

Molecular phylogeny of the yuhinas (Sylviidae: Yuhina): a paraphyletic group of babblers including Zosterops and Philippine Stachyris

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Abstract Mitochondrial sequences (2,379 bp) from also implies that an ancestor of Zosterops Philippine cytochrome b, ND3, 12s and 16s rRNA were analyzed in Stachyris derived the ability to disperse over long distances, order to reconstruct the phylogenetic relationships within so that it could fly over the sea and arrive at the Philippines. the yuhinas (Yuhina), including the chestnut-faced babbler Stachyris whiteheadi which is endemic to the Philippines, Keywords Phylogenetic analysis Mitochondrial DNA Yuhina Zosterops Stachyris the Japanese white-eye Zosterops japonicus, the chestnut-flanked white-eye Z. erythropleurus and the oriental white-eye Z. palpebrosus. The results showed strong support for the idea that S. whiteheadi and three white-eye species form a clade embedded within the Yuhina clade. The robustness of the Yuhina Philippine Stachyris Zosterops clade rejected the validity of the monotypic genus Staphida which was proposed for the striated yuhina Yuhina castaniceps based on its peculiar morphological traits. Sister group relationships between the striped-throated yuhina Yuhina gularis and the rufous-vented yuhina Yuhina occipitalis and between the whiskered yuhina Yuhina flavicollis and the white-naped yuhina Yuhina bakeri were discovered. The sympatric patterns of the above two sister groups in the Himalayas is most likely due to secondary contact. The molecular phylogeny also suggests that crestlessness was derived just once for the Zosterops Philippine Stachyris clade. The Zosterops Philippine Stachyris clade in our study

Introduction
Babblers (Timaliini) comprise more than 200 highly morphologically diverse species in South-East Asia and Africa. Although both morphological and molecular data have been used to assess the systematic relationships among major groups of Timaliini (Delacour 1946, 1950; Cibois et al. 1999, 2002; Cibois 2003), phylogenetic relationships of the babblers within genus are still poorly understood. Here we used mitochondrial DNA sequence data to re-evaluate the phylogenetic relationships within a group of Asia endemic birds, the yuhinas (Yuhina). The genus Yuhina includes eleven species that are distributed in the Himalayas, Indochina and the Sunda region (Sibley and Monroe 1990). Yuhinas inhabit broad-leaved, coniferous or bamboo forests. Their distributions range in altitude from several hundred meters to 4,000 m high (Sibley and Monroe 1990). Yuhinas are omnivorous, feeding on insects, flower honey, small berries and seeds (Ali and Ripley 1987).

Introduction

Recent molecular data had suggested that the genus Yuhina is not a monophyletic group; three species of Stachyris endemic to the Philippines are embedded within the Yuhina clade: S. whiteheadi, S. striata, S. dennistouni and are not related to other continental species Stachyris (Cibois et al. 2002). Furthermore, the white-bellied Yuhina Yuhina zantholeuca which has traditionally been placed among the yuhinas (Deignan 1964; Sibley and Monroe

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1990; Inskipp et al. 1996; Clements 2000), is not even a babbler (Cibois et al. 2002). Instead, it is closely related to the Vireonidae (Barker et al. 2004). Molecular data have also revealed that the Japanese white-eye *Zosterops japonicus* appears to be closely related to the yuhinas (Cibois 2003).

In this study, by including two more yuhina species (the white-naped yuhina *Yuhina bakeri* and the striated yuhina *Yuhina castaniceps*), we aimed to elucidate the phylogenetic relationship within *Yuhina* in more detail by re-sequencing four mitochondrial DNA fragments (2,379 bp in total). By adding three species of *Zosterops* and one species of *Stachyris*, which is endemic to the Philippines, we also attempted to elucidate their relationships to the yuhinas. While typical yuhinas are crested, the Philippine *Stachyris* and *Zosterops* are crestless. Molecular phylogeny of these birds was used to infer the evolution trend of the crest. Since some species sampled in the study are endemic to Southeast Asia, we also hoped that molecular phylogeny could provide a better basis for interpreting the origins and diversification of birds in Southeast Asia, which is still poorly understood (Moyle et al. 2005).

Methods

Taxa sampling, amplification, and sequencing

Due to the difficulty involved in choosing an appropriate outgroup, five species of Timaliini, the red-tailed minla *Minla ignotincta*, the blue-winged laughingthrush *Sarrulax squamatus*, the grey-throated babbler *Stachyris nigriceps*, the rufous-capped babbler *Stachyris rupestris*, the golden babbler *Stachyris chrysaea*, and a species of Acrocephalinae, the brownish-flanked bush-warbler *Gettia fortipes* were included as a composite outgroup. Yuhina species described, except for the Burmese yuhina *Yuhina humilis* were sampled. However, *Y. humilis* is considered to be closely related to the whiskered yuhina *Yuhina flavicollis* and is treated as a subspecies of *Y. flavicollis* by some authors (Deignan 1964; Dickinson 2003). Three species of *Zosterops* (the Japanese white-eye *Zosterops japonicus*, the chestnut-flanked white-eye *Z. erythropleurus*, and the oriental white-eye *Z. palpebrosus*) and one species of *Stachyris* endemic to the Philippines (the chestnut-faced babbler *Stachyris whiteheadi*) were also included. The taxa sampled in this study are summarized in Table 1.

