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Phylogeny of the genus *Hirundo* and the Barn Swallow subspecies complex

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ABSTRACT

The cosmopolitan Barn Swallow complex (*Hirundo rustica* and related *Hirundo* species) provides a model system for studies of mate choice, sexual selection, and related topics in behavioral ecology, but the phylogenetic and phylogeographic relationships within this group are not yet completely resolved. We reconstructed the phylogeny of all 14 species of *Hirundo* as well as all six Barn Swallow (*H. rustica*) subspecies using maximum likelihood and Bayesian methods based on sequences of mitochondrial DNA from six protein-coding genes (5217 bp) and one nuclear intron (1039 bp) for most taxa. We found four well-supported clades within the genus, but low support values for one node decreased our ability to determine the relationships among them. *H. rustica* is recently derived and has a wide geographic distribution, and its six subspecies form a monophyletic group with respect to other *Hirundo* species. These subspecies divide into two well-supported clades, geographically corresponding to Asia-America and Europe-Middle East. The former comprises two groups, an East Asian subspecies that is sister to Southeast Asian, American, and Northwest Asian subspecies. In the other clade, European and East-Mediterranean subspecies are intermixed and both show some divergence from the Egyptian subspecies.

1. Introduction

The Barn Swallow species group (genus: *Hirundo*) has long intrigued both scientists and the general public (reviewed in Møller, 1994; Turner, 2006), and it has become a model system for topics in evolutionary and behavioral ecology such as sexual selection, breeding biology and flight behavior. Understanding the historical origins of morphological, life-history and behavioral variation in this group requires a robust phylogenetic hypothesis for the Barn Swallow (*H. rustica*) subspecies that have been the focus of the most intensive research as well as other closely related, and often ecologically similar, species in the genus.

Hirundo is the most species-rich genus in the swallow family Hirundinidae, comprising 14 described species (Turner and Rose, 1989; Dickinson, 2003; Turner, 2004). Because this group of aerial insectivores is characterized by a relatively conservative morphology that provides few characters with which to explore phylogenetic relationships, most recent studies of its evolutionary history have employed molecular markers. Such studies have explored relationships at both the species and subspecies levels. At the species level, reconstructions based on mtDNA and nuclear sequences (Sheldon and Winkler, 1993; Sheldon et al., 2005) showed that the genus *Hirundo* is grouped within the "mud-nesting" swallows together with the crag martins (*Ptyonoprogne*), house martins (*Delichon*), cliff swallows (*Petrochelidon*) and red-rumped swallows (*Cecropis*). The most taxonomically complete phylogenetic survey (Sheldon et al., 2005) resolved many of the species relationships within *Hirundo*, but some nodes remained weakly resolved and two *Hirundo* species were not compared in that study.

Among *Hirundo* species, only the Barn Swallow (*Hirundo rustica*) has a broad Holarctic breeding distribution and exhibits substantial geographic variation. This often-abundant species usually builds its nest in human-made structures such as barns, bridges and buildings (Turner, 2006). There are six currently recognized subspecies (Peters, 1960; Cramp, 1988; Turner and Rose, 1989; Dickinson and Dekker, 2001; Dickinson, 2003). The nominate *H. r. rustica* breeds in Europe, North Africa and Western Asia. There are two Asian subspecies: *H. r. gutturalis* from South and East Asia (eastern Himalayas to Japan and Burma and Northeast Russia) and *H. r. tytleri* from Northwest Asia (Central Siberia and Northern Mongolia). Two subspecies have localized distributions: *H. r. transitiva* in the East-Mediterranean region (Israel, Lebanon, Jordan and Syria) and *H. r. savignii* along the Nile River in Egypt. The New World subspecies *H. r. erythrogaster* breeds throughout most of North America and recently established a growing breeding population in Argentina (Martínez, 1983). These subspecies vary in morphological traits such as body size, ventral coloration, extent of

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breast band, and tail streamer length, and in life-history traits such as migration patterns (Turner and Rose, 1989; Turner, 2004, 2006; Winkler, 2007) and mate choice signals (Møller, 1988, 1994; Safran and McGraw, 2004; Safran et al., 2005). The differential use of sexually selected plumage signals in different populations may contribute to reproductive isolation in areas where these populations come into contact.

A recent study explored the phylogeographic relationships of four of the six Barn Swallow subspecies using mitochondrial and nuclear intron sequence data, with robust sampling across the geographic ranges of several of the more widely distributed subspecies (Zink et al., 2006). That study suggested that the Barn Swallow complex is comprised of three groups: Europe, East Asia, and North America together with Northwest Asia. It also found intriguing evidence of a reverse-colonization of northwest Asia by the North American population, which had itself been derived earlier by colonization from the Old World (Zink et al., 2006).

Despite these recent investigations of *Hirundo* relationships at both the species and population levels, several questions remain open. Here, we augment the species-level phylogeny of the genus by adding the two rare and localized African species not sampled by Sheldon et al. (2005): the Black-and-rufous Swallow (*H. nigrorufa*) and White-tailed Swallow (*H. megaensis*). We also expand the number of mitochondrial markers to increase resolution of the mtDNA gene tree. At the population level within *H. rustica*, we increase sampling to include all recognized subspecies, including the Middle Eastern, *H. r. transitiva*, and the Egyptian, *H. r. savignii,* subspecies that were not sampled by Zink et al. (2006). In addition to providing a more comprehensive phylogenetic hypothesis for these taxa, these analyses allow us to test for the monophyly of *H. rustica* with respect to closely allied *Hirundo* species. Together, these phylogenetic and phylogeographic reconstructions will provide a useful historical framework for studying character evolution in this model avian group.

