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Genome-wide SNPs confirm plumage polymorphism and hybridisation within a *Cyornis* flycatcher species complex

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Abstract

Morphology has been a leading taxonomic guiding light to systematists for the last couple of hundred years. However, the genetic and – more recently – genomic revolution have produced numerous demonstrations of erroneous classifications that were based on labile morphological traits. We used thousands of genome-wide markers to shed light on evolutionary dynamics in a confusing and taxonomically obscure group of Asian *Cyornis* flycatchers. Using genomic data, we corroborated recent findings based on three mitochrondrial and five nuclear genes that the two taxa *hainanus* and *klossi* which were previously treated as separate species (*Cyornis hainanus* and *Cyornis rubeculoides klossi*, respectively) are genomically homogeneous and form a single species, *C. hainanus*. We also uncovered a novel case of interbreeding between *C. hainanus* and a non-sister species, *C. glaucicomans*, illustrating these flycatchers' ability to hybridise in marginal situations even after substantial times of divergence. Our study illustrates how genome-wide loci can shed light on complicated taxonomic problems, resulting in a better integration of phenotypic and genotypic data.

KEYWORDS

Cyornis, ddRADseq, introgression, phylogenetics, species complex

1 | INTRODUCTION

Animal radiations have produced a bewildering and confusing diversity of species that can be difficult to disentangle based on the methods of traditional taxonomy alone. A large number of phylogenetic studies have revealed strong disagreement between traditional classifications based on morphology and phylogenetic relationships inferred from DNA sequence data (e.g. Alström et al., 2011, 2013, 2018; Burns et al., 2014; Fuchs et al., 2019; Gibson & Baker, 2012; Toon et al., 2003). These discrepancies are the result of parallel and divergent evolution, which have produced distantly related species with similar appearances as well as closely related species with marked morphological differences. Some adaptive radiations have resulted in particularly diverse phenotypes, such as cichlid fishes (Wagner et al., 2012), Madagascar vangas (Jønsson et al., 2012; Reddy et al., 2012), Hawaiian honeycreepers (Lerner et al., 2011; Zuccon et al., 2012) and Darwin's finches (Lamichhaney et al., 2015). Conversely, traditional, morphology-based species delimitations have often failed to reveal the presence of cryptic species, which have been increasingly elucidated with the use of genetic and bioacoustic analyses (e.g. Alström et al., 2015, 2016; Alström & Ranft, 2003; Barrowclough et al., 2016; Fuchs et al., 2018; Gwee et al., 2017; Ng et al., 2016; Oliver et al., 2009; Rheindt et al., 2008; Shakya et al., 2019; Taylor et al., 2019). Although molecular markers have the potential to reveal unexpected relationships as well as cryptic diversity, they are regularly affected by processes such as lineage sorting and/or introgression, which may lead to erroneous conclusions when only a small number of loci are analysed (Harris et al., 2018; Rheindt & Edwards, 2011).

High-throughput sequencing has allowed for increasing molecular marker density at reduced cost in studies addressing the evolutionary dynamics of complicated radiations of non-model organisms (e.g. Garg et al., 2018, 2019; Gwee et al., 2020, 2021; Nater et al., 2015; Ng et al., 2017; Prost et al., 2019; Wagner et al., 2012). Genome-wide markers such as single-nucleotide polymorphisms (SNPs) are increasingly used to elucidate fine scale differences among species and delimit cryptic taxa by virtue of their great abundance throughout the genome and ease of modelling (Chattopadhyay et al., 2016; Garg et al., 2016; Gwee et al., 2020; Hess et al., 2011; Kjeldsen et al., 2016; Morin et al., 2004; Rheindt et al., 2014; Wagner et al., 2012). Such high-density markers can reveal high-resolution genomic information that was not available with the use of traditional markers such as microsatellites and mitochondrial DNA.

The Asian *Cyornis* flycatchers represent a complex songbird radiation with a checkered taxonomic history and multiple rearrangements in recent years (Gwee et al., 2019; Sangster et al., 2010, 2021; Singh et al., 2019; Zhang et al., 2016; Zuccon & Ericson, 2010). One particularly confusing case concerns the Hainan Jungle Flycatcher *Cyornis hainanus* (*C. h. hainanus* and *C. h. klossi*) and the Blue-throated Jungle Flycatcher *Cyornis rubeculoides* (*sensu* Zhang et al., 2016; Singh et al., 2019). *Cyornis h. klossi* was originally described as a subspecies of the

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Blue-throated Jungle Flycatcher *C. rubeculoides* (Gill & Donsker, 2018) based on plumage similarities – the presence of an orange breast but with a variable extent of orange on throat. However, Delacour (1932) was the first to highlight that *C. rubeculoides klossi* and *C. hainanus hainanus* are possibly two forms of a single species on the basis that *C. h. klossi* had a disjunct distribution from all remaining *C. rubeculoides* taxa. Furthermore, he also highlighted that female *C. h. klossi* was indistinguishable from female *C. hainanus*. In contrast, female *C. h. klossi* had a distinct plumage from the females of all other taxa within *C. rubeculoides*. In spite of the highlighted differences, Delacour's notion was rejected by Stresemann and de Schauensee (1936), which led to the long-standing treatment throughout the 20th century of classifying *hainanus* and *klossi* as belonging to separate species entities with a complex and incompletely known distribution range spanning across southern China and mainland Southeas Asia (Figure 1). Despite their pronounced differences in adult male plumage – *C. h. hainanus* having a blue or grey throat/breast and *C. h. klossi* having a rufous lower throat/breast (Figures 1 and 2) – Zhang et al. (2016) found that they were virtually undifferentiated in mtDNA and five nuclear introns, and both taxa were diverged from *C. rubeculoides* by at least 2.8 million years according to mitochondrial clock estimates. This genetic evidence supported Delacour's (1932) suggestion that *C. rubeculoides klossi* and *C. h. hainanus* belong to the same species, *C. hainanus*. This led to the recognition of *klossi* as a subspecies of *C. hainanus* by most recent checklists (Clement & Christie, 2020; Clements et al., 2019; del Hoyo & Collar, 2016; Gill et al., 2021).

