

# Disentangling avian diversity: South-East Asian mainland Oriental White-eye *Zosterops palpebrosus* constitutes two distinct lineages

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We examined the mainland South-East Asian taxa formerly grouped as part of the Oriental White-eye *Zosterops palpebrosus* complex. We amplified three mitochondrial loci, cytochrome *b*, NADH dehydrogenase 2 and NADH dehydrogenase 3, and one nuclear locus, the fifth intron of the nuclear gene transcription growth factor, through PCR. Evidence from both DNA and vocalisations indicated that individuals of inland forest populations *Z. p. siamensis* clustered with nominate *Z. p. palpebrosus*. On the other hand, the mangrove-dwelling taxa, *Z. [p.] williamsoni* and *Z. [p.] cf. erwini*, were most closely related to the migratory Japanese White-eye *Z. japonicus simplex* (100% posterior probability support). While *williamsoni* and *cf. erwini* apparently constituted distinct lineages, there was only 50% posterior probability support for this arrangement. Because *Z. j. simplex* is apparently also distinct from other *Z. japonicus* lineages, a more detailed examination of mainland and island-inhabiting *Zosterops* taxa in East and South-East Asia is required.

## INTRODUCTION

The widespread and relatively homogeneous bird family Zosteropidae consists of 135 species presently grouped in 12 genera, within the sylvioid assemblage colloquially known as babblers. Within the Zosteropidae, *Zosterops* is by far the most prolific genus, with 96 species currently recognized (del Hoyo & Collar 2016). The Zosteropidae have been described as a ‘great speciator’ or ‘explosive speciator’, with the maximum uncorrected pairwise divergence for the NADH dehydrogenase 2 (ND2) gene being only 7.1% among the members of the clade containing *Zosterops* (Moyle *et al.* 2009). This represents a combination of low molecular diversity yet high species diversity seldom found elsewhere among avian species. Radiation among *Zosterops* is relatively recent and took place mainly in the Pleistocene, with almost half of all known species occurring on islands in the West Pacific.

The Oriental White-eye *Zosterops palpebrosus*, one of the 11 currently recognised Sino-Oriental *Zosterops* species, comprises 11 subspecies distributed from Afghanistan, South Asia and the Bay of Bengal eastwards through the Himalaya and South China, throughout South-East Asia and the Greater Sundas to the Lesser Sundas (Mees 1957, del Hoyo & Collar 2016). Most of these subspecies inhabit inland forest and hilly country, sometimes alongside other *Zosterops* species; for example, *Z. p. egregius* occurs in montane and submontane forest in Sri Lanka alongside the endemic Ceylon White-eye *Z. ceylonensis* (Ali & Ripley 1983, Rasmussen & Anderton 2005, Wickramasinghe *et al.* 2017). Some subspecies of the Oriental White-eye in parts of South-East Asia and the Sunda region show broad geographic overlap with inland forest taxa assigned to Everett’s White-eye *Z. everetti* (van Balen 2017), although evidence of actual sympatry with that species is lacking. The migratory Japanese White-eye *Z. japonicus simplex* and Chestnut-flanked White-eye *Z. erythropleurus* occur in the mainland South-East Asian habitat occupied by *Z. palpebrosus* during the Palearctic winter (Lekagul & Round 1991).

A molecular review by Moyle *et al.* (2009) recovered *Z. p. palpebrosus* from Nepal and *Z. p. unicus* from Flores in deeply divergent clades, suggesting that *Z. palpebrosus* as presently understood is polyphyletic. Indeed, the Indonesian races of Oriental White-eye—*melanurus*, *unicus*, *buxtoni* and *erwini*—have also been tentatively split from mainland Oriental White-eye taxa as Sunda White-eye *Z. melanurus* by Eaton *et al.* (2016).

In mainland South-East Asia there are currently four recognised taxa in the *Z. palpebrosus* complex: *Z. p. palpebrosus* apparently

extends from the Himalaya to north, east and central Myanmar; further east, *Z. p. siamensis* inhabits inland forest in south and east Myanmar, Thailand and South-East Asia; *Z. p. williamsoni* occurs in coastal areas of the Gulf of Thailand, including the east coast of the Thai-Malay Peninsula; and *Z. p. auriverter* occurs on the Andaman Coast of the Peninsula to the west. Both *Z. p. williamsoni* and *Z. p. auriverter* primarily inhabit mangroves, the latter taxon extending to the east coast of Sumatra and the north-west coast of Borneo (Mees 1957, del Hoyo & Collar 2016). The pattern of distribution of these taxa is unusual in that the ranges of the mangrove and inland forest taxa in mainland South-East Asia presently united as *Z. palpebrosus* are widely disjunct, segregated by habitat, and do not come into contact with each other.

Wells (2017) has shown that the type series of *Z. (p.) auriverter* is a taxonomic composite. The holotype of *Z. auriverter* was recognisable on plumage and biometrics as a representative of the mainly Sunda Region inland forest taxon presently known as *Z. everetti*, while the paratypes had features typical of the population of mangrove-dwelling birds, to which Wells (2017) applied the name *Z. p. erwini*. We henceforth refer to these birds, distributed along the Andaman Coast of the Thai-Malay Peninsula, as *Z. p. cf. erwini*.

We investigated the taxonomic relations between the mangrove-dwelling birds and inland forest birds found in Thailand. Our hypothesis was that the mangrove-inhabiting taxa (*williamsoni* and *cf. erwini*) would probably be a species distinct from the inland forest taxon *siamensis*. A subsidiary objective was to investigate the apparent discontinuity between the east and west coast populations which is, as far as we know, unique among Thai birds.

## METHODS

We sampled 57 individual *Z. palpebrosus* using mist-nets at 10 sites in Thailand and one in Nepal. Sound recordings of single individuals were made at two further sites in Thailand where no birds were captured (Table 1). Of these 13 sites, seven were inland localities and six were coastal mangrove sites—three in the Upper Gulf, one on the east coast and two on the west coast of the Peninsula (Figure 1). Sampling was carried out over a protracted period between February 2009 and September 2016. Birds were caught when coming to water, while feeding, or in response to playback of vocalisations. All birds caught were individually ringed, measured, weighed and examined for wear and moult; images were taken in each case. One or two tail-feathers were collected from each individual and in some cases

**Table 1.** Sites where *Zosterops* were caught, ringed and sampled or sound-recordings were made. (All sites in Thailand except the first.)

Koshi, Nepal	27.05°N 87.30°E
Nam Kham Nature Reserve, Chiang Rai province	20.28°N 100.07°E
Ban Saen Jai, Chiang Rai province	20.20°N 99.77°E
Doi Phu Kha, Nan province	19.20°N 101.08°E
Doi Chiang Dao Wildlife Sanctuary, Chiang Mai province	19.35°N 98.92°E
Phu Luang Wildlife Sanctuary, Loei province	17.35°N 101.52°E
Doi Musoe, Tak province	16.78°N 98.92°E
Ban Nam Chiao, Trat province	12.17°N 102.57°E
Bang Khun Thien, Bangkok	13.57°N 100.45°E
Bang Ya Phraek, Samut Sakhon province	13.50°N 100.27°E
Pak Phanang, Nakhon Si Thammarat province	8.52°N 100.15°E
Ban Tha Maphrao, Krabi province	7.72°N 99.18°E
Ban Thung Rin, Satun province	6.73°N 99.92°E

blood samples were taken from the intertarsal vein before release using Number 27 needles, usually by SM (a qualified veterinarian). A single drop of blood, which was preserved in Longmire's lysis buffer or on filter paper, was adequate for DNA analysis.

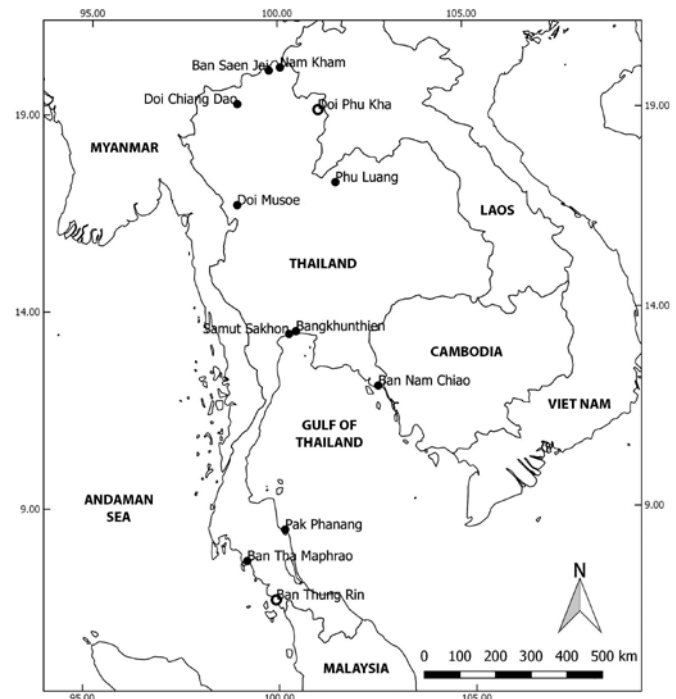
Songs or calls were recorded when heard, using a Sony Linear PCM D50 recorder in combination with a Sennheiser ME 66 microphone. Spectrograms and measurements of bioacoustic parameters were obtained using Adobe Audition 3.0 software.

