



Molecular evidence suggests radical revision of species limits in the great speciator white-eye genus *Zosterops*

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Abstract

White-eyes (*Zosterops* spp.) are a group of small passerines distributed across the Eastern Hemisphere that have become a textbook example of rapid speciation. However, traditional taxonomy has relied heavily on conservative plumage features to delimit white-eye species boundaries, resulting in several recent demonstrations of misclassification. Resolution of confused taxonomy is important in order to correctly delimit species and identify taxa which may require conservation, particularly in Asia where the songbird trade is decimating wild populations. In this study, we aim to untangle multiple instances of confused taxonomic treatment in three large, widespread Asian wastebasket species complexes of white-eye (Oriental White-eye *Zosterops palpebrosus*, Japanese White-eye *Zosterops japonicus* and Mountain White-eye *Zosterops montanus*) renowned for their conservative morphology. Using mitochondrial DNA from 173 individuals spanning 42 taxa, we uncovered extensive polyphyly in *Z. palpebrosus* and *Z. japonicus* and propose some radically revised species limits under which former members of *Z. palpebrosus* and *Z. japonicus* would be reassigned into four and two different species, respectively. The revised taxonomy results in a net loss of two previously recognized species and a net gain of two newly recognized species, leading to significant taxonomic change but a lack of additional species-level diversity. One of the newly elevated species, *Zosterops melanurus* from Java and Bali, is also the world's most heavily traded songbird and requires urgent conservation attention.

Keywords Cryptic speciation · Phylogenetics · Wastebasket species · Polyphyly · Taxonomy

Zusammenfassung

Molekulare Belege erfordern eine radikale Revision der Artgrenzen innerhalb der „Superartbildner“- Brillenvogelgattung *Zosterops*

Brillenvögel (*Zosterops* spp.) sind eine Gruppe kleiner Singvögel der östlichen Hemisphäre, die zu einem Paradebeispiel für schnelle Artbildung geworden sind. Allerdings stützte sich die traditionelle Taxonomie bei der Abgrenzung der Brillenvogelarten bisher vorwiegend auf konservative Gefiedermerkmale, was zu verschiedenen in neuerer Zeit aufgedeckten Falschklassifikationen führte. Eine Entwirrung der Taxonomie ist wichtig für eine korrekte Artabgrenzung und die Ermittlung von Taxa mit besonderem Schutzbedarf, speziell in Asien, wo der Handel mit Singvögeln die Wildpopulationen stark dezimiert. Ziel dieser Untersuchung war es, verschiedene Fälle verworrener taxonomischer Einordnung bei drei großen, weitverbreiteten asiatischen „Sammelsurium-Artkomplexen“ von Brillenvögeln (Gangesbrillenvogel *Z. palpebrosus*, Japanbrillenvogel *Z. japonicus* und Gebirgsbrillenvogel *Z. montanus*) aufzulösen, welche für ihre konservative Morphologie

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bekannt sind. Anhand von mitochondrialer DNA von 173 Individuen aus 42 Taxa entdeckten wir ein beträchtliches Maß an Polyphylie bei *Z. palpebrosus* sowie *Z. japonicus* und empfehlen eine radikale Revision einiger Artgrenzen, durch welche vormalige Angehörige der Arten *Z. palpebrosus* und *Z. japonicus* neu zu jeweils vier beziehungsweise zwei verschiedenen Arten gerechnet würden. Diese überarbeitete Taxonomie resultiert insgesamt in dem Verlust zweier vormals anerkannter Arten sowie einem Hinzugewinn von zwei neu etablierten Arten, was zwar zu einer signifikanten taxonomischen Veränderung, jedoch nicht zu zusätzlicher Diversität auf Artebene führt. Eine der neu anerkannten Arten, *Z. melanurus* von Java und Bali, ist zudem der meistgehandelte Singvogel der Welt und bedarf dringender Schutzmaßnahmen.

Introduction

The genus *Zosterops*, commonly referred to as ‘white-eyes’, is a group of small passerines comprising approximately 100 species with a distribution spanning the Eastern Hemisphere (Africa to Australasia) (van Balen 2017a). The genus is famously recognized for having one of the fastest diversification rates amongst birds, and possibly terrestrial vertebrates, and is thus known as a ‘great speciator’ (Diamond et al. 1976; Moyle et al. 2009; Cornetti et al. 2015). This trait has made the group a popular model for diversification and island biogeography studies (Diamond et al. 1976; Warren et al. 2006; Moyle et al. 2009; Cox et al. 2014; Cornetti et al. 2015; Linck et al. 2016; Wickramasinghe et al. 2017).

Despite their fast genetic diversification rate and a distribution spanning across land masses and islands around the entire Indian and Western Pacific oceans, the morphology and vocalisations of many *Zosterops* taxa have remained conserved (Eaton et al. 2016). Owing to similarities in plumage and song, species identification is often challenging especially with captive birds of unknown provenance, and different treatises have applied varying species delimitations (e.g. Eaton et al. 2016; Wells 2017a, b; van Balen 2017a). In this work, we adopt one of the more conservative *Zosterops* treatises (van Balen 2017a) as a baseline taxonomy, with the exception of the taxon *auriventer*: the latter taxon is widely considered a Sundaic subspecies of the Oriental White-eye *Zosterops palpebrosus* by van Balen (2017b) and other traditional inventories, but Wells (2017a, b) recently demonstrated that *auriventer* is the proper senior name applying to Sundaic populations of Everett’s White-eye *Zosterops everetti*, as circumscribed by van Balen (2018b), thereby replacing more junior names such as *wetmorei*. In return, the Oriental White-eye subspecies previously named *auriventer* assumes its next most senior name *erwini* (Wells 2017a, b).

Cryptic species-level lineages may lurk in the form of taxa currently classified as subspecies across multiple large wastebasket species complexes within *Zosterops*. Three such wastebasket species complexes are the Oriental White-eye *Zosterops palpebrosus*, the Japanese White-eye *Zosterops japonicus*, and the Mountain White-eye *Zosterops montanus*. As currently circumscribed, the Oriental White-eye is

one of the most widespread species within *Zosterops* and is conventionally divided into 11 subspecies ranging from those of the Indian subcontinent eastwards to Sundaland and the Lesser Sundas (Fig. 1a) (van Balen 2017b). The Japanese White-eye, ranging from the Japanese Archipelago through China and northernmost Southeast Asia, is conventionally divided into eight subspecies (Fig. 1a) (van Balen 2017c). Nine subspecies of the Mountain White-eye are currently widely accepted, ranging from Sumatra, Java and the Lesser Sundas northwards to Sulawesi and the Philippine archipelago (Fig. 1a) (van Balen 2017d).

These three wide-ranging species complexes have been identified as requiring more taxonomic attention. For instance, molecular sampling of two disjunct Oriental White-eye subspecies has demonstrated that the nominate form *palpebrosus* from India and *unicus* from the Lesser Sundas are not closely related within the genus (Moyle et al. 2009). These two taxa occupy the extreme ends of the distribution of the complex, leaving taxonomic arrangements across the Sundaic region largely unknown and intimating that the current Oriental White-eye complex constitutes a non-monophyletic composite. Molecular sampling in the Japanese White-eye has so far only covered Korea and Japan, leaving the affinities of populations across the rest of continental Asia largely unknown (Nishiumi and Kim 2004; Nagata and Kanetsuki 2006). The evolutionary affinities of taxa within the widespread Mountain White-eye *Z. montanus* complex have also received little attention over recent years except for a study that shed light on the complicated relationships among populations within the Philippine archipelago (Jones and Kennedy 2008).

