

Molecular phylogenetic analysis of the white-crowned forktail *Enicurus leschenaulti* in Borneo

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Comparison of 1017 nucleotides of mitochondrial ND2 and ND3 DNA sequences of 26 individuals of white-crowned forktail *Enicurus leschenaulti* from SE Asia revealed multiple evolutionary lineages within Borneo. Montane birds were genetically homogeneous across localities, but diverged by more than 4.3% from all other samples. Lowland birds formed two distinct clades, one consisting of individuals from northern Borneo, and the other including individuals from western Borneo, as well as Sumatra and Malaya. Relationships among the subspecies were not well resolved. These findings indicate another example of montane and north Bornean endemism, support the separation of the montane and lowland species, and define areas of conservation interest.

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Identifying populations with distinct genealogical histories is a fundamental goal in evolutionary biology. Whether this process of identification is used to refine classifications, study speciation, or infer biogeographic history, it is a cornerstone of systematic inquiry. In tropical birds, molecular comparisons have been especially powerful tools for assessing population structure and relationships. For example, molecular genetics has revealed unexpectedly high levels of divergence between individuals considered to be members of the same populations or subspecies (Bates et al. 1999, Marks et al. 2002), as well as surprisingly low levels of divergence between members of different species (Hackett and Lehn 1997). It has been used to test hypotheses of speciation and endemism (Roy 1997, Smith et al. 1997, Aleixo 2004), estimate the timing of population subdivision (Klicka and Zink 1997, Johnson and Cicero 2004), and illuminate conservation issues (Smith et al. 2000, Bates 2002).

As valuable as these molecular genetic studies are, it is surprising how few have been directed at the many evolutionary and taxonomic problems facing ornithology in SE Asia. This is particularly so, considering the prominent role that SE Asia has played in the history of bird biogeography and evolutionary biology in general. Most molecular studies have sampled only sparsely in SE Asia as part of projects aimed at resolving higher-level relationships (Cibois et al. 2002, Gamauf and Haring 2004), or Palearctic patterns (Price et al. 1997, Drovetski et al. 2004). In the absence of molecular comparisons, SE Asian taxonomy, biogeography, and conservation have necessarily relied on analyses of morphology and geographic distribution. Unfortunately, the information content in distributions is limited. Without the evolutionary hierarchy implicit in a phylogenetic hypothesis, the cohesive nature (monophyly) of the groups being compared, regardless of how similar or different they look, is unknown, and the temporal

sequence of historical events responsible for their distribution cannot be inferred accurately. On the Sunda Shelf (Borneo, Sumatra, Java, Malaya, and smaller islands), reliance solely on morphological and distributional data is exacerbated because bird diversity has been influenced by unusually complex historical events, including dramatic changes in climate, sea level, and land form (Hall and Holloway 1998). Without a phylogenetic perspective, reconstruction of the sequence and details of interaction responsible for SE Asian taxic diversity is purely speculative.

Here we report the results from genetic analysis of a widespread SE Asian bird species and discuss our findings in light of current knowledge of diversity and biogeography of the region. We compared mitochondrial DNA (mtDNA) sequences of the white-crowned fork-tail *Enicurus leschenaulti* from across its range in southern and SE Asia. This species is polytypic, with 6 described subspecies: *leschenaulti* from Java, *frontalis* from Sumatra, Malaya, and the lowlands of Borneo, *borneensis* from the mountains of Borneo, *chaseni* from the Batu Islands off west Sumatra, *indicus* from southern Indo-China, and *sinensis* from northern Indo-China. Our analyses and sampling focused on two issues. First, we assessed the genetic relationship between *frontalis* and *borneensis* on Borneo. These subspecies, which are distinguished by overall size (Harrison 1949) and tail length (Davison 1999, Smythies 1999), co-occur in Borneo but segregate elevationally. Despite ample opportunity for contact and inter-breeding at mid-elevations, the morphological differences between montane and lowland birds persist, suggesting the existence of two species rather than subspecies. Second, we reconstructed the phylogeny of the species to assess its biogeographic history.

Methods

Taxon sampling consisted of individuals from five of six named subspecies of *Enicurus leschenaulti* (lacking *E. l. chaseni* of the Batu Islands off West Sumatra). We sampled most densely within Borneo (Fig. 1), including multiple localities for both *E. l. borneensis* and *E. l. frontalis*, because we were particularly interested in the lowland-montane species issue. Four additional species of *Enicurus* were chosen as outgroup taxa for rooting the phylogeny (Table 1).