### Molecular analyses

We sampled DNA from 27 live individuals from 10 sites in Thailand and one in Nepal (Appendix 1). In addition to our targeted taxa within the *Z. palpebrosus* (*sensu lato*) complex, we sampled two Japanese White-eye *Z. j. simplex* from Phu Luang Wildlife Sanctuary and Nam Kham Nature Reserve, both in Thailand (Appendix 1). Representative examples of each taxon are shown in Plates 1–8. Our analysis covered three mitochondrial genes, cytochrome *b* (*cytb*), NADH dehydrogenase 2 (ND2) and NADH dehydrogenase 3 (ND3), and one nuclear locus, the fifth intron of the nuclear gene transcription growth factor (TGF).

Published sequences of *Z. palpebrosus* and *Z. japonicus* from GenBank were also included in the analyses (Appendix 1). Because there were no published sequences in GenBank for any subspecies of continental Asian *Zosterops* which covered all three mitochondrial loci that we used, we also constructed gene trees for each of these three genes separately. DNA was extracted using QIAamp DNA Mini Kit (Qiagen, Inc.) according to the manufacturer's protocol, with an additional 20 µl of 1 M Dithiothreitol added during the proteinase K incubation step. All loci were amplified through standard PCR (polymerase chain reaction) using taxon-specific primers (Appendix 2). The PCR products were purified using GeneJET PCR Purification Kit (Fermentas). Sequencing was carried out using an ABI 3730xl automatic sequencer following the ABI PRISM BigDye Terminator Cycle Sequencing protocol. Both strands were sequenced using the same primers as for PCR. Sequences were aligned and checked manually in MEGA 6.06 (Tamura *et al.* 2013). Bayesian gene trees for each mitochondrial locus, and a partitioned Bayesian gene tree using all three mitochondrial loci and one nuclear locus, were reconstructed using Bayesian inference with reversible jump Metropolis-coupled Markov chain Monte Carlo (MCMC) (Huelsenbeck & Rannala 2004), implemented in MrBayes version 3.2.3 (Huelsenbeck & Ronquist 2001, Ronquist & Huelsenbeck 2003, Ronquist *et al.* 2012). The best-fit substitution models for each locus were estimated using the Akaike information criterion implemented in Kakusan software (Tanabe 2007). The selected models were HKY + G for *cytb* and ND2, HKY + I for ND3 and K80 + G for TGF. Four Metropolis-coupled MCMC chains were run for one million iterations with tree topology sampled every 500 iterations. The MCMC output was analysed in Tracer version 1.5 (Rambaut & Drummond 2009) to assess the adequacy (>200) of posterior samples taken from the MCMC analysis. The first 25% of the generations were discarded

**Figure 1.** Map showing locations of study sites in Thailand.



(‘burn-in’) and the posterior probability (hereafter referred to as PP) was estimated for the remaining sampled generations.

Genetic divergence within and between major lineages from the inferred gene trees was estimated using the uncorrected *p*-distance model implemented in MEGA 6.06 (Tamura *et al.* 2013). Sequences of all birds sampled were deposited in GenBank.

### Morphology

Wings of live-caught birds were measured to the nearest 1 mm with a stopped wing-rule; tails were measured on the underside, following the method in Svensson (1992). Other measurements were made with a dial caliper to the nearest 0.1 mm. Univariate and multivariate statistical analyses were carried out in Minitab v.16. For univariate statistics, the level of significance was set at  $p \leq 0.05$ . Because males and females were not separated and the sample size was small, we opted for a robust, non-parametric Mann-Whitney U-Test when making pairwise comparisons between taxa.

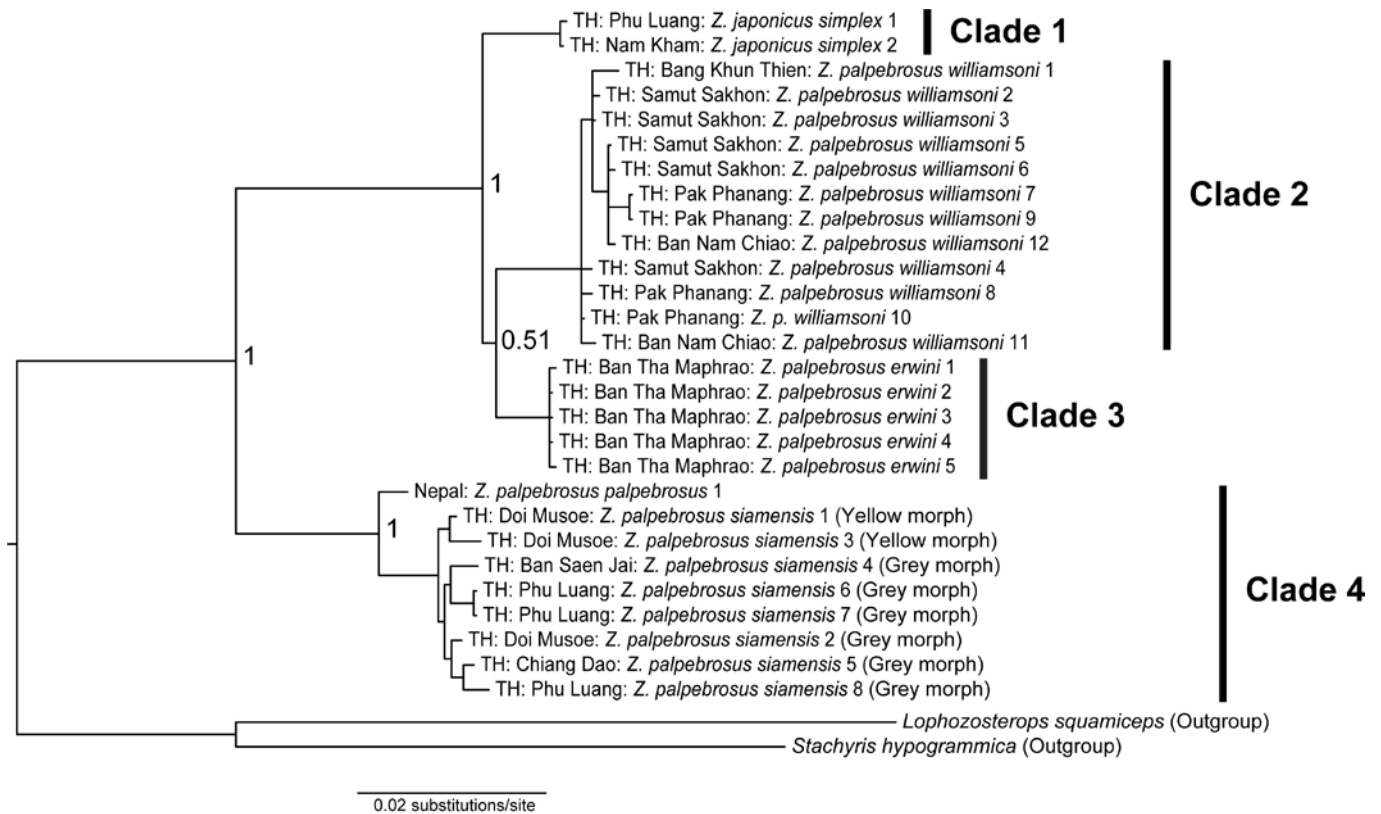
## RESULTS

### Phylogenetic analysis

The Bayesian multi-gene tree (Figure 2) showed two main lineages receiving 100% PP support: (i) the inland forest taxa *Z. p. siamensis* and *Z. p. palpebrosus* (Clade 4) and (ii) the mangrove-dwelling taxa from both coasts of the Peninsula and the Upper Gulf (Clades 2 and 3). We also found that *Z. j. simplex*, represented by two individuals (Clade 1), was sister to the mangrove lineage, receiving 100% PP support. Within the mangrove lineages the Upper Gulf and east coast birds clustered together, without any distinct internal structure within the group (Clade 2). They were apparently distinct from the west coast (Andaman Coast) birds which formed a second discrete cluster (Clade 3). However, there was only 50% PP support for this arrangement.

Uncorrected *p* genetic distance values among lineages (Table 2) indicated that *Z. palpebrosus*, comprising the inland forest taxa nominate *palpebrosus* (Nepal) and *siamensis* (Thailand)—Clade 4 in the tree (Figure 2)—was the most distinct lineage, showing 4.46–7.83% divergence from the other taxa for the three mitochondrial

**Figure 2.** Bayesian gene tree based on combined mitochondrial (Cytb, ND2 and ND3) and one nuclear locus (fifth intron TGF) estimated using MrBayes. Numbers at nodes indicate posterior probability.



loci. *Z. p. palpebrosus* and *Z. p. siamensis* were only 0.73–1.99% different from each other. The genetic distance values among the other three taxa (mangrove-dwelling *williamsoni* and cf. *erwini*, and migratory *Z. j. simplex*) were relatively low at 0.46–2.43%, indicating their close affinity. While *Z. j. simplex* appeared as a sister clade in the tree to the mangrove-dwelling taxa, the pairwise genetic distance between *simplex* and cf. *erwini* for cytochrome *b* was only 0.46% whereas that between *erwini* and *williamsoni* was 2.19%, and between *simplex* and *williamsoni* 2.39%. For ND2, the distance between *simplex* and cf. *erwini* was 1.97%, comparable with 2.18% between cf. *erwini* and *williamsoni*, whereas *simplex* and *williamsoni* differed by 2.43%. For ND3, pairwise distance from

*simplex* was 1.47% for *williamsoni* and 2.28% for *erwini*, and that between *williamsoni* and *erwini* was also 1.47% (Table 2). Whilst *simplex*, *williamsoni* and cf. *erwini* were each other's closest relatives, the precise pattern of branching among them was not fully resolved.