With the taxonomy of many Asian white-eyes in disarray, conservation may become a question of urgency in rare and endangered species-level taxa that may have mistakenly languished at the subspecific level so far. The primary threat to white-eyes is over-harvesting for the songbird trade coupled with widespread habitat destruction (Eaton et al. 2015). In Indonesia alone, the centuries-old tradition of bird-keeping has critically endangered many avian species in the wild (Eaton et al. 2015; Lee et al. 2016), with white-eyes being among the most popular cage bird species affected. To satisfy this demand, Oriental White-eyes are commonly

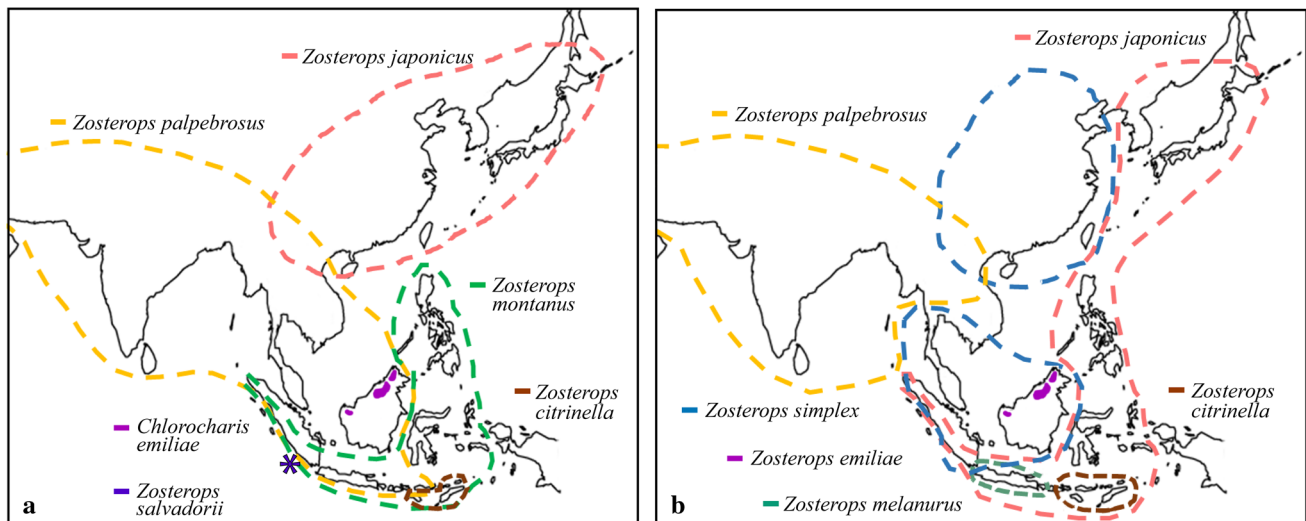


Fig. 1 a Range map of *Zosterops japonicus*, *Zosterops palpebrosus*, *Zosterops montanus*, *Zosterops citrinella*, *Zosterops salvadorii* and *Chlorocharis emiliae* based on traditional taxonomic classification (following van Balen 2017b, c, d, e, f, 2018a; Wells 2017a, b). **b**

Range map of revised species distributions based on the results of this study—*Zosterops palpebrosus*, *Zosterops japonicus*, *Zosterops melanurus*, *Zosterops simplex*, *Zosterops citrinella* and *Zosterops emiliae*

poached from their natural habitats across Sundaland, and massive numbers are sold in wildlife markets across the region (Chng et al. 2015; Chng and Eaton 2016; Lee et al. 2016; Eaton et al. 2017). Consequently, the Oriental White-eye and other *Zosterops* taxa have been identified as being at high risk of trade-driven local extinction (Lee et al. 2016), but the taxonomic uncertainty within these species complexes thwarts meaningful conservation attempts.

In the present study, we investigated taxonomic relationships within each of the three Asian white-eye wastebasket species complexes—*Z. palpebrosus*, *Z. japonicus* and *Z. montanus*. We used a combination of novel sequence data and published GenBank material for two mitochondrial genes in order to test conspecificity of taxa within each of the three complexes. Our genetic analysis is supplemented by qualitative information on key morphological characteristics in order to more accurately estimate and define boundaries between and within taxa.

Materials and methods

DNA sampling

DNA samples were obtained from a mixture of both live samples and museum loans (Table S1). In total, we obtained 27 samples from live birds, including ten blood samples of *Zosterops japonicus simplex* that we caught in Hong Kong and 17 blood samples of initially unidentified individuals of *Zosterops* from Wildlife Reserves Singapore's captive collection (Table S1). The latter samples had either been

donated to Wildlife Reserves Singapore by members of the Singaporean public or had been confiscated.

The museum specimens, in the form of either blood, feather or breast muscle tissue, were loaned from the Lee Kong Chian Natural History Museum (Singapore), the Museum Zoologicum Bogoriense (Cibinong, West Java, Indonesia), and the Burke Museum of Natural History and Culture (Seattle, Washington, DC). In total, 57 museum samples representing ten taxa were obtained for this study (Table S1). These ten taxa included an optimized selection of members of the three focal species complexes and sympatric and/or neighbouring congeneric taxa that may be closely related.

Our final sequence alignments included a total of 140 sequences of nicotinamide adenine dinucleotide (reduced; NADH) dehydrogenase subunit 2 (ND2) and 103 sequences of cytochrome *b* (*cytb*) across 42 taxa of *Zosterops* white-eyes (Tables 1, S1 and S2). In total, 54% of ND2 and 73% of *cytb* sequences constituted novel sequences generated for this study (Table S1). The remaining sequences were sourced from other *Zosterops* publications (National Center for Biotechnology Information GenBank) to ensure a more robust sampling (Table S2). The sequences of the two loci from the outgroup species, *Yuhina brunneiceps*, were retrieved from GenBank as well (Table S2).

Laboratory procedures

DNA extractions were performed with a Qiagen DNeasy Blood and Tissue Kit following the manufacturer's protocol for blood, tissue and feather samples in ethanol. Targeted

Table 1 Summary list of all the taxa and number of sequences generated for this study