The assignment of *C. hainanus* individuals to subspecies using male plumage alone is complex, as plumage in both *hainanus* and *klossi* is highly variable. Most typical male *hainanus* have a blue throat and breast, with a variable amount of grey or whitish mottling on the lower central breast (e.g. Figure 2l,m,n). However, some *C. hainanus* male individuals have a rather poorly demarcated pale grey or whitish wedge extending from the lower breast

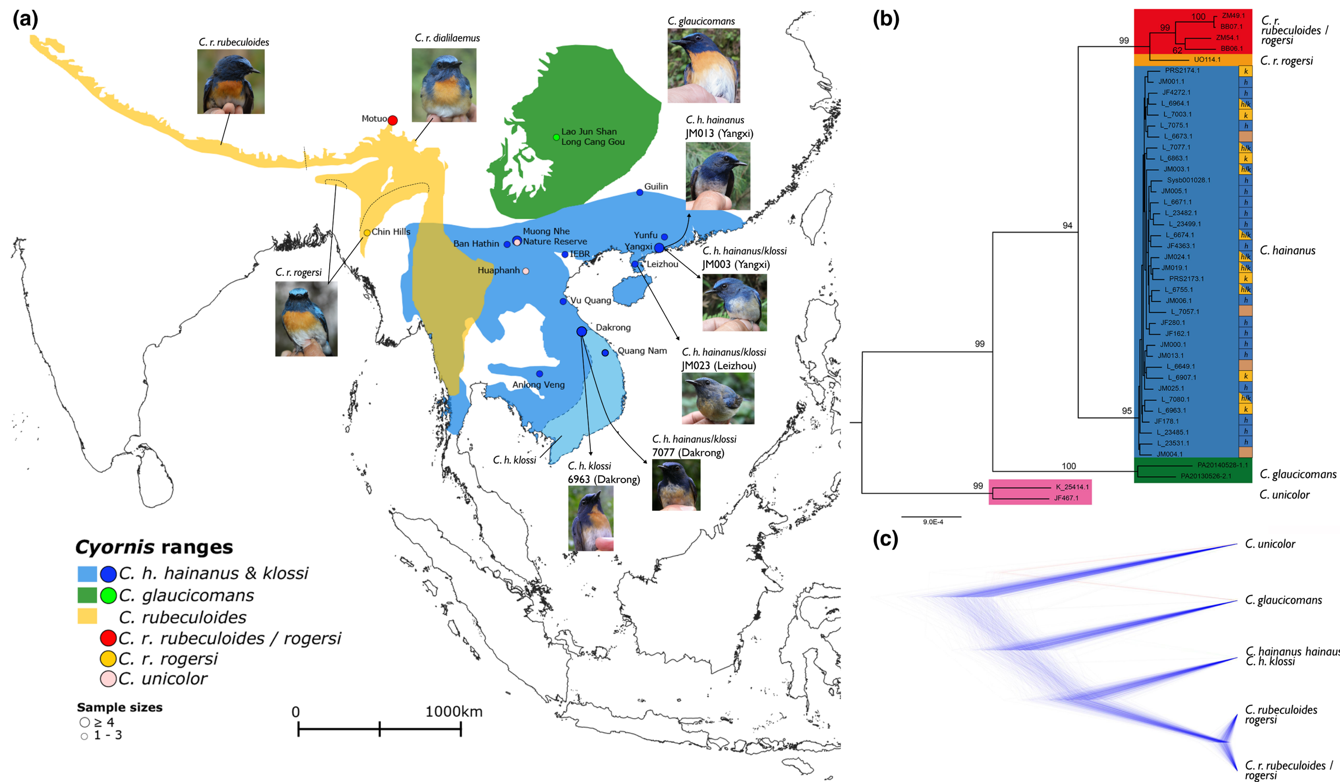

FIGURE 1 (a) Distribution range of *Cyornis rubeculoides* and *C. hainanus*. Sampling localities are indicated by coloured circles, and the size of the circles is proportional to sample size. Approximate distributions of subspecies are indicated with dashed lines, but it should be noted that the subspecies distributions are poorly understood, especially the range of *dialilaemus*. Photographs of *C. rubeculoides* by Ashutosh Singh are reproduced here with permission from Singh et al. (2019). (b) Maximum likelihood tree based on 21,283 SNPs, with bootstrap support indicated at major nodes. Colours represent different taxa: red, *C. r. rubeculoides*; orange, *C. r. rogersi*; blue, *C. hainanus hainanus* and *C. h. klossi*; green, *C. glaucicomans*; and pink, *C. unicolor unicolor* (outgroup). Plumage-type assignment for male *C. hainanus* is indicated by the squares, with typical *hainanus* in blue squares labelled with a *h*, typical *klossi*-type plumage in orange with a *k* and intermediate plumage types in both orange and blue squares and labelled with a *h/k*. Females have a brown square without any labels. (c) Species tree for all individuals generated with SNAPP

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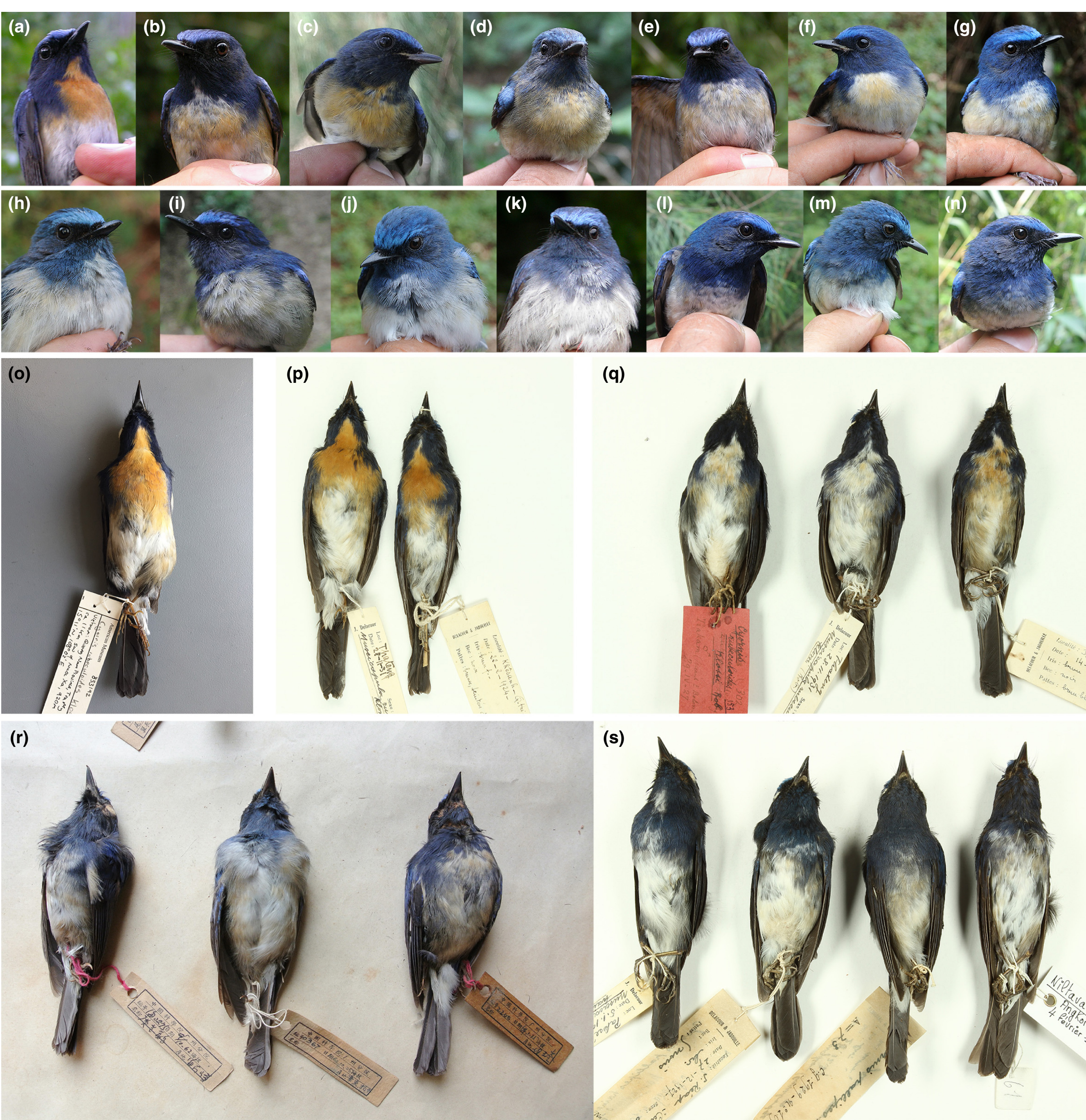