Single gene trees incorporating archival material for *cytb*, ND2 and ND3 (Figures 3–5) supported the findings from the concatenated tree based on live-caught birds. In all three trees *Z. p. siamensis* was recovered with other *Z. palpebrosus* races from India, Sri Lanka and Nepal. Our mangrove-caught birds, *williamsoni* and cf. *erwini*, clustered with *Z. j. simplex* from mainland China and Taiwan, although the single gene trees yielded no further insights into the precise branching pattern among these taxa. The topology of the cytochrome *b* tree (Figure 3), which incorporated nominate *Z. j. japonicus* and other island populations, *Z. j. insularis* and *Z. j. batanis* (the latter placed with *Z. japonicus* by Mees [1957] and with Lowland White-eye *Z. meyeri* by Parkes [1971]), indicated that these lay in a different and unrelated lineage from *Z. j. simplex*. A single *Z. japonicus* from mainland Japan (GenBank accession no. AY136599), which figured in the ND2 tree, was likewise recovered separately from *Z. j. simplex*. The appearance of a single supposed *Z. japonicus* from Vietnam (specimen DOT10981) in the *palpebrosus* clade in both the ND2 and ND3 trees may be accounted for by the specimen in question being a misidentified example of *Z. palpebrosus*.

**Table 2.** Mitochondrial pairwise genetic distances between major clades at 3 loci.

Values along the diagonal are the genetic distance within the clade. (n/c = not calculated since nominate *palpebrosus* was represented by only 1 individual)

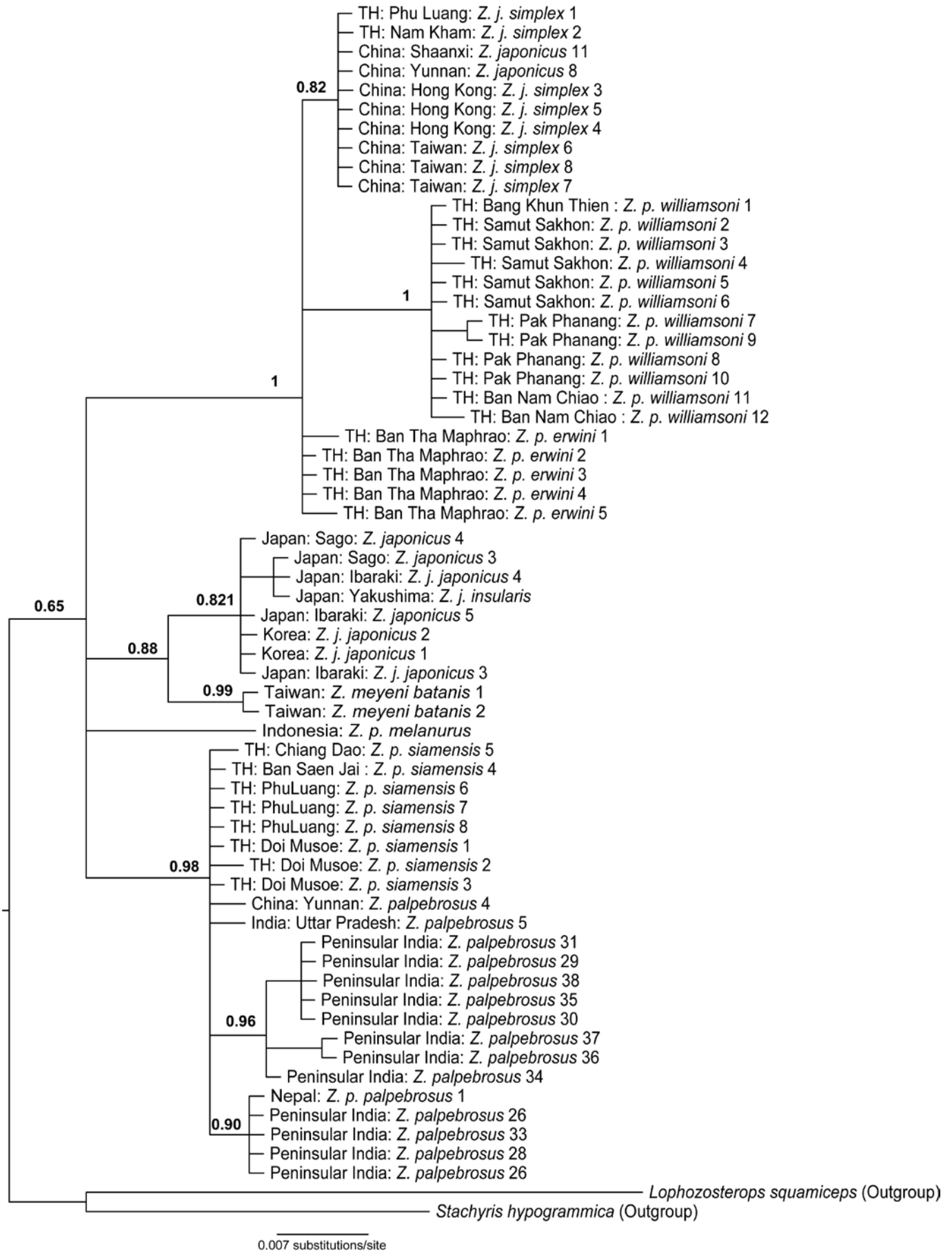
	<i>simplex</i>	<i>williamsoni</i>	<i>erwini</i>	<i>siamensis</i>	<i>palpebrosus</i>
<b>Cytb</b>					
<i>simplex</i>	<b>0.0000</b>	0.0239	0.0046	0.0529	0.0521
<i>williamsoni</i>		<b>0.0021</b>	0.0219	0.0567	0.0559
<i>erwini</i>			<b>0.0026</b>	0.0508	0.0502
<i>siamensis</i>				<b>0.0016</b>	0.0073
<i>palpebrosus</i>					n/c
<b>ND2</b>					
<i>simplex</i>	<b>0.0010</b>	0.0243	0.0197	0.0719	0.0648
<i>williamsoni</i>		<b>0.0017</b>	0.0218	0.0783	0.0715
<i>erwini</i>			<b>0.0000</b>	0.0695	0.0624
<i>siamensis</i>				<b>0.0036</b>	0.0139
<i>palpebrosus</i>					n/c
<b>ND3</b>					
<i>simplex</i>	<b>0.0000</b>	0.0147	0.0228	0.0452	0.0456
<i>williamsoni</i>		<b>0.0009</b>	0.0147	0.0446	0.0461
<i>erwini</i>			<b>0.0000</b>	0.0527	0.0541
<i>siamensis</i>				<b>0.0045</b>	0.0199
<i>palpebrosus</i>					n/c

**Morphometric analysis**

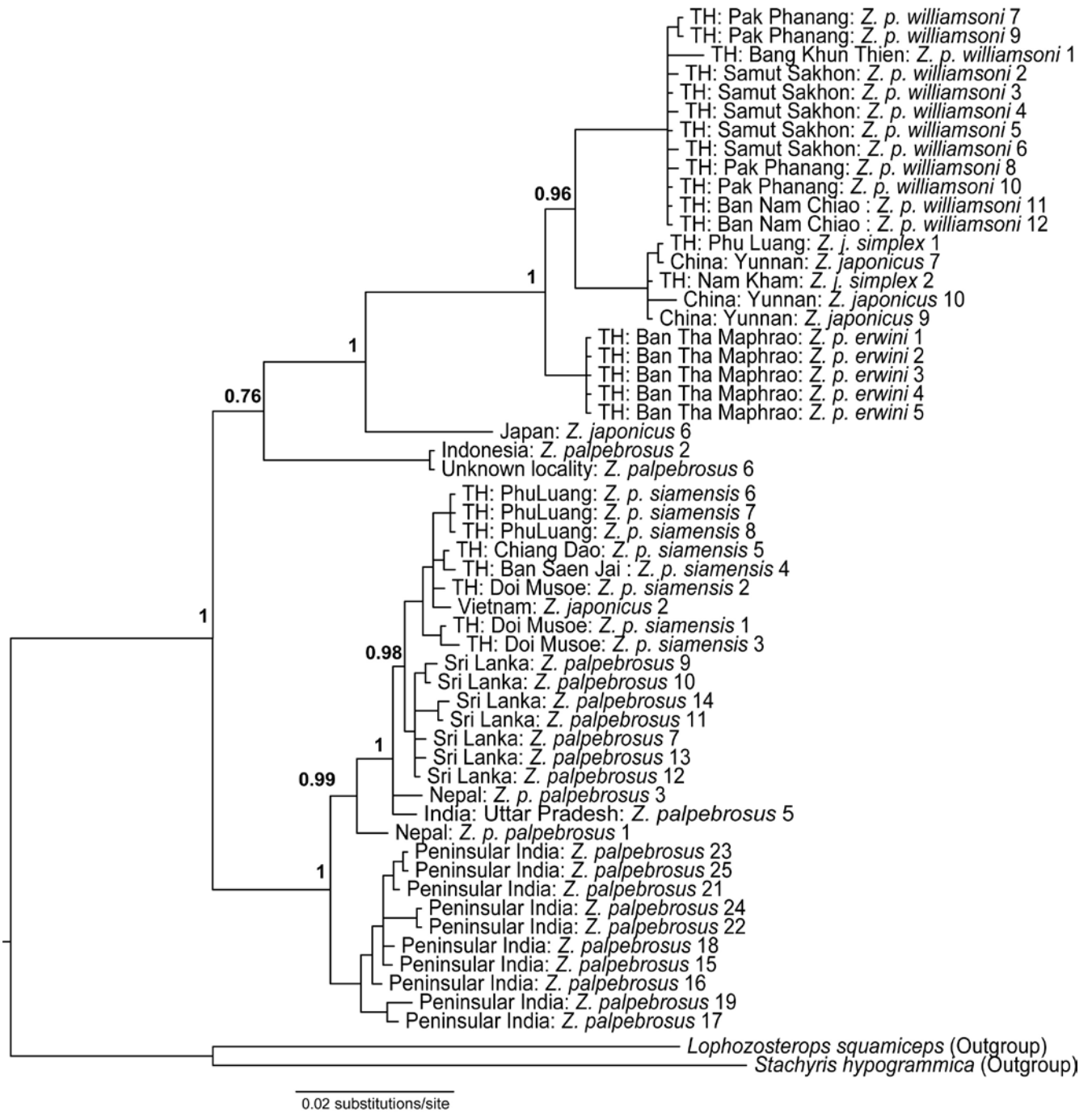
We present biometric data from all the birds caught and measured in Thailand. More individuals from each population were measured than were subjected to molecular sampling (Table 3) and the number of individuals measured for each biometric character varied because some birds sampled during July–December were in moult so that some wing formula parameters could not be measured.

