrestrial biota would predate the oldest exposed lava flows (0.13 Ma) and correspond to the estimated origin of the island at 0.5 Ma. Secondly, we also estimate the maximum age of the node (node 9, Fig. 3) separating the Moheli and Grande Comore populations of *N. notata* to be 0.5 Ma. Thirdly, nodes 5 and 6 (Fig. 4) represent the earliest and latest possible times for the colonization of the Aldabra archipelago by *N. souimanga* if one accounts for existing mitochondrial diversity within the ancestral population, the presence of the western Madagascar individual between these nodes in Fig. 3, and the possibility of double colonization of the Aldabra Group. Therefore the maximum age for the older node (node 5) is taken as the earliest possible colonization of the Aldabra Group since the last complete inundation of Aldabra Atoll (0.125 Ma). Because the level of sequence divergence between Madagascar lineages and those found in Aldabra archipelago (node 5) is low (maximum 1.1%), divergence estimates obtained from this calibration point are likely to be less reliable than those obtained from the dating of nodes 3 and 9 (3.8 and 3.0% divergence, respectively). However, node 5 does provide an independent calibration for mtDNA divergence because it is based on a different island and a different geological dating method than we used for calibrating the age of divergence across nodes 3 and 9.

A log likelihood test rejected the null hypothesis of rate constancy with all taxa included. Following the removal of *A. malacensis*, *Anthreptes orientalis*, and *A. boltoni*, the data failed to reject the null hypothesis ( $-\ln L$  clock-enforced tree = 6584.00,  $-\ln L$  unconstrained tree = 6563.89,  $\chi^2 = 40.22$ ,  $df = 28$ ,  $P > 0.05$ ). We therefore used the ML tree constructed under the assumption of a molecular clock with these taxa deleted as one method for estimating divergence times. The

divergence times obtained by calibrating a sunbird mtDNA molecular clock using the aforementioned age of nodes 3, 5, and 9 (Table 2) are 1.7–7.2 times younger than those obtained when we apply the commonly used passerine mtDNA clock figure of 2% divergence per million years (see Fleischer et al., 1998; Fleischer and McIntosh, 2001). While divergence calculations are dependent on the distance metric used, we calculate similar high sunbird mtDNA divergence rates whether we use absolute distances, maximum likelihood distances (based on the TIM + I + G model), or distances based on the Kimura 2-parameter model applied by Fleischer et al. (1998).

We subsequently compared absolute pairwise divergence rates between *N. humbloti* individuals on the geologically dated islands of Moheli and Grande Comore, and between *N. notata* individuals on the same two islands, for each of the gene regions independently. This strategy permits straightforward comparison to the commonly used passerine 2% mtDNA clock, which is principally based on data from the *cytb* gene. Between the *N. humbloti* individuals we observed 29/609 changes in ATPase6 (4.76%), 12/380 changes in ND3 (3.16%), and 9/320 changes in *cytb* (2.81%). Between *N. notata* individuals we observed 19/609 changes in ATPase6 (3.12%), and 11/380 changes in ND3 (2.89%). Despite the apparently high substitution rate, most of the changes in all three genes were silent. Differences in pairwise divergence between genes are difficult to test statistically, since each haplotype is represented in many pairwise comparisons, and therefore such a comparison contains high levels of autocorrelation due to the non-independence of pairwise distances. To avoid such biases, we compared ATPase6, ND3, and *cytb* divergence along branches of a NJ tree, rather than among pairs of individuals, since each branch can be considered a statistically independent estimate of relative nucleotide

Table 2  
Sunbird divergence times estimated from molecular clock and penalized likelihood methods calibrated using geologically determined island ages

	Node number	1	2	3	4	5	6	7	8	9
Clock-enforced ML tree	Genetic distance from node to tip	0.1021	0.0651	0.0267	0.0258	0.0049	0.0019	0.0792	0.0359	0.0166
Clock-enforced ML tree	Dating based on node 3 as 0.5 Ma	1.912	1.220	0.500	0.484	0.092	0.036	1.483	0.672	0.311
Node ages in Ma	Dating based on node 5 as 0.125 Ma	2.599	1.658	0.680	0.657	0.125	0.049	2.015	0.913	0.422
	Dating based on node 9 as 0.5 Ma	3.078	1.964	0.805	0.779	0.148	0.058	2.387	1.082	0.500
Penalized likelihood	Dating based on node 3 as 0.5 Ma	2.118	1.032	0.500	0.506	0.068	0.062	1.897	0.818	0.368
Node ages in Ma	Dating based on node 5 as 0.125 Ma	3.913	1.908	0.924	0.935	0.125	0.114	3.505	1.511	0.679
	Dating based on Node 9 as 0.5 Ma	2.880	1.404	0.680	0.688	0.092	0.084	2.580	1.112	0.500

