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PATTERNS OF GENETIC VARIATION IN THE LITTLE SPIDERHUNTER (*ARACHNOTHERA LONGIROSTRA*) IN SOUTHEAST ASIA

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ABSTRACT. **–** The aim of this study was to determine the intraspecific genetic variation of the mtDNA control region of the little spiderhunter (*Arachnothera longirostra*). Sixty-two individuals representing individuals collected from southern Thailand, peninsular Malaysia, Sabah, Sarawak, Palawan and Mindanao were sequenced, and 508 nucleotides of mtDNA-sequence were compared. Little differentiation was found between peninsular Malaysian and Bornean populations. However Thailand, Palawan and Mindanao populations were distinctly different from the core Sundaic populations. The pattern of divergence among the little spiderhunter populations is congruent with hypothesized land connection and separation during LGM in Southeast Asia, and it is likely that the Bornean and peninsular Malaysian populations probably underwent a recent population expansion. This study also revealed that long-term separation of subregions and islands (e.g., the Philippines from the Greater Sunda islands) promoted substantial differentiation among some of the little spiderhunter populations.

KEY WORDS. **–** Little spiderhunter; genetic variation; mitochondrial DNA control region; Last Glacial Maximum; phylogeography.

INTRODUCTION

The little spiderhunter (*Arachnothera longirostra*) is a member of the sunbird family (Nectariniidae) and is widely distributed in southern Asia. It ranges from India and China in the north and throughout insular Southeast Asia in the south, including Sumatra, Borneo, Java, Bali, and the southern Philippines (Cheke & Mann, 2008). It does not reach Lombok or Sulawesi, areas east of Wallace line. The little spiderhunter is divided into 13 subspecies (Rand, 1967; Dickinson 2003; Cheke & Mann, 2008): *A. l. longirostra* (India across northern Indochina to southwestern Yunnan), *A. l. sordida* (southern Yunnan and northeastern Indochina), *A. l. pallida* (southeastern Thailand and southern Indochina), *A. l. cinereicollis* (Malay Peninsula and Sumatra), *A. l. zharina* (Banjak Island off western Sumatra), *A. l. niasensis* (Nias Island off western Sumatra), *A. l. prillwitzi* (Java), *A. l. rothschildi* (northern Natuna islands), *A. l. atita* (southern Natuna islands), *A. l. buettikoferi* (Borneo), *A. l. dilutor* (Palawan), *A. l. flammifera* (Samar, Leyte, Bohol, Mindanao), and *A. l. randi* (Basilan). It can be found from lowlands to about 1,500 m above sea level (Sibley & Monroe, 1990) and occupies primary forests, second–growth forests (or secondary forests), farmlands, and gardens, wherever there are plenty of wild bananas and gingers for it to feed on (MacKinnon & Phillipps, 1993; Smythies, 1999). The little spiderhunter is an excellent species for phylogeographic study because it is common, has a wide geographic range, exhibits substantial geographic variation, and is unusually easy to catch in mistnets. Since the little spiderhunter inhabits both primary and secondary forests, it is likely that gene flow occurred during the Last Glacial Maximum (LGM), 10,000–20,000 Ma, when the Sunda Islands were connected to the mainland by land bridges.

During the LGM, the effects of lowered sea levels in Southeast Asia were extreme and resulted in the exposure of the Sunda continental shelf and the physical connection of major Sunda landmasses such as the Malay Peninsula, Sumatra, Java, Borneo, and many smaller islands, into a landmass known as Sundaland (Inger & Voris, 2001; Inger, 2005; Sathiamurthy & Voris, 2006). Palawan was also part

of Sundaland. It was connected to north Borneo when the sea level decreased to 160 m below its present level during the middle Pleistocene period (Heaney, 1986). However, the other Philippines islands, and Sulawesi and Lombok, were never part of Sundaland, even during the LGM (Heaney, 1991, Sathiamurthy & Voris, 2006). About 6.07 Ka, rapid marine transgression reduced land surface areas to their present limits (Inger & Voris, 2001; Sathiamurthy & Voris, 2006). Also during the LGM, changes in climatic conditions are presumed to have exerted a great influence on vegetation and thus habitats in the region (Heaney, 1991; Voris, 2000). Connected land masses during the LGM were most likely dominated by savanna-like vegetation, with gallery forests along rivers (Verstappen, 1975; Morley & Flenley, 1987; Sun et al., 2000; Sathiamurthy & Voris, 2006), which would have limited faunal movements, although some believe that there was still adequate habitat for corridors to permit movement of flora and fauna among present-day Sunda islands (Verstappen, 1975; Sun et al., 2000; Sathiamurthy & Voris, 2006). In any event, the historical events of the Quaternary, and especially of the Pleistocene, undoubtedly influenced many of the current patterns of avifaunal distribution in the Southeast Asian region (Heaney, 1991; Michaux, 1998).