towards (but not reaching) the bill (e.g. Figure 2h,j,k). In contrast, a typical male *klossi* has a clear-cut wedge on the throat (again not reaching the bill), which varies in colour from deep orange to very pale buffish or almost white (Figure 2a,o,p,q). Zhang et al. (2016) found that in one locality in central Vietnam (Dakrong), pure *hainanus*, pure *klossi* and intermediate phenotypes all co-occur. The study also unearthed individuals within the *C. h. hainanus* population in southern China with plumages approaching *C. h. klossi*, which is traditionally known to occur only in Southeast Asia; the exact distribution of such individuals in southern China is not well understood and is limited to the few individuals sampled in Zhang et al. (2016) and the current study.

Deep divergences among other subspecies of *C. rubeculoides* were also detected, and one of these deeply diverged subspecies of *C. rubeculoides*, i.e. the taxon *glaucicomans*, was suggested to be raised to species rank as Chinese Jungle Flycatcher *C. glaucicomans*, supported by its unique song (Zhang et al., 2016). This result was later corroborated by Gwee et al. (2019) based on an independent song analysis. Singh et al. (2019) found the situation to be even more complex, as *C. rubeculoides dialilaemus* (with a rufous lower throat/breast) was inferred to be sister to

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FIGURE 2 Photos of male *Cyornis hainanus* showing variability in breast coloration from typical *C. h. klossi* (a, o, p) to typical *C. h. hainanus* (l, m, n, s) and birds with intermediate characteristics in between. (a) Dakrong, Quang Tri, Vietnam, 25 March 2004; (b) Dakrong, Quang Tri, Vietnam, 4 April 2004; (c) Xitou, Guangdong, China, 29 September 2014 [IOZ-JM019]; (d) Leizhou, Guangdong, China, 24 October 2014 [IOZ-JM024]; (e) Dakrong, Quang Tri, Vietnam, 4 April 2004; (f) Leizhou, Guangdong, China, 13 November 2013; (g) Sanjia Shan, Guangdong, China, 20 April 2014 [IOZ-JM003]; (h) Weizhou Island, Guangxi, China, 15 April 2013; (i) Longtan, Guangxi, China, 01 June 2015; (j) Leizhou, Guangdong, China, 13 November 2013; (k) Vu Quang National Park, Ha Tinh, Vietnam, 13 March 2005; (l) Xitou, Guangdong, China, 27 September 2014 [JM013]; (m) Yunfu, Guangdong, China, 12 May 2014 [IOZ-JM005]; (n) Heweishan, Guangdong, China, 26 May 2014 [IOZ-JM006]; (o) Tra My, Quang Nam, Vietnam [AMNH833192]; (p) from left to right, Bolovens plateau, Thatèng, Xedong, Laos, 28 November 1931 [MNHN-ZO-MO-1933-72]; Quang Tri, Annam, Vietnam, 22 February 1924 [MNHN-ZO-MO-1924-665]; (q) Trakam, Laos, 29 April 1927 [MNHN-ZO-MO-1929-1100]; Bolovens plateau, Thatèng, Xedong, Laos, 28 November 1931 [MNHN-ZO-MO-1933-73]; Di Linh Plateau, Vietnam, 14 March 1927 [MNHN-ZO-MO-1928-385]; (r) Hainan, China, 9 October 1962 [GIABR-0275]; Ledong, Hainan, China 25 October 1962 [GIABR-0367]; Bawangling, Hainan, China 15 January 1964 [GIABR-2200]; (s) Paksé, Laos, 5 January 1932 [MNHN-ZO-MO-1933-74]; Siem Reap, Cambodia, 27 December 1927 [MNHN-ZO-MO-1929-1092]; Angkor, Siem Reap, Cambodia, December 1927 [MNHN-ZO-MO-1929-1093]; Angkor, Siem Reap, Cambodia, 4 February 1962 [MNHN-ZO-MO-61]. Photos in (a, b, e) by Peter Nilsson at Swedish Museum of Natural History; Photos in (c, d, f, g-j, l-n, p-s) by Jonathan Martinez; Photo in K by Ingrid Cederholm at the Swedish Museum of Natural History; Photo in (o) by Paul Sweet at the American Museum of Natural History. Abbreviations: AMNH – American Museum of Natural History, New York, USA; GIABR – Guangdong Institute of Applied Biological Resources; IOZ – Institute of Zoology, Beijing, China; MNHN – Muséum National d'Histoire Naturelle, Paris, France

C. h. hainanus/C. h. klossi mainly on account of mtDNA data, whereas *C. r. rogersi* and *C. r. rubeculoides* formed the sister clade to these, with an estimated divergence time of 2.6 million years based on widely used mtDNA clock rate of 2.1% divergence per million years (Weir & Schluter, 2008).

Given the uncertainty pertaining to the species complex, in the present study, we further explore the relationships between *hainanus* and *klossi* as well as other closely related taxa (e.g. *glaucicomans* and *rubeculoides*) through the use of double digest restriction enzyme-associated DNA sequencing (ddRADSeq) to conduct phylogenetic and population genetic analyses. Through this study using genome-wide data, we confirm that *hainanus* and *klossi* are genomically undifferentiated. Further, we also uncover a novel instance of interbreeding between two non-sister species, *C. hainanus* and *C. glaucicomans*.