When fresh tissue was available, genomic DNA was extracted from muscle using proteinase K digestion following the manufacturer's protocol (Dneasy tissue kit, Qiagen). Limited availability of tissues required us to fill gaps in geographic sampling by extracting DNA from museum study skins for select taxa. Protocols for DNA extraction from museum skins generally followed those for fresh tissues, with the addition of 10 µl of 1M

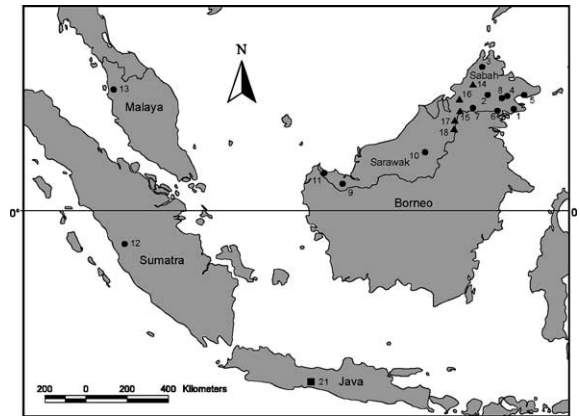


Fig. 1. Map of the Sunda region, displaying collecting localities (see Table 1 for key to numbers). ▲ denotes *E. l. borneensis* samples, ■ denotes *E. l. leschenaulti* samples, ● denotes *E. l. frontalis* samples. Localities in China and Vietnam are not displayed on this map.

dithiothreitol solution. The protocols also require special handling of specimens and prevention of contamination, as described by Mundy et al. (1997).

For this study, we compared the entire third subunit and a portion of the second subunit of mitochondrial nicotinamide adenine dinucleotide dehydrogenase (ND3 and ND2). To amplify and sequence DNA from fresh tissues, we used primer pairs 400 to 650 base pairs apart, but amplification of DNA from skin samples, which was often degraded, required the use of primer pairs <300 base pairs apart. External primers for the ND3 gene were L10755 and H11151 (Chesser 1999). Internal ND3 primers for the skin samples were ND3-180L (5'-GCCCGMCTBCCATTYTC-3') and ND3-220H (5'-AGGGCRATTTCTAGRTCRAA-3'). Primers for the ND2 gene were L5215 (Hackett 1996), H6313, L5758, and H5766 (Johnson and Sorenson 1998). Internal ND2 primers for the skin samples were ND2-96L (5'-TGARTCAYAGCCTGAG-CYGG-3'), ND2-160H (5'-TTGGTTGCMGCTTCAA-TGGC-3'), ND2-275L (5'-ATCACCCAATAACCT-GCCC-3'), ND2-285H (5'-ATGGCTGARGTTAGGATTA-3'), ND2-457L (5'-CATCACAATCACTAAACC-CAACCC-3'), ND2-477H (5'-TTCATCCNCTAGRG-CTGCAGATAG-3'), and ND2-667H (5'-AGTGTAGATAGTTTTAGGGTATT-3').

We purified PCR products with Perfectprep PCR cleanup kits (Eppendorf). Sequencing of purified PCR products was performed with BigDye Terminator Cycle Sequencing Kits (Applied Biosystems). Primers used for PCR were also used for cycle sequencing reactions, resulting in double stranded sequence for all taxa. Cycle sequencing products were run on an ABI Prism 3100 automated DNA sequencer (Perkin-Elmer Applied Biosystems). The computer program Sequencher 4.1 (Genecodes) was used to reconcile chromatograms of

Table 1. List of *Enicurus leschenaulti* and other *Enicurus* species samples used in the study. Asterisks (*) denote individuals for which fresh tissue was not available. DNA for these samples was extracted from museum study skins. Numbers next to localities cross-reference sites to Fig. 1. Source abbreviations are as follows: AMNH, American Museum of Natural History; LSUMNS, Louisiana State University Museum of Natural Science; WFVZ, Western Foundation of Vertebrate Zoology; SM, Sabah Museum; SarM, Sarawak Museum.