*Z. j. simplex* was the most morphologically distinct bird, being significantly larger, having a longer wing, longer tail and longer primary projection (extension of the wing point beyond the longest tertial) than any other taxon (Figure 6, Table 3).

**Figure 3.** Bayesian gene tree based on mitochondrial cytochrome *b* gene estimated using MrBayes. Numbers at nodes indicate posterior probability.



**Figure 4.** Bayesian gene tree based on mitochondrial ND2 gene estimated using MrBayes. Numbers at nodes indicate posterior probability.



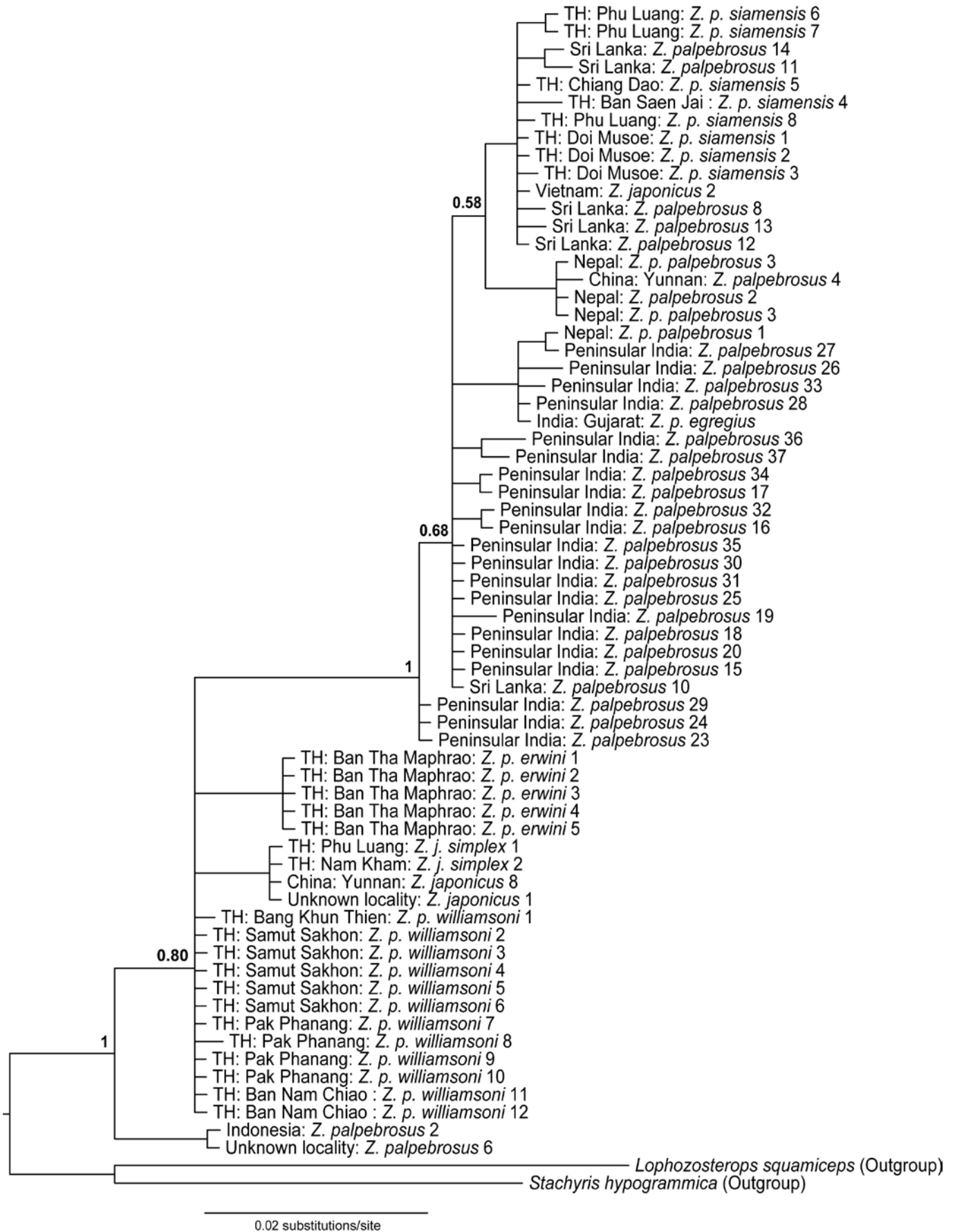
**Table 3.** Comparison of measurements (mm ± 1 SD), taken from live birds, of four *Zosterops* taxa.

Taxon	Wing	Tail	Tail:wing ratio	Bill	Tarsus	Primary projection	Primary projection:wing ratio
<i>Z. japonicus simplex</i> * (Chiang Rai)	57.2 ± 1.39 (55.0–59.0) n = 8	39.6 ± 1.33 (37.0–41.0) n = 8	0.691 ± 0.10 (0.673–0.707)	13.8 ± 1.47 (11.2–15.8) n = 8	16.0 ± 1.10 (14.5–17.2) n = 5	12.0 ± 1.04 (10.0–13.4) n = 8	0.208 ± 0.021 (0.172–0.244) n = 8
<i>Z. palpebrosus williamsoni</i> (Samut Sakhon and Trat combined)	54.0 ± 1.20 (52.0–56.0) n = 15	36.9 ± 1.16 (34.0–39.0) n = 15	0.684 ± 0.016	14.8 ± 0.77 (13.2–16.3) n = 14	15.4 ± 0.51 (14.6–16.2) n = 14	9.7 ± 1.36 (8.1–12.9) n = 12	0.180 ± 0.02 (0.149–0.230) n = 12
<i>Z. palpebrosus williamsoni</i> ** (Nakhon Si Thammarat)	51.3 ± 0.96 (50.0–52.0) n = 4	34.0 ± 1.41 (33.0–35.0) n = 4	0.668 ± 0.014	14.3 ± 0.13 (14.1–14.4) n = 4	15.2 ± 0.45 (14.6–15.7) n = 4	7.1, 7.6, 8.7 n = 3	0.137, 0.146, 0.170 n = 3
<i>Z. palpebrosus cf. erwini</i> ** (Andaman)	52.6 ± 0.95 (51.0–54.0) n = 11	33.6 ± 0.92 (32.0–35.0) n = 11	0.639 ± 0.012	14.2 ± 0.55 (13.5–15.1) n = 11	14.4 ± 0.49 (13.6–14.9) n = 11	9.9 ± 1.18 (8.0–11.4) n = 8	0.189 ± 0.024 (0.148–0.219) n = 8
<i>Z. palpebrosus siamensis</i> (Tak and Chiang Rai)	53.9 ± 1.25 (52.0–57.0) n = 19	37.3 ± 1.14 (36.0–40.0) n = 16	0.691 ± 0.02	13.5 ± 0.83 (12.1–14.8) n = 9	15.3 ± 0.25 (14.9–15.5) n = 7	10.1 ± 1.22 (9.1–11.7) n = 7	0.189 ± 0.024 (0.146–0.225) n = 7

\* Significantly longer wing, tail and primary projection than any other taxon.

\*\* Significantly smaller in wing and tail dimensions than *Z. [p.] williamsoni* from Upper Gulf.

**Figure 5.** Bayesian gene tree based on mitochondrial ND3 gene estimated using MrBayes. Numbers at nodes indicate posterior probability.



Both peninsular mangrove-dwelling taxa, *williamsoni* from the east coast and cf. *erwini* of the Andaman Coast, were significantly smaller in wing and tail dimensions than *williamsoni* from the Upper Gulf (Table 3).

