The Auk 117(2):355–365, 2000

PHYLOGENETIC RELATIONSHIPS OF MICRONESIAN WHITE-EYES BASED ON MITOCHONDRIAL SEQUENCE DATA

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ABSTRACT.—Using mitochondrial sequence data, we estimated phylogenetic relationships and genetic divergence among selected species of white-eyes (Zosteropidae). We focused on taxa endemic to islands in Micronesia, specifically *Zosterops conspicillatus, Z. semperi, Z. hypolais, Rukia oleaginea,* and *Cleptornis marchei.* We also included in our data set five additional species of *Zosterops* from the Indo-Australian region and three species from Africa, as well as additional passerine outgroups. Our mitochondrial sequence data revealed substantial genetic divergence (5.7 to 7.3%) among *Z. conspicillatus, Z. semperi,* and *Z. hypolais,* three taxa that formerly were treated as a single species. In addition, a sequence divergence of 6.5 ± SE of 1.7% was found between the population of *Z. conspicillatus* from Rota and "conspecific" populations on Guam, Tinian, and Saipan. The distinctiveness of the Rota population suggests that this taxon should be recognized as a distinct species, a result that bears on the conservation of this population because it has been declining dramatically in recent years. All optimal trees based on analysis of the mitochondrial sequence data place *Rukia oleaginea* within the genus *Zosterops.* In all optimal trees, *Cleptornis marchei* positions as the sister taxon to a clade including all other zosteropids included in this study. The trees based on our data strongly contradict the traditional classification of *Cleptornis* as a honeyeater (family Meliphagidae). Our data cannot resolve with any confidence the sister relationships of the insular endemic white-eyes, although the optimal trees suggest multiple colonizations of Micronesia by more than one white-eye lineage. *Received 10 February 1999, accepted 20 August 1999.*

THE FAMILY ZOSTEROPIDAE (white-eyes) includes about 100 species of small songbirds distributed throughout the Old World tropics and subtropics, including sub-Saharan Africa, islands in the Indian Ocean, India, Southeast Asia, Japan, and Australasia. The family includes 13 genera, although most of the species (75%) are in the nominate genus *Zosterops* (Sibley and Monroe 1990). Most species of white-eye have a rather plain plumage that typically is greenish above with white or yellow underparts and flanks. Sexes are monomorphic in plumage, although males average slightly larger in size. Most species sport a conspicuous ring of white feathers around their eyes (Mees 1969, Pratt et al. 1987).

This speciose and widely distributed family is interesting from several perspectives. One intriguing feature is the relatively high proportion of insular endemics in the family. White-eyes have colonized numerous small and remote islands in both the Indian Ocean and the tropical Pacific (Mees 1969). The origins of these island endemics are unknown. Identifying the colonizing lineages is necessary to test hypotheses regarding biogeography, speciation, rates of evolution, adaptation, and competitive exclusion. In addition, data on relationships and species limits are needed to aid in conservation decisions.

Resolving relationships among white-eye species is notoriously difficult. Traditional methods of taxonomy based on comparisons of museum skins have proven inadequate. Interspecific variation in external morphology is slight, except in characteristics of plumage, which appear to vary haphazardly. Indeed, in some cases species that occur on different continents can appear to be more similar than allopatric populations of a (presumed) single species (Mayr 1965, Mees 1969). For most species of white-eyes, additional data on behavior, song, and ecology are limited or lacking altogether.

This study is an investigation, based on mitochondrial sequence data, of the relationships and genetic divergence among a selected sample of white-eye taxa endemic to islands of Micronesia. We focus principally on the Bridled

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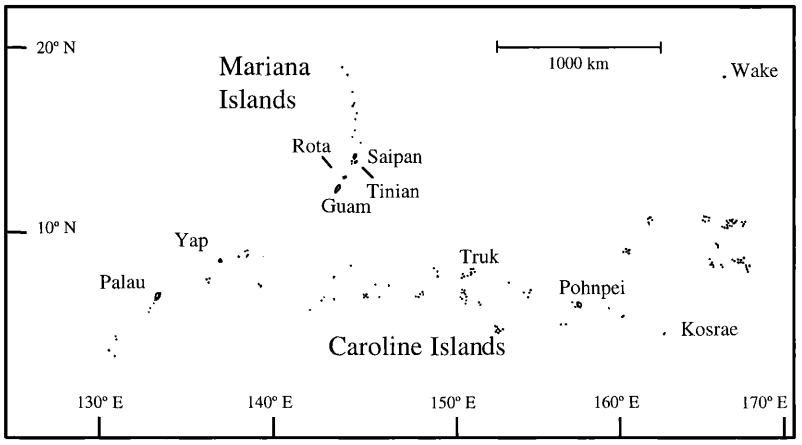

FIG. 1. Map of the southwestern Pacific, featuring the Mariana Islands and Caroline Islands.

White-eye (*Z. conspicillatus*) complex, including the Caroline Islands White-eye (*Z.* [*c.*] *semperi*) and the Plain White-eye (*Z.* [*c.*] *hypolais*), and we also address the relationships of two atypical Micronesian white-eyes, *Rukia oleaginea* from Yap Island and *Cleptornis marchei* from Saipan and Agiguan in the Mariana Islands. We give special attention to the taxonomic status of the Bridled White-eye population on Rota, which has declined drastically over the past 15 to 20 years (Fancy and Snetsinger 1996). Although considered conspecific with populations on Guam and other islands in the Marianas (Saipan, Tinian, and Agiguan), the Bridled White-eye on Rota is distinctive in plumage, vocalizations, and behavior (Pratt et al. 1987, Collar et al. 1994). We test the hypothesis that the Rota population is a distinct species, thus warranting increased protection under the Endangered Species Act.