2. Methods

2.1. Sampling and laboratory methods

We analyzed DNA sequences of 48 individuals representing all 14 generally recognized species in the genus *Hirundo* (Peters, 1960; Turner and Rose, 1989; Dickinson, 2003) and all six subspecies of the Barn Swallow, *Hirundo rustica* (Peters, 1960; Turner and Rose, 1989; Dickinson, 2003; Appendices A and B). *Ptyonoprogne fuligula* (Rock Martin), a member of the sister group to *Hirundo* (Sheldon et al., 2005), as well as *Petrochelidon fulva* (Cave Swallow) and *Tachycineta bicolor* (Tree Swallow) were employed as outgroups for the species-level reconstruction. In order to examine the monophyly of *H. rustica* we included *H. aethiopica* (Ethiopian Swallow), *H. angolensis* (Angolan Swallow) and *H. lucida* (Red-chested Swallow) in the *H. rustica* subspecies comparisons.

Genomic DNA was obtained from tissue using the DNAeasy tissue Extraction kit (Qiagen, Valencia, CA) and from blood using the Perfect gDNA Blood Mini kits (Eppendorf, Westbury, NY). When high-quality tissue was not available, DNA was extracted from museum-skin toe-pads via the ancient DNA protocols described in Lovette and Rubenstein (2007).

We amplified and sequenced six mitochondrial protein-coding regions including complete section of nicotinamide adenine dinucleotide dehydrogenase subunit 2 (ND2), complete section of cytochrome b (Cyt-b), partial section of cytochrome c oxidase subunit I (COI), complete section of cytochrome c oxidase subunit 2 (COII), complete section of ATPases 6 and 8, and one nuclear intron, β-fibrinogen intron 7 (βfib-7, complete section). Only ND2 and Cyt-b were sequenced from toe-pad samples (Appendices A and B). To amplify ND2 we used primers METb and TRPc (Hunt et al., 2001). To amplify Cyt-b we used primers ProgND5F (CACTCTGGCCTAATCAAGTCCTAC), ProgCBR (GGCAGTCTTCAATCT TTGGC), HnigritaCBF (CATCCTCATTATCCTCATATCAACAC), and HnigritaCBR (CTATTAGAGTTGGTTTAGAGTTTGAG). For COI we used primers AvianCOIF1 (seq) and AvianCOIR1 (seq); for COII, ATPases 6 and ATPases 8 we used IL8232I and ILLYSh (Lovette and Rubenstein, 2007) and GQL and HMH (Hunt et al., 2001); for βfib-7 we used FIB-BI7L and FIB-BI7U (Prychitko and Moore, 1997). Numerous primers were used to amplify short regions of ND2 and Cyt-b from toe-pad-derived DNAs, some of which were designed for specific swallow clades or taxa.

PCR amplifications followed the protocol described by Lovette and Rubenstein (2007). Ten microliters PCR amplifications included 1 μL undiluted DNA, 10 μM Tris–HCl, 50 μM KCl, 3–4 mM MgCl2, 0.25 mM of each nucleotide, 0.25 mM from each primer, and 0.025 U jumpstart Taq polymerase (Sigma). PCR amplification conditions were: initial denaturation at 95 °C for 4 min 30 s; 30–35 cycles of denaturating at 95 °C for 1 min, annealing at 54–62 °C for 1 min, and extension at 72 °C for 1 min 30 s–2 min; and a final extension at 72 °C for 4 min 30 s.

PCR products were purified using Exonuclease and Shrimp Alkaline Phosphatase enzymatic reactions (United States Biochemical). Purified products were cycle sequenced in both directions using amplification primers and ABI BigDye Terminator. For βfib-7 we designed internal primers to cross regions in which individuals were heterozygous for insertion/deletions. Sequencing products were cleaned using Sephadex columns and finally processed in an ABI 3730 Automated DNA Analyzer (Applied Biosystems). We aligned forward and reverse strands for each specimen and checked them using Sequencher 4.7 (Gene Codes Corp.). All sequence data are deposited in GenBank (Accession Nos. GU460206–GU460357, Appendices A and B).

2.2. Data analysis

New sequences were combined with data from previous studies of these swallows (Appendix A; Sheldon et al., 2005). To estimate phylogenies, we used maximum likelihood (ML) and Bayesian analysis methods implemented in in RAxML v7.0.3 (Stamatakis, 2006) and MrBayes v3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003), respectively. Because of the heterogeneous distribution of sequence data among taxa, we analyzed *Hirundo* interspecific relationships in three datasets: (1) ND2 and Cyt-b for all specimens; (2); all seven loci for 10 *Hirundo* species and 3 outgroups; and (3) a full analysis using sequences obtained for all species. For phylogenetic analysis of the *H. rustica* subspecies, we compared ND2 and Cyt-b sequences. We did an additional analysis of 13 *H. rustica* specimens (representing five subspecies) that were sequenced also for the nuclear intron βfib-7.

We assessed the compatibility of different gene partitions (6 mitochondrial DNA genes and βfib-7) using the partition homogeneity test (incongruence length difference, ILD) as implemented on PAUP v.4 (Swofford, 2003). Model parameters were estimated for each gene and mtDNA codon position separately. We analyzed the full dataset using two partitioning schemes and evaluated their effect on phylogeny reconstruction using likelihood ratio test (LRT): (1) two partitions, mtDNA and βfib-7; (2) four partitions, mtDNA by codon position and βfib-7. In all analyses deletions in βfib-7 were treated as missing data. We excluded a frame-shifted 10 bp overlap between ATPase 6 and ATPase 8, since each of these bases occupied two codon positions. Because of the short length of ATPase 8, we grouped the ATPases together as one locus in the partitioning schemes.

Maximum likelihood (ML) analyses were applied using the GTR + GAMMA + I model for each partition with 1000 bootstrap

replicates. The most appropriate model for each partition for Bayesian analyses was chosen by comparing their AICc scores in MrModeltest 2.3 (Nylander, 2004). In each analysis two independent runs with four chains were run for 10 million generations (sampling every 1000 generations). Convergence was assessed by examining stationarity in log-likelihood scores as the correlation of split frequencies between runs (AWTY; Nylander et al. 2008). The first 100 trees (100,000 generations) were discarded as burn-in, and the remainders were used to estimate tree parameters and topology.