2 | METHODS

2.1 | Study group, samples and morphology

Our baseline taxonomy followed Clement and Christie (2020) and Gill et al. (2021), who treated the taxon *klossi* as a subspecies of *C. hainanus* following Zhang et al. (2016). Henceforth, our use of the term *hainanus* refers to *C. h. hainanus* and *klossi* refers to *C. h. klossi*. The typical male plumage of *C. hainanus hainanus* is a deep blue on the head and upperparts, with white belly and undertail coverts (Figures 1 and 2). Male *C. h. klossi* has a similar plumage to *C. h. hainanus*, but with the presence of an orange wedge on the throat to the upper breast (Figures 1 and 2). Females of *hainanus* and *klossi* are inseparable. Male *C. rubeculoides* have plumage like *C. hainanus hainanus* but with an orange upper breast (without an orange wedge on throat as in *C. h. klossi*, Figure 2). Male *C. glaucicomans* has plumage like *C. h. klossi* and orange on breast that also extends to upper flanks (Figure 1). *Cyornis hainanus* samples with plumage intermediate between *hainanus* and *klossi* had subspecies left unassigned due to the ambiguity of the plumage.

The distribution of all included taxa follows Singh et al. (2019). Four newly collected samples from Motuo, southeast Tibet, China were initially identified as either *C. r. rubeculoides*, which breeds in the Himalayas (450–1200 m asl), or *C. r. rogersi*, which has recently been found to breed in the nearby Meghalaya and Nagaland, India, in higher-elevation northeast hill states (900–1500 m asl) and can apparently be very similar to *C. r. rubeculoides* on plumage (Singh et al., 2019; Table 1). Mitochondrial genes (cytb, ND2, and COI) have been capable of distinguishing between both subspecies and were applied here to determine the subspecies of the Motuo samples. Discussion pertaining to *C. r. dialilaemus* was withheld as we do not have samples from this subspecies and will defer to Singh et al. (2019) for its distribution.

2.2 | Molecular sampling and DNA extraction

A total of 45 *Cyornis* flycatcher samples were obtained from localities in South, Southeast, and East Asia, with the majority representing individuals of *C. h. hainanus* and *C. h. klossi*, with eight samples collected specifically for this study (Figure 1a, Table 1). Genomic DNA was

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TABLE 1 List of samples included in the present study

Sex	Taxon (based on plumage)	Retained reads	Institution	Sample number	Locality
♂	*Cyornis h. hainanus*[a]	5,306,276	IOZ	JM000	Yangxi, Sanjia Shan, Guangdong, China
♂	*C. h. hainanus*[a]	7,575,383	IOZ	JM001	Yangxi, Sanjia Shan, Guangdong, China
♂	*C. h. hainanus*[a]	5,927,351	IOZ	JM005	Yunfu, Yunwu Shan, Guangdong, China
♂	*C. h. hainanus*[a]	8,473,294	IOZ	JM006	Hewei Shan, Bajia, Guangdong, China
♂	*C. h. hainanus*	6,729,858	NRM	6671	Captive, Vietnam
♂	*C. h. hainanus*[a]	5,574,321	MNHN	33-JF162	Ban Hathin, Laos
♂	*C. h. hainanus*	8,652,747	MNHN	33-JF178	Ban Hathin, Laos
♂	*C. h. hainanus*	5,867,787	MNHN	34-JF280	Andom Vei, Cambodia
♂	*C. h. hainanus*	7,888,240	MNHN and IEBR	MNHN ZO2015-632; JF4272	Me Linh Biodiversity Center, Institute of Ecology and Biological Research, Vietnam
♂	*C. h. hainanus*	6,393,594	MNHN and IEBR	IEBR JF4363	Me Linh Biodiversity Center, Institute of Ecology and Biological Research, Vietnam
♂	*C. h. hainanus*	7,217,150	IEBR	23482; IEBR - 2012/ B283	Muong Nhe Nature Reserve, Vietnam
♂	*C. h. hainanus*	6,345,565	IEBR	23499; IEBR - 2012/ B299	Muong Nhe Nature Reserve, Vietnam
♂	*C. h. hainanus*	8,704,489	IEBR	23485; IEBR - 2012/ B387	Muong Nhe Nature Reserve, Vietnam
♂	*C. h. hainanus*	6,943,844	IEBR	23531; IEBR - 2012/ B412	Muong Nhe Nature Reserve, Vietnam
♂	*C. h. hainanus*[a,b]	8,982,495	IOZ	JM013	Yangxi Xitou, Guangdong, China
♂	*C. h. hainanus*[a,b]	7,239,920	IOZ	JM025	Beitan, Leizhou, Guangdong, China
♂	*C. h. hainanus*	11,306,890	Sysb	Sysb001028	Guilin, Guangxi, China
♂	*C. h. hainanus/C. h. klossi*[a]	7,232,623	NRM	20046964	Dakrong, Quang Tri Province, Vietnam
♂	*C. hainanus/C. h. klossi*[a]	9,269,395	NRM	20047057	Dakrong, Quang Tri Province, Vietnam
♂	*C. h. hainanus/C. h. klossi*[a]	7,597,321	NRM	20047077	Dakrong, Quang Tri Province, Vietnam
♂	*C. h. hainanus/C. h. klossi*[a]	7,471,625	NRM	20047080	Dakrong, Quang Tri Province, Vietnam
♂	*C. h. hainanus/C. h. klossi*[a]	4,291,127	IOZ	JM003	Yangxi, Sanjia Shan, Guangdong, China
♂	*C. h. hainanus/C. h. klossi*[a]	7,767,431	NRM	20056674	Vu Quang National Park, Ha Tinh Province, Vietnam
♂	*C. h. hainanus/C. h. klossi*[a]	7,757,275	NRM	20056755	Vu Quang National Park, Ha Tinh Province, Vietnam
♂	*C. h. hainanus/C. h. klossi*[a,b]	5,603,840	IOZ	JM019	Yangxi Xitou, Guangdong, China
♂	*C. h. hainanus/C. h. klossi*[a,b]	9,528,199	IOZ	JM024	Beitan, Leizhou, Guangdong, China
♂	*C. hainanus klossi*[a]	5,670,118	NRM	20046863	Dakrong, Quang Tri Province, Vietnam
♂	*C. hainanus klossi*	3,331,234	NRM	20046907	Dakrong, Quang Tri Province, Vietnam
♂	*C. hainanus klossi*[a]	5,553,949	NRM	20046963	Dakrong, Quang Tri Province, Vietnam
♂	*C. hainanus klossi*[a]	9,053,904	NRM	20047003	Dakrong, Quang Tri Province, Vietnam
♂	*C. hainanus klossi*[a]	11,937,978	AMNH	PRS2173 / DOT10738	Ngoc Linh Range, Tra My District, Quang Nam, Vietnam
♂	*C. hainanus klossi*[c]	11,976,914	AMNH	PRS2174 / DOT10739	Ngoc Linh Range, Tra My District, Quang Nam, Vietnam
♀	*C. hainanus*[a,d]	6,538,513	NRM	20056673	Vu Quang National Park, Ha Tinh province, Vietnam