Four mitochondrial fragments (cytochrome *b*, ND3, 12s and 16s rRNA) were used to infer the molecular phylogeny of *Yuhina* and its allies. Total genomic DNA was isolated from 95% alcohol-preserved tissues (muscle, liver, and blood) or the toe pads of museum skin specimens (for the black-chinned yuhina *Yuhina nigrimenta*). For tissue

samples, DNA was isolated by the standard phenol/chloroform extraction method (Sambrook et al. 1989); for toe pad samples, Puregene DNA purification kits (Gentra Systems, Minneapolis, MN, USA) was used; the manufacturer's recommendations were followed. All primer sequences are shown in Table 2. For the historical DNA template of *Y. nigrimenta*, four primer pairs were used to amplify overlapping fragments of the cytochrome *b* gene. We were careful not to contaminate the historical DNA during DNA isolation and PCR (polymerase chain reaction) amplification was carried out using the following parameters: 94°C for 5 min, followed by 35 cycles of 94°C (30 s), 45–52°C (40 s), 72°C (40 s), and final extension at 72°C (5 min). A touchdown PCR was used to amplify four fragments of the cytochrome *b* gene of *Y. nigrimenta*. Annealing temperature ranged from 64 to 67°C for the two terminal fragments and 62–65°C for the two internal fragments. Primer pairs L1549/H1991 (Desjardin and Morais 1990), L3214 (Hedges 1994)/H3783 (Hedges and Sibley 1994) and L10755/H11151 (Chesser 1999) were used to amplify the 12s, 16s rRNA, and ND3 genes. The same PCR amplification parameters used for cytochrome *b* were used to amplify the above fragments, except that the annealing temperature was changed to 50–65°C. PCR products were purified using a DNA purification kit (Sangon Inc., Shanghai, China) and sequencing of purified PCR products was performed with BigDye Terminator Cycle Sequencing Kits (Applied Biosystems, Foster City, CA, USA) on an ABI Prism 3100 automated DNA sequencer (Perkin-Elmer Applied Biosystems).

Phylogenetic analysis

DNA sequences were proofread by eye and then assembled using the software DNASTAR (DNASTAR Inc., Madison, WI, USA). All cytochrome *b* and ND3 sequences were aligned by using Clustal X with default parameters (Thompson et al. 1997). To avoid the alignment difficulties introduced by indels, we used Gblock 0.91b (Castresana 2000) to delete gaps within certain regions of the 12s and 16s rRNA. Pairwise distances (Tamura–Nei distance) and other characteristics of the sequence were calculated using MEGA3.0 (Kumar et al. 2004). A partition homogeneity test (ILD) and a test of stationary nucleotide composition across taxa were conducted in PAUP*4.0b10 (Swofford 2003).

Phylogenetic analysis was performed using the maximum parsimony (MP) and maximum likelihood (ML) algorithms implemented in PAUP*4.0b10. For the MP analysis, a weighted parsimony method was used by removing the transitions at the third codon position in both cytochrome *b* and ND3 genes to avoid the saturation effect. A heuristic search, with 1,000 replicates of random addition sequences of taxa and tree bisection reconnection