Species' identifying number	Recognised taxa (van Balen 2017a; Wells 2017a, b)	No. of samples				Proposed reclassification from this study ^a
		Nicotinamide adenine dinucleotide dehydrogenase subunit 2 (ND2)		Cytochrome <i>b</i> (<i>cytb</i>)		
		Novel	GenBank	Novel	GenBank	
1	<i>Zosterops palpebrosus palpebrosus</i>	–	2	–	1	<i>Zosterops palpebrosus palpebrosus</i>
2	<i>Zosterops palpebrosus nilgiriensis</i>	–	9	–	12	<i>Zosterops palpebrosus nilgiriensis</i>
3	<i>Zosterops palpebrosus salimalii</i>	–	–	–	–	<i>Zosterops palpebrosus salimalii</i>
4	<i>Zosterops palpebrosus egregius</i>	–	7	–	–	<i>Zosterops palpebrosus egregius</i>
5	<i>Zosterops palpebrosus siamensis</i>	–	1	–	–	<i>Zosterops palpebrosus siamensis</i>
6	<i>Zosterops palpebrosus nicobaricus</i>	–	–	–	–	<i>Zosterops palpebrosus nicobaricus</i>
7	<i>Zosterops palpebrosus williamsoni</i>	–	–	–	–	<i>Zosterops simplex williamsoni</i> ^b
8	<i>Zosterops palpebrosus erwini</i>	27	–	28	–	<i>Zosterops simplex erwini</i> ^b
9	<i>Zosterops palpebrosus buxtoni</i>	5	–	5	–	<i>Zosterops melanurus buxtoni</i> ^b
10	<i>Zosterops palpebrosus melanurus</i>	3	–	2	1	<i>Zosterops melanurus melanurus</i> ^b
11	<i>Zosterops palpebrosus unicus</i>	–	2	–	–	<i>Zosterops citrinella unicus</i> ^b
12	<i>Zosterops japonicus japonicus</i>	1	–	1	8	<i>Zosterops japonicus japonicus</i>
13	<i>Zosterops japonicus simplex</i>	15	3	16	6	<i>Zosterops simplex simplex</i> ^b
14	<i>Zosterops japonicus hainanus</i>	–	–	–	–	<i>Zosterops simplex hainanus</i> ^b
15	<i>Zosterops japonicus loochooensis</i>	–	–	–	–	<i>Zosterops japonicus loochooensis</i>
16	<i>Zosterops japonicus daitoensis</i>	–	–	–	–	<i>Zosterops japonicus daitoensis</i>
17	<i>Zosterops japonicus stejneri</i>	–	–	–	–	<i>Zosterops japonicus stejneri</i>
18	<i>Zosterops japonicus alani</i>	–	–	–	–	<i>Zosterops japonicus alani</i>
19	<i>Zosterops japonicus insularis</i>	–	–	–	–	<i>Zosterops japonicus insularis</i>
20	<i>Zosterops salvadorii</i>	1	–	1	–	<i>Zosterops simplex salvadorii</i> ^b
21	<i>Zosterops citrinella citrinella</i>	13	1	12	–	<i>Zosterops citrinella citrinella</i>
22	<i>Zosterops citrinella harterti</i>	5	–	5	–	<i>Zosterops citrinella harterti</i>
23	<i>Zosterops citrinella albiventris</i>	–	–	–	–	<i>Zosterops citrinella albiventris</i>
24	<i>Zosterops montanus montanus</i>	5	2	5	–	<i>Zosterops japonicus montanus</i> ^b
25	<i>Zosterops montanus whiteheadi</i>	–	2	–	–	<i>Zosterops japonicus whiteheadi</i> ^b
26	<i>Zosterops montanus halconensis</i>	–	–	–	–	<i>Zosterops japonicus halconensis</i> ^b
27	<i>Zosterops montanus parkesi</i>	–	–	–	–	<i>Zosterops japonicus parkesi</i> ^b
28	<i>Zosterops montanus pectoralis</i>	–	2	–	–	<i>Zosterops japonicus pectoralis</i> ^b
29	<i>Zosterops montanus diuatae</i>	–	2	–	–	<i>Zosterops japonicus diuatae</i> ^b
30	<i>Zosterops montanus vulcani</i>	–	–	–	–	<i>Zosterops japonicus vulcani</i> ^b
31	<i>Zosterops montanus difficilis</i>	–	–	–	–	<i>Zosterops japonicus difficilis</i> ^b
32	<i>Zosterops montanus obstinatus</i>	–	2	–	–	<i>Zosterops japonicus obstinatus</i> ^b
33	<i>Zosterops chloris</i>	–	2	–	–	<i>Zosterops chloris</i>
34	<i>Zosterops atrifrons</i>	–	1	–	–	<i>Zosterops atrifrons</i>
35	<i>Zosterops atricapilla</i>	–	2	–	–	<i>Zosterops atricapilla</i>
36	<i>Zosterops luteus</i>	–	1	–	–	<i>Zosterops luteus</i>
37	<i>Zosterops ceylonensis</i>	–	4	–	–	<i>Zosterops ceylonensis</i>
38	<i>Chlorocharis emiliae</i>	–	1	–	–	<i>Zosterops emiliae</i> ^b
39	<i>Zosterops abyssinica</i>	–	1	–	–	<i>Zosterops abyssinica</i>
40	<i>Zosterops erythropleura</i>	–	1	–	–	<i>Zosterops erythropleura</i>
41	<i>Zosterops nigrorum</i>	–	1	–	–	<i>Zosterops nigrorum</i>
42	<i>Zosterops virens</i>	–	1	–	–	<i>Zosterops virens</i>
43	<i>Zosterops maderaspatanus</i>	–	1	–	–	<i>Zosterops maderaspatanus</i>
44	<i>Zosterops senegalensis</i>	–	1	–	–	<i>Zosterops senegalensis</i>
45	<i>Zosterops metcalfei</i>	–	1	–	–	<i>Zosterops metcalfei</i>

Table 1 (continued)

Species' identifying number	Recognised taxa (van Balen 2017a; Wells 2017a, b)	No. of samples				Proposed reclassification from this study ^a
		Nicotinamide adenine dinucleotide dehydrogenase subunit 2 (ND2)		Cytochrome <i>b</i> (<i>cytb</i>)		
		Novel	GenBank	Novel	GenBank	
46	<i>Zosterops ugiensis</i>	–	1	–	–	<i>Zosterops ugiensis</i>
47	<i>Zosterops vellalavella</i>	–	1	–	–	<i>Zosterops vellalavella</i>
48	<i>Zosterops fuscicapillus</i>	–	1	–	–	<i>Zosterops fuscicapillus</i>
49	<i>Woodfordia superciliosa</i>	–	1	–	–	<i>Woodfordia superciliosa</i>
50	<i>Zosterops flavifrons</i>	–	1	–	–	<i>Zosterops flavifrons</i>
51	<i>Zosterops luteirostris</i>	–	1	–	–	<i>Zosterops luteirostris</i>
52	<i>Zosterops splendidus</i>	–	1	–	–	<i>Zosterops splendidus</i>
53	<i>Zosterops rendovae</i>	–	1	–	–	<i>Zosterops rendovae</i>
54	<i>Zosterops lateralis</i>	–	1	–	–	<i>Zosterops lateralis</i>
55	<i>Zosterops rennellianus</i>	–	1	–	–	<i>Zosterops rennellianus</i>
56	<i>Zosterops murphyi</i>	–	1	–	–	<i>Zosterops murphyi</i>

^aTaxon names under the proposed taxonomic revision following the results of this study

^bTaxon reclassifications recommended based on this study (no. of samples in *italic*)

mitochondrial genes were amplified via polymerase chain reaction. The *ND2* gene was amplified using the primers L5219Met (5'-CCCATACCCCGAAAATGATG-3') and H6313Trp (5'-CTCTTATTTAAGGCTTTGAAGGC-3') (Sorenson et al. 1999), and the *cytb* gene was amplified using L14833 (5'-CAGGCCTAATAAAAGCCTA-3') and H15487 (5'-GATCCTGTTTCGTGGAGGAAGGT-3') (Cibois et al. 1999; Dong et al. 2010). Samples were cycle-sequenced using the BigDye Terminator version 3.1 Cycle Sequencing Kit. Sequences were obtained by capillary electrophoresis using an Applied Biosystems 3730 96-capillary Genetic Analyzer.

Phylogenetic analysis

The forward and reverse sequences of both mitochondrial genes were assembled using CodonCode Aligner version 7.0 (CodonCode). The datasets were exported to MEGA7 (Kumar et al. 2016) for alignment via ClustalW (Thompson et al. 2002). Three datasets were used for phylogenetic analysis—the *ND2* gene (trimmed to 888 base pairs), the *cytb* gene (trimmed to 588 base pairs), and a concatenated gene sequence with 1476 base pairs.

RAXML (Stamatakis 2014) was employed to build phylogenetic trees using maximum likelihood (ML) with a GTR + Inverse + Gamma model on each of the three datasets. Each dataset was run for 1000 rapid bootstrap replicates and ten runs of thorough ML search. For Bayesian analysis, we employed both PartitionFinder version 1.1.1 (Lanfear et al. 2012) and jModelTest 2.1.10 (Guindon and

Gascuel 2003; Darriba et al. 2012) to determine the best evolutionary model for each locus and codon position. PartitionFinder resulted in the following models: *ND2* gene (codon position 1 and 2—HKY + Inverse + Gamma; codon position 3—GTR + Gamma); *cytb* gene (codon position 1—K80 + Inverse; codon position 2—F81 + Inverse; codon position 3—HKY + Gamma), while jModelTest provided a TIM2 + Inverse + Gamma model for the *ND2* gene and an HKY + Gamma model for the *cytb* gene (Darriba et al. 2012; Tamura and Nei 1993; Hasegawa et al. 1985). As trees resulting from jModelTest and PartitionFinder models were identical in topology and extremely similar in branch support, we only report on the results of jModelTest. In total, 1000,000 generations were run on MrBayes version 3.2 (Ronquist and Huelsenbeck 2003) for each dataset. The analysis sampled every 1000 steps, with 25% of the samples discarded as burn-in. The phylogenetic trees generated were then combined and modified using FigTree version 1.4.3 (Rambaut 2006). Additionally, DnaSP version 5.10.1 was used to calculate pairwise sequence divergences (Librado and Rozas 2009).