The classic studies of animal biogeography (e.g., Wallace, 1896; Mayr, 1944; Banks, 1937; Darlington, 1957) are insufficient to explain the processes of species dispersal, distribution, and diversification in Southeast Asia. Knowledge of such processes depends upon understanding historical (phylogenetic) relationships among taxa (Brooks, 1990). For higher-level taxa, such as genera, and families of animals, many methods have been developed to extract the signal of common biogeographic history from estimated phylogenies (Donoghue & Moore, 2003). For comparisons of lower-level taxa, such as closely related species or populations within a single species, the most appropriate method of inquiry is phylogeography (Avise, 2000). Based on molecular genetic comparisons, this method combines the strengths of phylogenetics and population genetics. Phylogeography can be used to unravel evolutionary questions such as demographic history of populations and species, the role of gene flow in uniting populations, and causes of geographic and genetic variation among populations (Avise et al., 1987; Avise, 1998; Arbogast & Kenagy, 2001; Modolo et al., 2005). Comparison of mitochondrial DNA (mtDNA) is the most common method of performing phylogeographic studies. Although somewhat limited as a single locus, this genome exhibits certain especially useful properties, including maternal inheritance leading to small effective population size, lack of recombination, and a generally rapid rate of evolution compared to single-copy nuclear loci (Avise et al., 1987).

In this study, we present an intraspecific phylogeographic analysis of the little spiderhunter by comparing sequences of the highly variable mtDNA control region. In particular, we focused on geographic variation of populations from Thailand, peninsular Malaysia, Borneo, Palawan and Mindanao. Given the substantial current separation between mainland and island sites, we could expect to see strong phylogeographic structure. If, however, populations were connected across the Sunda shelf during the LGM, the molecular diversity will be less geographically structured because of past gene flow, possibly with a signature of population expansion.

MATERIALS AND METHODS

Sample Collection

We sequenced DNA extracted from blood and tissue samples of 62 individuals of four subspecies of little spiderhunter from nine sites in Southeast Asia (Fig. 1; Table 1). On Thailand, we sampled 10 individuals of *A. l. pallida* from Thale Ban National Park. On peninsular Malaysia, we sampled 10 individuals of *A. l. cinereicollis* from Taman Negara National Park. On Borneo, 36 individuals of *A. l. buettikoferi* were sampled from four sites: Matang Wildlife Centre, Sarawak; Kubah National Park, Sarawak; Poring Hot Springs, Sabah; and Tawau Hills Park, Sabah). Robert S. Kennedy donated four samples of *A. l. flammifera* from east Mindanao (CMNH35871 and CMNH35811) and south Mindanao (CMNH36997 and CMNH36998), and two samples of *A. l. dilutor* from Palawan (CMNH34853 and CMNH34856) (CMNH is an abbreviation for Chicago Field Museum of Natural History). Blood samples from Thailand, peninsular Malaysia and Borneo were obtained by non-destructive methods (Taberlet & Luikart, 1999). The samples from Palawan and Mindanao were tissues from vouchered specimens. The thick-billed spiderhunter (*A. crassirostris*) (MR50tbsh – personal collection of Mustafa Rahman number 50), which was captured by mist-net at Lambir Hill National Park, Sarawak, served as outgroup.

Laboratory Methods

Phenol-chloroform (Sambrook et al., 1989) and salt precipitation methods were used to extract genomic DNA

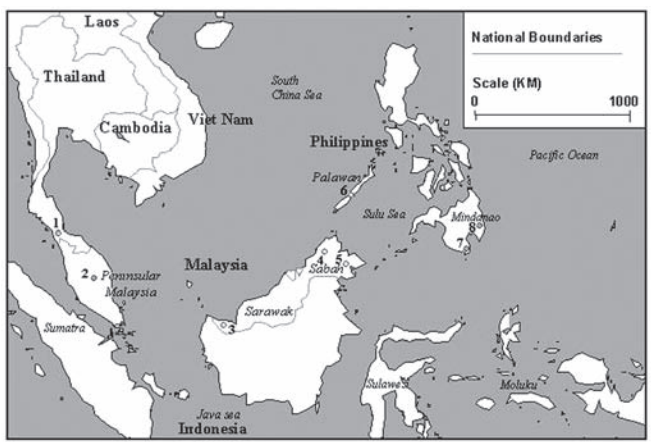

Fig. 1. Map showing sampling sites. The sites are numbered as follows: 1=Thale Ban National Park, Thailand; 2=Taman Negara National Park, Malaysia; 3=Matang Wildlife Centre and Kubah National Park, Sarawak; 4=Poring Hot Springs, Sabah; 5=Tawau Hills Park, Sabah; 6=Palawan; 7=South Mindanao; and 8=East Mindanao.

Table 1. Sampling sites and haplotype frequencies.