Uncorrected genetic distances (*p*-distances) from the combined mtDNA protein-coding loci were derived in PAUP v.4 (Swofford, 2003) and used to assess the relative divergence among *Hirundo* species and among *H. rustica* subspecies. One individual for each species or subspecies was used since variation within species or within subspecies was low.

A haplotype network using ND2 and Cyt-b sequence data for the six *H. rustica* subspecies (Appendix B) was estimated using statistical parsimony in the TCS v1.21 software package (Clement et al., 2000).

3. Results

3.1. Sequence characteristics

We aligned sequences at a total of 5217 mtDNA nucleotide sites (706–1041 bp for ND2, 927–1143 bp for Cyt-b, 1517 bp for COI, 684 bp for COII, 824–832 for ATPase 6 and ATPase 8 without the 10 bp overlap region) and 1039 sites (950–1005 bp not including indels) of the nuclear intron βfib-7 for most species. Sequences from two species (*H. nigrorufa* and *H. megaensis*) were derived from toe-pad samples and thus were limited to ND2 and Cyt-b. ND2 and Cyt-b sequences for *H. lucida* and *H. leucosoma* and for the outgroup *P. fuligula* were available from GenBank (Sheldon et al., 2005, Appendix A).

Characteristics of the sequences under the GTR + I + GAMMA model are presented in Table 1. Transitional substitutions predominated in the mitochondrial genes, and to a lesser extent in βfib-7. The proportion of invariant sites was higher in the second codon position compared to the first and third codon positions in protein-coding mitochondrial loci and similar to the overall proportion of invariant sites in βfib-7. The shape parameter (α) of the gamma distribution of rate variation among sites was higher for the nuclear intron and the second codon position of mitochondrial loci indicating lower coefficients of variation among sites. There was little difference among these parameters among mitochondrial loci, justifying the partitioning of mitochondrial loci by codon position.

3.2. Variation among partitions

Because of differences between mtDNA and βfib-7 and among codon positions of protein-coding mitochondrial loci (Table 2), we estimated the parameters for each of the partitions (gene, codon position, and gene and codon position) separately. βfib-7 was considered a separate partition in every analysis. The ILD test did not indicate significant conflict among the different partitions ($P = 0.928$), and thus we concatenated these partitions for combined analysis.

Substitution models chosen using MrModeltest 2.3 for fully concatenated mtDNA codon positions were GTR + I + G for the 1st and 3rd codon position and HKY + I + G for the second codon position, and HKY + I for βfib-7. The combined mtDNA dataset model was GTR + I + G.

Partitioning mtDNA codon position and βfib-7 generated a better fit of the tree to the *Hirundo* data (4 partitions, ML = 21396.9), compared to the mtDNA-βfib-7 partitioning (2 partitions, ML = −23275.7; LRT: $P < 0.00001$). However, the topological results were largely insensitive to the partitioning strategy, as the resulting branching pattern did not vary depending on the partitioning employed. Also, there were no notable differences in nodal support values among the partitioned analyses.

3.3. Genetic distances

We calculated uncorrected *p*-distances for combined mtDNA protein-coding genes using one individual from each species and one individual for each of the *H. rustica* subspecies. These results are summarized in Table 2. Interspecific *p*-distances ranged from 0.7% (*H. megaensis* to *H. dimidiata*) to 11.6% (*H. tahitica* to *H. nigrorufa*). *H. rustica* intraspecific *p*-distances ranged from 0.25% (*H. r. rustica* to *H. r. transitiva*) to 1.6% (*H. r. tytleri* to *H. r. transitiva*).

3.4. Phylogenetic results

3.4.1. Interspecific relationships within Hirundo

We analyzed multiple samples for some of the species (Appendix A), but in every case these conspecific replicates clustered closely together with respect to the more divergent samples of other

Table 1
Estimated model parameters for each of the six loci under the General Time Reversible model of sequence evolution with a gamma model for the rate of heterogeneity and an estimate of the proportion of invariable sites (GTR + I + G).

Locus	Relative substitution rate						Base frequencies				α	$P(I)$
	A–C	A–G	A–T	C–G	C–T	G–T	A	C	G	T		
ND2pos1	0.47	3.13	0.00	0.00	4.73	1.00	0.37	0.28	0.16	0.19	0.18	0.00
ND2pos2	0.56	20.21	0.64	0.00	4.79	1.00	0.17	0.33	0.10	0.40	1.33	0.81
ND2pos3	1.14	50.37	1.79	1.51	28.17	1.00	0.44	0.37	0.05	0.14	3.10	0.00
Cyt-bpos1	0.88	8.56	0.49	0.15	9.05	1.00	0.24	0.30	0.24	0.22	0.64	0.73
Cyt-bpos2	1.42	0.00	1.37	0.00	2.89	1.00	0.20	0.26	0.13	0.41	1000.30	0.92
Cyt-bpos3	0.97	69.19	3.96	2.29	47.38	1.00	0.43	0.42	0.04	0.11	3.10	0.06
COIpos1	0.00	1.06	0.00	0.00	18.46	1.00	0.26	0.24	0.29	0.21	0.77	0.83
COIpos2	4.53	0.00	0.00	0.00	9.52	1.00	0.18	0.27	0.15	0.40	1000.30	0.99
COIpos3	0.82	77.61	2.64	1.43	26.32	1.00	0.40	0.42	0.04	0.14	1.55	0.01
COIIpos1	0.89	3.25	0.96	0.21	15.51	1.00	0.26	0.26	0.29	0.19	0.87	0.72
COIIpos2	0.00	3.72	1.11	1.55	4.11	1.00	0.26	0.27	0.12	0.35	1250.14	0.91
COIIpos3	1.03	53.04	2.50	0.00	28.49	1.00	0.43	0.38	0.06	0.13	3.88	0.04
ATPasespos1	0.36	8.88	1.00	0.00	10.33	1.00	0.31	0.37	0.14	0.18	0.24	0.34
ATPasespos2	0.00	99.71	0.39	0.00	6.80	1.00	0.15	0.32	0.09	0.44	1000.30	0.93
ATPasespos3	1.03	58.81	4.00	0.00	46.17	1.00	0.47	0.36	0.06	0.11	2.91	0.07
βfib-7	0.74	2.49	0.62	2.05	2.07	1.00	0.32	0.18	0.17	0.33	1000.30	0.85

Table 2
Summary of molecular pairwise distances (uncorrected *p*-distances) for all mtDNA data combined among *Hirundo* species and among *H. rustica* subspecies.