G. F. Mees thoroughly reviewed the systematics of Indo-Australian zosteropids (Mees 1957, 1961, 1969). According to Mees (1969), the Bridled White-eye complex comprises seven subspecies, each endemic to a single island or archipelago in Micronesia (Fig. 1): *Z. c. conspicillatus* (Guam), *Z. c. saypani* (Saipan, Tinian, Agiguan), *Z. c. rotensis* (Rota), *Z. c. semperi* (Palau islands), *Z. c. owstoni* (Truk), *Z. c. takatsukasai* (Pohnpei), and *Z. c. hypolais* (Yap). These subspecies differ from each other slightly in size and substantially in plumage pattern and color. The classification proposed by Mees was disputed by Pratt et al. (1987), who separated the Bridled White-eye into three distinct species: *Z. conspicillatus*, including the three taxa on the Mariana Islands (*conspicillatus, saypani,* and *rotensis*); *Z. semperi*, including the three taxa on the Caroline Islands (*semperi, owstoni,* and *takatsukasai*), and *Z. hypolais*, the taxon from Yap. The split was motivated primarily by differences in song and secondarily by differences in behavior and plumage. Pratt et al. (1987) also argued that these three species (*Z. conspicillatus, Z. semperi,* and *Z. hypolais*) probably are not a monophyletic group. Based on song complexity, Pratt et al. (1987) hypothesized that *Z. hypolais* and *Z. conspicillatus* are derived from an ancestor that colonized from the Southeast Asian continent, whereas *Z. semperi* is derived from a Melanesian ancestor. Species of *Zosterops* from Southeast Asia have complex songs, whereas those from Melanesia tend to have simpler songs (like *Z. semperi*).

In addition to testing the views of Mees (1969) and Pratt et al. (1987), we also addressed the relationships of two atypical species in the family Zosteropidae: *Rukia oleaginea,* which is endemic to the island of Yap; and *Cleptornis marchei,* which is endemic to Saipan and Agiguan in the Marianas. The genus *Rukia* includes three species, each of which is restricted to a different island in Micronesia. The *Rukia* species are large, slender-billed white-eyes that are distinguished by an unusual brownish (not greenish) coloration. According to Mees (1969), *R. oleaginea* (Yap) and *R. ruki* (Truk) exhibit an overall similarity that is probably indicative of close relationship. The third species, *R. longirostra* (Pohnpei), is much more distinctive. We

have included only the species *R. oleaginea* in the present study.

Cleptornis marchei (the single species in the genus *Cleptornis*) has a distinctive appearance for a white-eye, being relatively large and having a bright, golden-yellow plumage and an orange bill and legs (Pratt et al. 1987). Traditionally, *C. marchei* has been considered to be a honeyeater in the family Meliphagidae (Oustalet 1889). Mees (1969) did not include *Cleptornis* in his review of the Indo-Australian Zosteropidae. However, H. D. Pratt advanced the hypothesis that *Cleptornis* is a species of white-eye, based on behavioral, ecological, and zoogeographical evidence (Pratt et al. 1987). The DNA-DNA hybridization data of Sibley and Ahlquist (1990) supported Pratt's hypothesis, placing *Cleptornis* as the sister taxon to several representative species of *Zosterops*. The *Zosterops* + *Cleptornis* clade is distantly related to the Meliphagidae in the Sibley and Ahlquist (1990) tapestry. The former falls within the parvorder Passerida, whereas the meliphagids fall within the parvorder Corvida.

Assessing the taxonomic status of populations of the Bridled White-eye in the Mariana Islands is critical from a conservation perspective. The Guam population (*Z. c. conspicillatus*) is extinct, a victim of predation by the brown tree snake (*Boiga irregularis*) that was accidentally introduced to Guam in the late 1940s (Savidge 1987). Thus far, brown tree snakes have not become established on other islands in the Marianas. Nonetheless, the Bridled White-eye population on Rota has been declining precipitously (Fancy and Snetsinger 1996) and determining its taxonomic status is of immediate importance.

Methods

Taxonomic sampling.—The data matrix includes representatives of *Z. conspicillatus* from Guam (n = 3), Saipan (n = 3), Tinian (n = 4), and Rota (n = 6); *Z. semperi* from Truk (n = 2) and Pohnpei (n = 1); *Z. hypolais* from Yap (n = 2); *C. marchei* (n = 2) from the Mariana Islands; and *R. oleaginea* (n = 2) from Yap (Table 1). To test the monophyly of the Bridled White-eye complex (*conspicillatus* + *semperi* + *hypolais*), we included five additional species of *Zosterops* from the Indo-Australian region and three from Africa. The former include *Z. palpebrosus* (Southeast Asia) and *Z. japonicus* (Japan), two species suggested by Mayr (1965) as possible ancestors of the insular species of *Zosterops* in Micronesia. To test whether *C. marchei* is more closely related to species in the Zosteropidae than to species in the Meliphagidae, we included representatives of several songbird families. Based on the results of Sibley and Ahlquist (1990), we included individuals of four species from the parvorder Corvida, including two species from the Meliphagidae (*Myzomela rubratra* and *Melidectes belfordi*) and seven species from the parvorder Passerida, representing four families in addition to the Zosteropidae (Table 1). Finally, we included two species of suboscine passerines (*Calyptomena viridis* and *Glyphorynchus spirurus*) as outgroups. These two species represent distantly related families within the suboscines, the Eurylaimidae of the Old World (*Calyptomena*) and the Furnariidae of the New World (*Glyphorynchus*).