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TABLE 1 (Continued)

Sex	Taxon (based on plumage)	Retained reads	Institution	Sample number	Locality
♀	*C. hainanus*[d]	7,638,479	NRM	6649	Dakrong, Quang Tri Province, Vietnam
♀	*C. hainanus*[a,d]	8,564,461	NRM	20047075	Dakrong, Quang Tri Province, Vietnam
♀	*C. hainanus*[a,d]	6,263,462	IOZ	JM004	Yangxi, Sanjia Shan, Guangdong, China
♂	*C. glaucicomans*	6,240,281	IOZ	PA20130526-2	Longcangguo, Sichuan, China
♂	*C. glaucicomans*	7,361,061	IOZ	PA20140528-1	Laojun Shan, Sichuan, China
♂	*C. rubeculoides rubeculoides/ rogersi*[b]	12,056,412	BNU	6771/BB06	Motuo, Xizang, China
♂	*C. rubeculoides rubeculoides/rogersi*[b]	8,478,606	BNU	6773/BB07	Motuo, Xizang, China
♂	*C. rubeculoides rubeculoides /rogersi*[b]	5,456,910	BNU	6577/ZM49	Motuo, Xizang, China
♂	*C. rubeculoides rubeculoides/ rogersi*[b]	5,299,496	BNU	6605/ZM54	Motuo, Xizang, China
♂	*C. rubeculoides rogersi*	6,914,908	DZUG	UO114	Mt Victoria, Chin hills, Myanmar
♂	*C. unicolor unicolor*	10,757,195	MNHN	ZO 2013–205	Camp Ban Napouak, Huaphanh, Laos
♂	*C. unicolor unicolor*	8,818,230	IEBR	25414; IEBR-2012/ B515	Muong Nhe Nature Reserve, Vietnam

Note: Institution codes: AMNH—American Museum of Natural History, New York; BNU—Beijing Normal University, Beijing China; DZUG—Department of Biodiversity and Environmental Sciences, University of Gothenburg, Sweden; IEBR—Institute of Ecology and Biological Resources, Hanoi, Vietnam; IOZ - Institute of Zoology, Beijing; MNHN—Muséum national d'Histoire naturelle, Paris, France; NRM—Swedish Museum of Natural History, Stockholm, Sweden; Sysb—Museum of Biology, Sun Yat-sen University, Guangzhou, China.

[a]Samples for which photographs are available (on request).

[b]Samples collected specifically for this study.

[c]Specimen saved as tissue and skeleton, noted to be similar to PRS2173/DOT10738 on plumage.

[d]Females could not be assigned to either subspecies or intermediate phenotype.

extracted using the DNeasy Blood & Tissue Kit (QIAGEN) according to the manufacturer's protocol.

2.3 | ddRADSeq library preparation and sequencing

We prepared ddRADSeq libraries using a modified version of the protocol by Peterson et al. (2012). Samples were digested with restriction enzymes *EcoRI* and *MspI*. Sera-Mag magnetic beads (Thermo Scientific) were used to select for 250–650 bp fragments and for clean-up steps. We pooled samples in equimolar volumes and performed a quality check on the final library using a Fragment Analyser (Advanced Analytical). The final multiplexed library was sequenced on an Illumina HiSeq 4000 platform to obtain 150 bp paired-end reads at Novogene Co., Ltd.

2.4 | SNP calling

We checked read quality using FastQC (Babraham Bioinformatics) to determine Phred scores across sequence reads, and demultiplexed and cleaned reads in Stacks v1.41 (Catchen et al., 2013) using settings to remove reads with ≥1 uncalled base and phred scores <10. We then trimmed sequences to 140 bp so that their interquartile Phred scores were at least 30. Quality controlled reads were mapped to the phylogenetically closest available reference genome (Sangster et al., 2010), the collared flycatcher *Ficedula albicollis* (Ellegren et al., 2012), using Burrows-Wheeler Aligner 0.7.12 (Li & Durbin, 2009). Reads were then sorted by coordinate order with Samtools 0.1.19 (Li et al., 2009).

We used the ref_map.pl pipeline to call SNPs with a stack depth of 10 (-m 10), while all other parameters were kept at default. Loci were then processed using the populations programme in Stacks v1.41 (Catchen et al., 2013) with the settings that at least 90% of all individuals in a population must have the loci (-*r* 0.9) and the loci must be in three out of four populations (-p 3) before it can be called. Only the first SNP from each locus was extracted to minimise the effect of linkage (-write-single-snp). All other parameters in the populations programme were left at default. SNPs called in the populations programme were further filtered for linkage disequilibrium in PLINK 1.90 (Chang et al., 2015) with a 25 bp window size, 10 bp

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step size and 0.9 r^2 threshold. We checked for neutrality of called SNPs in Bayescan 2.1 (Foll & Gaggiotti, 2008) at 5% false discovery rate. No locus was detected to be under selection.

2.5 | Population structure analysis

We calculated average heterozygosity in CERVUS 3.03 (Kalinowski et al., 2007) and performed a principal component analysis (PCA) using the unlinked SNPs in R 3.1.2 (R Core Team, 2014) with SNPRelate (Zheng et al., 2012) where missing genotypes are treated as a separate character. Admixture (Alexander et al., 2009) and fastSTRUCTURE 1.0 (Raj et al., 2014) were conducted to infer genetic clusters with Ks ranging from 1 to 10. Netview (Neuditschko et al., 2012), a high-definition network-visualisation approach, was used to detect fine-scale population structure from genome-wide patterns of variation. Genetic distances were calculated in plink. K values specified in Netview are not equivalent to K values defined in fastSTRUCTURE and admixture, instead K values in Netview represent the maximum number of nearest neighbours; i.e. at higher K, relationships at the population level between samples will be more apparent.

2.6 | Phylogenetic analyses

To prepare the data set for phylogenomic analysis, we used the pyRAD pipeline (Eaton, 2014) to obtain concatenated sequence alignments. We used the demultiplexed reads obtained from STACKS as input for this pipeline. For each individual we kept the minimum locus coverage at 10 reads and allowed for a maximum of four bases at Phred score < 20. The clustering threshold for within and between samples was set to 0.88 with default settings. We subsequently generated a concatenated data matrix for phylogenomic analysis without allowing for any missing data. The Pale Blue Flycatcher *Cyornis unicolor* was used as an outgroup.