Comparison	No. comparisons	Median	Range
Hirundo			
Within 'Pacific Swallow' clade	1	0.051	
Within 'Pearl-breasted Swallow' clade	3	0.081	0.007–0.083
Within 'Blue Swallow' clade	1	0.053	
Within 'Barn Swallow' clade	21	0.071	0.020–0.081
Within *angolensis-lucida-aethiopia*	3	0.025	0.020–0.026
Between *rustica* and *angolensis-lucida-aethiopia*	3	0.028	0.028–0.030
Between *nigrita* and *rustica-angolensis-lucida-aethiopia*	4	0.054	0.051–0.060
Between 'Blue Swallow' clade and 'Pacific Swallow' clade	4	0.097	0.092–0.116
Between 'Pearl-breasted Swallow' and 'Pacific Swallow' clades	6	0.097	0.088–0.111
Between 'Pearl-breasted Swallow' and 'Blue Swallow' clades	6	0.098	0.093–0.112
H. rustica			
Within all subspecies	15	0.014	0.003–0.016
Within the Asian-American clade	3	0.008	0.003–0.008
Within the European-Mediterranean clade	3	0.006	0.003–0.006
Between *H. rustica* and *H. transitiva*	1	0.003	
Between the Asian-American and the European-Mediterranean clades	9	0.015	0.013–0.016

species, and so we present here only one sample per species. Bayesian and ML analysis methods generated similar topologies in all analyses with only subtle differences in bootstrap and posterior probability support values (Fig. 1). In most reconstructions, the majority of the nodes received substantial support. However, separate analyses for each mtDNA gene generated low support for some of the nodes. Therefore, conclusions are based on the full data set. The only generally unresolved node connected the 'Blue Swallow', 'Pearl-breasted Swallow' and the joint 'Pacific Swallow' and 'Barn Swallow' clades.

3.4.2. H. rustica subspecies complex

We included all three species (*H. angolensis*, *H. lucida* and *H. aethiopica*) that are morphologically similar to—and phylogenetically closely allied to—the Barn Swallow in the mtDNA analysis investigating the monophyly of the *H. rustica* complex (Fig. 2a). Variations in analysis methods did not affect the resulting topology or change support values substantially. Our analysis confirmed that the Barn Swallow (*H. rustica*) is indeed monophyletic. An early divergence between the Asian-American and the European-Mediterranean subspecies was highly supported. The intraspecific hap-