Name	Locality	Source	ID/Voucher
Ingroup:			
<i>E. l. frontalis</i>	Tawau Hills, Sabah (1)	LSUMNS	B38580
<i>E. l. frontalis</i>	Tawau Hills, Sabah (1)	LSUMNS	B38581
<i>E. l. frontalis</i>	Imbak Valley, Sabah (2)	LSUMNS	B38612
<i>E. l. frontalis</i>	Serinsim, Sabah (3)	AMNH	BDM 920
<i>E. l. frontalis</i>	Serinsim, Sabah (3)	AMNH	RGM 613
<i>E. l. frontalis</i>	Serinsim, Sabah (3)	AMNH	RGM 617
<i>E. l. frontalis</i>	Bole River, Sabah* (4)	WFVZ	39822
<i>E. l. frontalis</i>	Brumas, Sabah* (5)	WFVZ	39829
<i>E. l. frontalis</i>	Silabukan, Sabah* (6)	SM	2068
<i>E. l. frontalis</i>	Simatuoh, Sabah* (7)	SM	2277
<i>E. l. frontalis</i>	Ulu Segama, Sabah* (8)	SM	215
<i>E. l. frontalis</i>	Gedong, Sarawak* (9)	SarM	10
<i>E. l. frontalis</i>	Usun Apau, Sarawak* (10)	SarM	11
<i>E. l. frontalis</i>	Telok Serabang, Sarawak* (11)	SarM	4896
<i>E. l. frontalis</i>	Korinchi, Sumatra* (12)	AMNH	577845
<i>E. l. frontalis</i>	Bukit Tangga, Malaya* (13)	AMNH	577848
<i>E. l. borneensis</i>	Mt. Trusmadi, Sabah (14)	LSUMNS	B36442
<i>E. l. borneensis</i>	Mt. Trusmadi, Sabah (14)	LSUMNS	B36452
<i>E. l. borneensis</i>	Muruk Miau, Sabah* (15)	SM	4770
<i>E. l. borneensis</i>	Muruk Miau, Sabah* (15)	SM	4775
<i>E. l. borneensis</i>	Mt. Lumaku, Sabah* (16)	SM	4041
<i>E. l. borneensis</i>	Bario, Sarawak* (17)	SarM	1013
<i>E. l. borneensis</i>	Bakelalan, Sarawak* (18)	SarM	W387(a)e
<i>E. l. indicus</i>	Quang Nam, Vietnam (19)	AMNH	PRS 2202
<i>E. l. sinensis</i>	Szechuan, China* (20)	AMNH	261552
<i>E. l. leschenaulti</i>	Karangbolong, Java* (21)	AMNH	577679
Outgroup:			
<i>Enicurus ruficapilla</i>	Tawau Hills, Sabah	LSUMNS	B38563
<i>Enicurus maculatus</i>	Ha Giang, Vietnam	AMNH	PRS 2402
<i>Enicurus scouleri</i>	Ha Giang, Vietnam	AMNH	CJV 49
<i>Enicurus schistaceus</i>	Ha Giang, Vietnam	AMNH	PRS 2464

complementary fragments and align sequences across taxa.

Congruence between phylogenetic signal in the two genes was tested with the incongruence length difference test (Farris et al., 1994, 1995), implemented in Paup* 4.0b10 (i. e. partition homogeneity test, Swofford, 2002). The test excluded constant characters and ran for 1000 bootstrap repetitions. We analyzed the data under maximum parsimony and maximum likelihood criteria using PAUP* 4.0b10 (Swofford 2002). In likelihood analyses, Modeltest 3.5 (Posada and Crandall 1998) determined both the appropriate model of nucleotide substitution and the parameter values. In parsimony analyses all characters were equally weighted. Support for nodes in the resulting phylogenetic hypotheses was assessed via non-parametric bootstrapping (Felsenstein 1985), and reanalysis of the resulting data (100 replicates).

Results

The aligned sequences yielded a matrix of 30 taxa (26 ingroup, 4 outgroup) and 1017 characters (351 ND3,

666 ND2). All sequences (Genbank AY878256-AY878315) appeared to be genuine mitochondrial sequence, rather than nuclear copies. Sequences contained no stop codons, overlapping fragments contained no conflicts, base composition was homogeneous across taxa, codon positions contained expected relative divergences ($3 > 1 > 2$), and there were no highly suspect relationships among the taxa. Base composition was biased towards adenine and cytosine ($A = 0.31$, $C = 0.32$, $G = 0.12$, $T = 0.25$), but consistent with base composition of these genes in other bird groups (Kirchman et al. 2001, Chesser 2004). The partition homogeneity test result was not significant ($P > 0.05$).