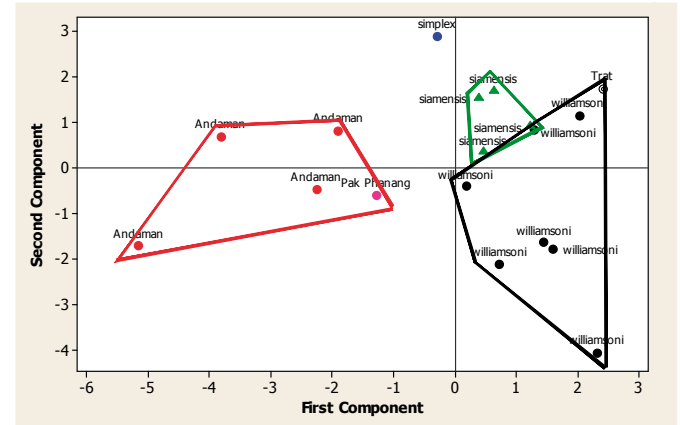
*Z. p. siamensis* had significantly larger wing and tail measurements than peninsular mangrove-dwelling *williamsoni*; but only the tail length was significantly different when *siamensis* was compared with cf. *erwini* (Table 3).

The only east coast bird for which a full set of biometric data was available appeared close to those of the Andaman Coast in a Principal Component Analysis (Figure 6). The first three factors explained only 55.2% of observed variation. In Factor 1, tail and tarsus length, and shortfall of some inner primaries (p5, p7, p8 and p10, numbered ascendantly) were of roughly equal importance. In Factor 2, bill depth, tarsus, wing length, the shortfall of p8 and p9 and primary projection contributed (Table 4).

*Z. p. siamensis* and *Z. p. williamsoni* of the Upper Gulf were morphologically less distinct from each other than either was from the peninsular birds, with limited overlap revealed by PCA (Figure 6).

**Figure 6.** Principal Component Analysis plot of scores for six different *Zosterops* taxa or populations.

- Z. japonicus simplex* = simplex
- Z. p. williamsoni* from Trat province, Upper Gulf = Trat
- Z. p. williamsoni* from Samut Sakhon province, Upper Gulf = *williamsoni*
- Z. p. williamsoni* from Nakhon Si Thammarat province (East Coast) = Pak Phanang
- Z. p. cf. erwini* from Krabi Province (West Coast) = Andaman
- Z. p. siamensis* from inland forest localities = *siamensis*



**Table 4.** Summary statistics for Principal Component Analysis of *Zosterops* taxa. The most important variables for the two factors shown in bold type.

Variable	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8
wing	0.194	<b>0.273</b>	-0.16	0.008	0.497	-0.353	0.006	0.167
tail	<b>0.361</b>	0.178	-0.015	0.024	0.299	-0.353	0.06	-0.035
bill	0.086	-0.099	-0.389	0.25	0.123	0.354	-0.054	0.474
biwi_prox	0.138	0.173	-0.254	0.266	-0.304	-0.016	0.227	-0.569
biwi_dist	0.189	0.143	-0.179	0.1	-0.606	-0.034	0.041	0.292
bidep	0.19	<b>0.369</b>	-0.002	-0.135	-0.082	-0.008	-0.474	-0.098
tarsus	<b>0.314</b>	<b>0.322</b>	-0.197	-0.026	-0.039	0.056	0.027	-0.058
hindclaw	0.146	0.064	0.156	-0.045	0.348	0.672	0.056	-0.235
p2	-0.192	-0.185	-0.43	0.142	0.164	-0.115	-0.239	-0.29
p3	-0.19	-0.009	-0.123	0.609	0.103	0.001	-0.231	-0.196
p4	0.112	0.211	-0.216	-0.263	-0.066	0.322	-0.386	-0.037
p5	<b>0.305</b>	-0.028	-0.133	0.143	0.069	0.137	0.559	0.041
p6	0.27	-0.238	-0.008	0.281	-0.015	-0.022	-0.288	0.279
p7	<b>0.375</b>	-0.247	0.169	0.097	-0.021	0.079	-0.078	-0.152
p8	<b>0.302</b>	<b>-0.327</b>	0.152	0.131	-0.009	-0.011	-0.131	-0.077
p9	-0.104	<b>0.319</b>	0.381	0.358	-0.051	0.075	-0.003	0.128
p10	<b>0.321</b>	-0.166	0.338	0.035	-0.086	-0.139	-0.179	-0.12
P projection	-0.129	<b>0.394</b>	0.296	0.344	0.053	0.056	-0.022	0.079
Eigenvalue	4.466	3.018	2.4459	1.8039	1.4476	1.1262	1.1088	0.9092
Proportion	0.248	0.168	0.136	0.1	0.08	0.063	0.062	0.051
Cumulative	0.248	0.416	0.552	0.652	0.732	0.795	0.856	0.907

**Plumage features (Plates 1–8)**

Although the population of *Z. p. siamensis* in western Thailand is dimorphic—yellow morphs with entirely yellowish underparts predominating at Doi Musoe (16.78°N 98.92°E) in five of six individuals trapped—comments for the purposes of comparison with other taxa are limited to the more widespread and typical ‘grey morphs’, showing mainly greyish-white underparts with no more than a narrow, broken or indistinct stripe of yellow suffusion along the midline of the breast and belly (Plates 2–4).

These were relatively easily distinguishable from mangrove-dwelling birds owing to their more yellowish-tinged green upperparts. In particular, a diffuse yellowish wash on the head was not merely confined to a supraloral streak but extended over the forehead. The mantle, lower back, rump and upper-tail coverts were a brighter, more yellowish-green than in *williamsoni* and other mangrove populations. The fringes of the wing-coverts and flight feathers of the wing and tail were similarly brighter and more yellow-green. The most striking feature was that the white spectacle of *siamensis* was much narrower than in *williamsoni* and other mangrove-dwelling birds. Features of the head are often obscured in study skins, which may be why this has not been remarked on previously in the literature.

**Table 5.** Characters of the long and complex songs of *Z. palpebrosus* inland forest taxa, *Z. p. palpebrosus* and *Z. p. siamensis*. Song of a single individual of each taxon analysed, except Tak province (two individuals).

	Maximum frequency (Hz)	Minimum frequency (Hz)	Verse duration (sec)	Number of strophes/verse	Strophe rate	Interval between verses (sec)
Koshi, Nepal ( <i>Z. p. palpebrosus</i> ) n = 9	4395 ± 170.1 (4158–4744)	3118 ± 66.4 (3037–3230)	3.5 ± 1.69 (1.71–7.25)	10.8 ± 3.94 (5–18)	3.2 ± 0.37 (2.48–3.68)	6.44 ± 2.528 (2.15–9.70)
Doi Chiang Dao ( <i>Z. p. siamensis</i> ) n = 7	4508 ± 125.8 (4305–4673)	3037 ± 117.8 (2861–3193)	3.2 ± 0.43 (2.12–3.77)	12.2 ± 1.48 (9–14)	3.8 ± 0.29 (3.26–4.25)	6.04 ± 0.918 (4.39–7.13)
Doi Phu Kha ( <i>Z. p. siamensis</i> ) n = 10	5461.1 ± 264.4 (5054.3–5719.9)	2609.8 ± 255.9 (2196.4–2881.5)	3.3 ± 0.56 (3.105–4.346)	12.9 ± 1.45 (10–15)	3.95 ± 0.24 (3.45–4.25)	4.954 ± 0.330 (4.512–5.428)
Phu Luang ( <i>Z. p. siamensis</i> ) n = 9	4656 ± 213.4 (4142–4869)	2923 ± 258.0 (2508–3135)	3.3 ± 0.74 (2.37–4.75)	11.1 ± 2.47 (8–16)	3.42 ± 0.235 (2.95–3.77)	5.64 ± 1.995 (3.57–8.32)
Tak ( <i>Z. p. siamensis</i> ) #1 n = 10	5205.2 ± 335.7 (4914.3–6115.8)	2972.9 ± 60.3 (2883.2–3045.1)	2.8 ± 0.40 (2.20–3.32)	9.7 ± 1.64 (8–13)	3.50 ± 0.18 (3.31–3.92)	5.156 ± 1.048 (3.702–6.085)
Tak ( <i>Z. p. siamensis</i> ) #2 n = 10	5341.9 ± 192.8 (5175.0–5793.8)	2557.3 ± 82.5 (2364.5–2688.3)	4.3 ± 0.93 (3.22–6.42)	14.4 ± 2.59 (11–20)	3.4 ± 0.19 (3.09–3.65)	5.4 ± 0.72 (4.516–6.403)

**Plates 1–8.** Images of White-eye taxa *Zosterops* sp. covered in this paper. All images by P. D. ROUND and all except Plate 1 are from Thailand.



**Plate 1.** Nominate *Z. p. palpebrosus*, Nepal, 9 May 2012.



**Plate 2.** *Z. p. siamensis*, grey-bellied morph, ventral view, Chiang Rai, ring no. 1A03899, 16 April 2010. Note the weak yellow mesial belly stripe, common to the grey-bellied morph of *siamensis* and variably present in the mangrove taxa depicted in Plates 5, 6 and 7.



**Plate 3.** *Z. p. siamensis*, grey-bellied morph, Doi Musoe, ring no. 1A01744, 15 February 2009.



**Plate 4.** *Z. p. siamensis*, yellow-bellied morph, Doi Musoe, ring no. 1A01742, 14 February 2009.



**Plate 5.** *Z. p. williamsoni*, Samut Sakhon, ring no. A33272, 8 December 2015.



**Plate 6.** *Z. p. williamsoni*, Pak Phanang, Nakhon Si Thammarat, ring no. A33808, 2 September 2016.



**Plate 7.** *Z. p. cf. erwini*, Ban Tha Maphrao, ring no. 1A04425, 13 July 2011.



**Plate 8.** *Z. japonicus simplex*, Nam Kham, Chiang Rai, ring no. 1A03830, 1 January 2010.



The yellowish supraloral streak extending above the base of the bill in Upper Gulf *williamsoni* contrasted with the crown which, together with the entire upperparts, was a colder green. These features resemble *Z. j. simplex* rather than *Z. p. siamensis*. The tail-feathers were blacker, lacking obvious green fringes, and the white eye-ring was much broader, roughly twice as broad as in *siamensis*.

The plumage features of *Z. p. williamsoni* from Pak Phanang, Nakhon Si Thammarat province, Thailand, appeared almost identical to those from the Upper Gulf (Plates 5 & 6). Andaman Coast birds were somewhat variable; in general, the loral streak was slightly weaker than in Upper Gulf *williamsoni*, although there was much variation. The yellow mesial streak on the lower breast and belly was weak in the mangrove-dwelling taxa, but less defined in *williamsoni* from the east coast and Upper Gulf, and somewhat more variable in Andaman Coast cf. *erwini*.

### Vocalisations

Without exception, at all locations where vocalisations were studied, inland forest populations of *Z. palpebrosus* in Nepal and Thailand had a long, obtrusive and relatively complex song. This followed a uniform pattern throughout (Table 5, Figures 7), consisting of a series of identical verses, each about 3 seconds long, usually uttered at intervals of 4–6 seconds, with up to 20 or more verses in any one song sequence. The minimum and maximum frequencies attained in each verse were between 2.5 KHz and about 5.5 KHz (Table 5). The number of distinct elements (strophes) in any one verse ranged from 6–16, usually 11 or 12. The song was of a recognisably similar pattern in all populations, but there was considerable individual variation. For example, the songs of two individual *Z. p. siamensis* from the same population in Tak province, Thailand, differed from each other about as much as they did from members of different

populations. This finding is important because it shows that there is concordance between both DNA and acoustic data between Himalayan *Z. p. palpebrosus* and Thai *Z. p. siamensis*.

No loud or complex song has yet been recorded or heard from any of the mangrove-dwelling populations, all these give simple ringing calls, typical of the genus. Ten calls from one inland forest *Z. p. siamensis* were at a slightly lower frequency than those given by individuals from three mangrove-dwelling populations (Table 6). However, comparing simple, apparently non-territorial, vocalisations of different taxa is problematical because calls which sound similar to the observer may be given during a variety of different activities (e.g. alarm response to predators, social contact, feeding, roosting) and be neither precisely analogous nor directly comparable.

### DISCUSSION

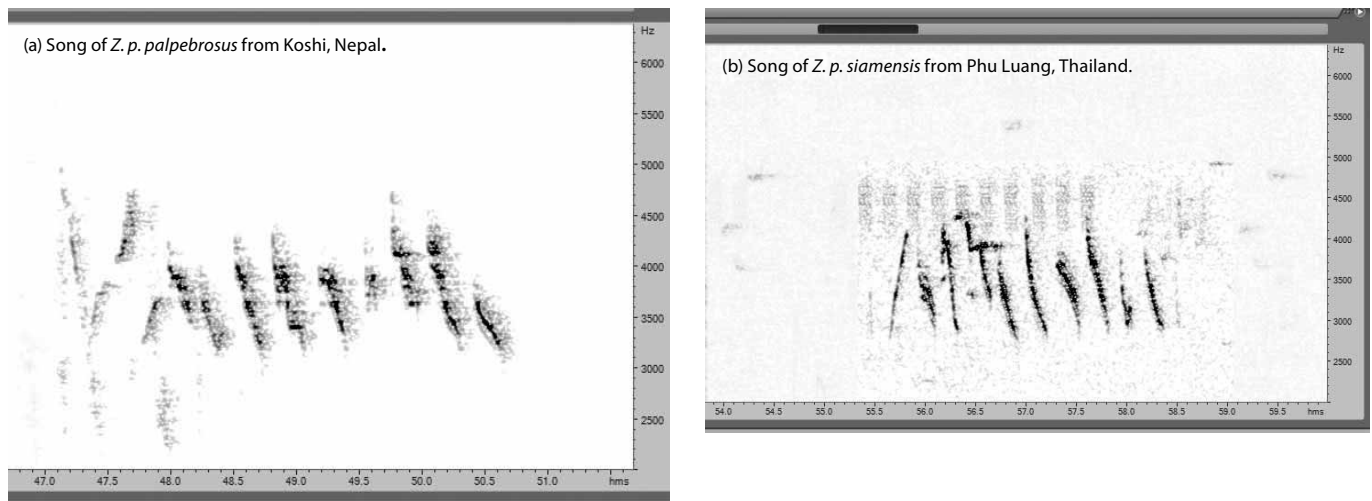
We present genetic, morphological and vocal evidence to indicate that *Z. p. siamensis*, the taxon inhabiting South-East Asian inland forests, is of a lineage distinct from the mangrove-dwelling taxa, *Z. (p.) williamsoni* and *Z. (p.) cf. erwini*. The concatenated multi-gene tree clearly shows that the inland forest and the mangrove-dwelling populations are 4.46–7.83% distinct from each other, reinforcing the evidence from habitat preferences and vocal data. The Upper Gulf and east coast populations *Z. (p.) williamsoni* together with the Andaman Coast population *Z. (p.) cf. erwini* are sister to the migratory Japanese White-eye *Z. j. simplex*, from which they differ only by 1.47–2.43% in the case of the three studied mt DNA loci (Table 2, Figure 2).

One obvious anomaly was that *Z. (p.) cf. erwini* was only 0.46% different from *Z. j. simplex* on cytochrome *b*, much less than the 2.19% difference between *Z. (p.) williamsoni* and *Z. (p.) cf. erwini* at

**Table 6.** Call parameters of four *Zosterops* populations (n = 10 in each case). In all cases, the vocalisations recorded were from a single individual/recording session.

	Peak (Hz)	Max (Hz)	Min (Hz)	Range (max-min) Hz	Duration (sec)	Interval between notes (sec)
<i>Z. p. siamensis</i> (Chiang Rai)	3733.1 ± 101.45	4092.3 ± 140.00	3257.6 ± 126.0	834.6 ± 134.0	0.338 ± 0.083	1.055 ± 0.665
<i>Z. p. williamsoni</i> (Samut Sakhon)	3905.6 ± 154.9	6627.6 ± 576.9	3323.9 ± 69.3	3303.7 ± 587.1	0.275 ± 0.034	1.1023 ± 0.777
<i>Z. p. williamsoni</i> (Nakhon Si Thammarat)	4474.2 ± 238.1	6987.8 ± 410.1	3759.2 ± 212.2	3228.6 ± 484.7	0.218 ± 0.020	0.833 ± 0.531
<i>Z. p. cf. erwini</i> (Satun)	4487.9 ± 391.0	5234.6 ± 333.4	3408.0 ± 229.5	1826.6 ± 500.0	0.143 ± 0.030	0.556 ± 0.469

**Figure 7.** Sonograms of songs of *Z. palpebrosus* inland forest taxa. *Z. p. palpebrosus* (Nepal) and *Z. p. siamensis* (north-east Thailand) sing recognisably similar songs. No complex song appears to be known for any mangrove-dwelling taxa presently assigned to *Z. palpebrosus* sp.



this locus. However, cytochrome *b* is the shortest, least informative locus and this finding was not replicated by the other mt DNA genes, in which pairwise distances among these three taxa were 1.47–2.43% different.

Robinson & Kloss (1919), when naming *Z. p. williamsoni*, compared it with *Z. p. simplex* (now *Z. j. simplex*) from South China, describing it as intermediate in upperpart colour between that taxon and *Z. p. aureiventer* [*sic*] (= cf. *erwini*). In fact, the resemblance between the mangrove-dwelling taxa and the migratory Japanese White-eye is striking. Not only do all populations of the mangrove-dwelling birds that we have examined possess colder green upperparts than *Z. p. siamensis*, they have a broader white spectacle, recalling *Z. j. simplex* rather than *Z. p. siamensis*, and in addition share with the former taxon a greenish forecrown and contrasting yellowish supraloral stripe. They differ from *simplex* in having a shorter, less pointed wing. The smaller size of both peninsular taxa relative to Upper Gulf *williamsoni* may possibly be attributed to Bergmann's Rule, which posits a decline in body size with decreasing latitude.