Table 1 Samples and sequences used in this study

Taxa	Museum, tissue number	Collecting locality	GenBank accession no.			
			Cytb	ND3	12s	16s
<i>Zosterops erythropleurus</i>	KIZ, GLGS1443	Yunnan, China	DQ837524	DQ837492	DQ837507	DQ837515
<i>Zosterops palpebrosus</i>	KIZ, GLGS1816	Yunnan, China	DQ837522	DQ837493	DQ837509	DQ837517
<i>Zosterops japonicus</i>	KIZ, GLGS2247	Yunnan, China	DQ837523	DQ837491	DQ837508	DQ837516
<i>Cettia fortipes</i>	KIZ, 05353	Yunnan, China	DQ837512	DQ837490	DQ837512	DQ837518
<i>Garrulax squamatus</i>	KIZ, GLGS1833	Yunnan, China	DQ092872	DQ837502	AF484900	AF484357
<i>Minla ignotincta</i>	KIZ, GLGS091	Yunnan, China	DQ092874	DQ837503	AF484918	AF484373
<i>Stachyris rupeps</i>	KIZ, GLGS494	Yunnan, China	DQ092876	DQ837505	AF376925 ^b	AF376905 ^b
<i>Stachyris chrysaea</i>	KIZ, 04035	Yunnan, China	DQ092875	DQ837506	AF376922 ^b	AF376902 ^b
<i>Stachyris whiteheadi</i>			AY124542 ^b		AF376928 ^b	AF094661 ^b
<i>Stachyris nigriceps</i>	KIZ, GLGS1854	Yunnan, China	DQ092873	DQ837495	AF376924 ^b	AF376904 ^b
<i>Yuhina castaniceps</i>	KIZ, 99172	Guangxi, China	DQ092879	DQ837488	DQ837510	DQ837514
<i>Yuhina everetti</i>	LSUMNS, B36457	Sabah, Malaysia	DQ092881	DQ837489	AF376917	AF376898
<i>Yuhina bakeri</i>	KIZ, 04323	Yunnan, China	DQ092877	DQ837499	DQ837511	DQ837513
<i>Yuhina saviacollis</i>	KIZ, GLGS1424	Yunnan, China	DQ092882	DQ837497	AF376918	AF376897
<i>Yuhina gularis</i>	KIZ, GLGS1248	Yunnan, China	DQ092883	DQ837498	AF376918	AF094659
<i>Yuhina diademata</i>	KIZ, 03026	Yunnan, China	DQ092880	DQ837496	AF376915	AF376896
<i>Yuhina occipitalis</i>	KIZ, 03031	Yunnan, China	DQ092885	DQ837494	AF376920	AF376900
<i>Yuhina brunneiceps</i>	TNU, T0620	Taiwan, China	DQ092878	DQ837501	AF376914	AF376895
<i>Yuhina nigrimenta</i>	KIZ, 012777	Yunnan, China	DQ092884	DQ837500	AF376919	AF376899
<i>Yuhina zantholeuca</i>	TNU, T0325	Taiwan, China	DQ092886	DQ837504	AF376921	AF376901

Taxonomic denomination followed Sibley and Monroe (1990)

^a Sequences new to this study

^b Sequences taken from GenBank database, published by Barhoum and Bourc (2003), Cibois et al. (2002), Cibois (2003)

KIZ Kunming Institute of Zoology, the Chinese Academy of Sciences; TNU Department of Life Science, Taiwan Normal University; LSUMNS Louisiana State University Museum of Natural History

Table 2 Primers used in this study

Gene	Primer name	Primer sequence (bp)	References	
Cyt b	External primers	L14827	CCACACTCCACACAGGCCTAATTAA	Helm-Bychowski and Cracraft (1993)
		H16065	GGAGTCTTCAGTCTCTGGTTTACAAGAC	Helm-Bychowski and Cracraft (1993)
	Internal primers	L15206	CACATCGGCCGAGGAATCTACTA	Cibois et al. (1999)
		H15224	GTACGACTCCGATGTTTCAGGTTTC	Designed in the study
		L15407	TGAGGTGGATTCTCAGTAGAC	Designed in the study
		H15487	GATCCTGTTTCGTGGAGGAAGGT	Cibois et al. (1999)
		L15664	CTCACTAGGAGACCCAGAAAACCTCAC	Designed in the study
		H15709	GTAGGCAAATAGGAAGTATC	Designed in the study
		L15383	GGACAAACACTAGTAGAATG	Cibois et al. (1999)
12s	External primers	L1549	GGGTTGGTAAATCCTGTGCCAGCCA	Desjardin and Morais (1990)
		H1991	GCTATACCTTGACCTGTCTT	Desjardin and Morais (1990)
16s	External primers	L3214	CGCCTGTTTATCAAAAACAT	Hedges (1994)
		H3783	CCGGTCTGAACTCAGATCACGT	Hedges and Sibley (1994)
ND3	External primers	L10755	GACTTCCAATCTTTAAAATCTGG	Chesser (1999)
		H11151	GATTTGTTGAGCCGAAATCAAC	Chesser (1999)

Note the letters L and H refer, respectively, to the light and heavy strands, and the number refers to the base position and the primer in the complete chicken mtDNA sequences established by Desjardins and Morais (1990)

^a SigniPeS primers for the specimen DNA

(TBR) branch swapping, was executed to obtain the MP mitochondrial partitions. Therefore, we combined sequence. We assessed the robustness of the MP tree with 1,000 replications of four mitochondrial fragments into a larger data set (2,379 bp). The characteristics of these sequences are summarized in Table 3. The pattern of base compositional bias was similar to that found in previous avian studies for the ML analysis. The model was subsequently used in Barhoum and Burns (2002, Perez-Emar 2005). The result of a chi-square test ($\chi^2 = 13.24, P = 1.00, df = 57$) supported the notion that nucleotide composition is stationary and TBR branch swapping. Reliability of the phylogenetic relationships was evaluated by performing 100 replicates of bootstrap analysis with taxa added as is. Because data on avian cytochrome b gene is commonly available, we calculated its divergence between our taxa for the purposes of comparison (Table 4). The average sequence divergence between the China clade and out-groups was 16.18%, but it was only 12.12% for the Yuhina clade and 11.85% for the Philippine clade. The Bayesian method was likewise used in the Bayesian analysis of Zosterops species (Ronquist 2001). The substitution model selected by the group was 16.18%, but it was only 12.12% for the Yuhina clade and 11.85% for the Philippine clade. The Bayesian analysis started with randomly generated trees; four Markov chains under default heating values were run for four million generations and sampled every 100 generations. The burn-in was determined by checking for the likelihood of being stationary. Posterior probabilities were estimated to assess the robustness of the tree.