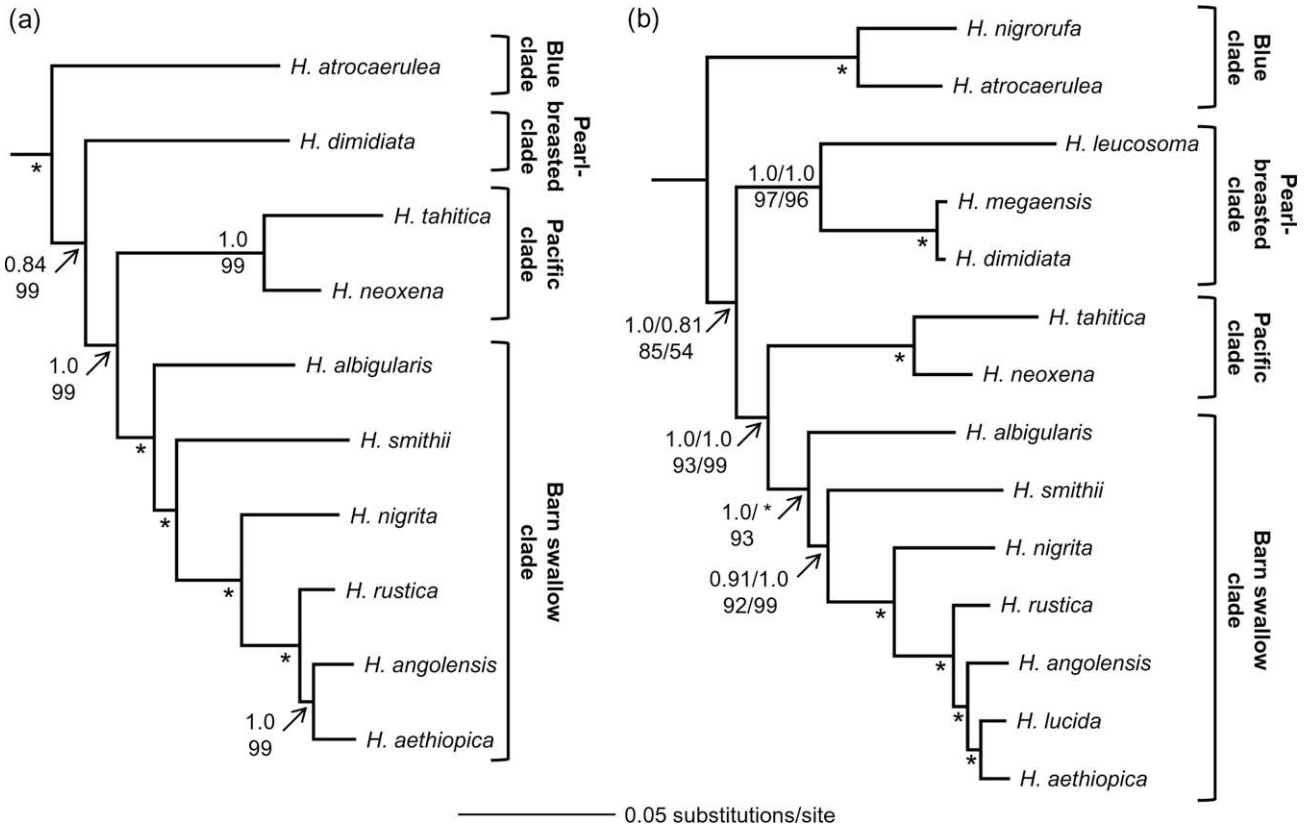

Fig. 1. Consensus trees of the genus *Hirundo*: (a) topology for the 10 *Hirundo* species for which we had complete data for all seven loci (six mtDNA and one nuclear), and (b) topology for all 14 *Hirundo* species based on ND2 and Cyt-b sequence comparisons (lefthand support values) or using all available data for all seven loci (righthand support values). Outgroups for these analysis were *Ptyonoprogne fuligula*, *Petrochelidon fulva* and *Tachycineta bicolor* (not shown). Numbers above nodes indicate the posterior probability from the Bayesian analysis and below nodes the ML bootstrap values of the associated clade. An asterisk indicates posterior probabilities of 1.0 and bootstrap values of 100.

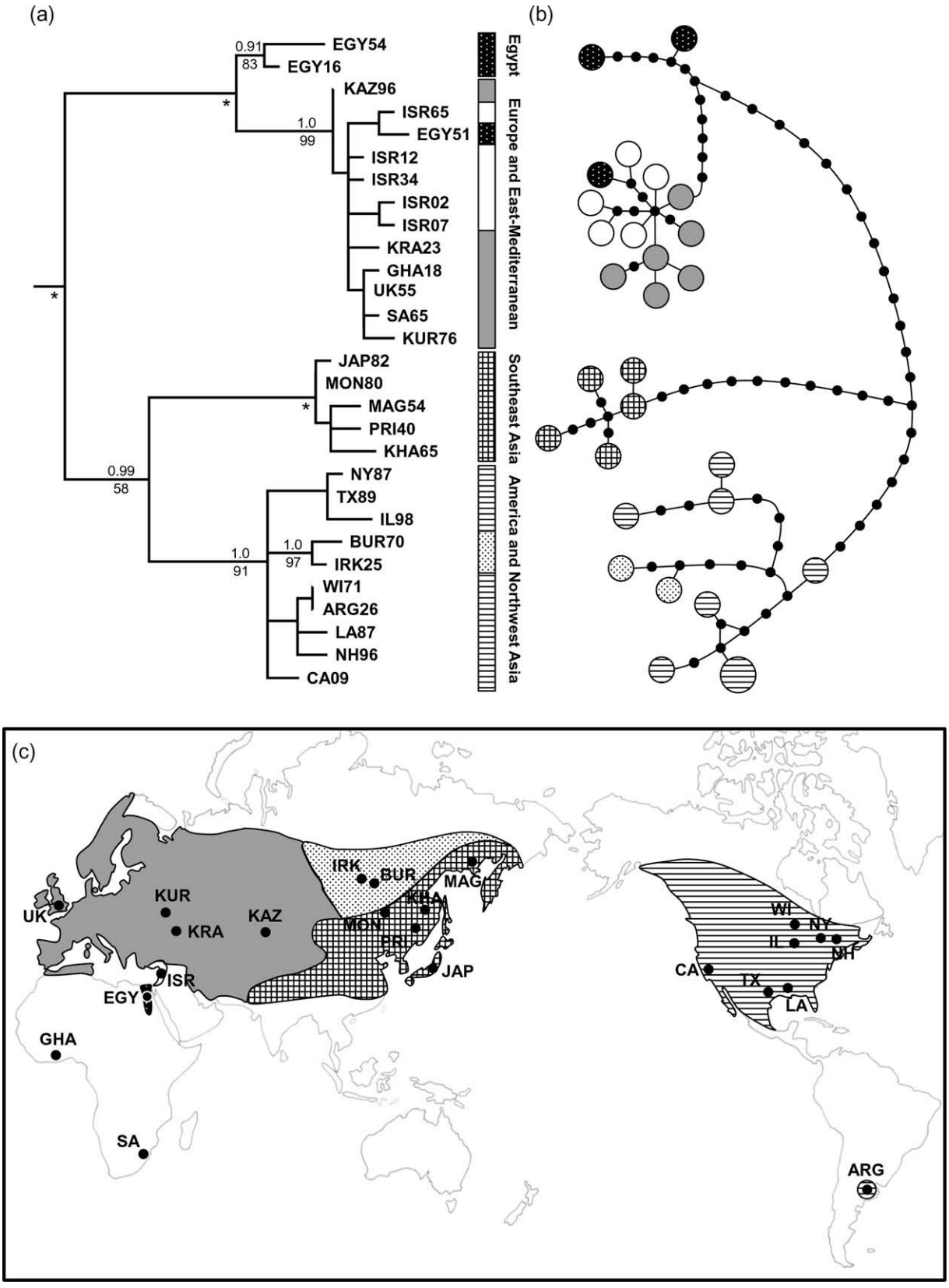

Fig. 2. Phylogeography of *H. rustica* subspecies complex: (a) consensus tree for all specimens from the six subspecies based on ND2 and Cyt-b sequence data. *H. angolensis*, *H. lucida* and *H. aethiopica* (not shown) were included in this analysis to determine the monophyly of *H. rustica*. Numbers above nodes indicate the posterior probability of the Bayesian analysis and below nodes the ML bootstrap values of the associated clade. An asterisk indicates posterior probability of 1.0 and bootstrap value of 100. (b) Unrooted parsimony haplotype network for *H. rustica* computed using TCS v1.21. Area of circles is proportional to number of individuals with that haplotype. (c) Map of the breeding ranges of the six subspecies (Turner, 2004) with sampling localities. Abbreviations for localities are listed in Appendix B. Samples from Ghana and South Africa are from migrating *H. r. rustica*. Patterns indicate the different subspecies: white: *transitiva*, grey: *rustica*, black with white dots: *savignii*, stripes: *erythrogaster*, grid: *gutturalis* and black dots: *tytleri*.

