

Novel insights into the historical biogeography of the streak-breasted scimitar babbler complex (Aves: Timaliidae: *Pomatorhinus ruficollis* complex)

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Abstract The Streak-breasted Scimitar Babblers of the *Pomatorhinus ruficollis* species complex are found in most of the forested habitats across southern and eastern Asia. The diversification history of this group is obscured by high plumage variation across populations and conflicting genetic signal across loci. We combined genetic and geographic data from several recent studies to investigate how these species diversified across China using both phylogenetic and ecological niche modeling analyses. These two lines of evidence are consistent in showing that two well-sampled species, *P. reconditus* and *P. nigrostellatus*, in central and southern China respectively, likely experienced a history of isolation and expansion as suitable habitat contracted during the last interglacial and expanded in patchy extent during the glacial maximum. The genetic analysis showed that populations of *P. nigrostellatus* on Hainan Island are very similar to the ones in nearby mainland southern China. We recovered two well-supported clades within *P. reconditus* that were not geographically structured with both containing individuals from the same localities across central China. This phylogenetic result corresponded to the ecological niche models that showed expansion from refugia since the last interglacial. This study illustrates the value of using an integrative approach and detailed geographic sampling to help understand the recent diversification of birds in China [*Current Zoology* 61 (5): 910–921, 2015].

Keywords Songbirds, Asia, Ecological niche modeling, Phylogenetics, Babblers

Cryptic or confusing variation in widespread species can cause difficulties when trying to decipher patterns of diversification. In this study, we examine the streak-breasted scimitar babblers (*Pomatorhinus ruficollis* complex), a widespread species complex inhabiting most of the forested and highland areas across southern and eastern Asia. Phenotypically, these populations vary in terms of plumage patterns, mainly in the intensity and coloration of the breast streaks. This variation has led to the recognition of 13–15 subspecies (Cheng, 1987; Dickinson et al., 2003). More recent analyses using DNA data as well as investigations of plumage variation across the geographic range have helped to illuminate the evolutionary history of this complex and confused group (Dong et al., 2010; 2014a; 2014b; Reddy and Moyle, 2011; Reddy et al., 2015). However, some confusion still exists in terms of how many distinct species are in this complex. Furthermore, the phylogenetic relationships of these species are also debated due to differences in interpreting the signal from various sources of data.

Babblers, or the family Timaliidae, have undergone

many taxonomic rearrangements in recent years. Several molecular phylogenetic studies have concluded that the traditional genus of scimitar babblers, *Pomatorhinus*, is non-monophyletic (Dong et al., 2010; Moyle et al., 2012; Reddy and Moyle, 2011). Moreover, many of the traditional species, hereby referred to as species complexes, were found to be multiple distinct lineages, which do not necessarily correspond to subspecies designations. The taxonomy of the lineages required serious overhaul in redefining species limits to reflect distinct units, not merely the trivial elevation of subspecies to species (see Reddy and Moyle, 2011; Reddy et al., 2015). These studies also uncovered a host of complications in the taxonomy of the species and subspecies, such that several subspecies and some species were polyphyletic. For example, three subspecies traditionally placed in the *P. schisticeps* complex grouped within the *P. ruficollis* complex in a phylogenetic analysis (Reddy and Moyle, 2011).

To be consistent in placing all distinct taxa at the same rank, Reddy and Moyle (2011) revised the *P. ruficollis* complex by using phylogenetic analyses and

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discrete plumage variation to delineate 11 diagnosably distinct species under the Phylogenetic Species Concept. Dong et al (2014) continued to use traditional subspecies delineations, many of which were paraphyletic or polyphyletic even in their phylogeny. However, using the revised species delineations resulted in a phylogenetic tree that is more fitting with biogeographic hypotheses of this region (Reddy et al., 2015). Nevertheless, there is still conflict in terms of interpreting the variation across plumage (Dong et al., 2010; Reddy et al., 2015) and gene-trees (Dong et al., 2014b). We hereby refer to the traditional classification of *P. ruficollis* as a species complex and the 11 distinct lineages within as species. Consistent across all these studies is that the *P. ruficollis* complex comprises five clades: *P. similis*, *P. saturatus*, *P. bakeri*, and *P. ruficollis* found in the Sino-Himalayan range; *P. reconditus* distributed widely in central China; *P. annamensis*, *P. humilis*, and *P. olivaceus* in Southeast Asia; *P. nigrostellatus* and *P. stridulus* in southern China and Hainan; and *P. musicus* in Taiwan. In most analyses of both mitochondrial and nuclear genes, the Sino-Himalayan and SE Asian clades are consistently found as sister groups. The relationships of *P. reconditus* are still unresolved – it is sister to the Sino-Himalayan + SE Asia clade in some analyses and alternatively sister to the southern China + Taiwan in others. Multispecies coalescent or gene-tree/species-tree analyses placed *P. musicus* from Taiwan as sister to all mainland populations (the remaining *P. ruficollis* complex; Dong et al. 2014a 2014b). This result is drastically different from concatenated total evidence analyses that show this species as nested within the complex and sister to the southern China clade (Reddy et al., 2015; Dong et al., 2014a).

Another important contribution to understanding speciation patterns in this group is the prediction of geographic diversification based on ecological niche models. Nyári and Reddy (2013) showed that the *P. ruficollis* complex occupied a climatic range that was substantially different from its congeners. It is the only species complex of *Pomatorhinus* to occur east of the Sino-Himalayas into southern and eastern China (although *Erythrogeus* [*Megapomatorhinus*] *erythrogeus* has a similar distribution). Moreover, compared to the other complexes, the *ruficollis* species appear to be a younger radiation that diversified within the last 1 million years (Nyári and Reddy, 2013). The impact of past climate change events, such as glacial cycles, in shaping the evolution history of this group, particularly in driving intraspecific variation and maintaining deeper diver-

gences, has yet to be explored.

In this study, we re-examine the *P. ruficollis* complex using information from phylogenetic analysis and ecological niche modeling. Here we focus on addressing the question of how the *ruficollis* complex diversified in central and southern China. We combined four genes across 90 samples to reconstruct phylogenetic relationships of the six species that occur in this region. Further, we built ecological niche models (ENMs) to predict how species' ranges may have shifted during the last glacial maximum and interglacial. In our analysis, we compared whether the results of the phylogenetic analysis fit the predictions of the ENMs.