Sampling sites	**Thale Ban National Park**	**Taman Negara National Park**	**Matang Wildlife Centre**	**Kubah National Park**	**Poring Hot Spring**	**Tawau Hills Park**	**Palawan**	**East Mindanao**	**West Mindanao**
N / haplotypes	10	10	10	10	6	10	2	2	2
S1	–	–	–	–	–	–	–	2	2
S2	–	–	3	4	1	–	–	–	–
S3	–	–	–	1	–	–	–	–	–
S4	–	–	1	–	–	–	–	–	–
S5	–	–	1	–	–	–	–	–	–
S6	–	1	–	–	–	–	–	–	–
S7	–	–	1	–	1	–	–	–	–
S8	–	–	–	–	–	1	–	–	–
S9	–	1	–	–	–	–	–	–	–
S10	–	1	–	–	–	–	–	–	–
S11	–	–	–	–	1	–	–	–	–
S12	–	–	–	–	–	1	–	–	–
S13	–	–	–	–	–	1	–	–	–
S14	–	–	–	–	–	1	–	–	–
S15	–	–	–	–	–	2	–	–	–
S16	–	–	–	–	–	1	–	–	–
S17	–	–	–	–	–	3	–	–	–
S18	–	1	–	–	–	–	–	–	–
S19	–	1	–	–	–	–	–	–	–
S20	–	–	–	1	–	–	–	–	–
S21	–	1	1	1	2	–	–	–	–
S22	–	–	–	1	1	–	–	–	–
S23	–	–	2	–	–	–	–	–	–
S24	–	1	–	–	–	–	–	–	–
S25	–	–	1	–	–	–	–	–	–
S26	–	1	–	–	–	–	–	–	–
S27	–	1	–	1	–	–	–	–	–
S28	1	–	–	–	–	–	–	–	–
S29	1	–	–	–	–	–	–	–	–
S30	1	–	–	–	–	–	–	–	–
S31	1	–	–	–	–	–	–	–	–
S32	1	–	–	–	–	–	–	–	–
S33	2	–	–	–	–	–	–	–	–
S34	2	–	–	–	–	–	–	–	–
S35	1	–	–	–	–	–	–	–	–
S36	–	–	–	1	–	–	–	–	–
S37	–	1	–	–	–	–	–	–	–
S38	–	–	–	–	–	–	2	–	–

from blood. For tissue samples, the Chelex 5% extraction method (Singer-Sam et al., 1989) was employed. Procedures for isolation and purification of mtDNA followed Sambrook et al., (1989) and Hillis et al., (1996). PCR amplifications were done in 25 µl reaction volumes, which included 2.5 µl 10X PCR buffer (Perkins Elmer), 2 µl $MgCl_2$ (25 mM), 0.5 µl of each primer (10 µm), 0.1 *Taq* polymerase (Amplitaq Perkins Elmer), 4 µl mtDNA template and 15.2 µl MilliQ water was added to the final volume of 25 µl. The amplifications were performed in the FTS-1 Thermal Sequencer (Corbett Research) and GeneAMP® PCR System 9700. The primers used were LND6-1 (5'-CCC ATA ATA CGG CGA AGG ATT AGA- 3') (kindly provided by Dr T. J. Parsons, Armed Forces DNA Identification Laboratory, Maryland, USA) and H417 (5'- ACG AGA ACC GAG CTA CT- 3') (Tarr, 1995). Both primers were used to amplify ≈ 711 bp of the first half of the hypervariable control region. The amplification began with initial denaturation for two minutes at 94°C, followed by reaction cycles consisting of denaturation for 45 seconds at 94° C, primer annealing for 45 seconds at 50°C, and extension for 15 seconds at 72°C. Cycles were repeated 35 times, with the final cycle including a final extension at 72°C for five minutes. PCR products were purified either by gel purification (Rahman, 2000) or Qiagen purification kit (Qiagen). Sequencing products were precipitated by applying the sodium-precipitation method. Gel separations of sequencing products were done on ABI model 377 DNA sequencer.

Data analysis

Chromatographs were aligned using SEQUENCHER v.4.1 (Genecodes). Both strands (heavy and light) were sequenced for approximately 600 bp of the total 711 bp fragment. Consensus sequences were then aligned using the Clustal X (Thompson et al., 1997). For phylogenetic analysis, we estimated phylogenetic trees using maximum likelihood (ML) and maximum parsimony (MP) methods implemented in PAUP* 4.0b10 (Swofford, 2002). MODELTEST 3.7 (Posada & Crandall, 1998) was used to determine an appropriate model of nucleotide substitution and parameter values for ML tree-building. Heuristic bootstrap analysis was performed with 1,000 replicates for MP and 100 replicates for ML. Then, 50% majority-rule consensus trees were computed from previous MP and ML bootstrap replicates. To investigate relationships among the 38 haplotypes generated from the 62 sequences (NCBI Accession Number AY968313 – AY968374), we constructed a Minimum Spanning Network (Kruskal, 1956; Prim, 1957) from the matrix of pairwise distances calculated between all haplotypes (Rohlf, 1973) using ARLEQUIN 3.11 (Excoffier, 2006). Values of nucleotide sequence diversity (π) within populations, and divergence among populations *Dxy* (Nei, 1987) were estimated using DnaSP version 4.0 (Rozas & Rozas, 1999).