PCR and sequencing.—Extraction of DNA followed one of two protocols, depending on the type of sample. A standard extraction protocol was followed for blood and frozen tissue. A small aliquot of sample (ca. 25 μL of blood or 0.1 g of tissue, finely minced) was placed in 500 μL of a lysis solution (15 mM glucose, 3 mM EDTA, 7.0 mM Tris, 0.1 mM NaOH, 1% SDS, 2 mg/mL proteinase K) and incubated overnight in a water bath at 55°C. After digestion, each sample was purified by two phenol extractions, followed by a single extraction with a chloroform and isoamyl (24:1) mix. DNA was precipitated from the supernatant by adding 0.1 volume of 3M NaAc and two volumes of cold 95% ethanol, mixing thoroughly, and spinning the samples in a microfuge at high speed (14K rpm) for 10 min. Following this spin, the ethanol was pipetted off, and each DNA pellet was washed with 70% ethanol and dried in a vacuum centrifuge. Each pellet was resuspended in 50 to 500 μL of 0.1X TE (10 mM Tris-HCL, pH 7.5; 1mM EDTA), the volume depending on pellet size. DNA samples were diluted 20× with sterile H_2O for use as PCR template.

Samples obtained from museum skins were extracted using protocols designed for degraded or "ancient" sources of DNA. The extractions were performed in a designated "ancient DNA" laboratory in a separate building from the primary genetics laboratory. No PCR amplifications are performed in the building housing the ancient DNA lab, and no PCR product is used or stored there. Stringent precautions are followed while extracting DNA and preparing PCR reactions involving ancient samples to avoid contamination. All surfaces in the laboratory are cleaned with a 10% bleach solution prior to initiating extractions. Pipettors are cleaned in bleach solution following each set of extractions, and only filter tips are used. All reagents are exposed to UV light (254 nm) for at least 20 min prior to use. Gloves, lab coat, and face mask are worn at all times during extraction and PCR preparation. Extractions are done in sets of six, including five samples and a blank extraction control.

TABLE 1. List of taxa included in this study. Subspecies designations follow Pratt et al. (1987) and Sibley and Monroe (1990).

Species	Sample no.	Sample type	Source[a]	Location	No. base pairs sequenced[b]
		Suboscine passerines			
Calyptomena viridis	1190	Tissue	ANSP	Sabah, Borneo	322
Glyphorynchus spirurus	1678	Tissue	ANSP	Ecuador	315
		Parvorder Corvida			
Myzomela rubratra	2187	Skin	BYU	Saipan	191
Melidectes belfordi	86	Blood	NZP	Papua New Guinea	320
Corvus brachyrhynchos	4207	Tissue	ANSP	Pennsylvania, USA	315
Rhipidura rufifrons	2134	Skin	BYU	Guam	191
		Parvorder Passerida			
Sturnus vulgaris	3689	Tissue	ANSP	Pennsylvania, USA	313
Dumetella carolinensis	3671	Tissue	ANSP	Pennsylvania, USA	314
Parus atricapillus	M3	Tissue	ANSP	Missouri, USA	316
Trichastoma bicolor	1205	Tissue	ANSP	Sabah, Borneo	316
Stachyris poliocephala	1078	Tissue	ANSP	Sabah, Borneo	313
Sylvia atricapilla	5355	Tissue	ANSP	Greece	315
Quiscalus quiscula	4207	Tissue	ANSP	Pennsylvania, USA	314
		Family Zosteropidae			
Z. c. conspicillatus	2165	Skin	BYU	Guam	191
	2166	Skin	BYU	Guam	191
	2168	Skin	BYU	Guam	191
Z. c. saypani	87656	Blood	USFWS	Tinian	313, ND2
	87657	Blood	USFWS	Tinian	313
	87665	Blood	USFWS	Tinian	313
	87666	Blood	USFWS	Tinian	313, ND2
	87647	Blood	USFWS	Saipan	313
	87654	Blood	USFWS	Saipan	313, ND2
	87655	Blood	USFWS	Saipan	313
Z. c. rotensis	1	Blood	NZP	Rota	313
	2	Blood	NZP	Rota	313
	3	Blood	NZP	Rota	313
	4	Blood	NZP	Rota	313
	5	Blood	NZP	Rota	313
	6	Blood	NZP	Rota	313, ND2
Z. c. hypolais	2160	Skin	BYU	Yap	191
	2161	Skin	BYU	Yap	191
Z. c. semperi	2169	Skin	BYU	Truk	313
	2170	Skin	BYU	Truk	191
	2244	Skin	BYU	Pohnpei	191
Z. japonicus	1	Blood	USFWS	Hawaii	313, ND2
	2	Blood	USFWS	Hawaii	313
	3	Blood	USFWS	Hawaii	313
	4	Blood	USFWS	Hawaii	313
	5	Blood	USFWS	Hawaii	313
	6	Blood	USFWS	Hawaii	313
Z. palpebrosus	1	Blood	R. Fleischer	Nepal	313
	2	Blood	R. Fleischer	Nepal	313
Z. erythropleurus	O2653	Blood	ZMUC	China	191
Z. nigrorum	O2666	Blood	ZMUC	Philippines	313
Z. montanus	O2660	Blood	ZMUC	Philippines	313
Z. poliogaster	O4421	Blood	ZMUC	Tanzania	313
Z. senegalensis	O4661	Blood	ZMUC	Tanzania	313
Z. kikuyuensis	O2654	Blood	ZMUC	Kenya	313
Rukia oleaginea	2140	Skin	BYU	Yap	191
	2143	Skin	BYU	Yap	191
Cleptornis marchei	2226	Skin	BYU	Saipan	191
	2227	Skin	BYU	Saipan	191