The observation that *Z. p. siamensis* on the one hand and the mangrove-dwelling taxa on the other are ecologically segregated by habitat, apparently not meeting anywhere in their ranges, would also be highly unusual if all were merely races of a single species. Previous understanding of the distribution of *Zosterops* taxa in mainland South-East Asia was complicated by the presence of other resident inland forest populations in some locations, including Khao Yai National Park, Thailand, which were widely assumed to be *Z. palpebrosus* (Dickinson 1967, McClure 1974, Lekagul & Round 1991). Only relatively recently was it confirmed that the Khao Yai resident population was in fact another species, Everett's White-eye *Z. everetti* (Pierce & Round 2006). Additionally, yellow morph apparent *Z. [p.] siamensis* was recorded by Hume & Davison (1878) 'in mangrove swamps south of Thatone', in a report on collections from Tenasserim (now Thanintharyi state), south-east Myanmar, where some specimens were collected (D. R. Wells *in litt.* to PDR May 2017). These, together with records including at least one specimen from Elephant Point (about 16.45°N 96.32°E), west of the Gulf of Mottama, Myanmar (D. R. Wells *in litt.* to PDR May 2017), have further confounded understanding of taxa distributions and relationships. However, mangrove forest and inland forest were probably closer together in the past than at present, perhaps facilitating dispersal between these habitats at both sites—certainly in the Zepathwe reserve, Elephant Point, where Stanford (1935) found *Zosterops* species.

Three mitochondrial gene loci of a single *Z. p. palpebrosus* sampled in Nepal were only 0.73–1.99% different from seven individuals of *Z. p. siamensis* sampled in Thailand. Moreover, the two populations sang very similar complex songs (Table 5, Figure 7). They are correctly judged to be races of the same species, with a range that extends from southern China and northern South-East Asia in the east and continues west along the Himalaya to Pakistan and north-east Afghanistan; the oldest name, *Zosterops palpebrosus* (Temminck, 1824) still stands as the species name. The affinities of the Sri Lankan and Indian lowland race *egregius* and the peninsular India race *nilgiriensis* of Oriental White-eye (race *salimalii* of south-east India was unrepresented in GenBank material) are also with nominate *palpebrosus* and *siamensis*, according to the single gene trees (Figures 3–5). It is notable, however, that many avian lineages from peninsular India are distinct from those found in the Himalaya and South-East Asia (Wells *et al.* 2003, Rasmussen & Anderton 2005, Moltesen *et al.* 2013) and the affinities of the races of *Z. palpebrosus* found in peninsular India may yet deserve further attention.

Application of the name *erwini* to Thai Andaman Coast birds (following Wells 2017) is necessarily tentative, particularly because Wells himself drew attention to plumage differences—namely the greater extent of mid-ventral yellow—in the *erwini* type series from

the Natuna Islands, Riau Islands province, Indonesia, off the north-west coast of Borneo. It is possible, however, that *erwini* individuals exhibit a cline of increasing mid-ventral colour saturation from north-west to south-east across the range. The molecular evidence, demonstrating the distinctiveness of the Thai Andaman Coast lineage (cf. *erwini*) compared with the east coast and Upper Gulf lineage (*williamsoni*), although with only 50% PP support, also deserves further investigation. Might these have arisen from two successive colonisation events by northern migrant *Z. j. simplex*? The question must arise whether these lineages meet and intergrade, given the relative continuity of the mangrove habitat in western Sundaland. Increased geographical sampling along both coasts of Peninsular Thailand and Malaysia might resolve the affinities of the Andaman Coast birds and east coast *williamsoni* and those from further south.

Given the relatively small genetic distance between *Z. j. simplex* on the one hand and cf. *erwini* and *williamsoni* on the other, it might be considered appropriate to treat all three as conspecific pending further resolution of their phylogenetic relations. The considerable morphological difference—particularly the shorter wing of the resident mangrove-dwelling taxa—and the ecological divergence of the northern migrant *simplex*, inhabiting forests and secondary growth, from the tropical resident inhabitants of mangroves, might militate against accepting such a treatment. Nominate *Z. j. japonicus* and some other island races are widely divergent from *Z. j. simplex* in the *cytb* tree, indicating that these clades are probably distinct at species level (Figure 3). A detailed examination of all East and South-East Asian mainland and island taxa, perhaps using more advanced methods such as whole genome sequencing, will be essential for a fuller understanding of *Zosterops* phylogeny.

## ACKNOWLEDGEMENTS

We thank Parames Laosinchai and Ekgachai Jeratthitikul for their help in interpreting our findings and David Wells for sharing his (then unpublished) *Zosterops* manuscript and his notes on specimens, for their many helpful comments on drafts of the present paper, and for drawing our attention to a key reference. Dr I. Nishiumi and Dr Trevor Price kindly provided some locality information on their white-eye specimens. Desmond Allen and Deepal Warakagoda also kindly commented. We thank two anonymous referees for their many valuable comments which led us to broaden our coverage to include single gene data from GenBank specimens. Supachaya Techachoochert prepared the accompanying map of localities. Feather samples were collected in collaboration with officials of the Thai Department of National Parks, Wildlife and Plant Conservation staff or on private land with the permission of the landowner.

Among those who assisted in the field, Panom Archarit, Woraphot Bunkhwamdi, Wichyanan Limparungpatthanakij, Yotin Meekaeo, Wicha Narungsri, Andrew J. Pierce, Ingkayut Sa-ar, Thanyarat Tatuwan and Kevin Thornton deserve special mention. The Wildlife Research Division, Department of National Parks, Wildlife and Plant Conservation supplied rings and archived our ringing data. This project was funded by the National Science and Technology Development Agency of the Thai government (grant #P-14-50576). Philip Round is very grateful to The Wetland Trust (UK) and to Stephen Rumsey for his generous support and encouragement.

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## Appendix 1.

### Bird samples included in this study.

Numbers in second column are either ring number for live-caught birds or specimen numbers/voucher numbers for sequences retrieved from GenBank. Abbreviations: AMNH – American Museum of Natural History, New York, USA; CMNH – Cincinnati Museum of Natural History, Ohio, USA.

Taxon	Voucher or ring number	Locality	cyt <b>b</b>	ND2	ND3	TGF
<i>Z. p. cf. erwini</i> 1	1A04426	TH: Ban Tha Maphrao	KY627634	KY627662	KY627610	KY627735
<i>Z. p. cf. erwini</i> 2	1A04429	TH: Ban Tha Maphrao	KY627635	KY627663	KY627611	KY627736
<i>Z. p. cf. erwini</i> 3	1A04421	TH: Ban Tha Maphrao	KY627636	KY627664	KY627612	KY627737
<i>Z. p. cf. erwini</i> 4	1A04416	TH: Ban Tha Maphrao	KY627637	KY627665	KY627613	KY627738
<i>Z. p. cf. erwini</i> 5	1A04422	TH: Ban Tha Maphrao	KY627638	KY627666	KY627614	KY627739

Taxon	Voucher or ring number	Locality	cytb	ND2	ND3	TGF
<i>Z. japonicus simplex 1</i>	1A08248	TH: Phu Luang	KY627631	KY627659	KY627607	KY627732
<i>Z. japonicus simplex 2</i>	1A03823	TH: Nam Kham	KY627632	KY627660	KY627608	KY627733
<i>Z. p. siamensis 1</i> (Yellow morph)	1A01741	TH: Doi Musoe	KY627653	KY627681	KY627604	KY627754
<i>Z. p. siamensis 2</i> (grey morph)	1A01744	TH: Doi Musoe	KY627654	KY627682	KY627605	KY627755
<i>Z. p. siamensis 3</i> (yellow morph)	1A01746	TH: Doi Musoe	KY627655	KY627683	KY627606	KY627756
<i>Z. p. siamensis 4</i> (grey morph)	1A03899	TH: Ban Saen Jai	KY627639	KY627667	KY627603	KY627740
<i>Z. p. siamensis 5</i> (grey morph)	1A04708	TH: Chiang Dao Wildlife Research Station	KY627630	KY627658	KY627602	KY627731
<i>Z. p. siamensis 6</i> (grey morph)	1A08209	TH: Phu Luang	KY627650	KY627678	KY627627	KY627751
<i>Z. p. siamensis 7</i> (grey morph)	1A08214	TH: Phu Luang,	KY627651	KY627679	KY627628	KY627752
<i>Z. p. siamensis 8</i> (grey morph)	1A04218	TH: Phu Luang	KY627652	KY627680	KY627629	KY627753
<i>Z. p. williamsoni 1</i>	1A12410	TH: Bang Khun Thien	KY627633	KY627661	KY627609	KY627734
<i>Z. p. williamsoni 2</i>	1A01818	TH: Samut Sakhon	KY627640	KY627668	KY627615	KY627741
<i>Z. p. williamsoni 3</i>	1A01819	TH: Samut Sakhon	KY627641	KY627669	KY627616	KY627742
<i>Z. p. williamsoni 4</i>	1A01821	TH: Samut Sakhon	KY627642	KY627670	KY627617	KY627743
<i>Z. p. williamsoni 5</i>	1A03729	TH: Samut Sakhon	KY627643	KY627671	KY627618	KY627744
<i>Z. p. williamsoni 6</i>	1A01814	TH: Samut Sakhon	KY627644	KY627672	KY627619	KY627745
<i>Z. p. williamsoni 7</i>	A33808	TH: Pak Phanang	KY627646	KY627674	KY627620	KY627747
<i>Z. p. williamsoni 8</i>	A33809	TH: Pak Phanang	KY627647	KY627675	KY627621	KY627748
<i>Z. p. williamsoni 9</i>	A33810	TH: Pak Phanang	KY627648	KY627676	KY627622	KY627749
<i>Z. p. williamsoni 10</i>	A33811	TH: Pak Phanang	KY627649	KY627677	KY627623	KY627750
<i>Z. p. williamsoni 11</i>	1A15154	TH: Ban Nam Chiao	KY627656	KY627684	KY627624	KY627757
<i>Z. p. williamsoni 12</i>	1A15155	TH: Ban Nam Chiao	KY627657	KY627685	KY627625	KY627758
<i>Z. p. palpebrosus 1</i>	NA0089	Nepal: Koshi	KY627645	KY627673	KY627626	KY627746
<b>Sequences retrieved from GenBank</b>						
<i>Z. japonicus 1</i>	B20880	From captivity			DQ328490	
<i>Z. japonicus 2</i>	DOT10981	Vietnam		FJ460801	FJ460869	
<i>Z. japonicus 3</i>	Tsushima-1	Japan: Sago	AB248939			
<i>Z. japonicus 4</i>	Tsushima-5	Japan: Sago	AB248940			
<i>Z. japonicus 5</i>	Ibaraki41	Japan: Tsukuba, Ibaraki	AB248938			
<i>Z. japonicus 6</i>	234850 (Tissue T1149)	Japan		AY136599		
<i>Z. japonicus 7</i>	GLG52246	China: Yunnan		KT310496		
<i>Z. japonicus 8</i>	GLG52247	China: Yunnan	DQ837523		DQ837491	
<i>Z. japonicus 9</i>	-	China: Yunnan		GU724482		
<i>Z. japonicus 10</i>	16-6F	China: Yunnan		DQ861968		
<i>Z. japonicus 11</i>	IOZ2538	China: Foping, Shaanxi	HQ608850			
<i>Z. japonicus insularis</i>	Yaku-13	Japan: Yakushima, Kagoshima,	AB248728			
<i>Z. japonicus japonicus 1</i>	ZJ131	Korea: Gyeongsangnam, Koje-do	AB159167			
<i>Z. japonicus japonicus 2</i>	ZJ133	Korea: Gyeongsangnam, Koje-do	AB159168			
<i>Z. japonicus japonicus 3</i>	ZJ9927	Japan: Tsukuba, Ibaraki	AB159165			
<i>Z. japonicus japonicus 4</i>	ZJ0102	Japan: Tsukuba, Ibaraki	AB159166			
<i>Z. japonicus simplex 3</i>	HK-101	Hong Kong: Mai Po N.R.	AB248718			
<i>Z. japonicus simplex 4</i>	HK-33	Hong Kong	AB248716			
<i>Z. japonicus simplex 5</i>	HK-42	Hong Kong	AB248717			
<i>Z. japonicus simplex 6</i>	Taiwan-12	Taiwan: Taipei	AB248715			
<i>Z. japonicus simplex 7</i>	A26867	Taiwan: Taitung	AB239512			
<i>Z. japonicus simplex 8</i>	A26869	Taiwan: Taitung	AB239513			
<i>Z. palpebrosus 2</i>	23218	Indonesia: Flores		FJ460807	FJ460875	
<i>Z. palpebrosus 3</i>	DOT5746	Nepal		FJ460806	FJ460874	
<i>Z. palpebrosus 4</i>	GLG51816	China: Yunnan	DQ837522		DQ837493	
<i>Z. palpebrosus 5</i>	F19141	India: Uttar Pradesh	KJ456519	KJ455705		
<i>Z. palpebrosus 6</i>	KUBI 23218			KC540183	KC540289	
<i>Z. palpebrosus 7</i>	MD18SS04	Sri Lanka: Galle		KY765098		
<i>Z. palpebrosus 8</i>	ME28SF02	Sri Lanka: Gampaha			KY765139	
<i>Z. palpebrosus 9</i>	MG175F05	Sri Lanka: Kotadeniyawa		KY765100		
<i>Z. palpebrosus 10</i>	MK22SF01	Sri Lanka: Gampaha		KY765099	KY765140	
<i>Z. palpebrosus 11</i>	NE11NW01	Sri Lanka: Nuwara Eliya		KY765094	KY765135	

Taxon	Voucher or ring number	Locality	cytb	ND2	ND3	TGF
<i>Z. palpebrosus</i> 12	NE09NW01	Sri Lanka: Nuwara Eliya		KY765095	KY765136	
<i>Z. palpebrosus</i> 13	NE09NW02	Sri Lanka: Nuwara Eliya		KY765096	KY765137	
<i>Z. palpebrosus</i> 14	NF02NW03	Sri Lanka: Nuwara Eliya		KY765097	KY765138	
<i>Z. palpebrosus</i> 15	Z51123	India: Munnar, Kerala		KY765101	KY765141	
<i>Z. palpebrosus</i> 16	Z51129	India: Munnar, Kerala		KY765102	KY765142	
<i>Z. palpebrosus</i> 17	Z51227	India: Munnar, Kerala		KY765103	KY765143	
<i>Z. palpebrosus</i> 18	Z51228	India: Munnar, Kerala		KY765105	KY765145	
<i>Z. palpebrosus</i> 19	Z51229	India: Munnar, Kerala		KY765106	KY765146	
<i>Z. palpebrosus</i> 20	Z51241	India: Munnar, Kerala			KY765144	
<i>Z. palpebrosus</i> 21	Z51241	India: Munnar, Kerala		KY765104		
<i>Z. palpebrosus</i> 22	Z51501	India: Periyar, Kerala		KY765108		
<i>Z. palpebrosus</i> 23	Z51507	India: Periyar, Kerala		KY765109	KY765148	
<i>Z. palpebrosus</i> 24	Z51509	India: Periyar, Kerala		KY765110	KY765149	
<i>Z. palpebrosus</i> 25	Z51603	India: Periyar, Kerala		KY765107	KY765147	
<i>Z. palpebrosus</i> 26	51037	India: Western Ghats	KJ702867		KP398709	
<i>Z. palpebrosus</i> 27	51041	India: Western Ghats	KJ702858		KP398708	
<i>Z. palpebrosus</i> 28	51074	India: Western Ghats	KJ702862		KP398704	
<i>Z. palpebrosus</i> 29	51104	India: Western Ghats	KJ702868		KP398705	
<i>Z. palpebrosus</i> 30	51105	India: Western Ghats	KJ702860		KP398713	
<i>Z. palpebrosus</i> 31	51123	India: Western Ghats	KJ702869		KP398711	
<i>Z. palpebrosus</i> 32	51129	India: Western Ghats			KP398703	
<i>Z. palpebrosus</i> 33	51201	India: Western Ghats	KJ702865		KP398707	
<i>Z. palpebrosus</i> 34	51227	India: Western Ghats	KJ702861		KP398706	
<i>Z. palpebrosus</i> 35	51241	India: Western Ghats	KJ702864		KP398714	
<i>Z. palpebrosus</i> 36	51255	India: Western Ghats	KJ702859		KP398712	
<i>Z. palpebrosus</i> 37	51269	India: Western Ghats	KJ702863		KP398710	
<i>Z. palpebrosus</i> 38		India: Western Ghats	KJ702866			
<i>Z. p. palpebrosus</i> 2	RF1	Nepal			DQ328449	
<i>Z. p. palpebrosus</i> 3	RF2	Nepal			DQ328448	
<i>Z. p. egregius</i>	1964.26.1	India: Gujarat			DQ328450	
<i>Z. p. melanurus</i>	Joyhon-1	Bird shop in Japan; imported from Java, Indonesia	AB248719			
<i>Z. meyeri batanis</i> 1	A26886	Taiwan: Orchid island	AB239514			
<i>Z. meyeri batanis</i> 2	A23889	Taiwan: Orchid island	AB239511			
<b>Outgroup</b>						
<i>Lophozosterops squamiceps</i> <sup>1</sup>	AMNH: DOT12549	Indonesia: Sulawesi	JN827094	FJ460793	FJ460861	FJ460930
<i>Stachyris hypogrammica</i> <sup>1</sup>	CMNH:37765	Philippines: Palawan	JN827193	JN826670	JN826940	JN826414

<sup>1</sup> Moyle et al. (2009)

## Appendix 2.

### Primers used in this study

#### Mitochondrial: Cytb, 308 bp

	5 > 3	Annealing T
L14841	AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA	50
H15149	AAACTGCAGCCCCCAGAATGATATTGTCCTCA	(Kocher et al. 1989)

#### Mitochondrial: Second subunits of mitochondrial nicotinamide adenine dinucleotide dehydrogenase (ND2), 1041 bp

	5 > 3	Annealing T
ND2F6	AGGGTCAGCTAAACAAGCTATCG	55
ND2R1141	GGCCTTCGGTTTAGGTGATCC	(Manawatthana et al. 2017)

#### Mitochondrial: Third subunits of mitochondrial nicotinamide adenine dinucleotide dehydrogenase (ND3), 351 bp

	5 > 3	Annealing T
ND3F	GACTTCCAATCTTTAAATCTGG	50
ND3R	GATTGTGAGCCGAAATCAAC	(Chesser 1999)

#### Nuclear: Fifth intron of the nuclear gene transcription growth factor (TGF), 590 bp

	5 > 3	Annealing T
TGF5	GAAGCGTCTCTAGATGCTG	65
TGF6	AGGCAGCAATTATCCTGCAC	(Primmer et al. 2002)