All dates are in millions of years before present. Nodes 1–9 have been labeled in Figs. 3 and 4.

substitution rate. Comparable ATPase6, ND3, and *cytb* branch lengths were identified by conducting NJ analyses for each gene region independently (using uncorrected *p* distances) with the topology constrained to match that of the ML tree for the combined data set (Fig. 4). Prior to analysis, branches with very short lengths were removed, resulting in a total of eight branch length comparisons. In this restricted data set, the lengths of ATPase6, ND3, and *cytb* branches did not

differ significantly (Friedman's method for randomized blocks;  $\chi^2 = 3.25$ ;  $df = 2$ ;  $P > 0.10$ ).

We also estimated divergence times from the non-ultrametric ML tree, using the data set of Fig. 3 after removing closely related or identical haplotypes representing the principal mtDNA lineages. We used the fixage option of the r8s 1.06 program (Sanderson, 2002), which assigns an age to one node in a tree and then estimates the age of all other nodes. Because the sunbird

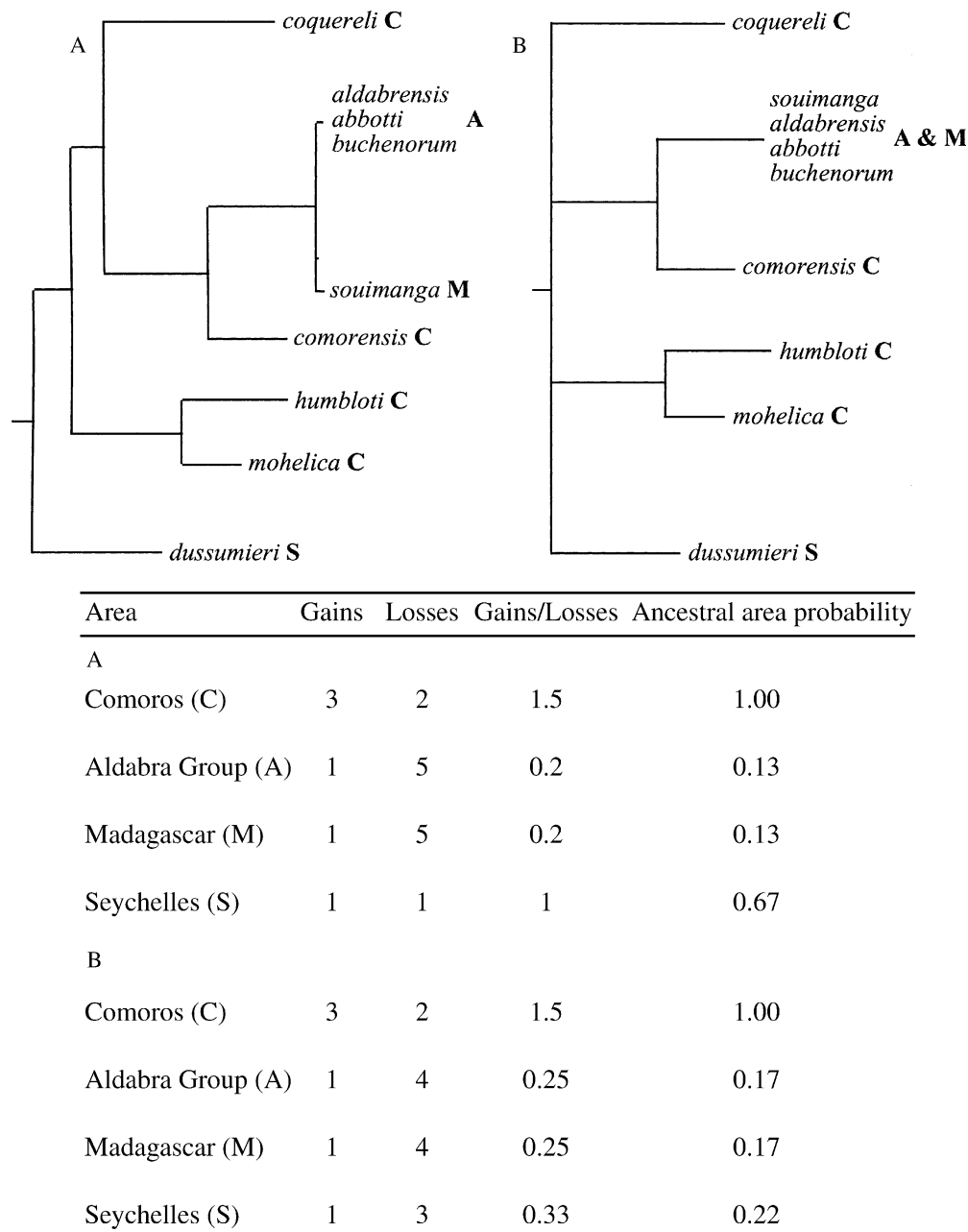


Fig. 6. Application of Bremer's (1992) ancestral areas method to: (A) the topology of the ML taxon tree of Fig. 4, regardless of the level of bootstrap support for nodes and (B) the topology of the ML taxon tree of Fig. 4, with nodes having less than 65% bootstrap support collapsed. C, Comoros; A, Aldabra Group; M, Madagascar; and S, Granitic Seychelles.

mtDNA data are relatively clocklike, a smoothing parameter of 1.0 was required to detect rate variation across the tree. Fixing the age of a terminal or near terminal node results in degenerate age estimates for internal nodes because there is no constraint on the age of the root. Thus we arbitrarily fixed the age of the root node and then scaled our divergence calculations according to the assigned ages of nodes 3, 5, or 9 (Table 2).