Samples from museum skins were first chopped finely with a sterile scalpel blade. Each chopped sample was added to 750μL of digestion buffer (10 mg/mL DTT, 1 mg/mL proteinase K, 1% SDS, 10 mM Tris, 2 mM EDTA, 10 mM NaCl2) and incubated overnight at 55°C in a rotating stand. Following digestion, each sample was purified with two phenol extractions and a single chloroform extraction. Each supernatant was then washed with sterile, UV-treated H_2O and concentrated, using Centricon 30 columns (Amicon, Inc). Each sample was brought to a final volume of 120 to 160 μL with sterile, UV-treated H_2O, aliquotted into four tubes of equal volume, and heated at 65°C in a heat block for 10 min (to kill any DNAse). Extracted DNA was stored at −20°C in the ancient laboratory. PCR reactions were prepared in the ancient laboratory, but PCR amplification and post-PCR procedures were performed in the primary genetics laboratory.

For DNA extracts obtained from blood or tissue samples, we amplified a piece of mitochondrial DNA (mtDNA) that yielded 313 base pairs (bp) of sequence, including 85 bp at the 3′ end of the COII gene, 70 bp of t-lysine, and 158 bp of the ATPase8 gene. We amplified the region using primers CO2GQL and A6MNH (Lovette et al. 1998). For samples obtained from museum skins, we amplified a smaller fragment that yielded 191 bp of sequence, including 33 bp of t-lysine and 158 bp of ATPase8. We could not amplify the larger fragment from the skin samples, presumably because the DNA was too degraded. To amplify the smaller fragment, we used primers L9051 (5′-CACCAGCACTAGCCTTTTAAG-3′) and H9241 (5′-TGGTCGAAGAAGCTTAGGTTCA-3′), where L and H designate the light and heavy mtDNA strands, respectively, and the numbers refer to the position of the 3′ end of the primers in the *Gallus gallus* mitochondrial sequence (Desjardins and Morais 1990). For a subset of samples (Table 1), we also amplified the mitochondrial ND2 gene using primers L5209 and H6113 (A. J. Baker pers. comm.), from which we obtained 309 bp of sequence.

Amplified products were sequenced using standard cycle sequencing and visualized with an ABI 373 automated sequencer. Sequences have been deposited in GenBank under accession numbers AF168425 to AF168459 (ATPase8 region) and AF168460 to AF168464 (ND2 region). Sequences were aligned using Sequencher 3.0. The program PAUP* (Swofford 1998) was used to calculate genetic distances and to estimate phylogenetic trees.

Phylogenetic analyses were performed separately on two subsets of taxa. To test the affinities of *C. marchei*, we analyzed a data matrix (313 bp) including nine representative taxa of *Zosterops* (*Z. c. saypani* [87665] *Z. c. rotensis* [1], *Z. c. semperi* [2169], *Z. japonicus* [1], *Z. kikuyuensis*, *Z. montanus*, *Z. nigrorum*, *Z. palpebrosus*, and *Z. poliogaster*; Table 1), *R. oleaginea*, *C. marchei*, and all other non-zosteropid species. To resolve relationships among the white-eyes, we analyzed a data set including *C. marchei*, *R. oleaginea*, and all species of *Zosterops*. Analyses of the latter data matrix were based on the 191-bp fragment common to all taxa, because we had sequenced only this smaller fragment for several species of *Zosterops*. Splitting the data matrix for analysis was necessary. If all taxa were included in a single analysis (based on 313 bp), the searches were slow and bootstrapping was impractical, presumably because of a low ratio of informative characters to taxa.

Maximum-parsimony analyses were performed with a step matrix that weighted transversions (tv) over transitions (ts) to model the more rapid accumulation of transitions (Lewin 1997). Analyses were run with two different tv:ts weightings: 5:1 and 10:1. The actual ratio of transitions to transversions is difficult to estimate accurately because of stochastic noise at low sequence divergence and saturation of transitions at high sequence divergence. For the sequence data in this study, the transition-transversion ratio ranges from 0.5 to 15.0 among species of *Zosterops*, excluding comparisons in which the number of transversions is zero. Our choice of step-matrix weights falls within this range, so the selected values seem reasonable.

We also performed maximum-likelihood analyses. The model and parameter values applied in the maximum-likelihood searches were selected by calculating the likelihood of the most-parsimonious trees under six different models. The models differed in the number of substitution categories (nst = 2 or 6) and the type of rate variation across sites (none; gamma-distributed; and site-specific, with different rates for noncoding regions and each codon position in coding regions). All parameter values were estimated to maximize the likelihood function. The model

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[a] ANSP, Academy of Natural Sciences of Philadelphia; BYU, Brigham Young University, Hawaii; NZP, National Zoological Park, Washington, D.C.; USFWS, United States Fish and Wildlife Service; ZMUC, Zoological Museum, University of Copenhagen.