We reconstructed the phylogeny for our concatenated sequence data under a Maximum Likelihood (ML) framework as implemented in RAxML GUI 1.3 (Silvestro & Michalak, 2012). We used the GTR + gamma model of sequence evolution as suggested in the manual for large data sets with few taxa and performed a single full maximum likelihood tree search. We used the rapid bootstrap algorithm with 1000 replicates. The final tree was viewed in FigTree 1.4 (http://tree.bio.ed.ac.uk/software/figtree/).

We used default settings in SNAPP (Bryant et al., 2012) to estimate a species tree from a random subset of 1000 unlinked SNPs with no missing data, classifying *C. unicolor*, *C. glaucicomans*, *C. r. rubeculoides*/*C. r. rogersi* as separate groups, but explicitly retaining phenotypically identified individuals of *klossi*, *hainanus* and hybrids as members of one group. This arrangement is based on SNAPP's assumption of no gene flow between groups, which would be violated if *klossi* and *hainanus* were kept separate. Analyses were checked for convergence using TRACER v1.6.0 (Rambaut et al., 2014), ensuring that Bayesian runs reached an effective sample size >200 after burn-in.

2.7 | Estimation of gene flow

We performed an ABBA-BABA test to confirm possible ancestral admixture between *C. hainanus* and *C. glaucicomans* using the D-statistic (Durand et al., 2011; Green et al., 2010). D-statistic was calculated in Dsuite v0.3 (Malinsky et al., 2020). The test of introgression was conducted between *C. glaucicomans* (set as P3) and a *hainanus* individual JM004 (P2). *Cyornis rubeculoides* was set as P1, and *C. unicolor* was set as the outgroup (P4; Figure S1).

2.8 | Cytochrome b tree

In the absence of genomic data for *C. r. rubeculoides* from the western part of the range, which includes the type locality of this taxon, we tried to determine the subspecific identity of the samples of *C. r. rubeculoides*/*C. r. rogersi* from Motuo using the mitochondrial cytochrome b (cyt b) gene (Singh et al., 2019; Zhang et al., 2016). Touchdown PCR amplification was done with primers mtF-NP (5′-GGYTTACAAGACCAATGTTT–3′; Fregin et al., 2009) and ND5-Syl (5′-GGCCTAATCA ARGCCTACYTAGG–3′; Stervander et al., 2015). PCR protocol involved initial denaturation at 95°C for 5 min, followed by 15 cycles at 95°C for 40 s, 56°C (0.5°C decrease per cycle) for 30 s, 72°C for 1 min, followed by another 20 cycles at 95°C for 40 s, 49°C for 30 s, 72°C for 1 min, and a final extension step was carried out at 72°C for 10 min. The amplified fragments were sequenced in both directions using 3730XL automatic sequencer (ABI).

Samples of all relevant taxa from Zhang et al. (2016) and Singh et al. (2019) were retrieved from GenBank, and a Maximum Likelihood tree was estimated by PhyML in Seaview 5.0.4 (Guoy et al., 2009; http://doua.prabi.fr/softw

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are/seaview) under the GTR model, with all parameters optimised and 100 bootstrap replicates.

3 | RESULTS

3.1 | Statistics summary

Forty-five samples were successfully sequenced, and a total of 358,856,842 reads were obtained. After quality filtering, a total of 339,560,151 reads (an average of 7,545,781 reads per individual) were retained. For population-genomic analyses, we obtained 28,943 unlinked SNPs using the STACKS pipeline. The overall level of missing data was less than 0.1%. After linkage filtering, 21,238 unlinked SNPs were retained; all SNPs passed the neutrality filtering step. For phylogenetic analyses, we used the pyRAD pipeline and obtained a total of 592,326 bases.

3.2 | Phylogeny

The ML tree based on 592,326 bases confirmed that *C. h. klossi* and *C. h. hainanus* do not form independent clades but are instead interspersed (Figure 1b). These two taxa are then sister to a monophyletic clade comprising *C. rubeculoides rogersi* and *C. r. rubeculoides*/*C. r. rogersi*. *C. glaucicomans* emerged sister to all aforementioned taxa. All relationships were strongly supported (94%–100% bootstrap support). The species tree (Figure 1c) was in agreement with the ML tree (Figure 1b).

3.3 | Population subdivision based on genomic data and plumage

In the Admixture analysis excluding *C. unicolor*, the taxa *C. h. klossi* and *C. h. hainanus* were undifferentiated at $K = 3$ (Figure 3a; fastSTRUCTURE and additional Admixture outputs are available in Figures S2 and S3, respectively), with only a single female *hainanus* (JM004) showing signs of admixture with *C. glaucicomans* (32%). The two *C. glaucicomans* emerged as a second cluster. *C. rubeculoides* samples emerged as a separate cluster that contained both *rogersi* and *rubeculoides*/*rogersi*. A Netview plot including only *C. h. hainanus* and *C. h. klossi* (Figure 3b) revealed that males with intermediate plumage were distributed across the genetic cluster. This also concerned individuals from far outside of the range of *klossi*. Moreover, the presumable hybrid female *hainanus* JM004 was separated from the main cluster. In PCA analysis (Figures 3c,d and S4), individuals of *hainanus* and *klossi* were also tightly clustered, with the female *hainanus* JM004 being significantly offset in the direction of *C. rubeculoides* and *C. glaucicomans*.

3.4 | D-statistics

Given that Admixture and PCA suggested gene flow between *C. glaucicomans* and a female *C. h. hainanus* individual (JM004), we ran an ABBA-BABA test specifically examining introgression between this female *hainanus* JM004 and *C. glaucicomans* (Figure S1). ABBA-BABA analyses showed a significantly positive *D*-value ($D = 0.410247$; p-value $= 0$), indicating genomic admixture between *C. glaucicomans* and *hainanus* individual JM004.

3.5 | Cytochrome b tree

The cytochrome b tree identified the four Motuo samples of *C. r. rubeculoides*/*C. r. rogersi* as part of the *C. r. rogersi* clade, and not of the *C. r. rubeculoides* clade, with good support (Figure S5).

4 | DISCUSSION

4.1 | Plumage polymorphism in one species has misled taxonomy

Using thousands of SNPs from across the whole genome, we found no significant genomic differences between the orange-breasted *klossi* phenotype and the predominantly blue/grey/white-breasted *hainanus* phenotype of the Hainan Jungle Flycatcher *C. hainanus*. Accordingly, our results corroborate those of Zhang et al. (2016) based on three mtDNA genes and five nuclear introns that *klossi* is genetically undifferentiated from *hainanus*.