#### 3.4. Estimation of direction and sequence of colonization in the *souimanga* clade

Basal nodes of the *souimanga* clade separating *N. coquereli*, *N. souimanga*, *N. dussumieri*, and *N. humbloti* lack bootstrap support, as do nodes separating taxa from Madagascar and the Aldabra archipelago. We have therefore compared the result obtained using the ML taxon tree (Fig. 6A) with the result obtained with a ML tree (Fig. 6B) formed by collapsing branches with less than 65% bootstrap support (65% served as a natural cutoff as there were no bootstrap values between 65

and 94%). In both analyses Bremer's (1992) method for determining ancestral areas suggests that the Comoros are the most probable ancestral area for the *souimanga* clade in the Indian Ocean (Fig. 6).

The Topology Plus Branch Length method of Thorpe et al. (1994) applied to the ML tree in Fig. 4 permitted two additional results regarding the colonization patterns of the *souimanga* clade (Fig. 7). Firstly, *N. humbloti* has colonized Grande Comore from Moheli. Secondly, *N. souimanga* has colonized Madagascar from Anjouan, the Madagascar population has subsequently colonized the Aldabra archipelago, and then sunbirds from Aldabra have colonized Cosmoledo. Both of these results conform to the geological history of the region and to the early occupation of the Comoros by the *souimanga* clade as inferred from the ancestral areas analysis. Grande Comore (with a maximum age of 0.5 Ma) is younger than the estimated date for the initial colonization of the Comoros from Africa (1.9–3.9 Ma, Table 2) and thus colonization from one of the older Comoro islands would have been predicted. Since the early