Of 1017 nucleotide positions, 153 were parsimony informative. Uncorrected divergence (p-distance) within *E. leschenaulti* ranged from 0% between several individuals to 5.8% between the single sample of *E. l. sinensis* and multiple other individuals. Little fork-tail *Enicurus scouleri* and slaty-backed fork-tail *Enicurus schistaceus* were more distant from *E. leschenaulti* than either chestnut-naped fork-tail *Enicurus ruficapillus* or spotted fork-tail *Enicurus maculatus*. Thus phylogenetic trees were rooted with the former two species and the latter two were allowed to “float” in the ingroup.

Modeltest (Posada and Crandall 1998) indicated a model of nucleotide substitution (TVM+G) that incorporated four classes of transversions, a single class of transitions, and gamma distributed rates across sites. Analysis of each gene separately produced the same model for ND2 (TVM+G) and a slightly simpler model for ND3 (HKY+G). Parameter estimates for the combined data were as follows: base frequencies (0.3389, 0.3253, 0.1137), rate matrix (6.2338 81.6251 3.7497 0.2726 81.6251), and shape (0.1637). Parsimony (five most parsimonious trees of 441 steps with consistency indices of 0.714) and likelihood (1 tree with $\ln L = 3386.61033$) analyses produced trees with no significant discrepancies (Fig. 2). Monophyly of *E. leschenaulti* was recovered, but with low bootstrap support. One of the outgroup taxa *E. maculatus* is genetically very close to *E. leschenaulti*, and in some bootstrap replicates caused the species to be paraphyletic. Relationships among the five subspecies of *E. leschenaulti* were not well resolved. All were 3.4% to 5.8% divergent from each other and bootstrap resampling did not support sister relationships

between any pairs of them. In contrast, relationships within the two densely sampled subspecies were well resolved. All seven samples of *E. l. borneensis*, from five different montane areas spanning 250 km, were united by high bootstrap support, and separated from one another by a maximum of 0.6% sequence divergence. They, in turn, were a minimum of 4.3% divergent from all other samples. Two clades were evident within *E. l. frontalis*. One clade included all of the lowland samples from Sabah (northeast Borneo). The other clade included lowland samples from southern Sarawak (Western Borneo), sister to the Sumatran and Malayan samples.

Discussion

Our analysis of mitochondrial DNA sequences from throughout the range of white-crowned fork-tail reconstructed relationships among populations of *E. l. frontalis* and *E. l. borneensis* with strong support. These two