Previous treatments of *klossi* as a subspecies of the orange-breasted *C. rubeculoides* were based on reliance on labile plumage traits. This taxonomic treatment is not supported by the results of Zhang et al. (2016) or the present study. As a consequence of the former study, most leading avian taxonomic authorities have switched to treating *klossi* as a subspecies of *C. hainanus* (Clements et al., 2019; del Hoyo & Collar, 2016; Gill et al., 2021). In our sample, pure *hainanus* and *klossi* phenotypes were mainly geographically separated, meeting at one locality in central Vietnam, whereas both *hainanus* and birds with intermediate phenotypes were found at two localities in central Vietnam and in southeast China (Figure 1; Table 1). The essentially parapatric distributions of pure *hainanus* and *klossi* phenotypes (also given by Clement

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FIGURE 3 (a) Admixture plot (K = 3) for *Cyornis hainanus hainanus*, *C. h. klossi*, *C. r. rubeculoides*/*C. r. rogersi* and *C. glaucicomans*. Plumage-type assignment for male *C. hainanus* is indicated by the squares, with typical *hainanus* in blue squares labelled with a *h*, typical *klossi* type plumage in orange with a *k* and intermediate plumage types in both orange and blue squares and labelled with a *h/k*. Females have a brown square without any labels. (b) Netview plot (k = 15) for *C. h. hainanus* and *C. h. klossi* and scoring of extent of red-breastedness in males. Blue circles denote *C. h. hainanus* male with typical plumage; orange circles denote individuals with a strong orange breast coloration typical of *C. h. klossi*; grey circles denote male *C. h. hainanus* with unknown breast coloration; mixed blue-and-orange circles denote individuals with an breast coloration intermediate between *klossi* and *hainanus*; brown circles denote female individuals. Individuals depicted below have black labels while all others are labelled in grey. (c, d) Principal component plots of (c) all individuals (*hainanus* and *klossi* samples highlighted by the red ellipse) and (d) only of *C. h. hainanus* and *C. h. klossi* individuals. The numbers refer to the sample numbers in Table 1, with the NRM numbers only indicated by the last four digits.

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& Christie, 2020) are in keeping with treatment of these as different subspecies. It should be noted that both *hainanus* and *klossi* are highly variable in appearance. Although most male *hainanus* have a blue throat and breast, with a variable amount of grey or whitish mottling on the lower central breast, some individuals have a rather poorly demarcated pale grey or whitish wedge extending from the lower breast towards (but not reaching) the bill (Figure 2; also see figure 5 in Zhang et al., 2016). Male *klossi* shows a clear-cut pale wedge on the breast/throat (again not reaching the bill), which varies in colour from deep orange to very pale buffish or almost white (Figure 2).

The throat/breast colour pattern in *C. hainanus* is obviously highly labile. As all taxa in the *C. hainanus-rubeculoides-glaucicomans* complex except *C. h. hainanus* itself show an orange breast, and the majority also display an orange wedge on the throat (Singh et al., 2019; Zhang et al., 2016), the most parsimonious explanation is that the orange breast is an ancestral trait that has been lost in *hainanus*. This loss of orange breast colouration in *hainanus* probably evolved in allopatry after separation of *hainanus* and *klossi* from a most recent common ancestor. The gene(s) coding for orange-breastedness may be retained in *hainanus* but inactivated (see Lopes et al., 2016). Another possibility is that there is a complex of loci that codes for the orange-breastedness that also interact epistatically, and that there is negative epistasis between these alleles found in *hainanus* and *klossi*. Alternatively, the throat/breast feathers might actually have similar underlying orange pigmentation in *hainanus* and *klossi*, but the microstructure of these feathers might differ, producing strikingly different visible colours (see Enbody et al., 2017; McCoy & Prum, 2019). Genomic data of larger population samples and analyses of feather microstructure will be needed to address these alternative hypotheses.

The overall lack of genomic divergence between *hainanus* and *klossi* could result either from the separation between these two taxa being so recent that only a single plumage trait, which may have been under strong sexual selection, has diverged substantially. Alternatively, interbreeding during secondary contact could have homogenised their genomes except at these plumage loci under selection. It seems likely that the marked plumage differences evolved during a period of geographical separation that was too short for reproductive barriers to evolve as suggested for other birds (e.g. Harris et al., 2018; Poelstra et al., 2014; Toews et al., 2016). Gene flow during secondary contact would have further homogenised their genomes, leading to a potential case of 'reverse speciation', i.e. the merging of two taxa (Kearns et al., 2018). If this is the case, an alternative explanation for the presence in southern China of birds with variably orange-breasted phenotypes might be that blue-breasted birds have expanded their range and infiltrated orange-breasted populations through rampant hybridisation. The orange breast of some Chinese birds might therefore represent 'ghost DNA' left behind by previous *klossi* populations (cf. Krosby & Rohwer, 2009; Toews et al., 2016; Zhang et al., 2019).

4.2 | Subspecific identification of *Cyornis rubeculoides* samples from Motuo, southeast Tibet

The previously unstudied population of *C. rubeculoides* from Motuo, southeast Tibet, was initially identified as either *C. r. rubeculoides* or *C. r. rogersi*. The former is generally considered to breed in the Himalayas and western to northeastern Myanmar and the latter in southwest Myanmar (Clements et al., 2019; del Hoyo & Collar, 2016; Gill et al., 2021). However, Singh et al. (2019) concluded, based on mtDNA and three nuclear introns, that a population of *C. rubeculoides* breeding at approximately 900–1500 m a.s.l. in Meghalaya, Nagaland and Mizoram, northeast India, was in fact *C. r. rogersi*. No genomic data are available for *C. r. rubeculoides* from the western part of the range, which includes the type locality. However, the cytochrome b tree places the Motuo samples in the *rogersi* clade with good support (Figure S5). Further studies will be needed to evaluate the geographical distributions of *C. r. rubeculoides* and *C. r. rogersi*.

4.3 | Interbreeding between *Cyornis hainanus* and *Cyornis glaucicomans*

Based on our population genomic analyses and ABBA-BABA tests, one female (JM004) was found to be genomically admixed with *C. glaucicomans* (Figures 3 and S1) as a probable backcrossed hybrid individual, with a *C. hainanus* matrilineage (Zhang et al., 2016). Given the abutting distribution between C. *hainanaus* and *C. glaucicomans*, it is not unexpected that populations close to the contact zone have introgression beyond just the first two generations, although the admixture event documented here is relatively recent. With climate or habitat change in *C. hainanus* historical distribution range, the species may be moving northwards and encroaching upon the distribution range of *C. glaucicomans*. These changes could explain the detection of an admixed individual.