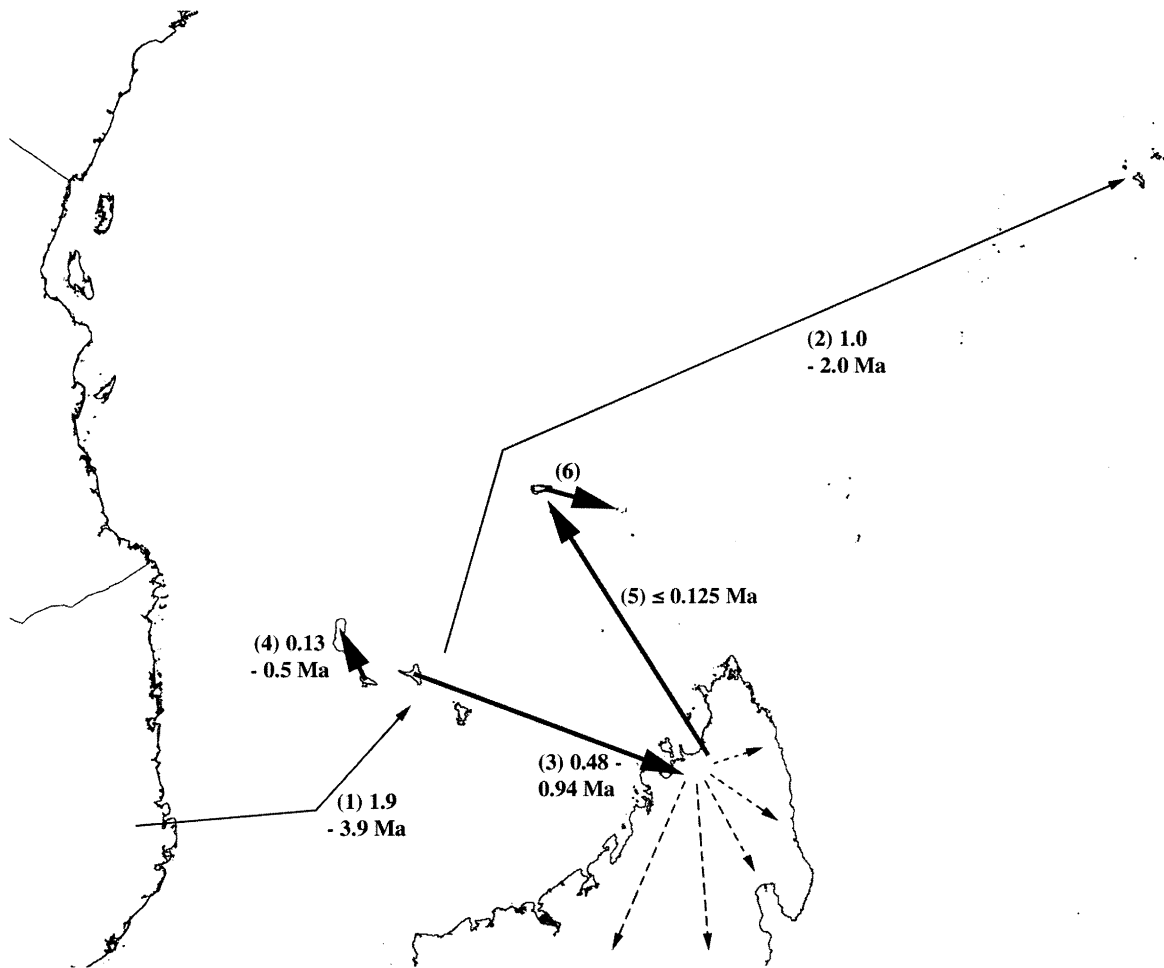


Fig. 7. Hypothesized pattern of colonization of the *souimanga* sunbird clade. Thick arrows depict colonization events inferred from the Topology Plus Branch Length method of Thorpe et al. (1994). Thin arrows depict colonization events inferred from other methods.

divergence of *N. s. comorensis* from the other *N. souimanga* taxa (within the interval of 0.48–0.94 Ma, node 4, Table 2) predates the last complete submergence of the Aldabra group (0.125 Ma), it follows that the Aldabra Group was colonized from Madagascar, and not vice versa. The single *N. souimanga* sampled from Assumption Island is more closely related to Madagascar birds (0.38% or 5 substitutions) than to populations on the neighboring islands in the Aldabra archipelago (0.53% or 7 substitutions).

#### 4. Discussion

##### 4.1. Phylogenetic relationships and historical origins of Indian Ocean sunbirds

Based on the mtDNA phylogeny, the origin of Indian Ocean sunbirds resulted from two independent colonizations of the region, which gave rise to the *notata* and *souimanga* clades depicted in Fig. 3. Furthermore, the null hypothesis of a monophyletic origin of Indian Ocean sunbirds was clearly rejected through application of the parametric bootstrap test. These results accord well with the divergent morphology between sunbirds comprising the *notata* and *souimanga* clades and traditional taxonomic views (Benson, 1960; Sibley and Monroe, 1990). Since the closest relatives of the *notata* clade (*N. senegalensis*, *N. adelberti*, *Nectarinia erythrocerca*, and *Nectarinia kilimensis*, see Fig. 3) are all African, our phylogenetic analysis provides strong evidence that the ancestor of this lineage colonized the Indian Ocean from Africa. The sister lineage to the *souimanga* clade (*N. bouvieri*, *N. venusta*, and *N. talatala*, see Fig. 3) contains only African species, and this combined Indian Ocean–Africa clade is further nested amongst African species. We therefore infer that the ancestor of the *souimanga* clade also colonized the Indian Ocean from Africa.

Since we found no evidence for nucleotide saturation in the *souimanga* clade, it is likely that the basal polytomy (separating the *N. dussumieri*, *N. coquereli*, *N. humbloti*, and *N. souimanga* lineages, Fig. 6B) is a hard polytomy resulting from the rapid expansion of this lineage across the breadth of its contemporary distribution in the Indian Ocean. The unambiguous inclusion of *N. dussumieri* in the monophyletic *souimanga* clade is satisfying, as the relative loss of sexual dimorphism in this species coupled to its geographic isolation in the granitic Seychelles had caused considerable uncertainty regarding its systematic placement (Benson, 1984; Louette, 1992; Sibley and Monroe, 1990). Generally speaking, different authors have had different ideas regarding the systematic relationships of the four species that we associate in the *souimanga* clade (Benson, 1960, 1984; Louette, 1992; Sibley and Monroe, 1990). For example,

our mtDNA analyses permit strong inference that *N. coquereli* is not very closely related to *N. venusta*, as suggested by Benson (1960), nor do our analyses support the alternative hypothesis of Deignan (in Benson, 1960) of a close relationship among the Indian Ocean *N. coquereli*, *N. humbloti*, and *N. souimanga* and the oriental species *Nectarinia jugularis* and *Nectarinia solaris*. The close phylogenetic relationship of *N. dussumieri*, *N. coquereli*, *N. humbloti*, and *N. souimanga* indicated by mtDNA analysis unites these four species in a monophyletic group for the first time.

##### 4.2. Divergence times

Rate variation across the sunbird phylogenetic tree is low and the mtDNA data do not reject a molecular clock. Thus, it is not surprising that the results obtained when dating nodes under the assumption of a molecular clock are close to those obtained under the Penalized Likelihood method, which does not assume ultrametricity. Much more surprising is the high level of sequence divergence observed between taxa on islands of known age. For example, if one assumes that the age of the youngest island provides the earliest date of divergence between island populations, application of the 0.5 Ma date to nodes 3 and 9 between Moheli and Grande Comore island populations yield absolute pairwise mtDNA divergence rates of 7.6 and 6.1% per million years, respectively. Comparing the absolute divergence rate per million years between genes shows that ATPase6 substitution rates (9.52%) exceed those of ND3 (6.32%), and *cytb* (5.63%) for the *souimanga* clade, while for the *notata* clade divergence in ATPase6 (6.24%) exceeds that of ND3 (5.79%) by a smaller margin. The variation in rate estimates depends both on the gene region compared and the particular pair of sunbird taxa analyzed, but in all cases exceeds commonly applied rates for avian mtDNA sequence evolution, most of which are based on *cytb* data (Fleischer et al., 1998; Krajewski and King, 1996; Tarr and Fleischer, 1993).

One possible explanation for the apparently high rate of sunbird mtDNA sequence evolution is that we have underestimated the age of Grande Comore, the younger island in our paired comparisons (across nodes 3 and 9). Grande Comore would have to be 2.7 Ma in order to bring the rate of sunbird mtDNA evolution down to the 2% tick rate of the commonly used passerine mtDNA *cytb* clock (Fleischer et al., 1998). Prevailing geological knowledge of Grande Comore indicates that the island is unlikely to be as old as 2.7 Ma. Both of the volcanoes on Grande Comore appear to have formed recently and the oldest exposed lava flows are only 0.13 Ma (Emerick and Duncan, 1982). Furthermore, none of the rocks sampled on Grande Comore are magnetically reversed, and therefore cannot be older than the 0.78 Ma Brun-



hes–Matuyama boundary (R. Duncan, personal communication). Nonetheless, buried strata have not yet been sampled (R. Duncan, personal communication), and on the other three islands of the Comoros a range of ages has been obtained from rocks corresponding to different phases of volcanic activity. Uncertainty regarding island age notwithstanding, we calculate a similar mtDNA evolutionary rate of 6.5% per million years if we base our calibration on the *N. souimanga* colonization of Aldabra (mean divergence across node 5, Fig. 4). Thorium–Uranium dating was used to age the Aldabra limestone, whereas Potassium–Argon dating was used to age the lava flows on Grande Comore. Thus, a high rate of sunbird mtDNA sequence evolution is supported by independent geological events and dating techniques.