We used the program Modeltest 3.6 (Posada and Crandall 1998) to choose an appropriate substitution model for the ML analysis. The model was subsequently used in Barhoum and Burns (2002, Perez-Emar 2005). The result of a chi-square test ($\chi^2 = 13.24, P = 1.00, df = 57$) supported the notion that nucleotide composition is stationary and TBR branch swapping. Reliability of the phylogenetic relationships was evaluated by performing 100 replicates of bootstrap analysis with taxa added as is. Because data on avian cytochrome b gene is commonly available, we calculated its divergence between our taxa for the purposes of comparison (Table 4). The average sequence divergence between the China clade and out-groups was 16.18%, but it was only 12.12% for the Yuhina clade and 11.85% for the Philippine clade. The Bayesian method was likewise used in the Bayesian analysis of Zosterops species (Ronquist 2001). The substitution model selected by the group was 16.18%, but it was only 12.12% for the Yuhina clade and 11.85% for the Philippine clade. The Bayesian analysis started with randomly generated trees; four Markov chains under default heating values were run for four million generations and sampled every 100 generations. The burn-in was determined by checking for the likelihood of being stationary. Posterior probabilities were estimated to assess the robustness of the tree.

In addition, Bayesian inference of phylogeny was performed using the program MrBayes 3.0b4 (Huelsenbeck and Ronquist 2001). The substitution model selected by the group was 16.18%, but it was only 12.12% for the Yuhina clade and 11.85% for the Philippine clade. The Bayesian analysis started with randomly generated trees; four Markov chains under default heating values were run for four million generations and sampled every 100 generations. The burn-in was determined by checking for the likelihood of being stationary. Posterior probabilities were estimated to assess the robustness of the tree.

Phylogenetic analysis

Results

Rooting the tree with *C. fortipes* parsimony analysis without transitions of the third codon position yielded only one MP tree (branch length = 1,277, consistency index = 0.5043, retention index = 0.5149, rescaled consistency index = 0.2597) (Fig. 1). In the MP analysis, the Yuhina clade was robust for the exclusion of *Zantholeuca* and three species of *Zosterops* formed a clade embedded within the Yuhina clade (99% bootstrap values). The white-collared Yuhina was found to be a sister group to the rest of the Yuhina (90% bootstrap values). Other terminal clades were also strongly supported: *castaniceps* was a sister group to *Y. everetti* (100% bootstrap values), and *Y. brunneiceps* was a sister group to *Y. nigrimenta* (94% bootstrap values). A sister group relationship between the stripe-throated Yuhina and the rufous-vented Yuhina was also supported (96% bootstrap values). This relationship was not significantly ($R = 0.896$) among the four *Z. japonica*, *Z. palpebrosus*, and *Z. erythropleurus* were

Sequence variation

We obtained a total of 50 sequences, and their GenBank accession numbers, together with those of 29 sequences published in Barhoum and Burns (2002), Cibois et al. (2002) and Cibois (2003), are listed in Table 1. We obtained complete cytochrome b (1,143 bp) and ND3 (351 bp) sequences for all species sampled except for the Yuhina diademata. We also obtained sequences of partial mitochondrial 12s (about 400 bp) and 16s (about 530 bp) rRNA genes for species omitted from the previous studies (Cibois et al. 2002, Cibois 2003). After removing the gaps, 386 bp of 12s rRNA and 499 bp of 16s rRNA was included in the subsequent phylogenetic analysis.

The ILD test revealed that phylogenetic signals discovered with strong support (96% bootstrap values) did not conflict significantly ($R = 0.896$) among the four *Z. japonica*, *Z. palpebrosus*, and *Z. erythropleurus* were

Table 3 Molecular characterization of the mitochondrial genes used in this study

Genes	Total sites	Variable sites	Informative sites	Variable sites by codon position			Nucleotide frequencies			
				First	Second	Third	%A	%C	%G	%T
Cytochrome b	1143	419 (36.7%)	338 (29.6%)	84 (20.0%)	21 (5.0%)	314 (75.0%)	28.5	33.4	13.2	24.9
ND3	351	157 (44.7%)	132 (37.6%)	44 (28.0%)	15 (9.6%)	98 (62.4%)	29.4	32.4	12.3	25.9
12s	386	81 (21.0%)	44 (11.4%)				33.1	25.7	19.4	21.8
16s	499	92 (18.4%)	53 (10.6%)				30	25.7	23.1	21.1

Table 4 Pairwise sequence divergence (Tamura-Nei distances) in cytochrome

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.Y. gularis																				
2.Y. occipitalis	0.0869																			
3.Y. flavicollis	0.0944	0.0840																		
4.Y. bakeri	0.0964	0.0839	0.0811																	
5.Z. palpebrosus	0.1225	0.1121	0.1134	0.1038																
6.Z. japonicus	0.1168	0.1126	0.1032	0.1072	0.0505															
7.Z. erythropeurus	0.1116	0.1099	0.1046	0.1028	0.0544	0.0491														
8.S. whiteheadi	0.1193	0.1148	0.1143	0.1143	0.1121	0.1147	0.1083	0.1000												
9.Y. nigrimenta	0.1176	0.1181	0.1212	0.1151	0.1367	0.1407	0.1322	0.1129												
10.Y. brunneiceps	0.0983	0.0876	0.0908	0.0908	0.0868	0.1097	0.1140	0.1032	0.1045	0.0967										
11.Y. everetti	0.1134	0.1079	0.1080	0.1080	0.1166	0.1263	0.1372	0.1307	0.1248	0.1372	0.1062									
12.Y. castaneiceps	0.1128	0.1070	0.1007	0.118	0.1285	0.1309	0.1267	0.1253	0.1253	0.1375	0.1012	0.0325								
13.Y. diademata	0.1317	0.1174	0.1228	0.1054	0.1379	0.1463	0.1331	0.1385	0.1310	0.1294	0.1322	0.1326								
14.M. ignortincta	0.1495	0.1502	0.1479	0.1601	0.1664	0.1682	0.1681	0.1679	0.1611	0.1492	0.1500	0.1436	0.1472							
15.G. squamatus	0.1241	0.1467	0.1357	0.1315	0.1568	0.1609	0.1475	0.1515	0.1416	0.1307	0.1493	0.1505	0.1537	0.1252						
16.C. forticeps	0.1498	0.1553	0.1465	0.1560	0.1701	0.1635	0.1650	0.1645	0.1689	0.1525	0.1654	0.1711	0.1509	0.1665	0.1727					
17.Y. zantholeuca	0.1611	0.1653	0.1636	0.1634	0.1732	0.1797	0.1734	0.1684	0.1534	0.1701	0.1787	0.1826	0.1676	0.1855	0.1690	0.1742				
18.S. rubiceps	0.1506	0.148	0.1606	0.1606	0.1842	0.1772	0.1761	0.1826	0.1449	0.1645	0.1676	0.1641	0.1732	0.1766	0.1570	0.1867	0.1652			
19.S. chrysaea	0.1583	0.1425	0.1677	0.1604	0.1689	0.1717	0.1742	0.1667	0.1688	0.1643	0.1705	0.1692	0.1681	0.1744	0.1639	0.1904	0.1678	0.0931		
20.S. nigriceps	0.1717	0.16	0.1757	0.1717	0.1807	0.1929	0.1790	0.1754	0.1248	0.1718	0.1825	0.1778	0.1648	0.1718	0.1690	0.1910	0.1787	0.1408	0.1527	

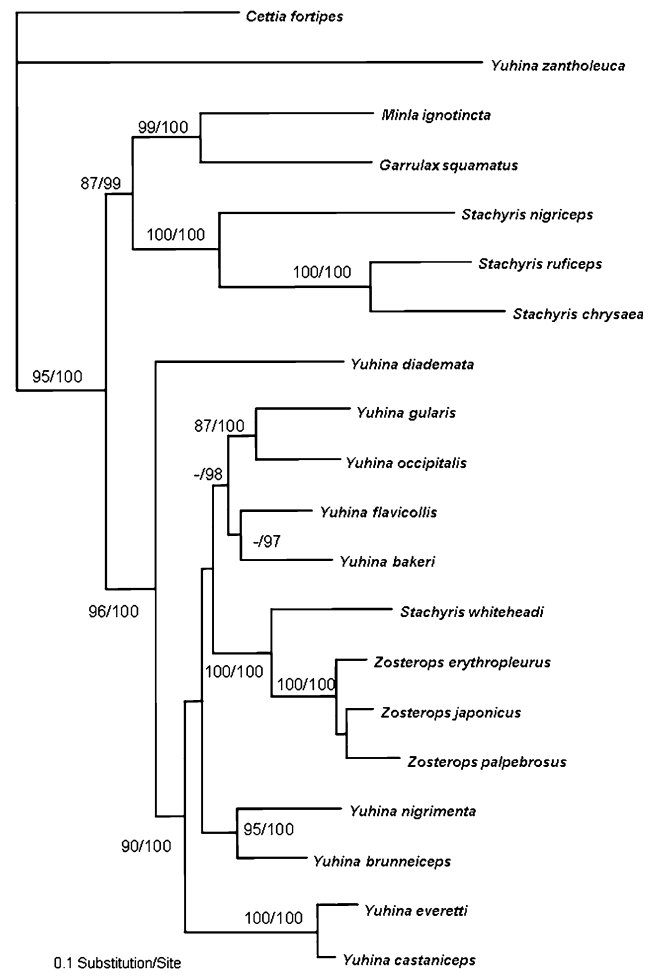
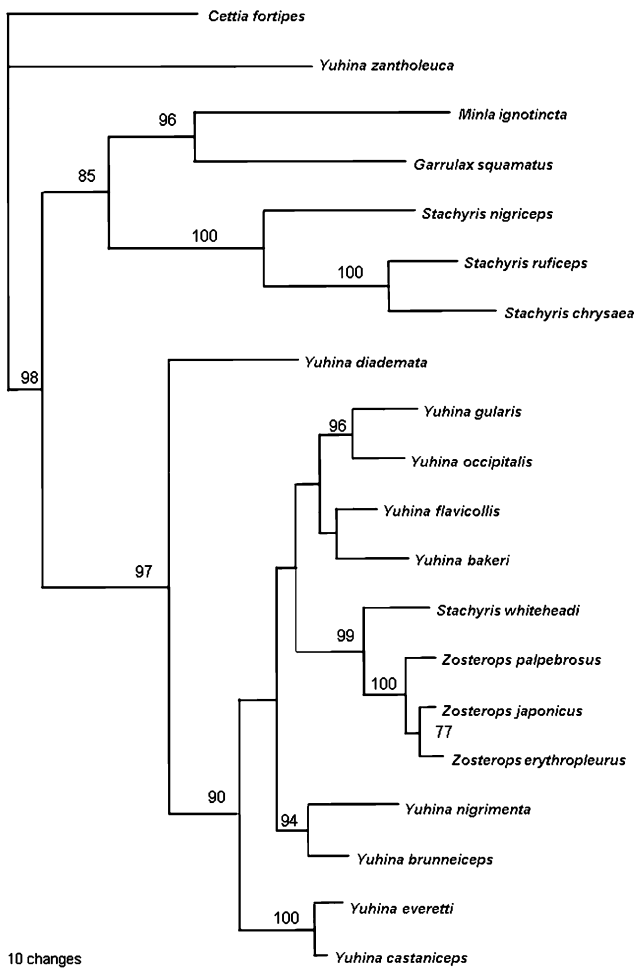


Fig. 1 Phylogenetic interspecies relationships of *Yuhina* based on cytb, ND3, 12s, and 16s sequences; maximum parsimony tree is shown with transitions of third position removed. Numbers represent bootstrap values; only those >70% are shown above the branches

Fig. 2 Phylogenetic relationships of *Yuhina* based on cytb, ND3, 12s and 16s sequences obtained with maximum likelihood and Bayesian analysis. Numbers represent bootstrap values/posterior probabilities, and are only shown when bootstrap values >70% and posterior probabilities >95%

found to form a clade (100% bootstrap values), *Z. palpebrosus* was a sister group to the clade made up of *Z. erythropleurus* and *Z. japonicus* and the relationship obtained strong support (77% bootstrap values).

Modeltest 3.6 selected the GTR + I + G model as the best substitution model for our combined data set. The parameters were estimated as follows: base frequencies: A = 0.3128, C = 0.3459, G = 0.1496, T = 0.1917; probabilities for the six different substitution types: $\Gamma_{matrix} = 1.8632, 6.6449, 2.3026, 0.2447, 17.5067, 1$, with 0.5857 sites invariable, and the substitution rates for variable sites followed a gamma distribution with a shape parameter of 0.7868. The ML and Bayesian trees were identical (Fig. 2) and they were almost identical to the MP tree, with the aforementioned nodes gaining strong support. Furthermore, the likelihood (log L = -13171.3846) was significantly better than the sister group relationship of *Y. flavicollis* and *Y. bakeri* at the 5% level than when the molecular clock was enforced (log L = -13199.7564) ($P = 0.000007$).

probability values). The clade of *Y. bakeri* and *Y. flavicollis* shared a common ancestor with *Y. gularis*, *Y. occipitalis* and obtained strong support in Bayesian analysis (97% posterior probability values). The topological difference between the MP and ML trees was within the *Zosterops* clade; in ML and Bayesian analysis, *Z. erythropleurus* was a sister group to the clade comprising *Z. palpebrosus* and *Z. japonicus* but this did not obtain strong support (with 59% bootstrap values in ML analysis and 80% posterior probability values in Bayesian analysis, not shown).

The hypothesis of constant rate within the alignment was rejected by a likelihood ratio test (LRT test) (Swofford et al. 1996) in PAUP*4.0b10. Under the GTR + I + G model of evolution, with no molecular clock enforced, the likelihood (log L = -13171.3846) was significantly better at the 5% level than when the molecular clock was enforced (log L = -13199.7564) ($P = 0.000007$).

Discussion

The likely close relationship of *Zosterops* and babblers

Our results revealed that the crested *Zosterops* and the Philippine *Stachyris* formed a clade embedded within the yuhinas with strong support (Figs. 2). *Zosterops* is a large genus including 76 species, which are mostly distributed in the old world (Sibley and Monroe 1990), and few studies of phylogenetic relationships with *Zosterops* have been reported (Warren et al. 2006). A close relationship between *Zosterops* and babblers has not previously been proposed by taxonomists on the basis of morphological or ecological characteristics (Cibois 2003). However, more and more molecular studies have suggested that some species of *Zosterops* are members of the Timaliini (Cibois 2003; Ericson and Johansson 2003; Barker et al. 2004; Voelker and Spellman 2004; Alstrom et al. 2006; Beresford et al. 2005; Fuchs et al. 2005). Four species of *Zosterops* have been proved to be members of the Timaliini mitochondrial cytochrome *b* gene between *Z. senegalensis*, the pale white-eye *Z. pallidus*, and the yellowish white-eye *Z. nigrorum* and *Z. japonicus*. Our study proved that another two species of *Zosterops*, *Z. palpebrosus* and *Z. erythropleurus* are closely related to the Timaliini. However, a larger sampling of *Zosterops* needed to verify the likely close relationship with babblers. Within *Zosterops*, the average sequence divergence (5.2%) was observed among *Z. japonicus*, *Z. palpebrosus* and *Z. erythropleurus*, which is consistent with their extremely similar plumage patterns.

Interspecies relationships among yuhinas

The middle nodes of the *Yuhina* clade have been poorly resolved in a previous study (Cibois et al. 2002). However, even when the length of the sequence used was doubled in the current study, these nodes were still difficult to resolve. This may be caused by the short branch lengths of the nodes (rapid diversification). Similar cases have been reported for numerous passerine birds (reviewed by Perez-Eman 2005). Adding more informative characters, especially nuclear loci, may shed light on this problem.

Systematic treatment of *Y. castaniceps* is considered to be controversial. Harrison (1986a, 1986b) proposed removing *Y. castaniceps* from the genus *Yuhina* and resurrecting its original monotypic genus *Staphida* (Gould 1871), as its tail is round-ended, which is different from the typical yuhina square-ended tail. Our data does not support this proposition; molecular phylogeny showed that all yuhinas (except *Y. zantholeuca*) along with *Zosterops* and the Philippine *Stachyris* formed a robust clade.

Yuhina castaniceps and *Y. everetti* formed a well-supported sister group in our study, while a debate regarding the species status of *Y. everetti* exists (Inskipp et al. 1996). In light of the phylogenetic species concept (PSC) (Cracraft 1983; Nixon and Wheeler 1990), diagnosability from molecular or morphological characters could justify the species status of *Y. everetti*. When we took all *Y. castaniceps* together with *Y. everetti* into account, we found that the morphological differences among the six taxa were obvious. The chestnut area on the head showed a trend for enlargement only in the ear-coverts for *Y. c. plumbeiceps* in the ear-coverts and a patch above the posterior supercilium for *Y. c. rubrigensis* in the ear-coverts and the sides of the neck. *Y. inc. striata* and in the ear-coverts, on the sides of the neck and the nape in *Y. c. torqueola*. *Y. c. castaniceps* is most similar to *Y. everetti* in appearance, but it is scalloped with pale grey on its forehead (Ali and Ripley 1987; Grimmett et al. 1999; Robson 2000). In addition, the Tamura–Nei distance of the *Y. castaniceps* and *Y. everetti* is 3.25%; the divergence level is comparable to that of well accepted sister groups in North American birds (Johnson and Cicero 2004).

Our study discovered a sister relationship between *Y. gularis* and *Y. occipitalis* with strong support in all trees. This is consistent with their morphological similarity; they share the same color pattern on the under-parts, except for some black stripes on the throat of *Y. gularis*. Interestingly, the current ranges of this species pair are highly sympatric; both are distributed in northern India, southeastern Tibet, northeastern Burma, and southwestern China, with *Y. gularis* also extending into northern Laos and Vietnam (Sibley and Monroe 1990). A sympatric sister species pattern was also found for the *Bavicolis*/*Y. bakeri* clade. These are both distributed in northern India, northern Burma and southwestern China, with *B. bavicolis* also extending into northwestern Thailand, northern Laos and northern Vietnam (Sibley and Monroe 1990). Although the four closely related species are currently sympatric in the Himalayas, it is hard to infer the cause of their distribution pattern. Due to their high mobility, the distributions of these birds would have easily adapted to the historical shift in vegetation (Losos and Glor 2003). However, their profound molecular divergence indicates independent evolutionary histories (8.11% for *Y. bavicolis*/*Y. bakeri*; 8.69% for *Y. gularis*/*Y. occipitalis*). The sympatric distribution patterns of these species were most likely caused by secondary contact.

Clade evolution

Including the results from the current study, the molecular phylogeny of the *Yuhina* and its allies has proved that the

contentious species. *Y. zantholeuca* is not a babbler but is instead closely related to the Vireonidae (Cibois et al. 2002; Barker et al. 2004). This suggests that the morphological similarities between *Y. zantholeuca* and other yuhinas, especially the presence of a crest, are probably caused by convergent evolution. In fact, when we carefully examined the crest from specimens of *Y. zantholeuca* and another seven yuhina species kept by the Kunming Institute of Zoology of the Chinese Academy of Sciences, we found that the crest of *Y. zantholeuca* is merely composed of crown feathers, while the crest of the other yuhina species comprises both crown and occipital feathers. It is the convergent crest characteristic that has caused this contentious species to be misclassified into *Yuhina*.