1 Materials and Methods

1.1 Phylogenetic Analysis

We combined data from three publications: Dong et al. (2014a), Dong et al. (2014b), and Reddy et al. (2015) to assemble a matrix of 90 samples for three mitochondrial genes and one nuclear intron – cytochrome *b* (CYTB), NADH dehydrogenase subunits 2 (ND2), and 3 (ND3), and aconitase 1 intron 9 (ACO1). We focused our analysis on individuals from southern and eastern China with data for at least three of these four genes to build the most thoroughly sampled matrix (Table 1) of this region. We included 6 of the 11 species in the *ruficollis* complex, which correspond to *P. reconditus*, *P. stridulus*, *P. similis*, *P. saturatus*, *P. nigrostellatus*, and *P. musicus*. We did not include 5 other species found outside of China (in the Himalayas and SE Asia) since the phylogenetic relationships of these species were well-resolved in previous studies and including them here would add a considerable amount of missing data to the matrix.

We reconstructed phylogenetic trees using maximum likelihood (ML) with RAxML 7.4.2 (Stamatakis, 2014) and Bayesian Inference (BI) with MrBayes 3.2 (Ronquist et al., 2012). We used PartitionFinder v.1.1.1 (Lanfear et al., 2012) to find the best partitioning scheme to separate subsets of loci that are evolving according to different evolutionary models. We examined all possible natural divisions of these loci and found the best partition according to the Bayesian Information Criterion to be 4 partitions: codon position 1 of CYTB, ND2, ND3; codon position 2 of CYTB, ND2, ND3; codon position 3 of CYTB, ND2, ND3; ACO1. In RAxML, we conducted an analysis of the combined, partitioned dataset using a GTRGAMMA model for each partition. We ran 100 bootstraps to estimate nodal support and used these to seed the ML searches for the optimal tree. In MrBayes,

Table 1 Sample information with voucher number, institution, locality, and Genbank accession numbers

Taxon	Voucher/ sample No.	Locality	Institution	CYTB	ND2	ND3	ACO1	Latitude	Longitude
<i>Pomatorhinus musicus</i>	NTNUT2331	Nantou, Taiwan	National Taiwan Normal University	GU724377	GU724455	-	GU724352		
<i>Xiphirhynchus superciliosus</i>	KIZGLGS5003		Kunming Institute of Zoology	GU724380	GU724458	-	GU724355		
<i>P. montanus</i>	LSUMNS B47063	Mt Kinabalu, Malaysia	Louisiana State University	HQ529174	HQ529075	JN826894			
<i>P. nigrostellatus [stridulus]</i>	GEL_S00096	Zhaocqing, Guangdong [ZQ]	Guangdong Entomological Institute	KF543375a	KF543426 a	KF749739	KF978024	23.17 N	112.55 E
<i>P. nigrostellatus [stridulus]</i>	GEL_S00097	Zhaocqing, Guangdong [ZQ]	Guangdong Entomological Institute	KF543376 a	KF543427 a	KF749740	-	23.17 N	112.55 E
<i>P. nigrostellatus [stridulus]</i>	GEL_S00098	Zhaocqing, Guangdong [ZQ]	Guangdong Entomological Institute	KF543377 a	KF543428 a	KF749741	KF978025	23.17 N	112.55 E
<i>P. nigrostellatus</i>	GEL_S00017	Hainan [HN]	Guangdong Entomological Institute	KF543381 a	KF543432 a	KF749745	KF978027		
<i>P. nigrostellatus</i>	GEL_S00078	Hainan [HN]	Guangdong Entomological Institute	KF543382 a	KF543433 a	KF749747	KJ608535	18.70 N	108.83 E
<i>P. nigrostellatus</i>	GEL_S00307	Hainan [HN]	Guangdong Entomological Institute	KF543383 a	KF543434 a	KF749746	KF978028	18.66 N	109.94 E
<i>P. nigrostellatus</i>	HNNU_LW04	Hainan [HN]	Hainan Normal University	KF543384 a	KF543435 a	KF749748	KF978033	-	-
<i>P. stridulus</i>	GEL_S00107	Hanzhong, Shaanxi [HZ]	Guangdong Entomological Institute	KF543385 a	KF543436 a	KF749749	KF978026	33.64 N	107.80 E
<i>P. reconditus [eidos]</i>	IOZ_LZP006	Yaan, Sichuan [YA]	Institute of Zoology	KF543391 a	KF543442 a	KF749750	KF978035	28.99 N	102.30 E
<i>P. reconditus [styani]</i>	NTNU_T0045	Hanzhong, Shaanxi [HZ]	National Taiwan Normal University	KF543392 a	KF543443 a	KF749751	KF978039	33.64 N	107.80 E
<i>P. reconditus [styani]</i>	NTNU_T0912	Hanzhong, Shaanxi [HZ]	National Taiwan Normal University	KF543393 a	KF543444 a	KF749753	KF978041	33.64 N	107.80 E
<i>P. reconditus [styani]</i>	KIZ_AH09079	Liuann, Anhui [LA]	Kunming Institute of Zoology	KF543394 a	KF543445 a	KF749754	KF978046	31.70 N	115.72 E
<i>P. reconditus [styani]</i>	KIZ_AH09044	Liuann, Anhui [LA]	Kunming Institute of Zoology	KF543395 a	KF543446 a	KF749755	KF978047	31.70 N	115.72 E
<i>P. reconditus [styani]</i>	KIZ_AH09048	Liuann, Anhui [LA]	Kunming Institute of Zoology	KF543396 a	KF543447 a	KF749756	KF978048	31.70 N	115.72 E
<i>P. reconditus [eidos]</i>	IOZ_LZP028	Yaan, Sichuan [YA]	Institute of Zoology	KF543398 a	KF543449 a	KF749758	KF978037	28.99 N	102.30 E
<i>P. reconditus [hunanensis]</i>	KIZ_LS002	Xiangxi, Hunan [XX]	Kunming Institute of Zoology	KF543399 a	KF543450 a	KF749759	KF978050	29.22 N	109.33 E
<i>P. reconditus [hunanensis]</i>	KIZ_LS003	Xiangxi, Hunan [XX]	Kunming Institute of Zoology	KF543400 a	KF543451 a	KF749760	KF978051	29.22 N	109.33 E
<i>P. reconditus [hunanensis]</i>	KIZ_LS004	Xiangxi, Hunan [XX]	Kunming Institute of Zoology	KF543401 a	KF543452 a	KF749761	KF978052	29.22 N	109.33 E
<i>P. reconditus [hunanensis]</i>	KIZ_DYS045	Laibin, Guangxi [LB]	Kunming Institute of Zoology	KF543402 a	KF543453 a	KF749762	-	24.03 N	110.31 E