Alternative explanations for the high divergence between sister sunbird taxa on Grande Comore and Moheli include the possibility of polymorphism in the ancestral populations that gave rise to these island populations of *N. humbloti* and *N. notata*. This alternative seems unlikely given the absence of evidence for intra-island polymorphism in present day sunbird populations across the Indian Ocean. Although our sample sizes per island are small, we have sampled at least two individuals from 11 island populations (treating both Madagascar and the granitic Seychelles as single islands) without observing within island divergence in excess of 0.71%. A second explanation is that the closest ancestors of the Grande Comore populations have gone extinct. However, this scenario requires the extinction of two unrelated taxa (one sister to *N. humbloti humbloti*, the other sister to *N. notata moebii*). It is also difficult to imagine where such sister taxa might have been located, since most land masses of suitable age are currently occupied by island lineages as old as the two putative Grande Comore–Moheli divergence events.

Is the sunbird mtDNA clock relatively fast, or has undue emphasis been placed on the 2% per million years tick rate of a passerine mtDNA *cytb* clock? Studies providing independent avian mtDNA rate calibrations are relatively few, and reported rates fall within a range of values: 0.9–5.2% (Fleischer et al., 1998; Krajewski and King, 1996; Nunn et al., 1996; Shields and Wilson, 1987; Tarr and Fleischer, 1993). Therefore the commonly used metric of 2% avian sequence divergence per million years ought to be viewed with reservation. Variation in reported rates of mitochondrial nucleotide substitution among birds probably results from multiple factors, including potential rate differences among avian lineages in nucleotide substitution, the use of different mitochondrial genes, nucleotide sample size and distance metrics, and error terms likely to be associated with dating nodes in a tree. However it is also interesting to note that the data from several studies which calibrate avian mtDNA clocks show an apparent increase in the

rate of molecular evolution with decreasing node age (Fleischer and McIntosh, 2001). The highest rate (5.2%) comes from a node calibrated at 0.4 Ma, close to our dating of nodes 3 and 9, while the lowest (0.87%) comes from a node dated at 15 Ma (Fleischer et al., 1998; Krajewski and King, 1996; Nunn et al., 1996; Tarr and Fleischer, 1993).

Applying the sunbird mtDNA clock, our estimated divergences of the *souimanga* and *notata* clades from African stocks would be no earlier than 3.9 and 3.5 Ma, respectively, and thus stand in strong contrast to the relatively old colonization and diversification patterns shown by a number of avian groups in the Indian Ocean. Non-passerine groups such as mesites, couas, elephant birds, ground rollers, and cuckoo rollers are Indian Ocean endemics at the family level, and were probably isolated early in the Cenozoic. The suboscine asities and oscine vangas may represent mid-Tertiary colonizations of Madagascar that may have coincided with the appearance of sea mount stepping stones in the Mozambique Channel at approximately 45–26 Ma (Fjeldså et al., 1999). The ancestor of the endemic Malagasy songbird radiation represented by the genera *Bernieria*, *Xanthomixis*, and *Hartertula* is posited to have colonized Madagascar more recently, probably 9–17 Ma (Cibois et al., 1999, 2001). Whether we apply the sunbird mtDNA clock or the 2% *cytb* clock, two points are clear. Firstly, sunbird colonizations of the Indian Ocean region substantially post-date the existence of sea mount stepping stones, and secondly, their expansion was roughly contemporaneous with the origin of the Comoros archipelago within the last 10 million years. Absolute dating aside, it is the *relative* timing of divergence events which is important to our interpretation of the geographic pattern of Indian Ocean sunbird colonization history.

#### 4.3. Phylogeography: a hypothesis of sunbird island colonization history

In contrast to biogeographic studies of continental radiations, where both vicariance and dispersal hypotheses are plausible, in this investigation vicariance can reasonably be rejected as a mechanism of diversification. None of the archipelagos in the western Indian Ocean show any evidence of having been joined to each other or to Madagascar, Africa, or India in the last 50 million years. Although the islands in the granitic Seychelles have been joined during periods of low sea level, this has occurred at regular intervals, the most recent probably being just 15–18,000 years ago. This history of connectedness is evidenced by the Seychelles sunbird *N. dussumieri*, which is found throughout this archipelago and shows no evidence of genetic divergence between island populations. All other islands under study have been isolated since their creation.