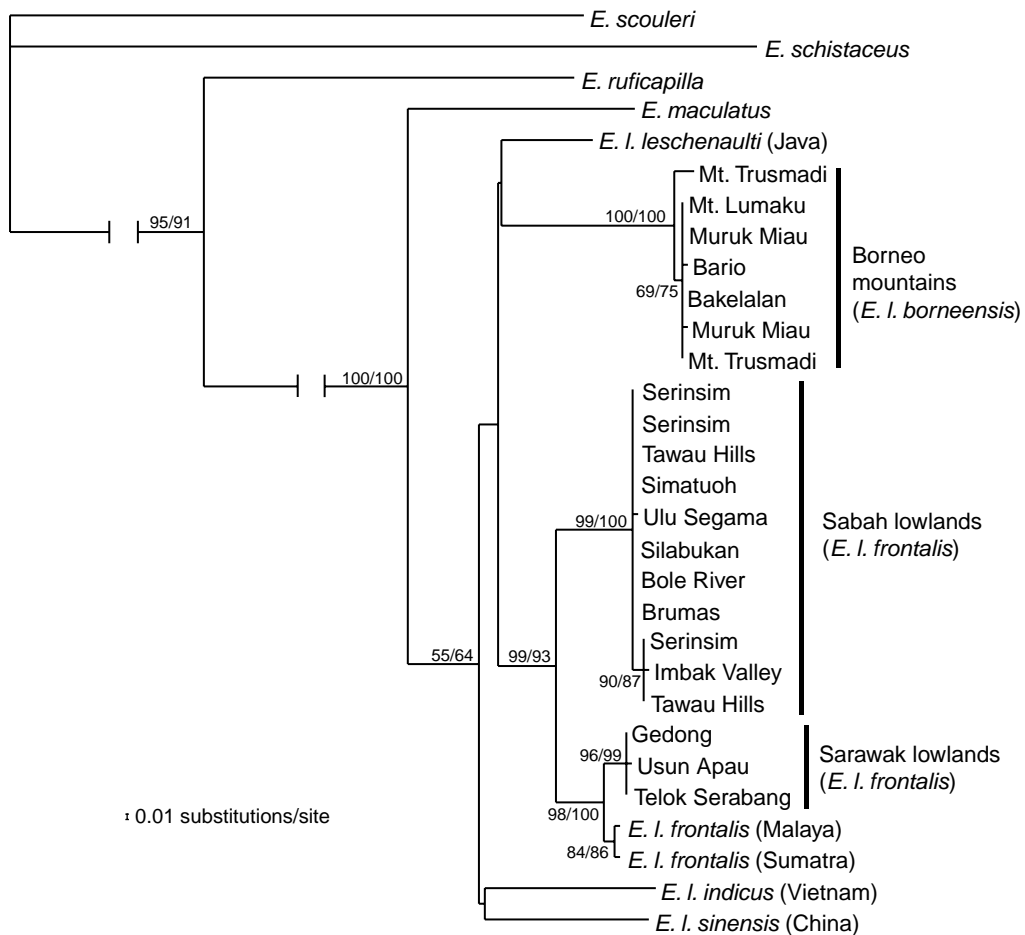


Fig. 2. Maximum likelihood tree based on the complete data set. Numbers above nodes indicate ML bootstrap proportions/MP bootstrap proportions. Unlabelled nodes received < 50% support in both ML and MP bootstrap replicates.

subspecies were monophyletic but not sister taxa, and their clades displayed distinct phylogenetic patterns. The widespread lowland taxon *frontalis*, which is found in Malaya, Borneo, and Sumatra, exhibited substantial phylogenetic structure corresponding to geographic distribution, both between islands and within Borneo, whereas the montane *borneensis* showed little geographic structuring. The subspecies *indicus*, *sinensis*, and *leschenaulti* were poorly sampled in our study, and their phylogenetic relationships were largely unresolved.

The phylogenetic patterns revealed within *E. leschenaulti* invite speculation about the processes that created them. A most notable result is the distinctiveness of the subspecies *borneensis*. This taxon is endemic to the mountains of Borneo and exhibits little haplotype diversity among isolated mountain regions, but substantial divergence from other subspecies, including individuals from adjacent lowland areas. This pattern indicates a barrier to gene flow exists between the populations, evidence that at least two species inhabit Borneo.

Similar patterns of montane endemism are well documented in the Greater Sunda Islands for vertebrates, invertebrates, and plants (MacKinnon et al. 1996). Roughly two-thirds of the endemic birds of Borneo are montane or lower montane specialists (Smythies 1999, Sheldon et al. 2001), and there are many examples of parapatric distribution of congeners altitudinally. A question in SE Asian biogeography is whether these parapatric congeners are sister taxa and whether there is a general pattern in which sister taxa are distributed altitudinally. If so, the biogeographic explanation for their distribution would be different than if the altitudinal pairs of taxa were more distantly related. In this study, the montane taxon (*borneensis*) is sister to an individual from Java, but bootstrap resampling provides little support for this relationship.

Another pattern apparent from the data is phylogenetic structure among populations of lowland *frontalis* corresponding to geographic distribution in Borneo, Sumatra, and Malaya. Although described as one subspecies, the haplotypes from these individuals segregate geographically and display divergences as high as 2.8%, indicating lengthy isolation. The main genetic division does not coincide with oceanic barriers, however. The basal split separates Sabah individuals from those of Sarawak, Sumatra, and Malaya. The latter group is further subdivided such that Sumatra and Malaya individuals form the sister group of Sarawak birds. This finding mirrors a less appreciated pattern of endemism in Borneo. Among birds and some mammal groups (e.g. tree shrews, Han 2000), some taxa are endemic to lowlands in the northeast part of the island, roughly coincident with the boundary separating Sabah from Sarawak and Kalimantan. Among birds, these include white-fronted falconet *Microhierax latifrons*,

white-crowned shama *Copsychus stricklandii*, and black-and-crimson pitta *Pitta ussheri*. It is unknown whether this pattern is static, the result of current geographic barriers to dispersal or gene flow, or a dynamic one in which population expansion or genetic introgression is occurring. Although some coastal lowland corridors seem to connect the populations in Sabah and Sarawak, much of the land in between is mountainous and may pose a barrier to dispersal. The close relationship between Sarawak, Sumatra, and Malaya is likely the result of intermittent land connections between those regions as recent as the last ice age. Sabah birds may be descendants of an original Bornean population that has lost ground (physically through competition and displacement, genetically through introgression, or a combination of the two) to an expanding population from the west. Only extensive geographic sampling across the lowlands of Borneo, Sumatra, and Malaya and analysis of unlinked molecular markers can differentiate these hypotheses.