Previous research (Singh et al., 2019; Zhang et al., 2016) as well as our genomic data indicate that *C. hainanus* and

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C. glaucicomans are not sister taxa, but instead that *C. hainanus* is more closely related to *C. rubeculoides*. At the same time, the incidence of an admixed individual among a panel of 38 birds suggests that hybridisation between *C. glaucicomans* and *C. hainanus* is probably not exceptional, although their very different songs would seem to act as a reproductive isolating barrier (Gwee et al., 2019; Zhang et al., 2016). Previous research has shown that hybrid zones are possible between non-sister species (Jacobsen & Omland, 2012). The genomic hybrid individual we identified was sampled in Guangdong, outside of the breeding range of *C. glaucicomans*, but was sampled on 20 April 2014, during the spring migration that would see many individuals from further north pass through this area.

Taken together, our results demonstrate the first instance of interbreeding between two non-sister *Cyornis* species with essentially parapatric breeding distributions. Further studies focusing on the potential area of contact (in areas between Yunnan, Guangxi and Guizhou) would be required to elucidate the extent of introgression between *C. hainanus* and *C. glaucicomans*.

5 | CONCLUSIONS

The classification of *Cyornis* flycatchers has undergone substantial change over the last two decades, resulting in taxonomic rearrangements, in particular the synonymisation of the genus *Rhinomyias* with *Cyornis* (Sangster et al., 2010; Zhang et al., 2016; Zuccon & Ericson, 2010). Our study demonstrates that population-genomic and phylogenomic methodologies can effectively be applied to disentangle the complicated evolutionary history of cryptic species complexes such as the *Cyornis* flycatchers. Our ddRADseq data set confirms the remarkable case of incongruence between plumage and genomic divergence that has misled previous taxonomists into erroneous classifications (Zhang et al., 2016). Our results also show a novel interbreeding event between two non-sister species.

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REFERENCES

Alexander, D. H., Novembre, J., & Lange, K. (2009). Fast model-based estimation of ancestry in unrelated individuals. *Genome Research*, *19*, 1655–1664. doi:10.1101/gr.094052.109

Alström, P., Barnes, K. N., Olsson, U., Barker, F. K., Bloomer, P., Khan, A. A., Qureshi, M. A., Guillaumet, A., Crochet, P.-A., & Ryan, P. G. (2013). Multilocus phylogeny of the avian family Alaudidae (larks) reveals complex morphological evolution, non-monophyletic genera and hidden species diversity. *Molecular Phylogenetics and Evolution*, *69*, 1043–1056. doi:10.1016/j.ympev.2013.06.005

Alström, P., Cibois, A., Irestedt, M., Zuccon, D., Gelang, M., Fjeldså, J., Andersen, M. J., Moyle, R. G., Pasquet, E., & Olsson, U. (2018). Comprehensive molecular phylogeny of the grassbirds and allies (Locustellidae) reveals extensive non-monophyly of traditional genera, and a proposal for a new classification. *Molecular Phylogenetics and Evolution*, *127*, 367–375. doi:10.1016/j.ympev.2018.03.029

Alström, P., Höhna, S., Gelang, M., Ericson, P. G. P., & Olsson, U. (2011). Non-monophyly and intricate morphological evolution within the avian family Cettiidae revealed by multilocus analysis of a taxonomically densely sampled dataset. *BMC Evolutionary Biology*, *11*, 352. doi:10.1186/1471-2148-11-352

Alström, P., & Ranft, R. (2003). The use of sounds in avian systematics and the importance of bird sound archives. *Bulletin of the British Ornithologists' Club*, *123A*, 114–135.

14636409, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/zsc.12568, Wiley Online Library on [31/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Alström, P., Rasmussen, P. C., Zhao, C., Xu, J., Dalvi, S., Cai, T., Guan, Y., Zhang, R., Kalyakin, M. V., Lei, F. M., & Olsson, U. (2016). Integrative taxonomy of the Plain-backed Thrush (*Zoothera mollissima*) complex (Aves: Turdidae) reveals cryptic species, including a new species. *Avian Research*, *7*, 1. doi:10.1186/s40657-016-0037-2

Alström, P., Xia, C., Rasmussen, P. C., Olsson, U., Dai, B., Zhao, J., Leader, P. J., Carey, G. J., Dong, L., Cai, T., Holt, P. I., Le Manh, H., Song, G., Liu, Y., Zhang, Y., & Lei, F. M. (2015). Integrative taxonomy of the Russet Bush Warbler *Locustella mandelli* complex reveals a new species from central China. *Avian Research*, *6*, 9. doi:10.1186/s40657-015-0016-z

Barrowclough, G. F., Cracraft, J., Klicka, J., & Zink, R. M. (2016). How many kinds of birds are there and why does it matter? *PLoS One*, *11*, e0166307. doi:10.1371/journal.pone.0166307

Bryant, D., Bouckaert, R., Felsenstein, J., Rosenberg, N. A., & RoyChoudhury, A. (2012). Inferring species trees directly from biallelic genetic markers: Bypassing gene trees in a full coalescent analysis. *Molecular Biology and Evolution*, *8*, 1917–1932. doi:10.1093/molbev/mss086

Burns, K. J., Shultz, A. J., Title, P. O., Mason, M. A., Barker, F. K., Klicka, J., Lanyon, S. M., & Lovette, I. J. (2014). Phylogenetics and diversification of tanagers (Passeriformes: Thraupidae), the largest radiation of Neotropical songbirds. *Molecular Phylogenetics and Evolution*, *75*, 41–77. doi:10.1016/j.ympev.2014.02.006

Catchen, J., Hohenlohe, P. A., Bassham, S., Amores, A., & Cresko, W. A. (2013). Stacks an analysis tool set for population genomics. *Molecular Ecology*, *22*, 3124–3140. doi:10.1111/mec.12354

Chang, C. C., Chow, C. C., Tellier, L. C. A. M., Vattikuti, S., Purcell, S. M., & Lee, J. J. (2015). Second-generation PLINK: Rising to the challenge of larger and richer datasets. *GigaScience*, *4*, 7. doi:10.1186/s13742-015-0047-8

Chattopadhyay, B., Garg, K. M., Kumar, A. V., Doss, D. P., Rheindt, F. E., Kandula, S., & Ramakrishnan, U. (2016). Genome-wide data reveal cryptic diversity and genetic introgression in an Oriental Cynopterine fruit bat radiation. *BMC Evolutionary Biology.*, *16*(1), 1–5. doi:10.1186/s12862-016-0599-y

Clement, P., & Christie, D. A. (2020). Hainan Blue-flycatcher (*Cyornis hainanus*). In J. del Hoyo, A. Elliott, J. