



Genetic and biometric variation across the fragmented range of Jerdon's Babbler, *Chrysomma altirostre*, a threatened Oriental grassland specialist

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Abstract

Jerdon's Babbler *Chrysomma altirostre* is a threatened Oriental grassland specialist passerine heavily affected by habitat loss. Its now-relictual range spans from Pakistan to Myanmar covering three poorly-studied subspecies. Our study of subspecific differentiation revealed limited range-wide mitochondrial divergences, suggesting that mountain ranges and other geographic barriers may not have been insurmountable obstacles to inter-subspecific Pleistocene connectivity of this highly specialized grassland bird. For the westernmost subspecies *scindicum*, we report on phenotypic differentiation consistent with Gloger's Rule, and ecomorphological adaptations to arid environments requiring increased mobility.

Keywords Grassland specialist · Ecomorphological adaptations · Gloger's rule · Jerdon's Babbler

Zusammenfassung

Genetische und biometrische Variation im fragmentierten Verbreitungsgebiet der Jerdontimalie *Chrysomma altirostre*, einer gefährdeten Grasland-Art der Orientalischen Region

Die Jerdontimalie *Chrysomma altirostre* ist eine auf Grasland spezialisierte, bedrohte Singvogelart der Orientalischen Region, welche stark unter Lebensraumverlusten leidet. Ihr verbliebenes Verbreitungsgebiet erstreckt sich von Pakistan bis Myanmar und umfasst drei kaum untersuchte Unterarten. Unsere Studie subspezifischer Differenzierung offenbarte begrenzte mitochondriale Abweichungen über das gesamte Verbreitungsgebiet, was nahelegt, dass Gebirgszüge und andere geografische Barrieren während des Pleistozäns unter Umständen keine unüberwindlichen Hindernisse für den Austausch zwischen den Unterarten dieses hochspezialisierten Graslandvogels dargestellt haben. Für die westlichste Unterart *scindicum* lässt sich sagen, dass deren phänotypische Differenzierung im Einklang mit der Gloger'schen Regel steht und ökomorphologische Anpassungen an eine aride Umwelt aufweist, welche eine erhöhte Mobilität erfordert.

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Introduction

Development across Asia has led to massive declines of a variety of habitat types and concomitant avian species loss (Wright et al. 2012). Grassland habitats and their avian biota have been affected across the entire Oriental Region for as long as the past 10,000 years after humans commenced crop cultivation (Zohary et al. 2012). These habitat conversions initially led to losses in megafauna, and have recently begun seriously impacting grassland avifauna (Wright et al. 2012).

Jerdon's Babbler *Chrysomma altirostre* is a sensitive grassland specialist with an extremely narrow historic range confined to riverine grasslands spanning from present-day Pakistan through Myanmar. It is currently classified as Vulnerable by the IUCN (BirdLife International 2017). Three subspecies are commonly recognized, each associated with the watershed of a great river, respectively: *C. a. altirostre* (Ayeyarwady), *C. a. griseigulare* (Ganga–Brahmaputra) and *C. a. scindicum* (Indus), with substantial morphological variation between them (Rasmussen and Anderton 2005). The nominate form from Myanmar, *C. a. altirostre*, was thought to be extinct (the last specimen seen and collected in 1941) until a small surviving population was rediscovered in 2014 (Rheindt et al. 2014). This relict population is under heavy pressure from regional development and habitat conversion.

Rheindt et al. (2014) suggested that the morphological and bioacoustic variation recorded among subspecies warrants further investigation to search for potential deeper differentiation. In this study, we set out to investigate levels of mitochondrial DNA and morphological differentiation across the range of this species.

Materials and methods

Field sampling

Mist-netting was conducted in Myanmar's Ayeyarwady floodplain, at grasslands near Udo (17.385°N, 95.858°E), Yangon region, in May 2014, where several Jerdon's Babblers of the nominate *altirostre* subspecies were detected and one individual was successfully captured (Rheindt et al. 2014). Standard morphological measurements were recorded and ~ 50 µl of blood obtained by brachial venipuncture using a 50-µl Drummond™ Short-Length Microcaps heparin-free capillary tube. Two individuals of the *scindicum* subspecies were similarly sampled in Pakistan.

Laboratory procedures

DNA extractions were carried using the DNEasy Blood & Tissue Kit (Qiagen, Hilden, Germany) using the manufacturer's recommended protocol. Polymerase chain reactions

(PCR) were carried out in a C1000 Thermal Cycler for the mitochondrial gene cytochrome b (cytb) as per Sadanandan and Rheindt (2015). PCR amplifications were conducted in 25-µl reaction volumes, which comprised 2.5 µl DreamTaq buffer, 0.5 µl dNTP mix (10 mM), 0.5 µl of each primer (10 µM), 0.125 µl DreamTaq polymerase, 2 µl mtDNA template and 18.8 µl molecular grade water. PCR product cleanup and sequencing followed Sadanandan and Rheindt (2015).