Continued Table 1

Taxon	Voucher/ sample No.	Locality	Institution	CYTB	ND2	ND3	ACO1	Latitude	Longitude
<i>P. reconditus</i> [hunanensis]	KIZ_DYS208	Laibin, Guangxi [LB]	Kunming Institute of Zoology	KF543405 a	KF543456 a	KF749765	KF978055	24.03 N	110.31 E
<i>P. reconditus</i> [eidos]	IOZ_LZP030	Yaan, Sichuan [YA]	Institute of Zoology	KF543406 a	KF543457 a	KF749766	KF978000	28.99 N	102.30 E
<i>P. reconditus</i> [eidos]	IOZ_LZP027	Yaan, Sichuan [YA]	Institute of Zoology	KF543407 a	KF543458 a	KF749767	KF978036	28.99 N	102.30 E
<i>P. similis</i>	KIZ_GLGS5820	Baoshan, Yunnan [BS]	Kunming Institute of Zoology	KF543409 a	KF543460 a	KF749769	KF978056	25.01 N	98.66 E
<i>P. similis</i>	KIZ_GLGS2140	Baoshan, Yunnan [BS]	Kunming Institute of Zoology	KF543410 a	KF543461 a	KF749770	KF978057	25.01 N	98.66 E
<i>P. similis</i> [laurentei]	KIZ_D06	Kunming, Yunnan [KM]	Kunming Institute of Zoology	KF543411 a	KF543462 a	KF749771	KF978061	25.09 N	102.00 E
<i>P. similis</i> [laurentei]	KIZ_D02	Kunming, Yunnan [KM]	Kunming Institute of Zoology	KF543412 a	KF543463 a	KF749772	KF978062	25.09 N	102.00 E
<i>P. similis</i>	KIZ_GLGS6546	Baoshan, Yunnan [BS]	Kunming Institute of Zoology	KF543413 a	KF543464 a	KF749773	KF978058	25.01 N	98.66 E
<i>P. similis</i>	KIZ_GLGS2166	Baoshan, Yunnan [BS]	Kunming Institute of Zoology	KF543415 a	KF543466 a	KF749775	KF978059	25.01 N	98.66 E
<i>P. saturatus</i> [albipectus]	KIZ_0907PE48	Puer, Yunnan [PE]	Kunming Institute of Zoology	KF543418 a	KF543469 a	KF749783	KF978064	22.56 N	101.17 E
<i>P. saturatus</i> [albipectus]	KIZ_0907PE47	Puer, Yunnan [PE]	Kunming Institute of Zoology	KF543419 a	KF543470 a	KF749778	KF978065	22.56 N	101.17 E
<i>P. saturatus</i> [albipectus]	KIZ_PE130	Puer, Yunnan [PE]	Kunming Institute of Zoology	KF543420 a	KF543471 a	KF749779	KF978066	22.56 N	101.17 E
<i>P. saturatus</i> [albipectus]	KIZ_PE043	Puer, Yunnan [PE]	Kunming Institute of Zoology	KF543421 a	KF543472 a	KF749780	KF978067	22.56 N	101.17 E
<i>P. nigrostellatus</i>	KUNHM 10007	Dawei Shan National Park, Hunan, China	University of Kansas	KM975205	KM975155	KM975102	KM97506	28.42 N	114.12 E
<i>P. nigrostellatus</i>	KUNHM 10069	Dawei Shan National Park, Hunan, China	University of Kansas	KM975206	KM975156	KM975103	KM975062	28.42 N	114.12 E
<i>P. nigrostellatus</i>	KUNHM 10087	Diding Headwater Nature Preserve, Guangxi, China	University of Kansas	KM975207	KM975157	KM975104	KM975063	23.12 N	105.96 E
<i>P. nigrostellatus</i>	KUNHM 10159	Diding Headwater Nature Preserve, Guangxi, China	University of Kansas	KM975208	KM975158	KM975105	KM975064	23.12 N	105.96 E
<i>P. nigrostellatus</i>	KUNHM 10255	Shiwandashan National Nature Preserve, Guangxi, China	University of Kansas	KM975211	KM975161	KM975108	KM975065	21.84 N	107.88 E
<i>P. nigrostellatus</i>	KUNHM 10256	Shiwandashan National Nature Preserve, Guangxi, China	University of Kansas	KM975212	KM975162	KM975109	KM975066	21.84 N	107.88 E
<i>P. nigrostellatus</i>	KUNHM 10297	Shiwandashan National Nature Preserve, Guangxi, China	University of Kansas	KM975213	KM975163	KM975110	KM975067	21.84 N	107.88 E
<i>P. nigrostellatus</i>	KUNHM 10324	Shiwandashan National Nature Preserve, Guangxi, China	University of Kansas	KM975214	KM975164	KM975111	KM975068	21.84 N	107.88 E

Continued Table 1

Taxon	Voucher/ sample No.	Locality	Institution	CYTB	ND2	ND3	ACO1	Latitude	Longitude
<i>P. nigrostellatus</i>	KUNHM 10325	Shiwandashan National Nature Preserve, Guangxi, China	University of Kansas	KM975215	KM975165	KM975112	KM975069	21.84 N	107.88 E
<i>P. nigrostellatus</i>	KUNHM 10431	Shiwandashan National Nature Preserve, Guangxi, China	University of Kansas	KM975216	KM975166	KM975113	KM975070	21.84 N	107.88 E
<i>P. nigrostellatus</i>	KUNHM 10448	Shiwandashan National Nature Preserve, Guangxi, China	University of Kansas	KM975217	KM975167	KM975114	KM975071	21.84 N	107.88 E
<i>P. reconditus</i>	KUNHM 11095	Kuan Kuo Shui Nature Reserve, Guizhou, China	University of Kansas	KM975218	KM975168	KM975115	KM975072	28.22 N	107.16 E
<i>P. reconditus</i>	KUNHM 11129	Kuan Kuo Shui Nature Reserve, Guizhou, China	University of Kansas	KM975219	KM975169	KM975116	KM975073	28.22 N	107.16 E
<i>P. reconditus</i>	KUNHM 11133	Kuan Kuo Shui Nature Reserve, Guizhou, China	University of Kansas	KM975220	KM975170	KM975117	KM975074	28.22 N	107.16 E
<i>P. reconditus</i>	KUNHM 11143	Kuan Kuo Shui Nature Reserve, Guizhou, China	University of Kansas	KM975221	KM975171	KM975118	KM975075	28.22 N	107.16 E
<i>P. reconditus</i>	KUNHM 11156	Kuan Kuo Shui Nature Reserve, Guizhou, China	University of Kansas	KM975222	KM975172	KM975119	KM975076	28.22 N	107.16 E
<i>P. reconditus</i>	KUNHM 11176	Kuan Kuo Shui Nature Reserve, Guizhou, China	University of Kansas	KM975223	KM975173	KM975120	KM975077	28.22 N	107.16 E
<i>P. reconditus</i>	KUNHM 11189	Kuan Kuo Shui Nature Reserve, Guizhou, China	University of Kansas	KM975224	KM975174	KM975121	KM975078	28.22 N	107.16 E
<i>P. reconditus</i>	KUNHM 11193	Kuan Kuo Shui Nature Reserve, Guizhou, China	University of Kansas	KM975225	KM975175	KM975122	KM975079	28.22 N	107.16 E
<i>P. reconditus</i>	KUNHM 11245	Dashahe Nature Preserve, Guizhou, China	University of Kansas	KM975226	KM975176	KM975123	KM975080	29.17 N	107.57 E
<i>P. reconditus</i>	KUNHM 11329	Dashahe Nature Preserve, Guizhou, China	University of Kansas	KM975228	KM975178	KM975125	KM975081	29.17 N	107.57 E
<i>P. reconditus</i>	KUNHM 11345	Dashahe Nature Preserve, Guizhou, China	University of Kansas	KM975229	KM975179	KM975126	KM975082	29.17 N	107.57 E
<i>P. reconditus</i>	KUNHM 13580	Libo, Guizhou, China	University of Kansas	KM975230	KM975180	KM975127	KM975083	25.48 N	107.88 E
<i>P. reconditus</i>	KUNHM 13612	Libo, Guizhou, China	University of Kansas	KM975231	KM975181	KM975128	KM975084	25.48 N	107.88 E
<i>P. reconditus</i>	KUNHM 13613	Libo, Guizhou, China	University of Kansas	KM975232	KM975182	KM975129	KM975085	25.48 N	107.88 E
<i>P. reconditus</i>	KUNHM 13619	Libo, Guizhou, China	University of Kansas	KM975233	KM975183	KM975130	KM975086	25.48 N	107.88 E
<i>P. reconditus</i>	KUNHM 13621	Libo, Guizhou, China	University of Kansas	KM975234	KM975184	KM975131	KM975087	25.48 N	107.88 E
<i>P. reconditus</i>	KUNHM 13628	Libo, Guizhou, China	University of Kansas	KM975235	KM975185	KM975132	KM975088	25.48 N	107.88 E
<i>P. reconditus</i>	KUNHM 13640	Libo, Guizhou, China	University of Kansas	KM975236	KM975186	KM975133	KM975089	25.48 N	107.88 E
<i>P. reconditus</i>	KUNHM 13733	Libo, Guizhou, China	University of Kansas	KM975238	KM975188	KM975135	KM975090	25.48 N	107.88 E
<i>P. reconditus</i>	KUNHM 13751	Libo, Guizhou, China	University of Kansas	KM975239	KM975189	KM975136	KM975091	25.48 N	107.88 E
<i>P. reconditus</i>	KUNHM 13826	Libo, Guizhou, China	University of Kansas	KM975240	KM975190	KM975137	KM975092	25.48 N	107.88 E
<i>P. reconditus</i>	KUNHM 13852	Libo, Guizhou, China	University of Kansas	KM975241	KM975191	KM975138	KM975093	25.48 N	107.88 E

Continued Table 1

Taxon	Voucher/ sample No.	Locality	Institution	CYTB	ND2	ND3	ACO1	Latitude	Longitude
<i>P. reconditus</i>	KUNHM 13874	Libo, Guizhou, China	University of Kansas	KM975242	KM975192	KM975139	KM975094	25.48 N	107.88 E
<i>P. reconditus</i>	KUNHM 13882	Libo, Guizhou, China	University of Kansas	KM975243	KM975193	KM975140	KM975095	25.48 N	107.88 E
<i>P. reconditus</i>	KUNHM 6711	Dongan Shun Huang Shan National Park, Hunan, China	University of Kansas	KM975245	KM975195	KM975142	KM975096	26.41 N	111.04 E
<i>P. reconditus</i>	KUNHM 6717	Dongan Shun Huang Shan National Park, Hunan, China	University of Kansas	KM975247	KM975197	KM975144	KM975097	26.41 N	111.04 E
<i>P. reconditus</i>	KUNHM 6721	Dongan Shun Huang Shan National Park, Hunan, China	University of Kansas	KM975248	KM975198	KM975145	KM975098	26.41 N	111.04 E
<i>P. reconditus</i>	KUNHM 6722	Dongan Shun Huang Shan National Park, Hunan, China	University of Kansas	KM975249	KM975199	KM975146	KM975099	26.41 N	111.04 E
<i>P. reconditus</i>	KUNHM 6734	Dawei Shan National Park, Hunan, China	University of Kansas	KM975250	KM975200	KM975147	KM975100	28.42 N	114.12 E
<i>P. nigrostellatus</i>	KUNHM 9973	Dawei Shan National Park, Hunan, China	University of Kansas	KM975252	KM975202	KM975148	KM975101	28.42 N	114.12 E
<i>P. reconditus</i> [<i>siyani/siridulus</i>]	NTNU_T0030	Hanzhong, Shaanxi [HZ]	National Taiwan Normal University	GU724358 b	GU724456 b	KF749752	KF978040	33.64 N	107.80 E
<i>P. similis</i>	KIZ_GLGS1835	Baoshan, Yunnan [BS]	Kunming Institute of Zoology	GU724382 b	GU724460 b	KF749776	KF978060	25.01 N	98.66 E
<i>P. nigrostellatus</i>	GEI_S00311	Hainan [HN]	Guangdong Entomological Institute	KF750010c	KF749834 c	KF749922	KF978029	18.66 N	109.94 E
<i>P. nigrostellatus</i>	HNNU_LW01	Hainan [HN]	Hainan Normal University	KF750011 c	KF749835 c	KF749923	KF978030	-	-
<i>P. nigrostellatus</i>	HNNU_LW02	Hainan [HN]	Hainan Normal University	KF750012 c	KF749836 c	KF749924	KF978031	-	-
<i>P. nigrostellatus</i>	HNNU_LW03	Hainan [HN]	Hainan Normal University	KF750013 c	KF749837 c	KF749925	KF978032	-	-
<i>P. nigrostellatus</i>	HNNU_LW06	Hainan [HN]	Hainan Normal University	KF750014 c	KF749838 c	KF749926	KF978034	-	-
<i>P. reconditus</i> [<i>siyani</i>]	NTNU_T0041	Hanzhong, Shaanxi [HZ]	National Taiwan Normal University	KF750072 c	KF749896 c	KF749984	KF978038	33.64 N	107.80 E
<i>P. reconditus</i> [<i>siyani</i>]	KIZ_AH09046	Liu'an, Anhui [LA]	Kunming Institute of Zoology	KF750025 c	KF749849 c	KF749937	KF978042	31.69 N	115.72 E
<i>P. reconditus</i> [<i>siyani</i>]	KIZ_AH09047	Liu'an, Anhui [LA]	Kunming Institute of Zoology	KF750026 c	KF749850 c	KF749938	KF978043	31.69 N	115.72 E
<i>P. reconditus</i> [<i>siyani</i>]	KIZ_AH09074	Liu'an, Anhui [LA]	Kunming Institute of Zoology	KF750022 c	KF749846 c	KF749934	KF978044	31.69 N	115.72 E
<i>P. reconditus</i> [<i>siyani</i>]	KIZ_AH09078	Liu'an, Anhui [LA]	Kunming Institute of Zoology	KF750024 c	KF749848 c	KF749936	KF978045	31.69 N	115.72 E
<i>P. reconditus</i> [<i>humanensis</i>]	KIZ_LS001	Xiangxi, Hunan [XX]	Kunming Institute of Zoology	KF750032 c	KF749856 c	KF749944	KF978049	29.22 N	109.33 E
<i>P. reconditus</i> [<i>humanensis</i>]	KIZ_LS007	Xiangxi, Hunan [XX]	Kunming Institute of Zoology	KF750042 c	KF749866 c	KF749954	KF978053	29.22 N	109.33 E