Deep structure within seemingly homogeneous populations across the lowlands of Borneo has important conservation implications. Destruction of forests in the Sunda region is progressing at an alarming rate, with all major blocks of lowland forest in Indonesia predicted to be gone or severely degraded by 2010 (Jepson et al. 2001, Curran et al. 2004). That this destruction of forest can lead to local extinction of species has also been documented (Brook et al. 2003). For most species, however, the possibility of cryptic diversity has never been considered, let alone quantified genetically, so "local" extinctions may in fact extirpate entire cohesive evolutionary units.

More genetic work in the Sunda region is vital to understanding its patterns of diversity and endemism. In this study, only two of the six subspecies were sampled densely, and even those had gaps. Extending the sampling of *E. leschenaulti* to cover the vast Indonesian portion of Borneo and denser sampling of the other subspecies is required to fully understand the evolutionary history of this species. Also, additional markers must be utilized to help compensate for the stochastic nature of the coalescent process. Finally, additional species must be analyzed in a similar way so that comparisons among them can be made. This is especially true as we try to come to grips with the cause of montane endemism in the Indonesian archipelago.

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References

- Aleixo, A. 2004. Historical diversification of a Terra-Firme forest bird superspecies: a phylogeographic perspective on the role of different hypotheses of Amazonian diversification. – *Evolution* 58: 1303–1317.
- Bates, J. M., Hackett, S. J. and Goerck, J. 1999. High levels of mitochondrial DNA differentiation in two lineages of Antbirds (*Drymophila* and *Hypocnemis*). – *Auk* 116: 1093–1106.
- Bates, J. M. 2002. The genetic effects of forest fragmentation on five species of Amazonian birds. – *J. Avian Biol.* 33: 276–294.
- Brook, B. W., Sodhi, N. S. and Ng, P. K. L. 2003. Catastrophic extinctions follow deforestation in Singapore. – *Nature* 424: 420–426.
- Chesser, R. T. 1999. Molecular systematics of the rhinocryptid genus *Pteroptochos*. – *Condor* 101: 439–446.
- Chesser, R. T. 2004. Molecular systematics of New World suboscine birds. – *Mol. Phylogenet. Evol.* 32: 11–24.
- Cibois, A., Kalyakin, M. V., Lian-Xian, H. and Pasquet, E. 2002. Molecular phylogenetics of babblers (Timaliidae): reevaluation of the genera *Yuhina* and *Stachyris*. – *J. Avian Biol.* 33: 380–390.
- Curran, L. M., Trigg, S. N., McDonald, A. K., Astiani, D., Hardiono, Y. M., Siregar, P., Caniogo, I. and Kasischke, E. 2004. Lowland forest loss in protected areas of Indonesian Borneo. – *Science* 303: 1000–1003.
- Davison, G. W. H. 1999. Notes on the taxonomy of some Bornean birds. – *Sarawak Mus. J.* 54: 289–299.
- Drovetski, S. V., Zink, R. M., Fadeev, I. V., Newsterov, E. V., Koblik, E. A., Red'kin, Y. A. and Rowher, S. 2004. Mitochondrial phylogeny of *Locustella* and related genera. – *J. Avian Biol.* 35: 105–110.
- Farris, J. S., Källersjö, M., Kluge, A. G. and Bult, C. 1994. Permutations. – *Cladistics* 10: 65–76.
- Farris, J. S., Källersjö, M., Kluge, A. G. and Bult, C. 1995. Testing significance of incongruence. – *Cladistics* 10: 315–319.
- Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. – *Evolution* 39: 783–791.
- Gamauf, A. and Haring, E. 2004. Molecular phylogeny and biogeography of Honey-buzzards (genera *Pernis* and *Henicopernis*). – *J. Zool. Syst. Evol. Res.* 42: 145–153.