lotype network (Fig. 2b) suggests a minimum of 24 nucleotide changes between those clades (*H. r. savignii* to *H. r. erythrogaster*). The connection between the Southeast Asian subspecies (*H. r. gutturalis*) and the Northwest Asian (*H. r. tytleri*) and American subspecies (*H. r. erythrogaster*) was less supported. The European (*H. r. rustica*) and the East-Mediterranean subspecies (*H. r. transitiva*) are intermingled, with no apparent mitochondrial divergence between them (Fig. 2). Two specimens of the Egyptian subspecies (*H. r. savignii*) form a separate group, while a third specimen falls within the haplotype cluster of the European (*H. r. rustica*) and the East-Mediterranean subspecies (*H. r. transitiva*) (Fig. 2).

The analysis of βfib-7 among 13 *H. rustica* specimens generated 6 haplotypes (nucleotide diversity; $\pi = 0.00031$, haplotype diversity; $h = 0.282$). One haplotype was shared by five individuals representing four subspecies (KUR76, ISR02, IRK25, BUR70, JAP82); three haplotypes were shared by two individuals, each from different subspecies (UK55, ISR65; KAZ96, MAG54; MON80, ISR12); and two haplotypes were unique to individuals from America (ARG26; NY87).

4. Discussion

4.1. Phylogenetic relationships among Hirundo species

Our phylogenetic hypothesis for *Hirundo* includes all 14 species in the genus (Fig. 1). This tree includes four well-supported clades. The largest group, the 'Barn Swallow' clade, is comprised of the Barn, Angolan, Red-chested, Ethiopian, White-throated Blue, Wire-tailed and White-throated swallows. Relationships among all the species within this clade are also well supported. The second group, 'Pacific Swallow' clade, includes the Pacific and Welcome swallows. A close relationship between these two species has long been recognized (Peters, 1960; Turner and Rose, 1989) and was confirmed by Sheldon et al. (2005). The additional data presented here allow us to place the 'Pacific Swallow' clade as sister to the 'Barn Swallow' clade. The third group, the 'Pearl-breasted Swallow' clade, is comprised of the Pearl-breasted, White-tailed and Pied-winged swallows. The close relationship between Pied-winged and Pearl-breasted swallows was also found by Sheldon et al. (2005). Phylogenetic relationships of the White-tailed Swallow (*H. megaensis*), a species endemic to a small region in southern Ethiopia, have not been previously examined, but based on plumage traits it was expected to be close to the Pearl-breasted Swallow (Sibley and Monroe, 1990; Turner and Rose, 1989), and our analysis confirmed this affinity. Indeed, the genetic divergence between *H. megaensis* and *H. dimidiata* (0.7%) is by far the smallest existing among species in the genus (otherwise, range = 2.0–11.6%) and is equivalent to the divergence among populations of the polytypic *H. rustica* (range 0.25–1.6%). *H. megaensis* and *H. dimidiata* inhabit similar habitats, but are separated by a range disjunction of about 1,500 km, and they differ morphologically in tail coloration (white in *H. megaensis* and blue–black in *H. dimidiata*). The fourth group, the 'Blue Swallow' clade, is comprised of the Blue and Black-and-rufous swallows. These two taxa have been thought to be closely related based on their similar morphology (Turner and Rose, 1989; Turner, 2004; but see Brooke, 1974).

Although we were able to link 'Pacific Swallow' and 'Barn Swallow' clades as sisters, we were not able to determine the exact branching topology among the 'Blue Swallow', 'Pearl-breasted Swallow' and joint 'Pacific Swallow' and 'Barn Swallow' clades. This is true despite the substantial sequence length of our mitochondrial markers.

Given these interspecific relationships, the most parsimonious explanation for the current geographic distribution of *Hirundo* is an African origin with subsequent expansion to Asia, Europe, the Pacific islands and Australia. Most (10 of 14) *Hirundo* species are presently restricted to Africa, including all members of the 'Blue Swallow' and 'Pearl-breasted Swallow' clades. The four species that can be found outside Africa belong to the more recent 'Barn Swallow' and 'Pacific Swallow' clades. Two species have broader distributions that also encompass Africa: *H. smithii* in Africa and southern Asia and *H. rustica*, which breeds in most of the northern hemisphere (including North Africa). Only the two species in the 'Pacific Swallow' clade have distributions that do not include Africa: *H. tahitica* in Southeast Asia and the Pacific islands and *H. neoxena* in Australia and New Zealand.

4.2. Phylogeography of the Barn Swallow (H. rustica) subspecies complex

This is the first phylogeographic study to include all six subspecies of *H. rustica* (Fig. 2). Combined analysis of specimens from all subspecies together with the Barn Swallow's closest relatives (*H. angolensis*, *H. lucida* and *H. aethiopica*) has confirmed that the Barn Swallow (*H. rustica*) is monophyletic. This assumption required confirmation since the morphological similarities between those closely related species are remarkable (Turner and Rose, 1989).

Our analysis suggests that the Barn Swallow complex is comprised of two primary clades. The first includes the nominate subspecies (*H. r. rustica*), which has a broad distribution across Europe and central Asia, and two subspecies with Middle Eastern distributions: *H. r. savignii* from Egypt and *H. r. transitiva* from the eastern Mediterranean. The second clade includes the American subspecies, *H. r. erythrogaster*, together with the two exclusively Asian subspecies: *H. r. gutturalis* of Southeast Asia and *H. r. tytleri* from northwest Asia. This division of the Barn Swallow into two major groups is further supported by a conspicuous plumage trait, the extent of the dark breast band; this band is broad and complete in all subspecies within the Europe-Middle East clade, and it is narrow and sometimes incomplete in the subspecies within the Asia-America clade.