we conducted a search of two runs with 4 chains each of 20,000,000 generations, sampling each 1,000th generation, with the substitution parameters unlinked across partitions. We checked for stationarity and convergence across runs using Tracer (Rambaut et al., 2014) and topological convergence using AWTY (Nylander et al., 2008). We also examined haplotype networks using SplitsTree 4 (Huson, 2005) for each of the genes. For ACO1, we first phased all heterozygous sequences before building networks. We used MEGA 6.0 (Tamura et al., 2013) to calculate uncorrected p-distances between and within species groups.

1.2 Paleodistribution models

Our occurrence dataset consisted of 330 unique localities for the entire *Pomatorhinus ruficollis* species complex. Localities were sourced from previously published research of the species complex (Dong et al., 2014a; Nyári and Reddy, 2013; Reddy and Moyle, 2011). Occurrence points (Fig. 1) were quality controlled by verifying their geographic position against the environmental dataset and to check for sufficient geographic coverage, outliers, erroneous georeferencing, and fall-off along coastlines.

Ecological variables in the form of bioclimatic GIS layers (<http://www.worldclim.org>) covering the entire

distributional extent of the group were used to summarize aspects of temperature and precipitation from the latter half of the 20th century (Hijmans et al., 2005) as well as for the Last Glacial Maximum (LGM; ~21,000 years before present [BP; CCSM and MIROC scenarios]) and Last Interglacial (LIG; ~120,000 - 140,000 years BP; Otto-Bliesner et al., 2006). Spatial resolution of our environmental datasets was scaled to 2.5 arc-minutes. To reduce dimensionality across environmental spaces and time scales, we used a subset of seven of the 19 bioclimatic layers: annual mean temperature (Bio1), mean diurnal range (Bio2), maximum temperature of warmest month (Bio5), minimum temperature of coldest month (Bio6), annual precipitation (Bio12), and precipitation of wettest (Bio13) and driest months (Bio14).

Lastly, we established a biogeographic hypothesis that takes into account the geographic accessibility of the species complex. Here, we explicitly defined areas that this taxon is unable to access based on a 500km radius buffer around each occurrence point. This buffer was further refined to exclude inaccessible areas such as high-elevation Himalayan plateaus, non-contiguous islands, and beyond the Isthmus of Kra on the Malay Peninsula.

Prior to running our final ecological niche models, we performed an initial model evaluation by dividing

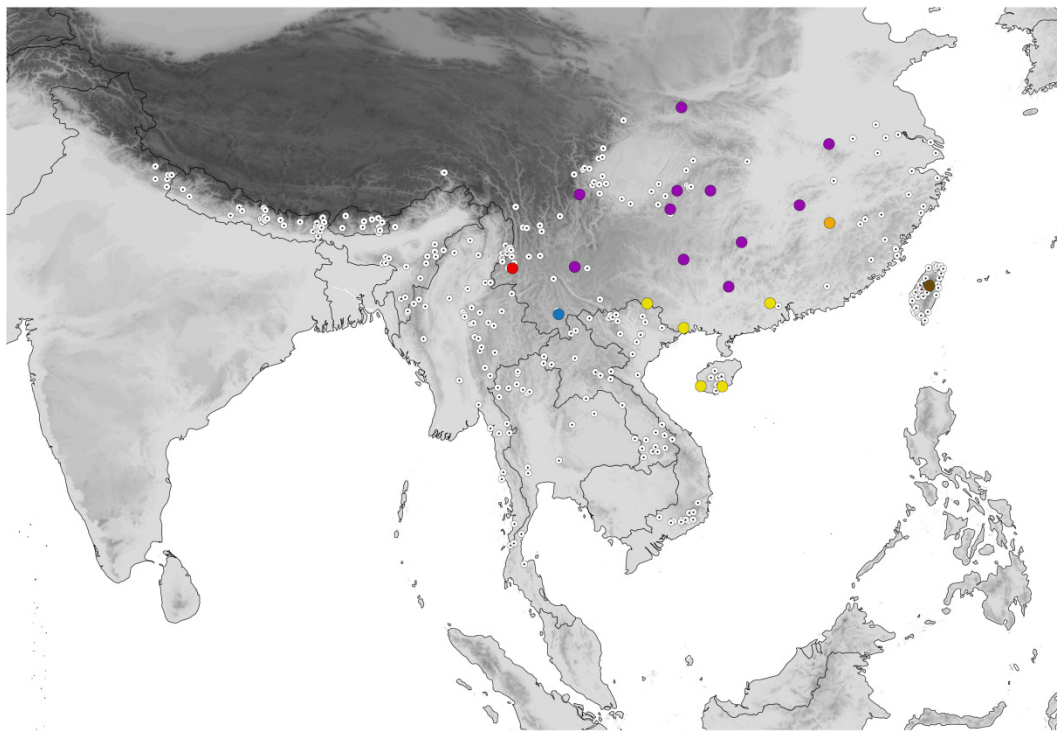


Fig. 1 Sampling localities of the *Pomatorhinus ruficollis* complex used in this study

White circles indicate localities used for ecological niche models. Colored circles indicate DNA samples: brown for *P. musicus* (Taiwan), orange for *P. stridulus* (Fujian), yellow for *P. nigrostellatus* (S China and Hainan), blue for *P. saturatus* (SE China), red for *P. similis* (Yunnan), purple for *P. reconditus* (central China).

our total number of occurrence records into two random halves for calibration and evaluation. Subsequently, ecological niche models were constructed with MaxEnt ver. 3.3.3 (Phillips et al., 2006) using only the calibration occurrences and were evaluated based on the evaluation occurrences via partial ROC analysis (Peterson et al., 2008). After model evaluation we developed our final models for the species complex using all available occurrence data and the above-mentioned environmental layers at present, LGM (MIROC and CCSM scenarios), and LIG time frames.

2 Results

2.1 Phylogenetic analysis

Average uncorrected *p*-distances of mitochondrial DNA (Table 2) indicate that divergences between these species are relatively shallow, as indicated in previous studies, since all of these speciation events apparently occurred within the last 1 million years (Nyári and Reddy, 2013; Reddy et al., 2015). Analysis of the combined and partitioned analyses showed similar topology and nodal support using both ML and BI methods (Fig. 2). These trees are also similar to previously published trees (Dong et al. 2014; Reddy et al. 2015) and reconstructed the *ruficollis* complex as split into two groups, the first corresponding to the central and Sino-Himalayan species: *P. reconditus* sister to a clade of *P. similis* + *P. saturatus*; the second composed of the species in southern China: a clade of *P. nigrostellatus* + *P. stridulus* sister to *P. musicus*. *Pomatorhinus reconditus*, which has a wide geographic range across central China (north of the Xi Jiang River), is split into two well-supported but geographically mixed clades with individuals from the same localities grouping in both. This study with expanded sampling corroborated the finding of *P. nigrostellatus* populations in Hainan being very similar and nested within populations from mainland southern China (as in Reddy et al. 2015).

The haplotype network of ACO1 showed little geo-

graphic signal (Fig. 3). Haplotypes of *Pomatorhinus reconditus* were diverse and appeared widely across the network, perhaps indicative of recent expansion. The network reconstruction of the 4 gene concatenated dataset using Splitstree showed the same species divisions and areas of uncertainty as in the phylogenetic analysis (Fig. 4).

2.2 Geographic analysis

The ecological niche models for current, last glacial maximum, and last interglacial predicted drastic changes in suitable habitat for the *ruficollis* complex (Fig. 5). During the LIG, the range of *ruficollis* species was highly fragmented and reduced. Most suitable habitat appeared to be restricted to parts of the Sino-Himalayan range and the extreme southern coast of China. During this time, the Hainan and Taiwan populations were isolated from those of the mainland.

The LGM predictions using CCSM and MIROC models were similar in showing connections of suitable conditions across the land bridges between Taiwan and Hainan to the mainland, but they differed in the extent of these connections. During this period, suitable habitat persisted along the coast for *P. nigrostellatus* and *P. stridulus*. The appearance of a broad patch of suitable habitat in central China probably allowed for the range expansion of *P. reconditus*. This result fits the prediction based on genetic models of recent expansion for this species (see Reddy et al., 2015) and further indicates that this has been occurring at least since LIG rather than LGM.

3 Discussion

Genetic and geographic analyses support similar patterns of diversification in the *ruficollis* complex. Genetic data show recent expansion of *P. reconditus*, which is further supported with ENMs that indicate the suitable habitat in central China was highly restricted in the LIG and expanded through LGM to the present. Although we were unable to model ranges of these species prior to LIG, the genetic data suggest separate waves of ex-

Table 2 Average uncorrected *p*-distances of mitochondrial DNA (lower triangle) and ACOI (upper triangle), within taxon distances are shown in diagonal

	<i>P. musicus</i>	<i>P. nigrostellatus</i>	<i>P. reconditus</i>	<i>P. saturatus</i>	<i>P. similis</i>	<i>P. stridulus</i>
<i>P. musicus</i>	-	0.001	0.001	0.001	0	0
<i>P. nigrostellatus</i>	0.028	(0.006/0.002)	0.002	0.002	0.001	0.001
<i>P. reconditus</i>	0.027	0.031	(0.006/0.001)	0.001	0.001	0.001
<i>P. saturatus</i>	0.026	0.032	0.023	(0.003/0.001)	0.001	0.001
<i>P. similis</i>	0.026	0.032	0.021	0.009	(0.004/0)	0
<i>P. stridulus</i>	0.027	0.008	0.029	0.03	0.029	-