[b] Base-pair fragments from 313 to 320 correspond to positions 8930 to 9239 in the *G. gallus* mitochondrial genome (Desjardins and Morais 1990); the 191-bp fragment corresponds to positions 9052 to 9241; and "ND2" indicates samples for which we also obtained 309 bp of ND2 sequence. Sequences including the ATPase8 gene are of unequal length due to insertions.

and parameter values yielding a significantly higher likelihood than any other model (determined by the likelihood ratio test) were selected for the maximum-likelihood search. For the data set that included the non-zosteropid taxa (313 bp), the model consisted of six substitution types (Rmatrix = 3.14, 15.01, 1.90, 2.76e–08, 10.50) and gamma-distributed rate variation across sites (alpha = 0.33). For the data set including *C. marchei*, *R. oleaginea*, and all species of *Zosterops*, the model consisted of two substitution types (t ratio = 6.5) and site-specific rate variation based on codon position (relative rates: noncoding = 0.29; codon position 1 = 0.64; codon position 2 = 0.36; codon position 3 = 2.34).

Heuristic searches were performed with a random addition sequence of taxa (10 reps), TBR branch-swapping, and the MULPARS option in effect. To assess the strength of support for nodes in the maximum-parsimony trees, data sets were also bootstrapped (1,000 replicates) with a 5:1 step matrix imposed and a simple addition sequence of taxa.

RESULTS

Sequence variation.—All sequences of 313 bp contained the expected TAA stop codon for the COII gene and the anticodon for t-lysine (TTT), and all sequences contained the expected methionine start for the ATPase8 gene. We translated the nucleotide sequences to amino acids, and no unexpected stop codons or unusual amino-acid changes were found. Within coding regions, variable sites were distributed as expected among codon positions. With all taxa included, 50% of the variable sites occur at third-codon positions, 30% at first positions, and 20% at second positions. This pattern of variation across codon positions is consistent with published patterns for other mitochondrial genes compared across avian species (e.g. cytochrome *b*; Randi 1996, Griffiths 1997; ND2, Johnson and Sorenson 1998). However, variability appeared to be more evenly distributed across codon positions for the ATPase8 gene than for the cytochrome-*b* or ND2 genes. The correspondence between observed and expected levels of variability across codon positions and the absence of unlikely amino-acid substitutions suggest that our sequences are mitochondrial sequences, not nuclear homologues (Arctander 1995).

Among species of *Zosterops* (including *R. oleaginea*), uncorrected sequence divergence (SE calculated according to Nei 1987: equation 4.2) between species ranged from 2.3 ± 1.1% (*Z.* [*c.*] *rotensis* to *Z. montanus*) to 8.6 ± 2.0% (*R. oleaginea* to *Z. erythropleurus*). Between *Cleptornis marchei* and all species of *Zosterops* (+ *R. oleaginea*), sequence divergences ranged from 9.8 ± 2.1 to 15.7 ± 2.6% ($\bar{x}$ = 13.5%). Sequence divergence values between *Zosterops* species (+ *R. oleaginea*) and other passerines averaged as follows: 18.8 ± 1.2% to oscine passerines in the parvorder Passerida (seven species; Table 1), 21.5 ± 1.0% to oscine passerines in the parvorder Corvida (four species; Table 1), and 28.6 ± 1.2% to suboscine passerines (two species; Table 1). All divergence values are based on the 191-bp fragment sequenced for all taxa (between bases 9052 and 9240 in the *G. gallus* mitochondrial sequence; Desjardins and Morais 1990); nine contiguous bases in the t-lysine region were excluded because of alignment uncertainty.

All six Rota Bridled White-eye sequences were identical and distinct from sequences of other Bridled White-eye populations. The Bridled White-eye populations from Guam, Tinian, and Saipan showed little differentiation. The three individuals from Saipan had identical sequences, which were also shared by two Tinian birds (87666 and 87656) and one Guam bird (2165). The remaining two Tinian birds (87665 and 87657) were identical in sequence and differed by a single G-to-A transition from the other Tinian haplotype. The two remaining Guam birds differed by two transitions from each other and two transitions from the shared Saipan/Tinian/Guam haplotype. Based on the 191 bp of sequence in common among all individuals, the mean uncorrected divergence among the Saipan, Tinian, and Guam sequences was 0.6 ± 0.5%, which is a typical value for within-species variation in mtDNA in songbirds (e.g. Burns 1998, Cicero and Johnson 1998). The Rota birds differ by 5.2 to 5.8% sequence divergence (uncorrected) from the Saipan and Tinian individuals ($\bar{x}$ = 5.4 ± 1.6%) and 5.2 to 6.9% from the Guam individuals ($\bar{x}$ = 6.0 ± 1.7 %). Compared with other Micronesian white-eyes, the Rota birds differed by 6.1 ± 1.7% from *Z. hypolais* from Yap and 7.5 ± 1.9% from *Z. semperi* from Truk and Pohnpei. The divergence between the Bridled White-eyes from Rota and their "conspecifics" was comparable to the divergence between the Rota birds and other species of *Zosterops*.

To verify the substantial sequence diver-

gence between the Bridled White-eye from Rota and conspecific populations on other islands in the Marianas, we also sequenced a portion of the mitochondrial ND2 gene (309 bp) for one individual of *Z. conspicillatus* from Rota (Rota 6), two individuals from Tinian (87666, 87656), one individual from Saipan (87654), and one Japanese White-eye (*Z. japonicus* [1]). The average uncorrected sequence divergence between the Japanese White-eye and the Bridled White-eyes was 6.8 ± 2.8% for the Rota bird and 6.4 ± 2.8% for the Tinian and Saipan birds. Among the Tinian and Saipan birds, the mean divergence was only 0.2 ± 0.5%. Between the Rota and Tinian + Saipan birds, the mean divergence was 5.4 ± 2.6%, confirming the genetic distinctiveness (in mtDNA) of the Rota population compared with the other Bridled White-eyes in the Mariana Islands.

For the Caroline Islands White-eye, *Z. semperi,* three individuals were sequenced for the smaller (191 bp) ATPase8 fragment, two from Truk and one from Pohnpei. All three yielded a different haplotype, differing from 0.6 to 1.2 ± 0.8%. For *Z. hypolais,* the Plain White-eye, two individuals from Yap Island were sequenced for the 191 bp ATPase8 fragment. The two had different haplotypes, differing by 0.5 ± 0.5%. Six individuals of the Japanese White-eye (from the introduced population on Hawaii) were sequenced for the larger (313 bp) fragment including the ATPase8 gene. Two haplotypes were found, each occurring in three individuals; the haplotypes differed by 0.6 ± 0.5%. Two individuals of the following three species were sequenced for the ATPase8 gene region, and within species each yielded identical sequences: *Z. palpebrosus* (313 bp), *R. oleaginea* (191 bp), and *C. marchei* (191 bp).

Phylogenetic analyses.—To assess the affinities of *Cleptornis marchei,* we analyzed a data matrix that included nine species of *Zosterops, R. oleaginea, C. marchei,* and 13 non-zosteropid species. The data matrix included 321 characters, 155 of which were variable and 121 of which were parsimony informative. Heuristic searches yielded 24 most-parsimonious (MP) trees (10:1 step matrix) and 23 MP trees (5:1 step matrix; Fig. 2). A maximum-likelihood (ML) search yielded three optimal trees. In all MP and ML trees, *C. marchei* was the sister to a clade including *R. oleaginea* and all species of *Zosterops.* The monophyly of the latter clade had strong bootstrap support (MP, >90%), and the pairing of *C. marchei* with this clade also was strongly supported (MP, >95%). A maximum-parsimony search was conducted in which *C. marchei* and the two meliphagid species (*Myzomela rubratra* and *Melidectes belfordi*) were constrained to form a monophyletic group. The search (5:1 step matrix) yielded 14 MP trees of length 1,263, which was 62 steps longer than the unconstrained MP trees. The likelihood of the MP trees obtained in the constrained and unconstrained searches was calculated using the same model as in the ML search and with parameter values optimized for each tree. The trees obtained under the constrained search yielded a significantly lower likelihood than the unconstrained MP trees, as judged by the Kishino-Hasegawa test (Kishino and Hasegawa 1989) in PAUP* (Swofford 1998). Thus, the hypothesis that *Cleptornis* is a member of the Meliphagidae was strongly rejected.

The second data set included *C. marchei, R. oleaginea,* and all *Zosterops* taxa (i.e. all distinct haplotypes). The data matrix included 182 characters, 52 of which were variable and 23 of which were parsimony informative. Heuristic searches yielded the same five MP trees for both step matrices (5:1 and 10:1). A maximum-likelihood search yielded a tree identical to the MP tree depicted in Figure 3. In all optimal trees, individuals of the Bridled White-eye from Guam, Tinian, and Saipan grouped as a clade with strong bootstrap support (MP, > 90%). The Guam/Tinian/Saipan clade did not pair as the sister taxon to the Bridled White-eye from Rota in any of the optimal trees. In all of the optimal trees, the immediate sister group to the Guam/Tinian/Saipan clade was a species from Africa, *Z. senegalensis;* bootstrap support for this pairing was moderate (60%; Fig. 3). In the semi-strict consensus of the five MP trees, the Bridled White-eye from Rota paired with *Z. montanus.*

We conducted an MP search (5:1 step matrix) with a constraint that forced monophyly of the Bridled White-eyes from Guam, Tinian, Saipan, and Rota. The search yielded eight MP trees of length 132, which was five steps longer than the MP trees obtained in the unconstrained search. The likelihood of the constrained and unconstrained MP trees was calculated using the same model as in the ML search, with parameter values optimized for