Owing to the dispersal-based history of diversification in Indian Ocean sunbirds, we used Bremer's (1992) ancestral areas analysis and Topology Plus Branch Length method (Thorpe et al., 1994) to infer the points of sunbird entry into the Indian Ocean. For the *notata* clade, the Bremer (1992) method does not resolve the point of initial colonization, whereas the Thorpe et al. (1994) approach suggests that this sunbird lineage first colonized Madagascar. The two Comoro taxa are more closely related to each other than either is to the Madagascar taxon, suggesting that Grande Comore was colonized from Moheli or vice versa. The Topology Plus Branch Length analysis indicates that the *notata* clade expansion proceeded from Moheli to Grand Comore. Dating of node 7 suggests that the *notata* clade colonized the Indian Ocean from Africa approximately 1.5–3.5 Ma.

In the *souimanga* clade, we estimate an age of 1.9–3.9 Ma for the initial colonization of the Indian Ocean (node 1, Fig. 3). The ancestral areas method (Bremer, 1992) supports the Comoros as the center of origin of this clade. This is a plausible scenario given the geographical location of the Comoros as 'stepping stones' equidistant from Africa and Madagascar. The ancestor of the *souimanga* clade probably colonized one of the older Comoro islands of Moheli, Anjouan, and Mayotte, rather than Grande Comore, the island closest to the source. Grande Comore is ruled out by its young age (0.5 Ma) and the Topology Plus Branch Length (Thorpe et al., 1994) analysis, which establishes Moheli as the ancestral source of *N. humbloti* on Grande Comore (Fig. 7). The basal polytomy in the *souimanga* clade (Fig. 6B) indicates that the expansion of this lineage across its contemporary distribution was rapid, beginning roughly 1.0–2.0 Ma (node 2, Fig. 3) and gave rise to the four main lineages that we know today (*N. dussumieri*, *N. coquereli*, *N. humbloti*, and *N. souimanga*).

Given the early colonization of the granitic Seychelles from the Comoros by *N. dussumieri*'s ancestor (Fig. 7), it is worth emphasizing that this lineage is currently absent from the intervening islands between these two points (the Aldabra Group, Farquhars, and Amirantes). The absence of the *N. dussumieri* lineage on these islands can be reasonably explained by the fact that they 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. That at least some of these islands are suitable for sunbirds is evidenced by the occupation of the Aldabra archipelago by *N. souimanga*, which appears to have colonized the archipelago from Madagascar since the last total submergence of Aldabra 0.125 Ma. Why *N. souimanga* colonized Aldabra and not *N. dussumieri* may well be explained by differences in the relative population productivity of the source populations (*N. souimanga* is common throughout Madagascar, which at 621,600 km<sup>2</sup>, is much larger

than the 218 km<sup>2</sup> granitic Seychelles), and by the greater distance of the granitic Seychelles from Aldabra.

It is interesting to note that the ancestor of *N. souimanga* colonized Madagascar from Anjouan relatively late in the expansion of the *souimanga* clade (0.48–0.94 Ma; node 4, Table 2). We speculate that colonization of Madagascar may have been delayed owing to processes opposing to community invasion, perhaps competitive displacement by *N. notata*, which appears to have been an earlier resident (Table 2). Alternatively, earlier colonization by the *souimanga* clade may have given rise to forms which are now extinct.

Our data suggest that Madagascar has given rise to two independent colonizations of the Aldabra archipelago. In addition to the previously discussed colonization of Aldabra Island (and Cosmoledo and Astove in turn), the single *N. souimanga* sampled from Assumption Island is more closely related to Madagascar birds than to populations on the neighboring islands in the Aldabra archipelago. Given the greater genetic divergence of the Aldabra and Cosmoledo populations from Madagascar than the Assumption population, it is likely that the Assumption population has been more recently founded than those of Aldabra, Cosmoledo, and Astove.