The Asian-American clade can be further divided into two groups: (1) the Southeast Asian subspecies (*H. r. gutturalis*) and (2) the American (*H. r. erythrogaster*) together with the northwestern Asian subspecies (*H. r. tytleri*). The sister relationship between *H. r. tytleri* and *H. r. erythrogaster* was well supported by Zink et al. (2006), who suggested that it resulted from a secondary dispersal from North America back into Asia. The population in Argentina (Martínez, 1983) is closely related to North American populations and was apparently recently established by wintering birds from the North (Billerman, unpublished data).

Relationships within the European-Middle Eastern clade are more complicated. The European (*H. r. rustica*) and the East-Mediterranean subspecies (*H. r. transitiva*) form an intermixed group (Fig 2). Two specimens of the Egyptian subspecies (*H. r. savignii*) form a separate group, while a third *H. r. savignii* specimen falls well inside the intermixed *H. r. rustica–H. r. transitive* clade (Fig. 2b). One possibility is that this *H. r. savignii* specimen represents a misidentification of a migrant from the European or the East-Mediterranean subspecies, as the specimen was collected during the period when northern birds were migrating through Egypt in large numbers. However, inspection of the voucher skin specimen showed that it most closely matches the dark plumage coloration expected for the Egyptian subspecies, rather than the lighter coloration typical of the migratory forms. The differing haplotype affinities of the *savignii* specimens may therefore represent either a case of incomplete lineage sorting in this population, or a signature of continued introgressive gene flow into the Egyptian population. In any case, it is most likely that an African ancestor established the Egyptian population and from there it expanded

Appendix A

Specimens, sequences, localities and accession numbers from the genus *Hirundo* included in this study.

Species	Common name	Type[2]	Collection locality	Museum[3]	Sample #	ND2[6]	Cyt-b[6]	COI	COII	ATPases	βfib-7[6]
H. aethiopica	Ethiopian Swallow	T	Cameroon	LSUMNS	B27161	AY826023	AY825964	GU460322	GU460340	GU460206	AY827433
H. albigularis[1]	White-throated Swallow	T	South Africa	LSUMNS	B14070	GU460289	GU460224	GU460323	GU460341	GU460207	GU460266
H. albigularis	White-throated Swallow	T	South Africa	FMNH	SA045	GU460290	GU460225	GU460324	GU460342	GU460208	GU460267
H. angolensis[1]	Angolan Swallow	T	Uganda	FMNH	346239[4]	AY826024	AY825965	GU460325	GU460343	GU460209	AY827434
H. angolensis	Angolan Swallow	T	Uganda	FMNH	384845	GU460291	GU460226	GU460326	GU460344	GU460210	GU460268
H. atrocaerulea	Blue Swallow	T	Tanzania	LSUMNS	B64211[5]	AY826030	AY825971	GU460327	GU460345	GU460211	AY827438
H. dimidiata[1]	Pearl-breasted Swallow	T	South Africa	LSUMNS	B14126	GU460292	GU460227	GU460328	GU460346	GU460212	GU460269
H. dimidiata	Pearl-breasted Swallow	T	South Africa	LSUMNS	B14130	GU460293	GU460228	GU460329	GU460347	GU460213	GU460270
H. leucosoma	Pied-winged Swallow	G	Ivory coast	Sheldon et al.		AY826031	AY825972				
H. lucida	Red-chested Swallow	G	Gambia	Sheldon et al.		AY826022	AY825963				
H. megaesis	White-tailed Swallow	S	Ethiopia	AMNH	348889	GU460294	GU460229				
H. neoxena[1]	Welcome Swallow	T	Australia	LSUMNS	B14189	AY826027	AY825968	GU460330	GU460348	GU460214	AY827436
H. neoxena	Welcome Swallow	T	Australia, NSW	UWBM	77055	GU460295	GU460230	GU460331	GU460349	GU460215	GU460271
H. nigrita	White-throated blue Swallow	T	Equatorial Guinea	UKNHM	8620	GU460296	GU460231	GU460332	GU460350	GU460216	GU460272
H. nigrita[1]	White-throated blue Swallow	T	Equatorial Guinea	YUPM	100555	GU460297	GU460232	GU460333	GU460351	GU460217	GU460273
H. smithii[1]	Wire-tailed Swallow	T	South Africa	LSUMNS	B14117	AY826028	AY825969	GU460334	GU460352	GU460218	AY827439
H. smithii	Wire-tailed Swallow	T	Ghana	FMNH	396521	GU460298	GU460233	GU460335	GU460353	GU460219	GU460274
H. tahitica	Pacific Swallow	T	Papua New Guinea	LSUMNS	B25390	AY826026	AY825967	GU460336	GU460354	GU460220	AY827435
H. nigrorufa	Black and rufous Swallow	S	Congo, Katanga	AMNH	764769	GU460299	GU460234				
H. rustica rustica	Barn Swallow	T	Russia, Kursk	UWBM	49276	DQ176513	GU460237	GU460337	GU460355	GU460221	GU460276
Ptyonoprogne fuligula	Rock Martin	G	South Africa	Sheldon et al.	B14114		AF074581				AY827451

(*continued on next page*)

Appendix A (*continued*)

Species	Common name	Type[2]	Collection locality	Museum[3]	Sample #	ND2[6]	Cyt-b[6]	COI	COII	ATPases	βfib-7[6]
Ptyonoprogne fuligula	Rock Martin	G	Cameroon	Sheldon et al.	B27169	AY826021					
Petrochelidon fulva	Cave Swallow	T	USA, NY	CUMV	51713	GU460300	GU460235	GU460338	GU460356	GU460222	GU460275
Tachycineta bicolor	Tree Swallow	T	USA, NY	CUMV	50502	GU460301	GU460236	GU460339	GU460357	GU460223	