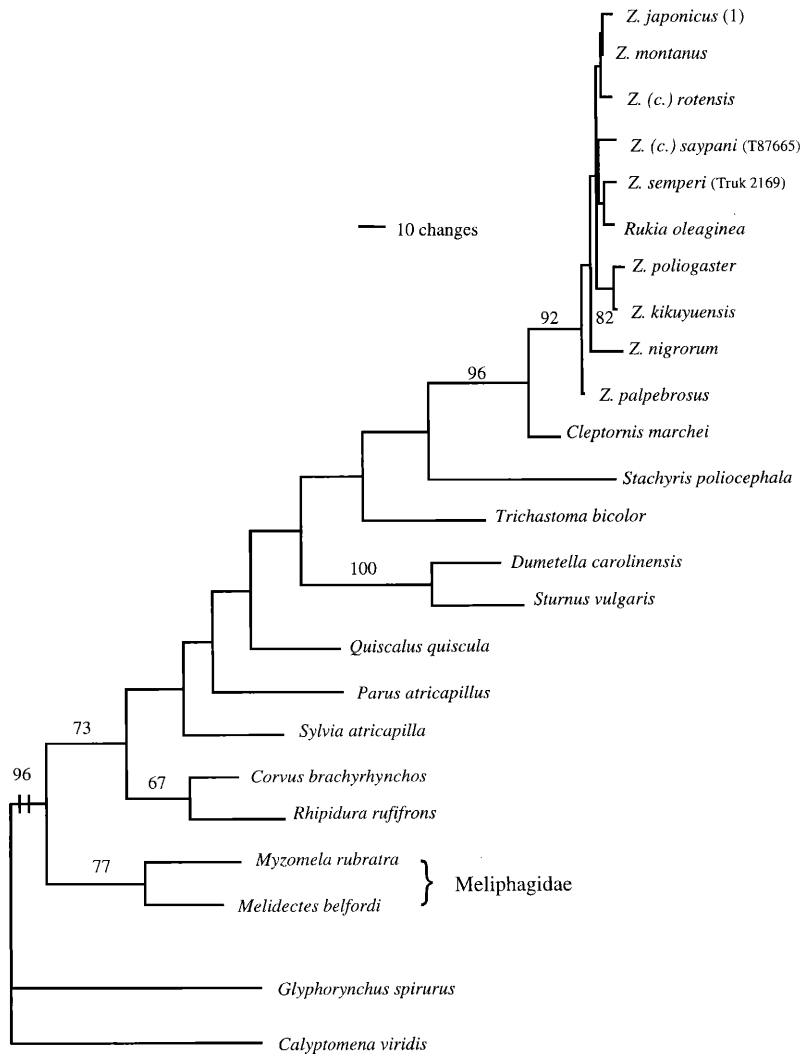

FIG. 2. One of 23 most-parsimonious trees obtained with a heuristic search (10 addition-sequence replicates, TBR branch swapping) and a step matrix that weights transversions over transitions by a factor of five. Bootstrap percentages (1,000 reps) greater than 50 are shown on the branches.

each tree. The likelihood of the constrained MP trees was not significantly different from that of the unconstrained trees, based on the Kishino-Hasegawa test in PAUP* (Swofford 1998). Thus, monophyly of *Z. conspicillatus* (Guam, Tinian, Saipan) and *Z. rotensis* (Rota) could not be rejected based on these sequence data.

In all optimal trees, individuals of *Z. semperi* from Truk and Pohnpei grouped together; one individual from Truk (2169) and one from Pohnpei (2244) paired as sister taxa, and a second individual from Truk (2170) paired as their sister. Thus, the two island populations were not separately resolved. In all optimal trees, *Rukia oleaginea* paired with the *Z. semperi* clade. The monophyly of the *Z. semperi* clade had strong bootstrap support (87%), and the pairing of *R. oleaginea* and *Z. semperi* had moderate bootstrap support (53%).

DISCUSSION

Comparison with previous estimates of relationships.—The phylogenetic trees and genetic distances derived from our mitochondrial sequence data imply that the Bridled White-eye

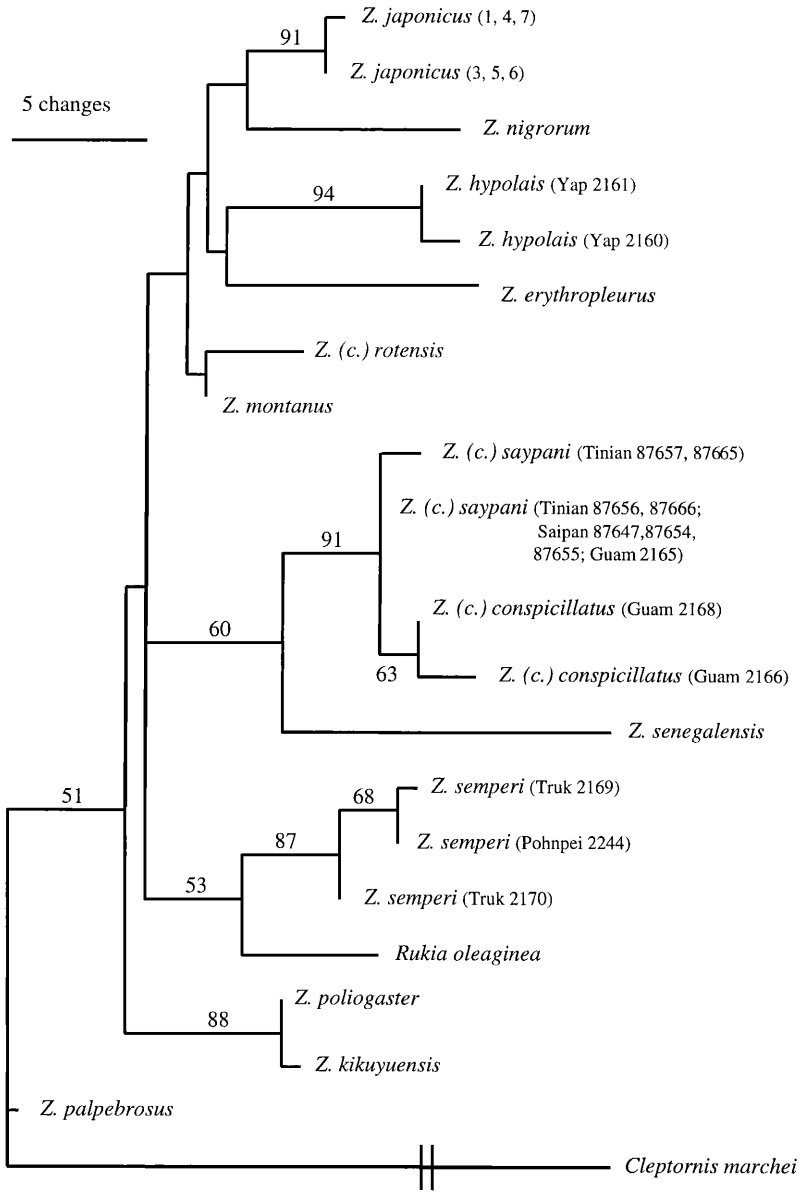

FIG. 3. One of five most-parsimonious trees obtained with a heuristic search (10 addition-sequence replicates, TBR branch swapping) and a step matrix that weights transversions over transitions by a factor of five. Bootstrap percentages (1,000 reps) greater than 50 are shown on the branches. The same topology also was obtained in a maximum-parsimony search with a 10:1 step matrix and in a maximum-likelihood search. Sample numbers of individuals having the same haplotype are listed in parentheses after the taxon name.

as defined by Mees (1969) is polyphyletic. Based primarily on differences in behavior and song, Pratt et al. (1987) suggested splitting the Bridled White-eye complex of Mees into three species: *Z. conspicillatus* (Marianas), *Z. semperi* (Truk, Pohnpei, Palau), and *Z. hypolais* (Yap). Our data support the recognition of *Z. semperi* and *Z. hypolais* as distinct species. Genetic divergence within these two taxa is low (although sample sizes are small), whereas divergences between *semperi, hypolais,* and *conspicillatus* are substantial and comparable to dis-

tances between other white-eye species. The sequence data also indicate that *Z. conspicillatus* (Mariana Islands) is comprised of two genetically well-diverged clades: the Rota population and the populations on Guam (extinct), Tinian, and Saipan. Within each of these two clades, divergence is small (<2.3%), whereas divergence between the clades is comparable to that between other species of *Zosterops* (6.5 ± 1.7%). The distinctiveness of the Rota Bridled White-eye was recognized previously by Pratt (Collar et al. 1994).

The optimal trees based on the sequence data weakly resolve relationships among the Micronesian white-eye clades. Nonetheless, in all most-parsimonious trees, the following three clades each pair with a different sister taxon, suggesting that each was derived from a different colonizing ancestor: *Z. hypolais*; *Z. conspicillatus* from Rota; and *Z. conspicillatus* from Guam, Tinian, and Saipan. However, a more exhaustive sampling of species of *Zosterops*, particularly those from the Indo-Australian region, is needed to identify the lineages that colonized the Micronesian islands. Also, our data set did not include the following white-eye species from Micronesia: *Z. cinereus* (Pohnpei and Kosrae), *Z. finschii* (Palau), *Rukia ruki* (Truk), *Rukia longirostra* (Pohnpei), and *Megazosterops palauensis* (Palau).

In all of the optimal trees, *Rukia oleaginea* is embedded among other species of *Zosterops*, suggesting that its placement in a separate genus is unwarranted (which agrees with results of Sibley and Ahlquist [1990]). *Rukia oleaginea* pairs with *Z. semperi* in all of the optimal trees from our study. Both species occur in the Caroline Islands: *R. oleaginea* is endemic to Yap, and *Z. semperi* occurs on Truk, Pohnpei, and Palau but not on Yap. The morphological attributes that distinguish *R. oleaginea* from other white-eyes, most notably its large size and melanistic plumage coloration, might reflect rapid differentiation in an island population.

The optimal trees and genetic distances both support a close relationship between *Cleptornis marchei* and species in the family Zosteropidae. In all optimal trees, *C. marchei* was the sister taxon to a clade including all species of *Zosterops* (and *R. oleaginea*). Sibley and Ahlquist (1990) found a sister relationship between *Cleptornis* and a clade including *R. oleaginea* and three species of *Zosterops* (*pallidus, palpebrosus,* and *lateralis*) based on nuclear DNA-DNA hybridization. *Cleptornis marchei* was originally described as an aberrant member of the Meliphagidae (Oustalet 1889). This classification persisted until Pratt et al. (1987) suggested a closer relationship to the white-eyes based on behavioral, ecological, and zoogeographical considerations. However, *C. marchei* is distinct in having a large 10th primary, whereas in other white-eyes the 10th primary is reduced or absent. Based on the nuclear DNA-DNA hybridization data of Sibley and Ahlquist (1990) and the mtDNA data from our study, *C. marchei* is closely related to the white-eyes and perhaps should be considered as a basal member of the family.

Conservation issues.—The Rota population of the Bridled White-eye has declined drastically and mysteriously over the past 14 years. Our mitochondrial sequence data support previous suggestions that the Rota population is a distinct species from the Bridled White-eye on the other Mariana Islands. Thus, the population on Rota could warrant increased protection as a full species under the Endangered Species Act. Another conservation issue regards the recently extirpated population of *Z. conspicillatus* on Guam. If the brown tree snake can be eradicated or controlled on Guam, then reintroduction of *Z. conspicillatus* from populations on Tinian or Saipan (rather than from neighboring Rota) is warranted on genetic grounds. The populations on Guam, Tinian, and Saipan shared a common mitochondrial haplotype, suggesting that the three populations either exchanged migrants recently, or the populations are too young for lineage sorting of mitochondrial genes to have gone to completion.

ACKNOWLEDGMENTS

We thank the following people for providing samples essential to this work: A. Marshall, J. Lepson, L. Sileo, J. Savidge, P. Bruner (Brigham Young University, Hawaii), and J. Fjeldså (Zoological Museum of Copenhagen). We are grateful to K. Rosa (USFWS) for lending logistical support and facilitating sample collection. We thank A. Neumann for laboratory assistance. H. D. Pratt provided helpful comments on the manuscript. Financial support for this project was provided by the United States Fish and Wildlife Service and Friends of the National Zoo. B. Slikas was supported by a Smithsonian postdoctoral fellowship while working on this project.

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Associate Editor: R. M. Zink