## 5. Conclusions

Existing molecular phylogenies of other recently diverged island organisms frequently conform to one of two generalized patterns. Radiations which are contemporary with island formation commonly show a stepwise 'island colonized as it emerges' pattern from older to younger islands in the group. This has been observed among Hawaiian island birds (Fleischer et al., 1998) and crickets (Shaw, 1996), as well as Canary island lizards (González et al., 1996; Thorpe et al., 1994), skinks (Brown and Pestano, 1998), geckos (Nogales et al., 1998), and beetles (Emerson et al., 2000; Juan et al., 1997, 1998). By contrast, several studies of radiation which post-date island formation have shown relatively rapid expansion and speciation, with a relatively short coalescence time within an archipelago. This can be seen among Darwin's finches (Freeland and Boag, 1999; Sato et al., 1999, 2001), as well as among birds of the Lesser Antilles (Hunt et al., 2001; Lovette et al., 1999; Lovette and Bermingham, 1999). The western Indian Ocean sunbirds show elements of both patterns—periods of rapid expansion, as well as the colonization of new islands from older ones—reflecting the wide range of island ages in the region. Sunbird radiation is contemporary with the origin of the younger islands, while greatly post-dating the origin of the older ones.

Of the two Indian Ocean sunbird clades, the *souimanga* clade is characterized by two periods of appar-

ently rapid range expansion and diversification, separated by a relatively long period of evolutionary independence with a lack of gene flow between islands. The *notata* clade exhibits a much more restricted geographical expansion which is roughly coincident with the first of the two dispersion events in the *souimanga* clade. Sunbirds appear capable of rapid range expansion and colonization in response to a relatively sudden change in their environment, be it falling sea level exposing a coral atoll, the emergence of a new island through volcanic activity, the opening of 'new space' as a result of the extinction of a neighboring species or population, or evolutionary changes in ecological traits in founding populations. This suggests that sunbirds are relatively mobile within the Indian Ocean region, and that chance dispersal events between islands are frequent in an evolutionary timeframe, at least for certain lineages.

Nevertheless, sympatry between sunbird species occurs on only three islands in the western Indian Ocean: Madagascar, Grande Comore, and Moheli. Genetic divergence between sympatric species is high, since in each case one species belongs to the *souimanga* clade while the other belongs to the *notata* clade. We therefore interpret these cases of sympatry as the result of double colonization, rather than sympatric speciation, conforming to the general absence of sympatric speciation substantiated for island birds (Coyne and Price, 2000). Given the evidence for high evolutionary mobility of sunbirds, the fact that all three cases of Indian Ocean sympatry involve divergent species may be an indication that colonists and residents interbreed at lower levels of divergence. Alternatively, it may reflect low survival of small colonizing populations in competition with a larger resident population in the absence of sufficient niche partitioning. Each of the three sympatric sunbird species pairs show marked differences in size (especially bill length), perching level, vegetation preference, and feeding strategy (Bijnens et al., 1987; personal observation). Within the *souimanga* and *notata* lineages, intraclade differences are smaller, suggesting that the potential for ecological segregation between more closely related taxa may be insufficient for sympatry. Competitive exclusion might also explain the peculiar absence of sunbirds from the Mascarene islands, 665 km from the East coast of Madagascar. The western islands of La Réunion and Mauritius each possess an endemic species of white-eye (*Zosterops olivaceus* and *Zosterops chloronothus*, respectively) which appear to occupy the niche filled by sunbirds in other Indian Ocean islands—both species have abnormally long bills for *Zosterops*, and have sunbird-like nectarivorous habits with constant movement between flowers (Cheke, 1987a,b; Gill, 1971; personal observation).

Existing Indian Ocean phylogenies have largely focused on the origins of insular Malagasy forms. Endemic Malagasy songbirds (Cibois et al., 1999, 2001)

and nonpasserine birds (Fjeldså et al., 1999; Johnson et al., 2000; Kirchman et al., 2001), as well as tortoises (Caccone et al., 1999), tenrecs (Douady et al., 2002), and primates (Marivaux et al., 2001; Martin, 2000; Yoder et al., 1996), all probably post-date the separation of Madagascar from Africa and Greater India and arrived in Madagascar by dispersal. Nonetheless, they all show long histories of isolation on the island, and probably diverged from continental forms in the region of 9–80 Ma. By contrast, the sunbirds represent a younger radiation within the region, with our oldest estimate of divergence from African forms being 3.9 Ma, and have dispersed and diversified over a much wider area. The sunbird phylogeny suggests that since the origin of the Comoros in the last 10–15 Ma, these islands have played a key role as 'stepping-stones' for dispersal between Africa, Madagascar, and other Indian Ocean islands.

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