Similarly, the broad-billed sapayoa *Sapayoa aenigma* and the Tibetan ground-jay *Pseudopodoces humilis* have recently been proven by molecular systematics to have been misclassified into different families (Fjeldså et al. 2003; James et al. 2003; but see Fjeldså et al. 2005). Overall morphological similarities could not reflect the true relationships of these contentious birds.

Our phylogeny also suggested that crestlessness was a derived character of the *Zosterops* Philippines *Stachyris* clade, and was only derived once. The general evolutionary trend of the crest showed that the crest was not stable in the true *Yuhina* clade, which made *Yuhina* a paraphyletic group. It has been suggested that, once evolved, similar color patterns can easily disappear and re-appear independently in the leaf warblers *Phylloscopus* (Price and Palvelka 1996). Therefore, extreme caution should always be taken when applying any morphological traits to avian systematics, otherwise the conclusions could be totally misleading.

The dispersal route for yuhinas and their allies

Geologically, the Philippine archipelago had never been connected to the Asian continent or to Sunda land directly (Hall 1998). In addition, no species of yuhinas is known for its long distance dispersal or migration. Therefore, the origin of the Philippine *Stachyris* is not straightforward. Seasonal migration and long distance dispersal are, however, well documented for several *Zosterops* species. For example, *Z. lateralis* have been found to frequently disperse from New Zealand to its nearby islands (Mees 1969).

Zosterops japonicus and *Z. erythropleurus* were found to migrate between northern and southeastern Asia seasonally (Sibley and Monroe 1990). It is possible that an ancestor of *Zosterops* Philippines *Stachyris* was able to disperse from its Indochinese or Himalayan origins into the Sunda region and subsequently into the Philippines. This is also consistent with the Borneo origin hypothesis proposed for the

Philippine *Stachyris* (Rand 1970; but see Cibois et al. 2002; Barker et al. 2004). This suggests that the morphological similarities between *Y. zantholeuca* and other yuhinas, especially the presence of a crest, are probably caused by convergent evolution. In fact, when we carefully examined the crest from specimens of *Y. zantholeuca* and another seven yuhina species kept by the Kunming Institute of Zoology of the Chinese Academy of Sciences, we found that the crest of *Y. zantholeuca* is merely composed of crown feathers, while the crest of the other yuhina species comprises both crown and occipital feathers. It is the convergent crest characteristic that has caused this contentious species to be misclassified into *Yuhina*.

Zusammenfassung
Molekulare Phylogenie der Yuhinas (Sylviidae: Yuhina), einer paraphyletischen Gruppe von Brillenvögeln aus Zosterops und Philippinischer *Stachyris*. Um die phylogenetischen Zusammenhänge innerhalb der Yuhinas (*Yuhina*) zu rekonstruieren, wurden Mitochondriensequenzen (2379 bp) von Cytochrom-b Genen, ND3, 12s und 16s rRNA analysiert. Die Yuhinas beinhalten die Brillentimalie *Stachyris whiteheadi* einen Endemiten der Philippinen, den Japanbrillenvogel *Zosterops japonicus* den Rotßanken-Brillenvogel *Z. erythropleurus* sowie den Indischen-oder Ganges-Brillenvogel *Z. palpebrosus*. Die Ergebnisse unterstützen stark die Hypothese, wonach *S. whiteheadi* und drei Brillenvogelarten eine phyletische Einheit innerhalb des *Yuhina*-Zweigs bilden. Die Robustheit des Zweiges *Yuhina*/Philippinische *Stachyris*/*Zosterops* lehnt die Gültigkeit der monotypischen Gattung *Staphida* ab, die die Rotohr-Yuhina *Yuhina castaniceps* auf Grund besonderer morphologischer Merkmale vorgeschlagen worden war. Eine Neuentdeckung stellt das Schwesterntaxa-Verhältnis zwischen Kehlstreifen-Yuhina *Yuhina gularis* und Roststeiß-Yuhina *Yuhina occipitalis* dar, ebenso wie das zwischen Gelbnacken-Yuhina *Yuhina bairdii* und Rotkopf-Yuhina *Yuhina bakeri*. Das sympatrische Verbreitungsmuster der beiden oben genannten Schwesterngruppen in der Himalaya-Region entstand höchstwahrscheinlich im Zuge eines sekundären Kontakts. Unsere molekulare phylogenetische Systematik zeigt auch, dass das Fehlen einer Haube ein einmalig abgeleitetes Eigenmerkmal für den Zweig aus *Zosterops* und Philippinischer *Stachyris* darstellt. Die monophyletische Einheit aus *Zosterops* und Philippinischer *Stachyris* in unserer Arbeit bedeutet außerdem, dass ein gemeinsamer Vorfahr von *Zosterops* und Philippinischer *Stachyris* als Autapomorphie die Fähigkeit zur Langstrecken-Dispersion entwickelt hat, was ihm ermöglichte, über See zu fliegen und die Philippinen zu erreichen.

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