Analysis of molecular variance (AMOVA) in ARLEQUIN 3.11 (Excoffier, 1995; Excoffier et al., 2006) was used to estimate the distribution of variation within and among populations using the settings of pairwise difference and 1000 permutations. First, AMOVA was performed for all six populations (Mindanao, Palawan, Sabah, Sarawak, peninsular Malaysia, and Thailand). All the six populations were lumped together as one group for first analysis. Subsequent analysis was performed by grouping the populations into two groups according to their geographical locations (the Malay Peninsula versus Borneo, excluding Thailand). To compare demographic histories of the three subspecies based on the phylogenetic analysis, we performed mismatch distribution analyses (Rogers & Harpending, 1992) in ARLEQUIN 3.11.

RESULTS

Phylogenetic Analyses

Analysis of mtDNA variation among the little spiderhunter samples was based on 508 nucleotides (the shortest sequence after editing) of the first (5') half of the control region. Across all sequences, 63 of the 508 sites were polymorphic (12.4%). A total of 38 haplotypes, differing by 1 to 38 changes from each other, were identified using Collapse 1.2 (Posada, 1999) (Table 1). Gaps were considered a fifth base. The most appropriate ML model determined by MODELTEST was HKY+G (Hasegawa et al., 1985), with the following parameters: base frequencies, A=0.2846, C=0.2999, G=0.1657, T=0.2498; gamma distribution α=0.2802, and transition/transversions ratio 1.5041. Both MP and ML analyses produced nearly the same majority-rule consensus topologies (Fig. 2), which indicate little structure between peninsular Malaysia and Bornean haplotypes. The Thailand population was, however, distinct, as were the Palawan and Mindanao populations.

Table 2 presents the sequence divergence among the populations. The most remarkable differences were between Mindanao and other populations (~8%). Bornean and peninsular Malaysian populations differed only by 0.39%–0.48%. Thailand and peninsular Malaysia populations differed by 1.11% across the Isthmus of Kra.

Population Genetics

In the minimum spanning network (Fig. 3), the phylogenetic pattern of the peninsular Malaysia and Bornean populations resembles a star phylogeny, in which eight haplotypes (S3-S5 and S7-S11) stem as slight variants from the widespread haplotype S21. This haplotype, shared by five individuals, occurred in four individuals from three sampling sites in Borneo and one individual from peninsular Malaysia. The most abundant haplotype, S2 (N = 8), which is shared by eight individuals in Borneo, is connected via haplotype S6 from peninsular Malaysia, to form a sub-cluster with haplotype S21. This qualitative pattern which is based on the number of haplotypes and the distances between them, suggests a history of recent connection and population expansion across Borneo and peninsular Malaysia (Slatkin & Hudson, 1991). The Thailand population, consisting of haplotypes

Table 2. Matrix of nucleotide diversity, π (above diagonal), and sequence divergence, D*xy* (below diagonal), among subregions respectively.

	Mindanao	Palawan	Sabah	Sarawak	Peninsular Malaysia	Thailand
Mindanao	–	0.050	0.031	0.027	0.027	0.032
Palawan	0.076	–	0.010	0.009	0.009	0.014
Sabah	0.080	0.020	–	0.005	0.004	0.009
Sarawak	0.080	0.020	0.006	–	0.004	0.008
Peninsular Malaysia	0.079	0.019	0.005	0.004	–	0.009
Thailand	0.087	0.011	0.012	0.012	0.011	–

S28-S35, forms a separate cluster, which suggests that the population may have been isolated from peninsular Malaysia and Borneo.

Statistical Analyses

Measures of sequence divergence (D*xy*) and nucleotide diversity (π) within and among the six selected populations are shown in Table 2. AMOVA revealed a significant difference between all populations (Table 3). Differences among populations within groups accounted for 71.88% of the variance, whereas 28.12% was referred to the partitions within populations (or attributed to variation among individuals within populations). *Fst*, estimated at 0.72, signified low gene flow among the six populations. When the analysis was repeated excluding the distinct Thailand population (yielding peninsular Malaysia versus Borneo), 9.09% of the variation occurred between Borneo and peninsular Malaysia and the *Fst* dropped to 0.19 (Table 4), signifying much higher gene flow.