- Hackett, S. J. 1996. Molecular phylogenetics and biogeography of tanagers in the genus *Ramphocelus* (Aves). – *Mol. Phyl. Evol.* 5: 368–382.
- Hackett, S. J. and Lehn, C. A. 1997. Lack of genetic divergence in a genus of Neotropical birds (*Pteroglossus*): The connection between life-history characteristics and levels of genetic divergence. – In *Studies in Neotropical Ornithology Honoring Ted Parker, Remsen, J. V. Jr.* (ed.) *Ornithol. Monogr.* 48: 267–280.
- Hall, R. and Holloway, J. D. 1998. Biogeography and geological evolution of SE Asia. – Backhuys, Leiden.
- Harrisson, T. 1949. A note on *Enicurus leschenaulti*. – *Sarawak Mus. J.* 5: 149–152.
- Han, K. H. 2000. Phylogeny and biogeography of tree shrews (Scandentia: Tupaiidae). – Ph.D Dissertation, Louisiana State University, Baton Rouge.
- Jepson, P., Jarvie, J. K., MacKinnon, K. and Monk, K. A. 2001. The end for Indonesia's lowland forests? – *Science* 292: 859–861.
- Johnson, K. P. and Sorenson, M. D. 1998. Comparing molecular evolution in two mitochondrial protein coding genes (cytochrome *b* and ND2) in the dabbling ducks (Tribe: Anatini). – *Mol. Phyl. Evol.* 10: 82–94.
- Johnson, N. K. and Cicero, C. 2004. New mitochondrial DNA data affirm the importance of Pleistocene speciation in North American birds. – *Evolution* 58: 1122–1130.
- Kirchman, J. J., Hackett, S. J., Goodman, S. M. and Bates, J. M. 2001. Phylogeny and systematics of ground rollers (Brachypteraciidae) of Madagascar. – *Auk* 118: 849–863.
- Klicka, J. and Zink, R. M. 1997. The importance of recent ice ages in speciation: a failed paradigm. – *Science* 277: 1666–1669.
- MacKinnon, K., Hatta, G., Halim, H. and Mangalik, A. 1996. The ecology of Kalimantan: Indonesian Borneo. – Singapore Periplus Editions Ltd.
- Marks, B. D., Hackett, S. J. and Capparella, A. P. 2002. Historical relationships among Neotropical lowland forest areas of endemism as determined by mitochondrial DNA sequence variation within the wedge-billed woodcreeper (Aves: Dendrocolaptidae *Glyphorhynchus spirurus*). – *Mol. Phylogenet. Evol.* 24: 153–167.
- Mundy, N. I., Unitt, P. and Woodruff, D. S. 1997. Skin from feet of museum specimens as a non-destructive source of DNA for avian genotyping. – *Auk* 114: 126–129.
- Posada, D. and Crandall, K. A. 1998. Modeltest: testing the model of DNA substitution. – *Bioinformatics* 14: 817–818.
- Price, T. D., Helbig, A. J. and Richman, A. D. 1997. Evolution of breeding distributions in the Old World leaf warblers (genus *Phylloscopus*). – *Evolution* 51: 552–561.
- Roy, M. S. 1997. Recent diversification in African greenbuls (Pycnonotidae: *Andropadus*) supports a montane speciation model. – *Proc. R. Soc. Lond. B* 264: 1337–1344.
- Sheldon, F. H., Moyle, R. G. and Kennard, J. 2001. Ornithology of Sabah: history, gazetteer, annotated checklist, and bibliography. – *Ornithol. Monogr.* 52: 1–278.
- Smith, T. B., Wayne, R. K., Girman, D. J. and Bruford, M. W. 1997. A role for ecotones in generating rainforest biodiversity. – *Science* 276: 1855–1857.
- Smith, T. B., Holder, K., O'Keefe, K., Larison, B. and Chan, Y. 2000. Comparative avian phylogeography of Cameroon and Equatorial Guinea mountains: implications for conservation. – *Mol. Ecol.* 9: 1505–1516.
- Smythies, B. E. 1999. *The Birds of Borneo* Fourth Edition, revised by Geoffrey W. H. Davison. – Kota Kinabalu, Natural History Publications, Borneo.
- Swofford, D. L. 2002. PAUP*. Phylogenetic Analysis Using Parsimony (*and other methods). – Sunderland, Massachusetts, Sinauer Associates.

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