Phylogenetic analysis

DNA sequences were assembled with CodonCode Aligner v.4.1 and aligned with MEGA 6.06 using ClustalW (Larkin et al. 2007, Tamura et al. 2013). An individual of the Yellow-eyed Babbler *Chrysomma sinense* was included in the analysis to provide a congeneric baseline, while the Black-throated Parrotbill *Paradoxornis nipalensis* was used as an outgroup (Table S1).

MEGA 6.06 was employed to construct phylogenetic trees using maximum parsimony. We applied settings of 1000 bootstrap replicates, all sites used for gap treatment, Tree-Bisection-Reconnection as the tree search method and other settings in default mode. Maximum likelihood phylogenetic inference was performed using RAxML (Stamatakis 2014). A rapid bootstrap analysis and search for best-scoring maximum-likelihood tree (-f a) were run for 1000 iterations (-N). The General Time Reversible plus Gamma model (GTR-GAMMA) was utilized as the nucleotide substitution model.

Raw sequence divergence values between sampled taxa were generated with MEGA 6.06 employing a bootstrap replicate setting of 1000 (Table 1).

Morphological measurements and analysis

We recorded biometric measurements of 28 specimens across all three recognized subspecies, including from the type specimens of each subspecies (Table S2). This material was made available by the Natural History Museum at Tring (Hertfordshire, UK). Wing chord measurements were taken from the carpal joint to the tip of the longest primary, while the bill was measured from the tip of the rostrum to the base of the forehead. A Bonferroni-corrected *p* value of 0.008 was applied to test for significance in pairwise comparisons of wing and bill measurements (Table 2).

Results

Mitochondrial phylogeny

A total sequence length of 1041 base pairs was obtained for our cytb alignment of five individuals. Six single nucleotide

Table 1 Matrix displaying raw sequence divergences (p-distances) between samples for the mitochondrial gene cytochrome b from Jerdon’s Babbler *Chrysomma altirostre*

	<i>Chrysomma altirostre altirostre</i> (UT1)	<i>Chrysomma altirostre scindicum</i> (242-11)	<i>Chrysomma altirostre scindicum</i> (242-09)
<i>Chrysomma altirostre scindicum</i> (242-11)	0.005		
<i>Chrysomma altirostre scindicum</i> (242-09)	0.006	0.001	
<i>Chrysomma sinense</i>	0.099	0.098	0.099

Table 2 Results of unpaired *t* tests between taxa for bill (above diagonal) and wing measurements (below diagonal) of Jerdon’s Babbler *Chrysomma altirostre*

	<i>C. a. altirostre</i>	<i>C. a. griseigulare</i>	<i>C. a. scindicum</i>
<i>C. a. altirostre</i>	59.60/11.55	0.0967	0.8495
<i>C. a. griseigulare</i>	0.1308	61.75/11.91	0.0288
<i>C. a. scindicum</i>	0.0003*	0.0010*	65.00/11.51

Significant *p* values are indicated by an asterisk (*). Mean wing and bill measurements are recorded along the diagonal, respectively (in bold)

polymorphisms (SNP) were recorded between the two sequenced subspecies *altirostre* and *scindicum*, two of which constituted synonymous substitutions. In contrast, 95 SNPs containing eight synonymous substitutions were recorded between Jerdon’s Babbler and the congeneric Yellow-eyed Babbler.

Our two *scindicum* samples from the westernmost terminus of the range formed a close-knit, fairly highly supported clade with minimal mtDNA divergence based on all three phylogenetic search strategies (Fig. 1; Table 1). Our nominate sample from the easternmost terminus of the range emerged sister to the *scindicum* clade (Fig. 1), but was diverged at an unexpectedly low level, ranging from 0.5 to 0.6% (Table 1).

Morphological differentiation

Only wing length measurements yielded significant differences in the unpaired *t* test comparisons between taxa, with the westernmost subspecies, *C. a. scindicum*, differing significantly from the other two subspecies (Table 2). Wing length appeared to show a clinal pattern, increasing towards the westernmost subspecies. Raw bill length measurements seemed to display a slight leapfrog pattern, with the eastern and western subspecies displaying shorter lengths than the

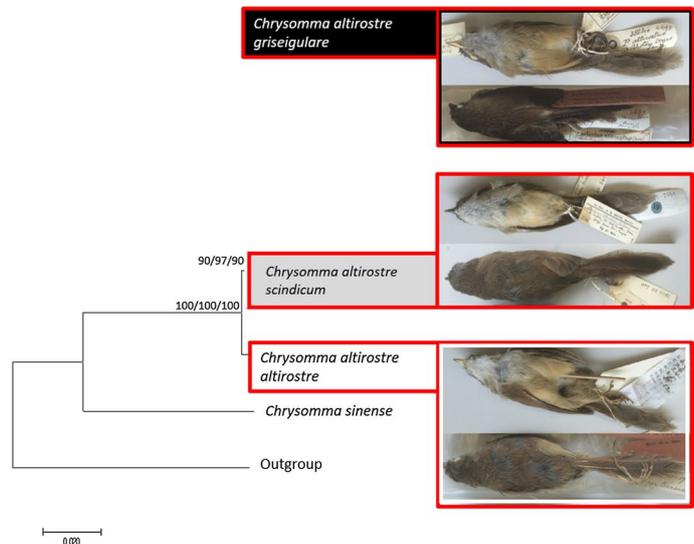
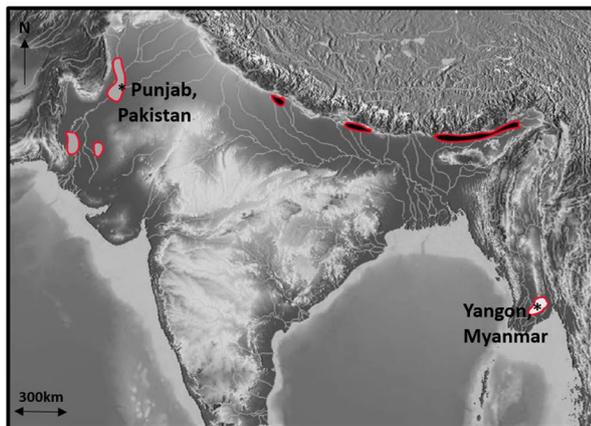


Fig. 1 Phylogram based on cytochrome b for Jerdon’s Babbler *Chrysomma altirostre* collected from either end of their range, and map displaying present ranges of all three subspecies, with sampling localities annotated. Phylogram topology based on maximum likelihood analysis. Nodes are annotated with bootstrap support from

neighbor-joining, maximum parsimony and maximum-likelihood analyses, respectively. Photographs of dorsal and ventral features of museum specimens of all three subspecies are included on the right of the phylogram

central subspecies, *C. a. griseigulare* (Table 2), but these differences were not significant and hence inconclusive.

Discussion

Jerdon's Babbler is a rare and poorly-known grassland specialist passerine with a range spanning from Myanmar to Pakistan. Its populations are now highly relictual given immense developmental pressures in the region (Fig. 1). Marked subspecific differentiation in both coloration and song has been noted by several authors (Rasmussen and Anderton 2005; Rheindt et al. 2014). In this study, we examined genetic divergence between subspecies from opposite ends of its range using the mitochondrial gene *cytb*, as well as biometric variation across all three subspecies.

We detected low levels of mitochondrial divergence between nominate *C. a. altirostre* and *C. a. scindicum* (Table 1) that are consistent with subspecific differentiation across a wide range of avian barcoding studies using mtDNA coding genes with similar evolutionary rates (e.g., Hebert et al. 2004; Kerr et al. 2007). Based on the approximate mitochondrial clock rate of ~ 2% divergence per million years (Lovette 2004), which has been validated for a wide range of avian taxa, it would appear that gene flow last occurred between the two subspecies from Myanmar and Pakistan ~ 240,000–280,000 years ago (Table 1), although such estimates are extremely approximate and should only be interpreted as rough guides. The shallow mitochondrial differentiation between Jerdon's Babbler subspecies from either terminus suggests some level of Pleistocene connectivity across this species' vast Oriental Region range, despite the presence of considerable geographic barriers (e.g., Chin Hills, Naga Hills) that would seem to permanently obstruct gene flow between populations that require shallow grassland plains. Alternatively, the phylogenetic signal obtained could also be a result of rapid post-glacial expansion from a single small refuge.

Intraspecific variation in coloration in Jerdon's Babbler is comparatively high in contrast to other members of the family. Plumage tone of upper- and underparts vary widely across the three subspecies, with *C. a. scindicum* being paler overall (Rasmussen and Anderton 2005; Fig. 1). Our biometric analysis showed that *scindicum* significantly differed from the other two subspecies in its longer wings, while no significant biometric differences were detected between the other two subspecies (Table 2).

The longer wings and paler coloration of *scindicum* are consistent with ecomorphological adaptations to a drier environment. Arid-adapted populations across many animal species are less richly pigmented than their counterparts from less xeric zones (Gloger's Rule; Gloger 1833), while longer-winged passerines are widely known to be more adept

at migration or nomadism than their short-winged counterparts with a higher home range fidelity (Winkler and Leisler 1992). In a taxonomic sense, these bioclimatic adaptations are appropriately reflected as subspecific variation.

Conclusion

We found limited mitochondrial divergence between subspecies from either end of the sizeable range of the elusive and range-restricted Jerdon's Babbler. Our biometric analyses revealed a westward increase in wing length. Given such shallow differentiation, mountain ranges (e.g., Chin Hills, Naga Hills) and other geographic barriers between subspecies do not seem to have been insurmountable obstacles to connectivity over the Pleistocene, although the westernmost subspecies *scindicum* appears to have attained eco-morphological adaptations equipping it for a more arid environment.

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