[1]Specimen that were excluded from the final analysis presented in this paper.
[2]Specimen type: B, blood; G, sequence from GenBank; S, toe-pad from museum-skin; T, frozen or buffered-preserved tissue.
[3]Institutional Source of samples: AMNH: American Museum of Natural History, New York, NY, USA; CUMV: Cornell University Museum of Verterbrates, Ithaca, NY, USA; FMNH: Field Museum of Natural History, Chicago, IL, USA; LSUMNS: Louisiana State University Museum of Natural science, Baton Rouge, LA, USA; UKNHM: University of Kansas Natural History Museum, Lawrence, KS, USA; UWBM: University of Washington Burke Museum, Seattle, WA, USA; YUPM: Yale University Peabody Museum of Natural History, New Haven, CT, USA. Sheldon et al. (2005)—details for sequences from GenBank.
[4]Sample 346239 is referred to as JCK1418 in Sheldon et al. (2005).
[5]Sample B64211 is referred to as LSU160566 in Sheldon et al. (2005).
[6]ND2, Cyt-b and βfib-7 accession numbers begin with AY/AF are from Sheldon et al. (2005) and accession numbers begin with DQ are from Zink et al. (2006).

Appendix B

Specimens, sequences, localities and accession numbers of Barn Swallow (*H. rustica*) included in this study.

Species	Type[1]	Collection locality	Museum[2]	Sample #	Abbreviation	ND2[3]	Cyt-b	βfib-7
H. rustica rustica	T	Russia, Kursk	UWBM	49276	KUR76	DQ176513	GU460237	GU460276
H. r. rustica	B	UK, Exeter		759755	UK44	GU460302	GU460238	GU460277
H. r. rustica	T	Kazakhstan, Almati	UWBM	46396	KAZ96	DQ176536	GU460239	GU460278
H. r. rustica	T	South Africa	UWBM	53165	SA65	GU460303	GU460240	
H. r. rustica	T	Russia, Krasnodar	UWBM	64723	KRA23	DQ176527	GU460241	
H. r. rustica	T	Ghana	FMNH	396518	GHA18	GU460304	GU460242	
H. r. transitiva	B	Israel		11765	ISR65	GU460305	GU460243	GU460279
H. r. transitiva	B	Israel		26402	ISR02	GU460306	GU460244	GU460280
H. r. transitiva	B	Israel		11612	ISR12	GU460307	GU460245	GU460281
H. r. transitiva	B	Israel		11707	ISR07	GU460308	GU460246	
H. r. transitiva	B	Israel		11734	ISR34	GU460309	GU460247	
H. r. savignii	S	Egypt	FMNH	250551	EGY51	GU460310	GU460248	
H. r. savignii	S	Egypt	FMNH	256316	EGY16	GU460311	GU460249	
H. r. savignii	S	Egypt	AMNH	559854	EGY54	GU460312	GU460250	
H. r. erythrogaster	B	USA, CA		1941-07809	CA09	GU460313	GU460251	
H. r. erythrogaster	B	USA, NY		3121-84187	NY87	GU460314	GU460252	GU460282
H. r. erythrogaster	T	USA, NH	LSUMNS	B19196	NH96	GU460315	GU460253	
H. r. erythrogaster	T	USA, LA	LSUMNS	B20587	LA87	GU460316	GU460254	
H. r. erythrogaster	T	USA, TX	LSUMNS	B28989	TX89	GU460317	GU460255	
H. r. erythrogaster	B	Argentina		1851-44326	ARG26	GU460318	GU460256	GU460283
H. r. erythrogaster	T	USA, IL	FMNH	431798	IL98	GU460319	GU460257	
H. r. erythrogaster	T	USA, WI	FMNH	436171	WI71	GU460320	GU460258	
H. r. tytleri	T	Russia, Buryatiya	UWBM	46470	BUR70	DQ176557	GU460259	GU460284
H. r. tytleri	T	Russia, Irkutsk	UWBM	51725	IRK25	DQ176567	GU460260	GU460285

H. r. gutturalis	T	Japan	LSUMNS	B16982	JAP82	GU460321	GU460261	GU460286
H. r. gutturalis	T	Russia, Khabarovsk	UWBM	47365	KHA65	DQ176550	GU460262	
H. r. gutturalis	T	Mongolia, Dornod Aymag	UWBM	60080	MON80	DQ176542	GU460263	GU460287
H. r. gutturalis	T	Russia, Magadan	UWBM	43854	MAG54	DQ176551	GU460264	GU460288
H. r. gutturalis	T	Russia, Primor'ye	UWBM	72140	PRI40	DQ176547	GU460265	

[1]Specimen type: B, blood; S, toe-pad from museum-skin; T, frozen or buffered-preserved tissue.
[2]Institutional Source of samples: AMNH: American Museum of Natural History, New York, NY, USA; CUMV: Cornell University Museum of Verterbrates, Ithaca, NY, USA; FMNH: Field Museum of Natural History, Chicago, IL, USA; LSUMNS: Louisiana State University Museum of Natural science, Baton Rouge, LA, USA; UKNHM: University of Kansas Natural History Museum, Lawrence, KS, USA; UWBM: University of Washington Burke Museum, Seattle, WA, USA; YUPM: Yale University Peabody Museum of Natural History, New Haven, CT, USA.
[3]ND2 accession numbers begin with DQ are from Zink et al. (2006).

further east and north to occupy the Middle Eastern and European regions.

Genetic distances among mtDNA sequences of *H. rustica* taxa were very low, and it is therefore not surprising that our analysis of a nuclear intron did not find meaningful nuclear differentiation among these taxa. These low divergences suggest that the rapid expansion of *H. rustica* throughout the world occurred recently, consistent with Zink et al.'s (2006) estimate of around 100,000 years before present. Therefore, the morphologically differentiated *H. rustica* subspecies may represent a radiation in the early stages of differentiation and potential later speciation.

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