Dating analysis

We employed BEAUti and BEAST (Drummond et al. 2012) to estimate the divergence times within our sampled taxa. We selected the *ND2* gene dataset for this dating analysis as it encompassed the largest sample size with the highest number of taxa. We assumed a Yule speciation process (Yule 1925; Gernhard 2008) for this model together with a relaxed

molecular clock with lognormal distribution and a calibrated rate of 4.94%/M years obtained from the age of Ranongga Island for *Zosterops splendidus* (Moyle et al. 2009). We ran Markov chain Monte Carlo chains for 10 million generations and discarded the first 25% as burn-in. Finally we employed Tracer version 1.6 (Rambaut et al. 2014) to ensure stationarity and examine respective parameters, including the effective sample size value.

Results

The final datasets included 140 samples for the ND2 alignment and 103 samples for the cytb alignment, plus one out-group sequence for each alignment. All three analyses (ND2, cytb, and concatenated) resulted in similar tree topologies,

with no conflict among highly supported clades (Fig. 2, S1). Based on consensus trees, both *Z. palpebrosus* and *Z. japonicus* constitute polyphyletic species (Fig. 2, S1). Deep pairwise nucleotide divergences within these traditional species complexes yielded additional support for their polyphyly (Table 2).

In the *Zosterops palpebrosus* species complex, we discovered deep divergences between the nominate subspecies group (represented by *palpebrosus*, *egregius*, *nilgiriensis* and *siamensis*, ranging from India and Sri Lanka to North Vietnam) and all other races in Southeast Asia. We found the Greater Sundaic race *erwini* [sensu Wells (2017a, b); formerly *auriventer* sensu van Balen (2017b)] to be closely related to *Zosterops salvadorii* from Enggano Island and to *Zosterops japonicus simplex* from mainland China. The Javan races *Zosterops palpebrosus melanurus* and *Zosterops*

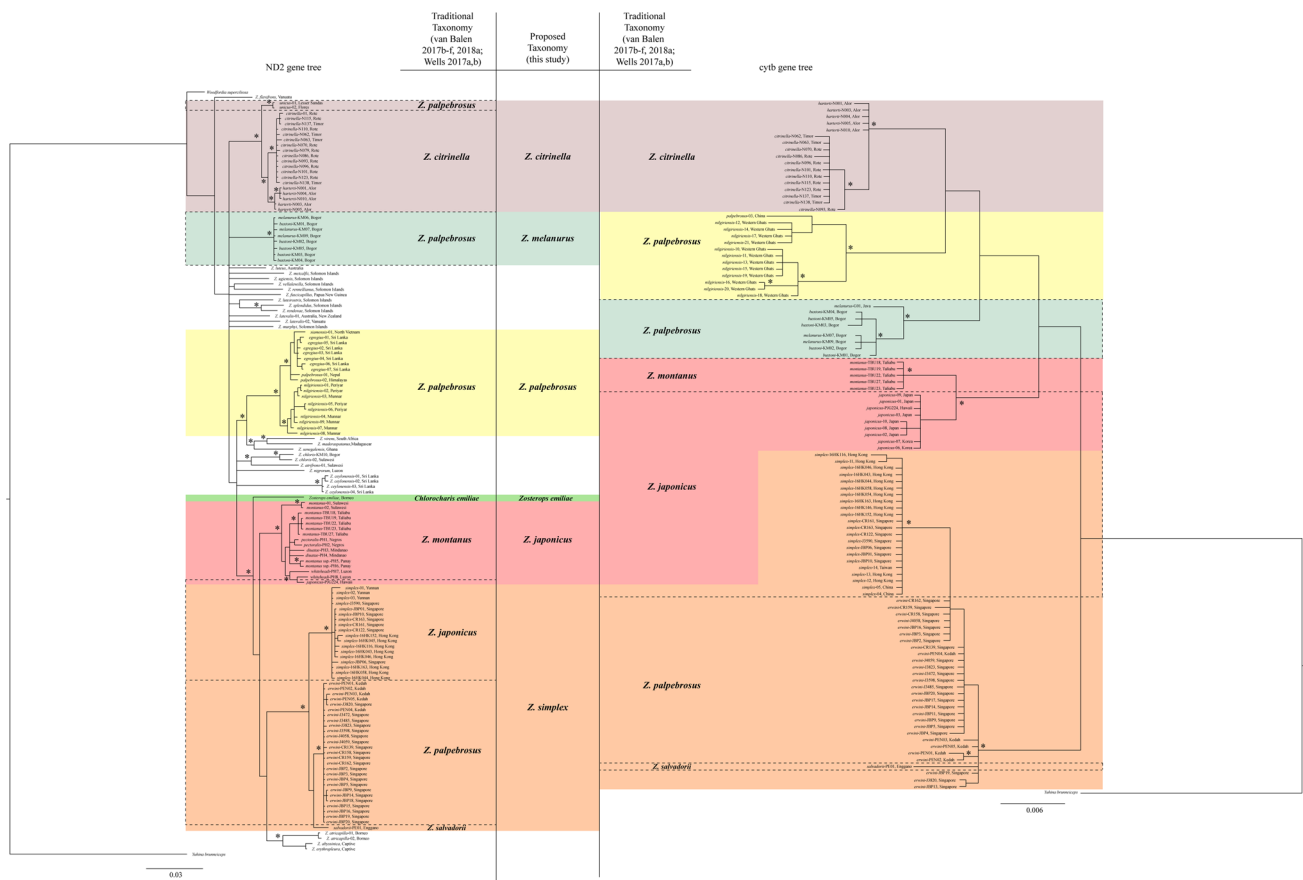


Fig. 2 Bayesian tree topology for nicotinamide adenine dinucleotide (reduced) dehydrogenase subunit 2 (*ND2*; left) and cytochrome *b* (*cytb*; right). Tip labels for newly sequenced samples (this study) are denoted by the terminal taxon name and an identifier corresponding to Table S1. Tip labels for Genbank-derived samples have a numeral identifier corresponding to Table S2 unless they are unique for their species. The colour-shaded areas of the figure indicate the newly proposed species delimitation as presented in the central column. Traditional species delimitation (as in, e.g. van Balen 2017b, c, d, e, f, 2018a, b; Wells 2017a, b) is indicated in the two columns bordering

the central column to the left and to the right, respectively, with stippled lines assisting in delineating some of the species blocks. Branch support values are indicated by an asterisk at each node if high (Bayesian posterior probability > 0.95/maximum likelihood > 0.7) but omitted otherwise. Each terminal taxon name at the tip of the tree is either the full scientific name or a subspecies epithet (for the key species complexes). The initially unidentified *Zosterops* samples (*JBP*) from Wildlife Reserves Singapore were identified to species level in this tree from the results obtained

Table 2 Average pairwise nucleotide divergences between *Zosterops* taxa in this study (%)