The minimum spanning network (Fig. 3) suggests that the Bornean and Malay Peninsula populations may have

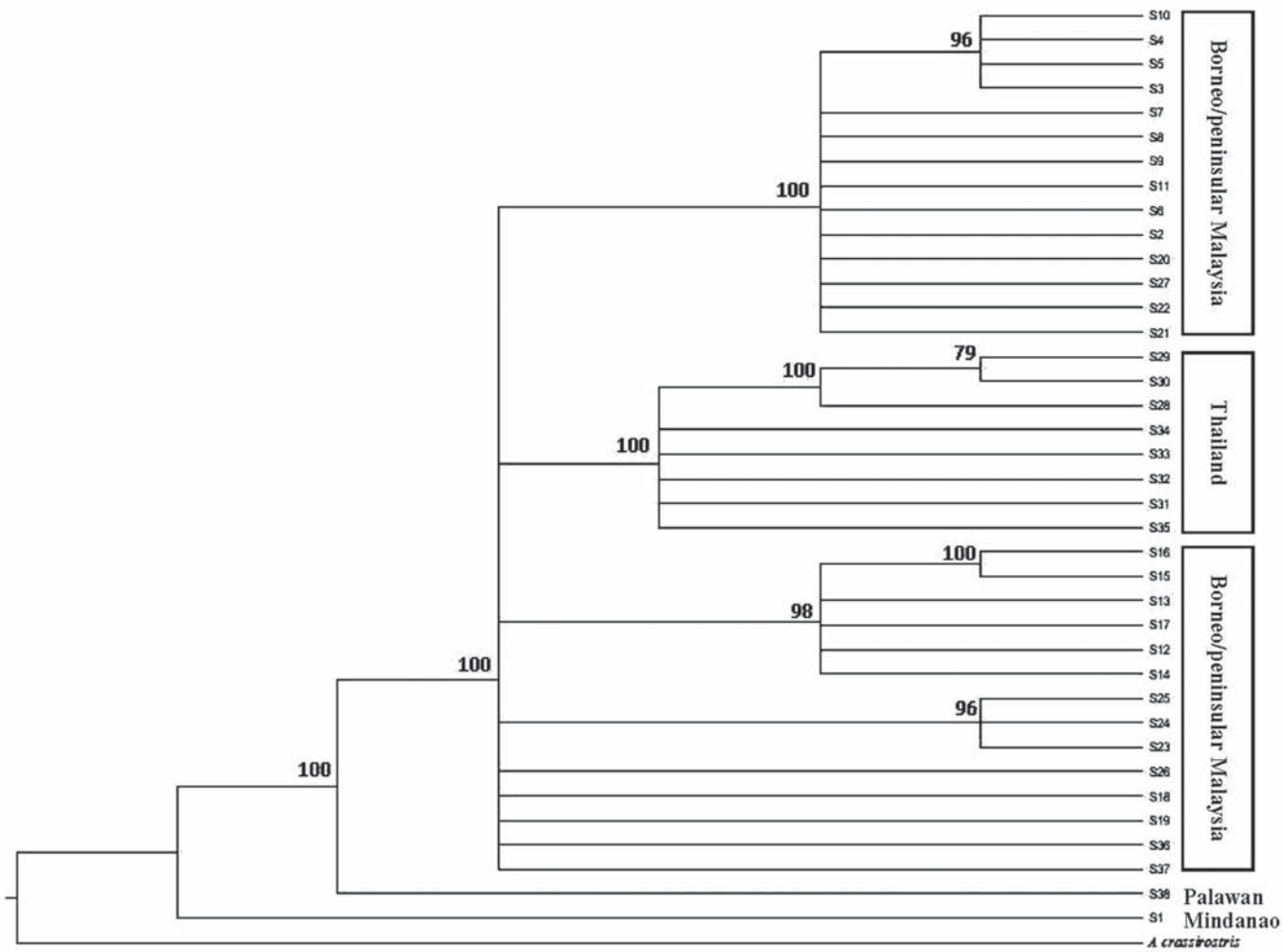

Fig. 2. Maximum parsimony majority rule consensus tree for all haplotypes of little spiderhunter and for the outgroup (*A. crassirostris*). Bootstrap values estimated for maximum parsimony are shown above node.

Table 3. Hierarchical analysis of variance (AMOVA) of six populations of little spiderhunter (Mindanao, Palawan, Sabah, Sarawak, peninsular Malaysia, and Thailand).

Hierarchical structure	Source of variation	Sum of squares (df)	Variance component	*p*-value	Fixation index	Percentage of variation
1	Among populations	227.88 (3)	4.09	< 0.00001		71.88
2	Within populations	86.63 (54)	1.56	<0.00001	F_{ST} = 0.72	21.12

Table 4. Hierarchical analysis of variance (AMOVA) of two groups: Borneo, comprising Sabah and Sarawak, and peninsular Malaysia (excluding Thailand).

Hierarchical structure	Source of variation	Sum of Square (*df*)	Variance component	*p*-value	Fixation index	Percentage of variation
1	Among groups	4.193 (1)	–0.18	0.82	F_{CT} = –0.09	–9.09
2	Among population Within groups	19.233 (3)	0.29	< 0.00001	F_{SC} = 0.26	28.30
3	Within populations	63.900 (41)	1.56	< 0.00001	F_{ST} = 0.19	80.79

undergone historical expansion. Sudden expansion is supported by mismatch distribution analysis (Table 5). A test of goodness of fit (SSD and Harpending's Raggedness Index test) indicated no significant difference between observed and expected mismatch counts, leading to acceptance of the hypothesis of sudden population expansion. This conclusion is further supported by the mismatch distribution plot of pairwise differences (Fig. 4), which was unimodal (Schneider & Excoffier, 1999).

DISCUSSION

Population structure of the little spiderhunter

At present, populations of the little spiderhunter from the Malay Peninsula are physically separated from their Bornean conspecifics by the South China Sea. However, these two land

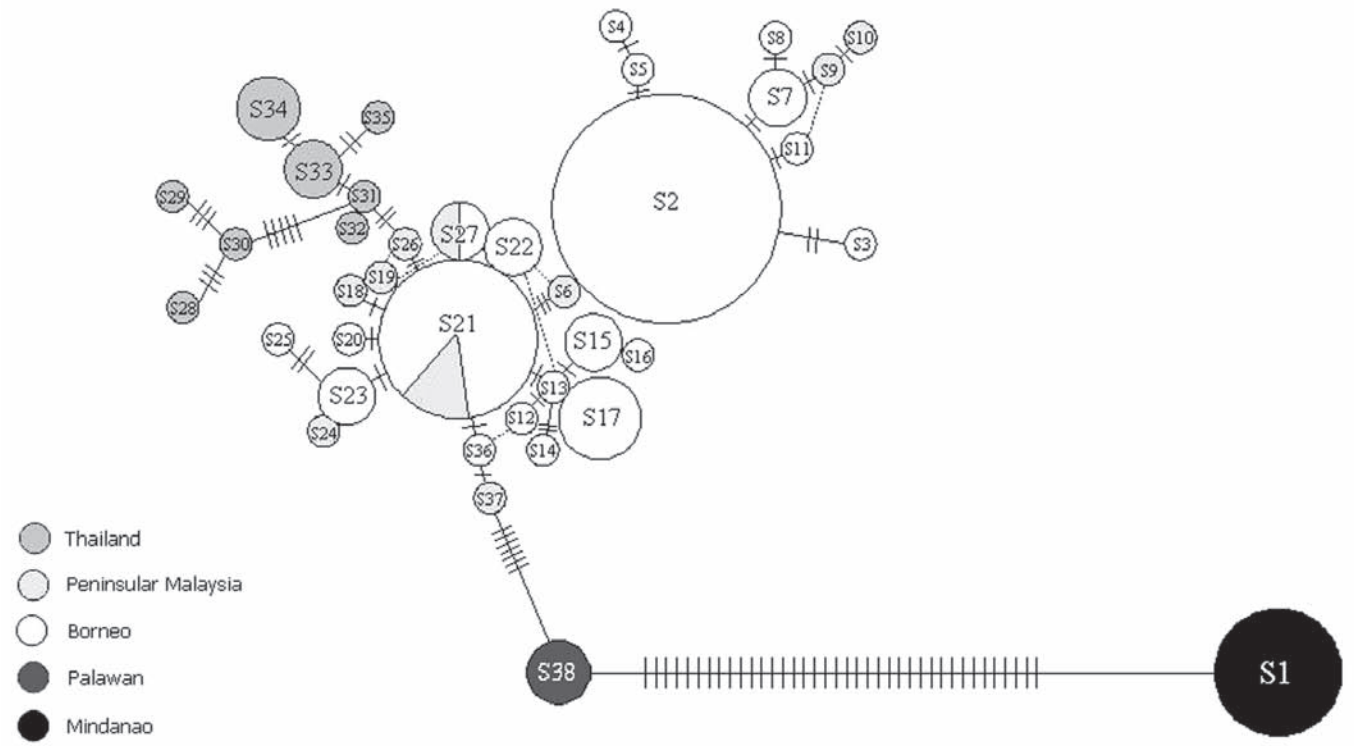

Fig. 3. Minimum spanning network of 38 haplotypes found by sequencing the mtDNA control region of little spiderhunter. Each hatch on branches corresponds to one mutation. Numbers in circles represent haplotype numbers (Table 1). The area of the circles in the network reflects haplotype frequency, with the smallest circle equal to one and the largest equal to eight. Haplotypes are shaded to match respective populations on the map (Fig. 1). Haplotypes without intervening branches differ by gaps.

Table 5. Mismatch analyses results.

Group	τ (Low bound; Upper bound)	Observed	θ_0 (Low bound; Upper bound)	θ_1 (Low bound; Upper bound)	Raggedness (*p* value)	SSD (*p* value)
Peninsular Malaysia and Borneo	4.203 (1.928; 5.713)	3.726	0.002 (0.000; 2.081)	38.672 (12.852; 99999)	0.0168 ($p > 0.05$)	0.00202 ($p > 0.05$)

Note: Parameters such as τ (unit of mutational time), the observed mismatch mean (Observed), the mutation parameter before ($\theta_0 = 2N_o\mu$) and after ($\theta_1 = 2N_1\mu$) population expansion, Harpending's raggedness index (Raggedness), and the sum of standard deviation (SSD) were estimated under a sudden demographic expansion model for both peninsular Malaysia and Bornean haplotypes, according to the minimun spanning network (Fig. 3) and phylogeny (Fig. 2). Values in parentheses refer to 95% (1- α) confidence intervals. Lower and upper bounds represent $\alpha/2$ and 1- $\alpha/2$ percentiles of the distribution (Efron & Tibshirani, 1993), respectively. The alpha (α) value used for this analysis was 0.05.

masses were connected to one another several times during the Pleistocene when sea levels fell as a result of glaciation in the Northern Hemisphere. The last connection was believed to have taken place in the Wurmian glacial maximum during the late Pleistocene (about 18,000–10,000 years ago), when the sea level dropped to 120 m below the present level (Sartono, 1973; Heaney, 1991; Inger & Voris, 2001).

Historical connectivity between the peninsular Malaysian and Bornean populations is evident from their low sequence divergence (0.39% to 0.56%), shared haplotypes, and low Fst values. One haplotype in particular, S21, is probably ancestral among the two populations. On the other hand, the Thai population differs from the peninsular Malaysian and Bornean populations. Its haplotypes form a monophyletic cluster (Fig. 2 and Fig. 3) differing from the peninsular Malaysian haplotypes is 1.11%.

Speculation on historical forest refugia during the LGM

Although the Malay Peninsula and Borneo are currently separated by the South China Sea, the low genetic divergence between Taman Negara National Park and the Bornean populations suggests that these two populations were likely connected during the LGM. Together with the strong signature of population expansion, we suggest that the little

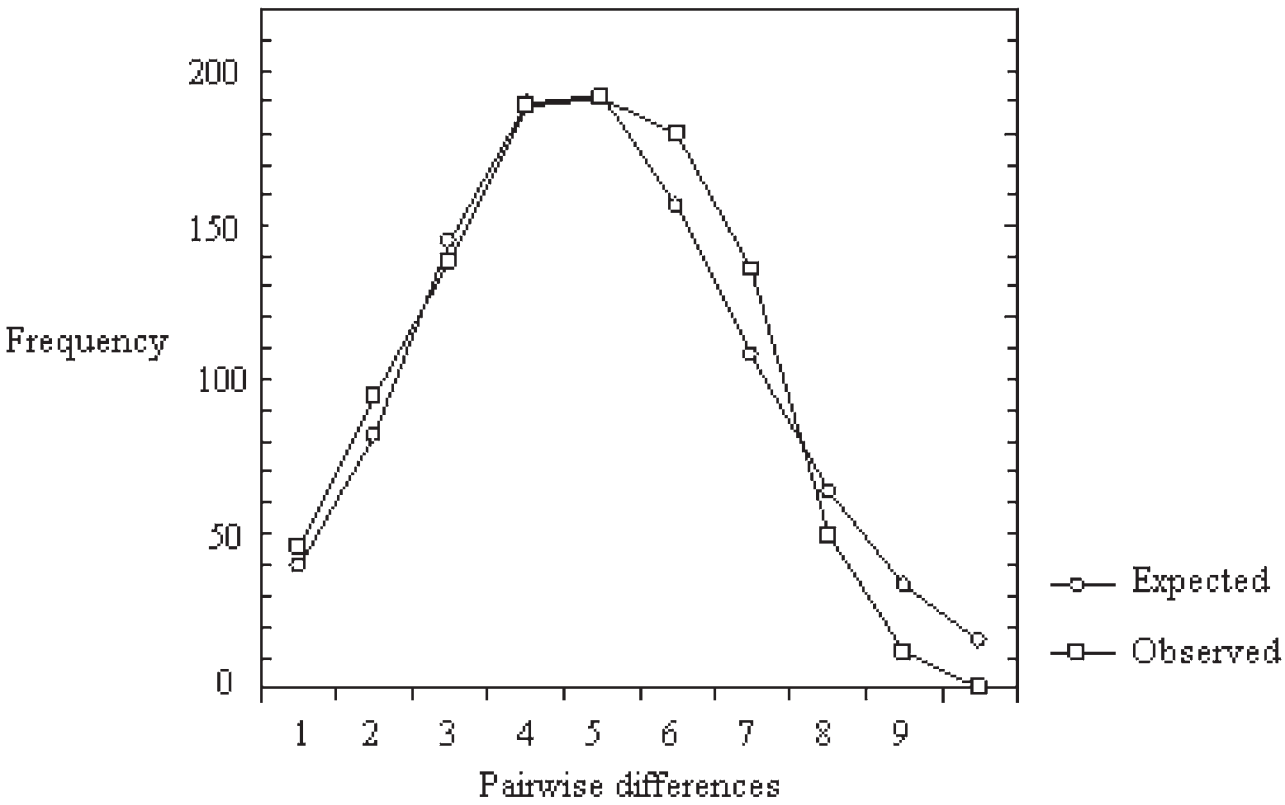

Fig. 4. Frequency distribution of pairwise sequence differences among little spiderhunter individuals from the Malay Peninsula and Borneo populations.

spiderhunter was largely reduced to a single refugial area on the Sunda shelf during the LGM. The relative genetic distinctiveness of the southern Thailand and Palawan populations suggests that they could have occupied distinct refugia. However, our sampling scheme does not allow us to determine the geographic locations of the refugia. One way to identify possible refugial sites is by relating them to present-day endemic areas, which are assumed to have been areas of relative ecological stability (Fjeldsa & Lovett, 1997; Taylor et al., 1999). In Southeast Asia, avian endemic areas are found in the heart of Borneo, Palawan, the west-central part of peninsular Malaysia, and in northern and western part of the Sumatra. Another approach is to model the potential range of the species or its rainforest habitat under relevant paleoclimatic scenarios (Hugall et al., 2002), though this is beyond the scope of the current study.

Divergence among the little spiderhunter subspecies

MtDNA control region sequence comparisons revealed substantial divergence in two of the four subspecies of little spiderhunter. Most striking is the genetic divergence between the Mindanao *A. l. flammifera* and the other subspecies: 7.6%–8.7% when compared to the Malay Peninsula *A. l. cinereicollis* and Bornean *A. l. buettikoferi*, and 7.6% to the Palawan *A. l. dilutor.* Although these differences cannot be compared directly to the better known mtDNA divergences of cytochrome *b* and ND2 genes (Johns & Avise, 1998; Zou et al., 2007), they still signal substantial genetic differentiation. The population on Mindanao would be expected to have diverged substantially from the Palawan and Bornean populations for two main reasons. First, Mindanao has never been connected to Palawan, and thus little gene flow is expected between the islands. Second, the Mindanao population of little spiderhunter must have been colonized by long distance dispersal. Thus, the founding population would have been small and could have differentiated relatively rapidly due to founder effects. The genetic distinctness of the Mindanao populations combined with morphological differences (observed by Tweeddale when he classified the Mindanao population as subspecies *A. l. flammifera* in his manuscript published in Proceedings of Zoological Society, London 1878), it would seem to indicate that *A. l. flammifera* should be elevated to full species level. The same may also be true of *A. l. dilutor* on Palawan. Although this taxon differs from the Bornean and peninsular Malaysian populations by only 2.7%, it is highly distinctive morphologically, having generally drab plumage with a bright yellow eye ring (R. Moyle pers. comm.). Palawan has been separated from Borneo for a longer period of time than Borneo has from mainland Asia, possibly for as long as 160,000 years (Heaney & Rickart, 1990), and this isolation has apparently allowed for substantial differentiation between Palawan and Borneo.

On the other hand, the subspecific designations for the Bornean and Thai-Malay Peninsula populations do not indicate natural genetic boundaries of taxa. The close relationship between the Bornean *A. l. buettikoferi* and the Thai-Malay Peninsula *A. l. cinereicollis* (particularly the population from Taman Negara National Park in peninsular Malaysia) suggests that the current subspecific designations (Rand, 1967; Dickinson, 2003) do not reflect major historical subdivisions within the species (Zink, 2004). This is particularly apparent when the distinctness of the Thailand and peninsular Malaysian populations (both members of *A. l. cinereicollis*) are considered. If indeed these populations have recently diverged, then the morphological differences that merit the populations to be classified into different subspecies among the Bornean and Thai-Malay Peninsula populations have been established rapidly.

CONCLUSIONS

In conclusion, the pattern of divergence among populations of the little spiderhunter is congruent with hypothesized land connections and separation between Malay Peninsula, Borneo, Palawan, and Mindanao. There was recent divergence between peninsular Malaysia and Bornean populations. This study also reveals that the historical separation among the subregions and islands has promoted the differentiation between some of the little spiderhunter subspecies. Substantial divergence occurred between subspecies *A. l. flammifera* (Mindanao) and *A. l. dilutor* (Palawan). Both of these taxa probably merit full species status. Taxonomic status of other two subspecies, *A. l. buettikoferi* on Borneo and *A. l. cinereicollis* on the Malay Peninsula, need to be reconsidered in light of the close relationship between Bornean and peninsular Malaysian populations, and the distinctness of the southern Thailand population.

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