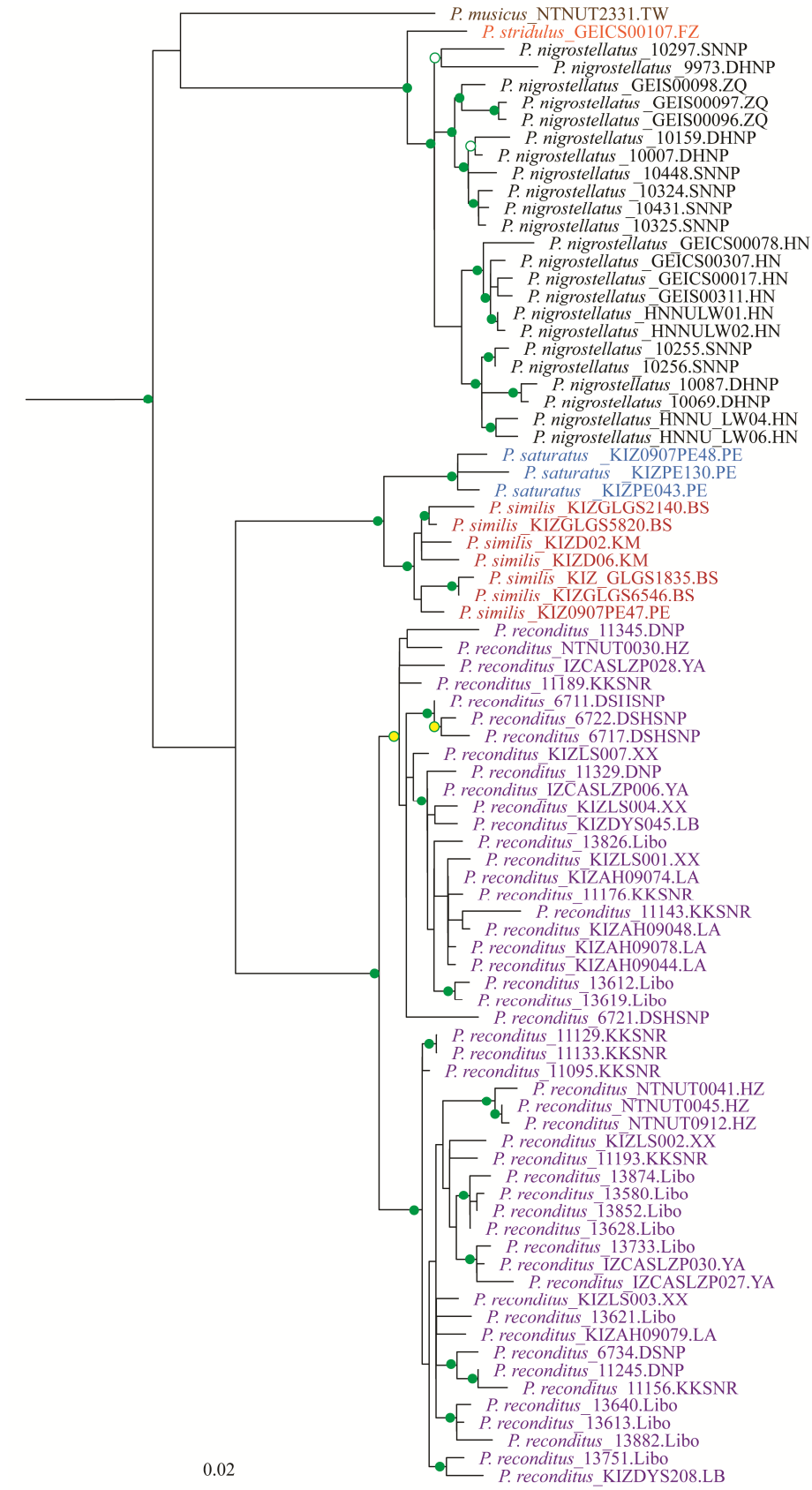


Fig. 2 Phylogenetic tree of combined, partitioned data showing ML/Bayes topology with ML branch lengths

Solid green circles at nodes indicate high support in ML (bootstrap of 70% or higher) and posterior probabilities (0.9 or higher); open circles indicate high ML bootstrap only; and yellow circles indicate high posterior probability.

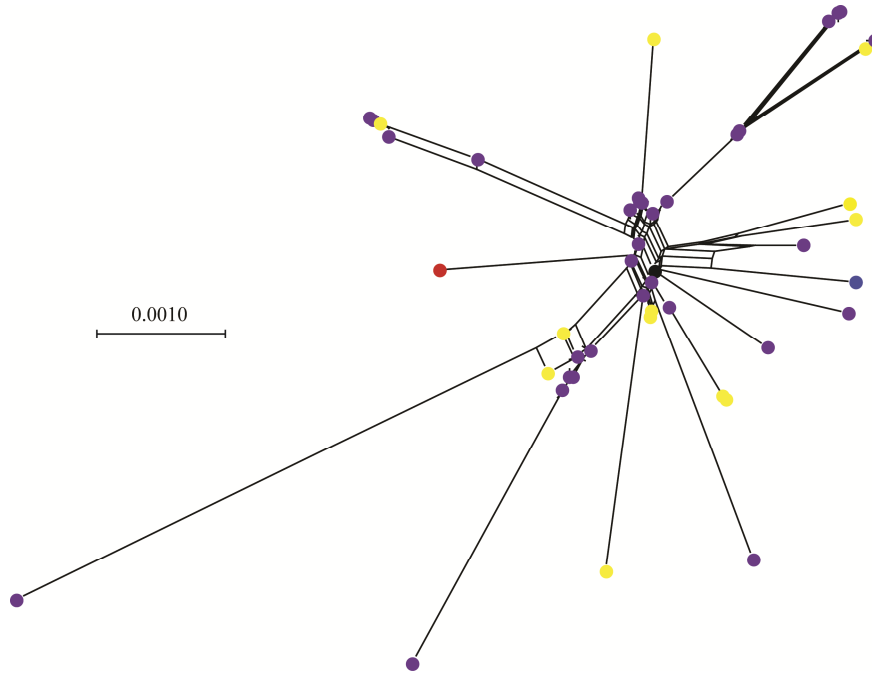


Fig. 3 Haplotype network of ACO1
Nodes are colored to match species as in Figure 1.

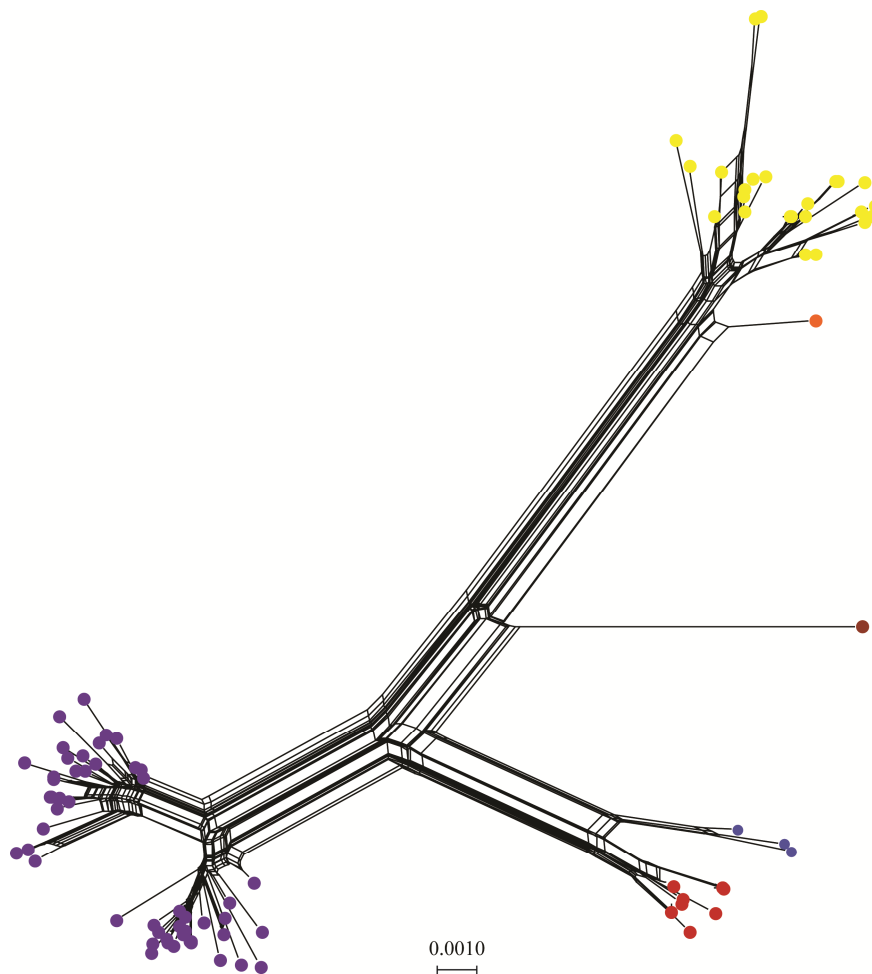


Fig. 4 Network reconstruction of the four gene concatenated dataset using Splitstree 4

pansion in *P. reconditus*, which was split into two well-supported clades with both including individuals from the same localities across central China. Population contractions during periods of glacial maxima have been documented in temperate and tropical taxa (Hewitt, 2004; Waltari et al., 2007; Peterson and Nyári, 2008; Peterson and Ammann, 2012). However, the reverse pattern of range contraction during interglacial periods, followed by range expansion during glacial maxima has been also documented (Galbreath et al., 2009; Hewitt, 2004; Lessa et al., 2003; Peterson and Ammann 2012). Our study lends support for the latter scenario, where ranges of scimitar babblers were more restricted and localized into potential refugia during the LIG, witnessing an expansion trend during the LGM that persisted towards the present time.

P. nigrostellatus was previously described as confined to the island of Hainan. However a recent study (Reddy et al., 2015) showed that it was genetically indistinguishable from populations in southern China and northern Vietnam. Our results also supported this pattern both with phylogenetic and ENM analyses that indicate a model of cyclical isolation during interglacials and then reticulation during glacial maxima and matched with the shallow genetic divergences between mainland

and island samples. While some Hainan samples form a monophyletic group, others were more closely related to samples from Guangxi and suggested that either *P. nigrostellatus* colonized Hainan at least two different times or that these small divergences are the result of ancestral polymorphisms retained after recent isolation.

Phylogeographic studies are highly sensitive to geographic sampling, therefore access to specimens from different sources are critical for conducting more informative analyses. This study was able to further support some of the new and sometimes unexpected discoveries reported in recent papers. Unfortunately, our analysis did not have enough samples to address other crucial questions about diversification within the *ruficollis* complex, such as how species interact when they come in contact in the suture zone described in Dong et al. (2014a) and Reddy et al. (2015). Also we do not have enough samples to address the matter of the relationships of *P. musicus* (see Dong et al., 2014b). To address these and related matters, we feel that future studies should aim to coordinate gathering more compatible genetic data across these different sampling points in order to more completely tackle the enormous task of comparing the diversification of species across this vast and heterogeneous landscape.

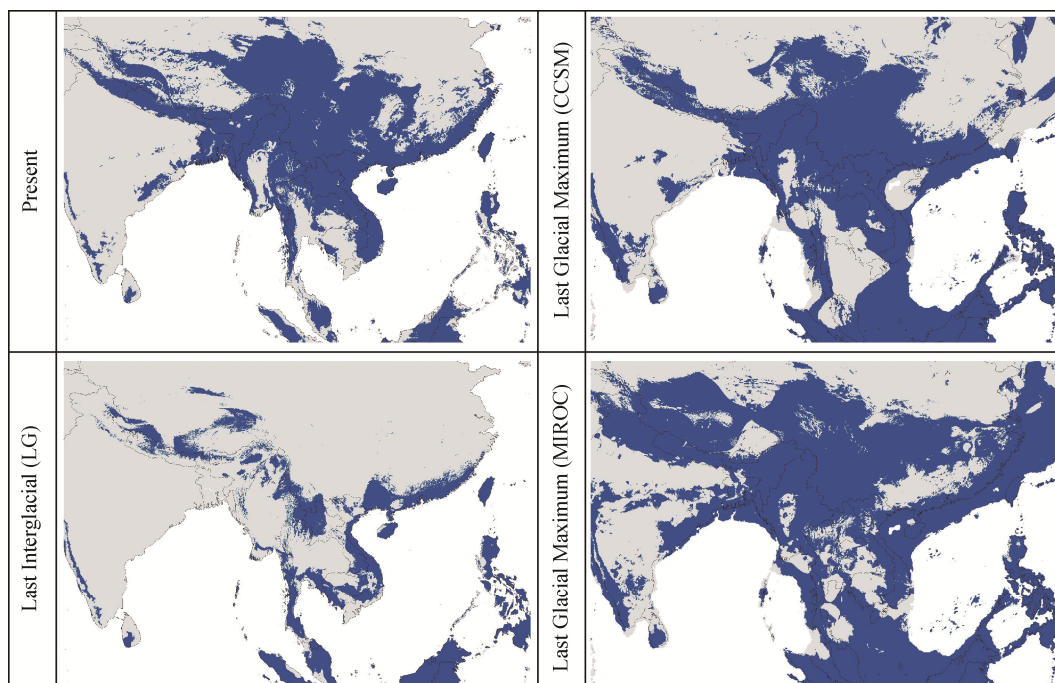


Fig. 5 Ecological niche model projections of the suitable habitat for the *Pomatorhinus ruficollis* complex in the present (top left), last interglacial (bottom left), last glacial maximum (right top and bottom)

Blue areas indicate 95% or higher probability of occurrence.

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