	Zos-terops palpe-brosus	Zos-terops palpe-brosus nilgir-tensis	Zos-terops palpe-brosus egre-gius	Zos-terops palpe-brosus siamen-sis	Zos-terops palpe-brosus erwini	Zos-terops simplex salvadorii	Zos-terops simplex rinella cit-rinella	Zos-terops simplex cit-rinella har-terti	Zos-terops mela-nurus	Zos-terops mela-nurus mela-nurus	Zos-terops emiliae	Zos-terops japoni-cus japoni-cus	Zos-terops japoni-cus white-headi	Zos-terops japoni-cus diuatae	Zos-terops japoni-cus pecto-ralis	Zos-terops japoni-cus monta-nus	Zos-terops japoni-cus ssp.
<i>Zosterops palpe-brosus</i>	2.21	–	–	–	4.59	4.59	4.59	4.08	4.59	4.93	–	–	–	–	–	–	–
<i>Zosterops palpe-brosus</i>	1.80	–	–	–	5.78	6.12	4.76	4.25	4.42	4.42	–	–	–	–	–	–	–
<i>Zosterops palpe-brosus nilgir-tensis</i>	0.90	1.58	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Zosterops palpe-brosus egre-gius</i>	1.13	2.03	0.90	–	–	–	–	–	–	–	–	–	–	–	–	–	–
<i>Zosterops simplex erwini</i>	6.99	6.98	6.98	7.43	1.36	1.36	5.10	4.59	5.10	5.44	–	4.08	–	–	–	–	–
<i>Zosterops simplex salvadorii</i>	7.21	7.32	7.21	7.66	2.59	2.04	5.44	4.93	4.76	5.10	–	4.42	–	–	–	–	–
<i>Zosterops simplex rinella cit-rinella</i>	6.87	6.31	6.87	7.32	1.46	2.59	5.78	5.27	5.44	5.78	–	4.42	–	–	–	–	–
<i>Zosterops cit-rinella</i>	5.63	5.18	5.41	5.86	6.76	6.64	6.64	0.51	3.74	4.08	–	4.42	–	–	–	–	–
<i>Zosterops cit-rinella harterri</i>	5.41	4.96	5.18	5.63	6.76	6.64	1.13	–	3.23	3.57	–	3.91	–	–	–	–	–

Table 2 (continued)

Zos- terops palpe- brosus palpe- brosus nilgir- tensis	Zos- terops palpe- brosus egre- gius	Zos- terops palpe- brosus siamen- sis	Zos- terops simplex erwini	Zos- terops simplex sabva- dorii	Zos- terops cit- rinella cit- rinella	Zos- terops cit- rinella har- terti	Zos- terops cit- rinella unicus	Zos- terops mela- nurus mela- nurus	Zos- terops mela- nurus mela- nurus	Zos- terops emiliae	Zos- terops japoni- cus japoni- cus	Zos- terops japoni- cus white- headi	Zos- terops japoni- cus diuatae	Zos- terops japoni- cus pecto- ralis	Zos- terops japoni- cus monta- nus	Zos- terops japoni- cus ssp.
<i>Zosterops</i> 5.52	5.07	5.29	5.74	6.31	6.64	6.19	1.46	1.46	–	–	–	–	–	–	–	–
<i>cit- rinella</i>																
<i>unicus</i>																
<i>Zosterops</i> 5.52	5.07	5.29	5.74	6.87	7.88	7.21	4.96	4.73	4.39	1.02	–	–	–	–	4.59	–
<i>mela- nurus</i>																
<i>mela- nurus</i>																
<i>Zosterops</i> 5.18	4.73	4.96	5.41	6.76	7.77	6.87	4.62	4.39	4.05	0.34	–	–	–	–	4.93	–
<i>mela- nurus</i>																
<i>nurus</i>																
<i>Zosterops</i> 6.08	5.63	5.86	6.31	4.28	5.74	4.62	5.41	5.18	5.29	5.97	4.62	–	–	–	1.53	–
<i>mela- nurus</i>																
<i>buxtoni</i>																
<i>Zosterops</i> 6.42	6.19	6.19	6.64	5.29	6.53	5.52	6.19	6.19	5.86	6.42	6.08	–	–	–	–	–
<i>emiliae</i>																
<i>Zosterops</i> 6.08	5.63	5.86	6.31	4.28	5.74	4.62	5.41	5.18	5.29	5.97	5.63	–	–	–	1.53	–
<i>japoni- cus</i>																
<i>japoni- cus</i>																
<i>Zosterops</i> 5.97	5.52	5.74	6.19	4.73	5.97	4.84	5.52	5.29	5.29	5.86	5.52	4.96	–	–	–	–
<i>japoni- cus</i>																
<i>white- headi</i>																
<i>Zosterops</i> 6.08	5.41	5.63	6.08	5.18	6.19	5.29	5.18	5.18	5.29	5.63	5.29	4.84	–	–	–	–
<i>japoni- cus</i>																
<i>diuatae</i>																
<i>Zosterops</i> 5.74	5.29	5.52	5.74	4.73	5.74	4.84	5.07	4.84	4.96	5.86	5.52	4.62	–	–	–	–
<i>japoni- cus</i>																
<i>pecto- ralis</i>																

Table 2 (continued)

Zos-terops palpe-brosus palpe-brosus nigritens	Zos-terops palpe-brosus palpe-brosus nigritens	Zos-terops palpe-brosus palpe-brosus nigritens	Zos-terops palpe-brosus palpe-brosus nigritens	Zos-terops palpe-brosus palpe-brosus nigritens	Zos-terops palpe-brosus palpe-brosus nigritens	Zos-terops palpe-brosus palpe-brosus nigritens	Zos-terops palpe-brosus palpe-brosus nigritens	Zos-terops palpe-brosus palpe-brosus nigritens	Zos-terops palpe-brosus palpe-brosus nigritens	Zos-terops palpe-brosus palpe-brosus nigritens	Zos-terops palpe-brosus palpe-brosus nigritens	Zos-terops palpe-brosus palpe-brosus nigritens	Zos-terops palpe-brosus palpe-brosus nigritens	Zos-terops palpe-brosus palpe-brosus nigritens	Zos-terops palpe-brosus palpe-brosus nigritens	Zos-terops palpe-brosus palpe-brosus nigritens	Zos-terops palpe-brosus palpe-brosus nigritens	Zos-terops palpe-brosus palpe-brosus nigritens	Zos-terops palpe-brosus palpe-brosus nigritens
Zosterops japonicus montanus	5.86	5.63	5.63	5.63	5.63	5.63	5.63	5.63	5.63	5.63	5.63	5.63	5.63	5.63	5.63	5.63	5.63	5.63	5.63
Zosterops japonicus ssp.	5.97	5.74	5.74	5.74	5.74	5.74	5.74	5.74	5.74	5.74	5.74	5.74	5.74	5.74	5.74	5.74	5.74	5.74	5.74

Cells above the diagonal refer to cytb and cells below the diagonal refer to ND2. A dash represents cells in which the taxon was not represented for the gene in question

palpebrosus buxtoni were discovered to constitute a distinct lineage not closely related to other ‘‘Oriental white eye’’ taxa. The Lesser Sundaic race *Zosterops palpebrosus unicus*, on the other hand, emerged as closely related with and poorly diverged from members of *Zosterops citrinella* (Fig. 2; Table 2).

In the *Z. japonicus* species complex, we uncovered deep divergence between the continental Asian race *simplex* and the nominate subspecies group from the Japanese archipelago. The continental *Z. japonicus simplex* emerged as closely related to *Z. palpebrosus erwini* and *Z. salvadorii*, with high support and shallow divergence (Table 2; Fig. 2). On the other hand, the Japanese nominate subspecies emerged as embedded within the Mountain White-eye *Z. montanus* with high support (Fig. 2).

The results obtained from the BEAST dating analysis provided approximate divergence times within the species complexes (Fig. 3). Various members of the traditional *Z. palpebrosus* species complex emerged as polyphyletic and were generally ~1.2–2 million years removed from one another. Similarly, the two main taxon groups forming the traditional *Z. japonicus* species complex were relatively distantly related and ~1.4 million years apart from each other. We caution that these estimates can only be interpreted as rough approximations because they depend on a molecular clock rate calibrated by using island age estimates for divergences between Solomon island taxa of *Zosterops* (Moyle et al. 2009). This particular clock rate (4.94%/million years) is the only one thus far presented in the literature for *Zosterops* evolution, but is ~2.4 times slower than the widely used passerine mitochondrial clock rate of 2.1%/million years (e.g. Weir and Schluter 2008; Lovette 2004; Peterson 2006), implying that our actual divergence estimates may be twice as old as here presented. More importantly, our phylogenetic tree’s topology and nodal support demonstrate that these *Zosterops* wastebasket species are in fact composed of artificially merged lineages which are often not closely related to one another within the genus.

Discussion

Taxonomic revision

The resulting phylogenetic trees provide new insights into the diversification of white-eyes across Asia, suggesting the non-monophyly of multiple wide-ranging *Zosterops* wastebasket species. Unsurprisingly, low support values were obtained on some of the basal nodes of the phylogenetic trees, reflecting the explosive and sudden nature of *Zosterops* diversification (Moyle et al. 2009). This pattern mirrors the results of whole-genome studies of the evolutionary

history across avian families, which have similarly found low support or unresolved branches during periods of rapid diversification (Jarvis et al. 2014; Prum et al. 2015).

Traditional *Zosterops* taxonomy has doubtless been based on an over-reliance on conservative plumage features, without taking vocal or molecular evidence into account. Our molecular results redefine species limits for five traditionally circumscribed species, which are re-arranged into five newly circumscribed species: the ‘*palpebrosus*’, ‘*simplex*’, ‘*melanurus*’, ‘*citrinella*’ and ‘*japonicus*’ groups, with taxon ranges based on van Balen (2017b, c, d, e, f) and Wells (2017a, b) (Fig. 1b; Table 1).

The ‘*palpebrosus*’ group

The Oriental White-eye *Zosterops palpebrosus* was first described by Temminck in 1824 on the basis of a type specimen originating from Bengal, India. A further 11 taxa have been classified as its subspecies (van Balen 2017b) ranging from South Asia eastwards to Sundaland and the Lesser Sundas. However, our mitochondrial DNA (mtDNA) analysis revealed deep divergences and non-sister relationships among South Asian taxa and most races further east (Fig. 2; Table 2), corroborating and considerably extending earlier results (Moyle et al. 2009; Wickramasinghe et al. 2017). Among the Southeast Asian subspecies we sampled, only *siamensis* clustered closely with *palpebrosus*, whereas all

other Southeast Asian subspecies do not emerge as embedded within or even remotely sister to the *palpebrosus* cluster, and hence must be separated from it (Fig. 2; Fig. S1). Geographically, the newly circumscribed *Z. palpebrosus* ranges from Arabia through India eastwards to North Vietnam. A single GenBank sample (AMNH DOT10981) from North Vietnam that had been lodged as *Zosterops japonicus simplex* emerged within the *palpebrosus* cluster, doubtless constituting a misidentified individual of *Zosterops palpebrosus siamensis*. The sample did not align with the other 18 genuine *simplex* samples sequenced in this study and was collected in late May when wintering *simplex* should be absent from Vietnam whilst the breeding *siamensis* should be present.

For South Asian taxa, close relationships were confirmed among *Zosterops palpebrosus palpebrosus*, *Zosterops palpebrosus egregius* and *Zosterops palpebrosus nilgiriensis* (Fig. 2) (Warren et al. 2006; Wickramasinghe et al. 2017), corroborating their conspecificity. The sole remaining unsampled taxon *Zosterops palpebrosus salimalii*, which is embedded within the range of *Zosterops palpebrosus egregius*, is unlikely to be an exception and is provisionally retained under *Z. palpebrosus* because of morphological similarity and geographic proximity.

The taxonomic status of the subspecies *Zosterops palpebrosus nicobaricus* is more contentious, but we here provisionally retain it under *Zosterops palpebrosus* given an

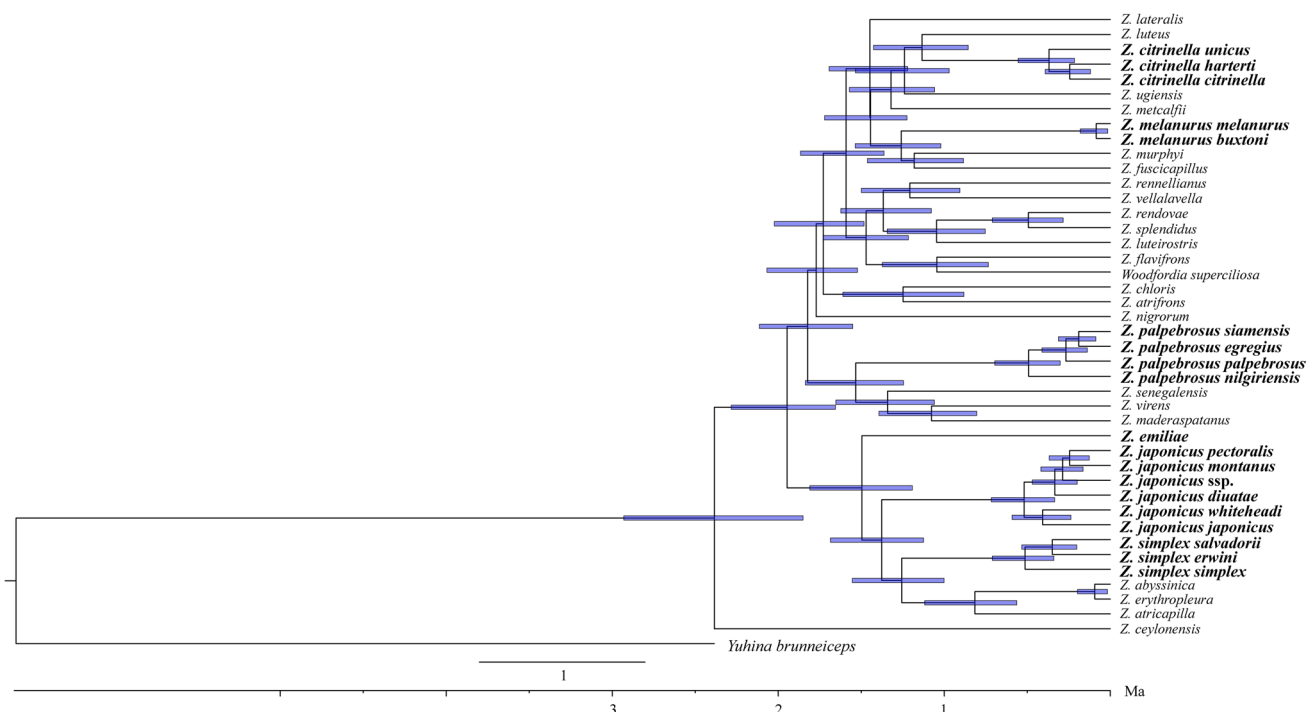


Fig. 3 The divergence times of *Zosterops* taxa with horizontal blue bars indicating 95% highest posterior density intervals. Taxa in bold are the focal species of this study. *Yuhina brunneiceps* was the outgroup species for this analysis

absence of DNA material and pending its incorporation into phylogenetic research. This taxon is restricted to the Nicobar Islands; nearby populations on the adjacent Andaman Islands are currently considered undescribed but may belong with *nicobaricus* (Rasmussen & Anderton 2012). Biogeographically, the Nicobar and Andaman Islands exhibit a greater similarity with Southeast Asia than with South Asia (Woodruff 2010), implying that *nicobaricus* may share close affinities with the adjacent taxon *erwini* from Sumatra and the Thai-Malay Peninsula rather than with the nominate India-centred *Z. palpebrosus* complex as here defined. Plumage descriptions of this poorly known taxon imply that *nicobaricus* should have a less yellow-bright and bronzier (= more olive) body coloration than Indian taxa (Rasmussen and Anderton 2012), which would equally suggest a closer affinity with *erwini* than with Indian *palpebrosus*. A merger of *nicobaricus* with *erwini* would have great nomenclatural consequences as *nicobaricus* would become the oldest name (proposed by Blyth in 1845) among all the taxa known to be closely related to *erwini* (including *erwini* itself). Our phylogeny demonstrates conclusively that *erwini*, along with a number of other East Asian taxa, is not even closely related to *Z. palpebrosus* across the genus (see below). Even so, it seems premature to base the species name for this East Asian species on a taxon that has not even been included in the study. Hence, we provisionally retain *nicobaricus* under *Zosterops palpebrosus* but note that it may soon become necessary to merge it with the East Asian taxa *erwini*, *simplex* etc. (see below), rendering the name of that newly constituted species *Zosterops nicobaricus*.

We provisionally include the following subspecies within the newly circumscribed *Z. palpebrosus*:

- *Zosterops palpebrosus*—Indian White-eye.
- *Zosterops palpebrosus palpebrosus* Temminck, 1824—southeast Arabia through northern India east to southwest Sichuan, Yunnan, and Myanmar.
- *Zosterops palpebrosus nilgiriensis* Ticehurst, 1927—southern Western Ghats in southwest India.
- *Zosterops palpebrosus salimali* Whistler, 1933—southern Eastern Ghats in southeast India.
- *Zosterops palpebrosus egregius* Madarász, 1911—lowlands of peninsular India and Sri Lanka.
- *Zosterops palpebrosus siamensis* Blyth, 1867—southern Myanmar to northwest Indochina and northern Vietnam.
- *Zosterops palpebrosus nicobaricus* Blyth, 1845—Andaman Islands and Nicobar Islands.

The 'simplex' group

The Sundaic taxon *erwini* [sensu Wells (2017a, b); formerly named *auriventer* sensu van Balen (2017b)], previously subsumed under the Oriental White-eye *Z. palpebrosus*,

emerged as deeply diverged from the *palpebrosus* cluster and in a polyphyletic placement on the tree (Fig. 2; Table 2). In fact, *Z. palpebrosus* as here redefined (see above), emerged as more closely related to a number of African species with high support (Figs. 2, 3), demonstrating the inappropriateness of retaining *erwini* as a member of *Z. palpebrosus*. Instead, *erwini* turned out to be closely related to *simplex*, a taxon predominantly from China previously classified erroneously under the Japanese White-eye *Z. japonicus*. While a deep division between *simplex* from the Chinese mainland and *japonicus* from the Japanese Archipelago has previously been proposed on a tentative basis (van Balen 2017c), the association between Chinese *simplex* and *erwini* from Southeast Asia is a novel insight. mtDNA sequence divergence between *erwini* and *simplex* is extremely low (Table 2). Given that they are also united by close phenotypic and vocal similarities (personal observations), the most conservative course of action appears to be a merger of these two forms into one species, the senior name of which would be *simplex* (described by Swinhoe in 1861).

Further to these two forms, we assume that *williamsoni* from the Gulf of Thailand also falls within this complex, based on geographical adjacency to and close morphological similarity with *erwini* (Wells 2017a, b). The taxon *salvadorii* from Enggano Island west of Sumatra is also revealed to have an extremely low mtDNA divergence from *erwini* and *simplex* (Table 2) and to be embedded within those two (Fig. 2). It has long been considered a weak monotypic species, Enggano White-eye, with a poorly known morphological distinctness (Eaton et al. 2016). Its unremarkable differentiation strongly suggests its incorporation with *Z. simplex*, shifting the burden of proof to those who would wish to continue to regard it at the species level.

We have commented above on the contentious status of *nicobaricus*, whose DNA has never been included in any phylogenetic research. To remain conservative, we have provisionally included this taxon with *Z. palpebrosus* (see above), despite contrary circumstantial evidence based on biogeography and morphology. It may well form a species-level lineage of its own or necessitate inclusion in the present *Z. simplex*. In the second case, the species name would need to change to *Z. nicobaricus* because of the priority of the latter name.

In summary, we propose the following taxonomic arrangement for *Z. simplex*:

- *Zosterops simplex*—Swinhoe's White-eye.
- *Zosterops simplex simplex* Swinhoe, 1861—eastern China, Taiwan and extreme northeast Vietnam; non-breeding in Thailand and Indochina.
- *Zosterops simplex hainanus* E. J. O. Hartert, 1923—Hainan.

- *Zosterops simplex erwini* Chasen, 1934—Thai-Malay Peninsula, lowland Sumatra, Riau Island, Bangka, Natuna Island and lowland Borneo.
- *Zosterops simplex williamsoni* Robinson & Kloss, 1919—Gulf of Thailand coast.
- *Zosterops simplex salvadorii* A. B. Meyer & Wiglesworth, 1894—Enggano Island.

The ‘*melanurus*’ group

Mitochondrial evidence revealed *melanurus* and *buxtoni*, from eastern and western Java, respectively, to form a tight-knit group that is deeply diverged from and distantly related to both *Z. palpebrosus* and *Z. simplex* (see above; Figs. 2, 3; Table 2). Instead, the Javan forms are embedded within a clade that comprises mostly Malenesian and eastern Indonesian species (Fig. 3). The vocal impression of Javan populations in the field is also different from that of adjacent *erwini* from Sumatra (personal observation). This Javan subspecies group displays an atypical range of diversity in phenotype, with two distinct belly colours—yellow (in *melanurus*) and grey (in *buxtoni*). These two phenotypes have long been recognised to meet in a hybrid zone in West Java, where flocks with pure and intermediate phenotypes are widespread around the city of Bogor and adjacent areas. mtDNA analysis shows extremely limited divergence between the grey and yellow-bellied forms on Java, suggesting conspecificity within one polytypic species, *Zosterops melanurus*. This newly constituted species, *Z. melanurus*, may indeed be endemic to Java and Bali. The West Javan subspecies *buxtoni* is reported to extend in range into montane Sumatra (van Balen 2017b), but photographic evidence of populations in montane Sumatra strongly suggests the presence of a population of the montane species Hume’s White-eye, *Zosterops auriventer* [sensu Wells (2017a, b); equals *Zosterops everetti tahanensis* sensu van Balen (2018b)], that has hitherto been misidentified (unpublished data), and we follow this preliminary evidence here in excluding montane Sumatran populations from the range of *buxtoni*. The separation of *Z. melanurus* (including *buxtoni*) as an independent species has important conservation implications as this taxonomic treatment renders it the most heavily trapped bird species on Earth (Chng et al. 2015; Chng and Eaton 2016; Lee et al. 2016; Eaton et al. 2017).

In summary, we propose the following taxonomic arrangement for *Zosterops melanurus*:

- *Zosterops melanurus*—Sangkar White-eye.
- *Zosterops melanurus melanurus* Hartlaub, 1865—eastern and central Java, Bali.
- *Zosterops melanurus buxtoni* Nicholson, 1879—West Java.

The ‘*citrinella*’ group

The Ashy-bellied White-eye *Zosterops citrinella*, native to the eastern Lesser Sunda Islands (East Nusa Tenggara), was revealed to exhibit an extremely shallow mtDNA divergence from *Zosterops palpebrosus unicus*, a former member of the ‘Oriental White-eye’ complex that was found to be unrelated to *Z. palpebrosus* already by Moyle et al. (2009) (Table 2). The traditional classification of *unicus* has clearly been erroneous. Based on our mtDNA data, we propose to unite *unicus* with *Z. citrinella*, of which it is an allopatric vicariant in the region. Its yellowish belly colour sets it apart from other more ashy-bellied members of *Z. citrinella*. However, this difference in belly colour amongst members of the same species is not unusual in *Zosterops* as seen in the Java/Bali endemic *Z. melanurus* (see above) and Indochinese populations of *Z. palpebrosus siamensis* (Robson 2005).

In summary, we propose the following taxonomic arrangement for *Zosterops citrinella*:

- *Zosterops citrinella*—Ashy-bellied White-eye.
- *Zosterops citrinella citrinella* Bonaparte, 1850—Sumba, Savu, Timor, Semau and Roti.
- *Zosterops citrinella harterti* Stresemann, 1912—Lembata and Alor.
- *Zosterops citrinella albiventris* Reichenbach, 1852—Gunungapi, Wetar, Romang, Damar, Teun, Kisar, Leti, Moa, Luang, Sermata, Babar, Tanimbar Islands; islands in Torres Strait and islets off extreme north-eastern Australia.
- *Zosterops citrinella unicus* E. J. O. Hartert, 1897—Sumbawa and Flores.

The ‘*japonicus*’ group

Our analyses corroborate prior preliminary suggestions (e.g. van Balen 2017c) of a division of the former Japanese White-eye *Z. japonicus* into an archipelagic group centred around the Japanese nominate *japonicus* and a Chinese mainland group centred around the subspecies *simplex*. Extending these preliminary suggestions, we demonstrated and discussed above that the new *Z. simplex* additionally includes Southeast Asian taxa that had erroneously been subsumed under *Z. palpebrosus* or had been treated as independent species. However, the status of the Japanese nominate group is also more complicated than merely a simple split. Our mtDNA analysis revealed members of the nominate subspecies group of *Z. japonicus* from the Japanese archipelago, South Korea and an introduced population in Hawaii to be embedded with samples of the Mountain White-eye *Z. montanus* (on the basis of ND2) or in a shallow sister relationship with it

(based on cyt**b**; Fig. 2). Price et al. (2014) reported on a deep split between *Z. japonicus* and *Z. montanus*, but a search regards their sample's locality showed it to be from eastern China, thereby confirming its identity as *Z. simplex*, leaving the actual relationship between *japonicus* and *montanus* uninvestigated until now. Merging *japonicus* and *montanus* results in a new composite species, *Zosterops japonicus*, according to nomenclatural priority. Beyond the results of our mtDNA analysis, this merger is also suggested by vocal impressions [distinct flight call (personal observation)] and their shared pale iris, which is unusual in most other *Zosterops* species in the region. The Mountain White-eye as traditionally circumscribed ('*Zosterops montanus*') is known to have great dispersal capabilities, with a fairly uniform morphology across an insular mountain distribution from Sumatra all the way to the Moluccas and Philippines. Therefore, an extension of this vast range northwards to include the Japanese archipelago is unsurprising. All the remaining unsampled subspecies refer to insular forms within the nominate cluster of *Z. japonicus* or to Philippine island subspecies of former *Z. montanus* which are unlikely to fall outside of their respective main clade, prompting us to retain them with their traditional taxonomic alliances.

In summary, we propose the following taxonomic arrangement for *Z. japonicus*:

- *Zosterops japonicus*—Mountain White-eye.
- *Zosterops japonicus japonicus* Temminck & Schlegel, 1845—Sakhalin, Japan and coastal Korean Peninsula.
- *Zosterops japonicus insularis* Ogawa, 1905—extreme northern Ryukyu Islands.
- *Zosterops japonicus lochooensis* Tristram, 1889—main Ryukyu Islands.
- *Zosterops japonicus daitoensis* Nagamichi Kuroda, 1923—Borodino Island.
- *Zosterops japonicus stejneri* Seebohm, 1891—Oshima (Izu Island) south to Torishima (Nanpo Archipelago).
- *Zosterops japonicus alani* E. J. O. Hartert, 1905—Iwo (Volcano) Island.
- *Zosterops japonicus montanus* Bonaparte, 1850—mountains in Sumatra, Java, Bali, Lesser Sundas, Sulawesi and southern Moluccas.
- *Zosterops japonicus whiteheadi* E. J. O. Hartert, 1903—highlands of Luzon.
- *Zosterops japonicus halconensis* Mearns, 1907—Mindoro.
- *Zosterops japonicus parkesi* duPont, 1971—mountains of Palawan.
- *Zosterops japonicus pectoralis* Mayr, 1945—Negros.
- *Zosterops japonicus diuatae* Salomonsen, 1953—northern Mindanao.
- *Zosterops japonicus volcani* E. J. O. Hartert, 1903—central Mindanao.
- *Zosterops japonicus difficilis* Robinson & Kloss, 1918—Mount Dempo in south Sumatra.
- *Zosterops japonicus obstinatus* E. J. O. Hartert, 1900—Ternate, Tidore, Bacan, Seram.

Generic reassignment of *Chlorocharis emiliae*

The Bornean Mountain Black-eye *Zosterops emiliae* was first described in 1888 by Sharpe and was initially given monotypic genus status under *Chlorocharis*. Our results confirmed previous findings (Moyle et al. 2009) showing this Bornean endemic to be embedded within *Zosterops*, and basal to our newly circumscribed *Z. japonicus* and *Z. simplex* species (Fig. 2), with which it is allopatric.

Conservation implications

Our taxonomic rearrangements have led to the elevation of threatened lineages to species level, foremost *Zosterops melanurus*. In Java, the white-eye trade has already led to the endangerment of previously recognised endemic species such as the Javan white-eye *Zosterops flavus* (Eaton et al. 2015), which is suffering a decreasing population trend with a current International Union for Conservation of Nature (IUCN) 2016 status of 'Vulnerable' (BirdLife International 2018). With its elevation to species level, *Z. melanurus* urgently requires IUCN classification for conservation priority. *Z. melanurus* must be the most heavily traded bird species on Earth with records of 2393 individuals in single market surveys around West Java (Chng et al. 2015; Eaton et al. 2015; Chng and Eaton 2016). As the common white-eye on the island, this species seems to fuel Java's deeply entrenched tradition of bird keeping (Eaton et al. 2015). Given the documented exploitation levels (Chng et al. 2015; Chng and Eaton 2016), we believe a ~30% reduction in global population size over the past 10 years is a reasonable—if not conservative—assumption, and hence propose Vulnerable A2d status under the 2001 IUCN Red List criteria (version 3.1; available at www.iucnredlist.org) for *Z. melanurus*.

On the small island of Singapore, both the native *Z. simplex erwini* and escaped individuals of the locally traded *Z. simplex simplex* (Jeyarajasingam 2012; Eaton et al. 2017) are found in the wild, as confirmed by our analyses (Fig. 2). Under previous taxonomic treatments, *erwini* and *simplex* would have been considered members of different species (Oriental White-eye *Z. palpebrosus* and Japanese White-eye *Z. japonicus*, respectively). However, our genetic data show that these two taxa are closely related to each other, and the possibility of secondary gene flow in the wild is very likely (Fig. 2; Table 2). Future studies using nuclear DNA sources

will be necessary to assess the level of gene flow between native and introduced white-eyes on Singapore.

Conclusion

Using mitochondrial data, we have uncovered the non-monophyly of several wide-ranging white-eye species complexes across Australasia—*Z. palpebrosus*, *Z. japonicus* and *Z. montanus*. Our molecular results redefine species limits for five traditionally circumscribed species, which were previously based predominantly on conservative plumage features. The new taxonomic classification may help direct conservation attention to relevant groups, particularly *Z. melanurus* from Java and Bali, which represent the most heavily traded bird species on earth and may be at risk of trade-driven extinction.

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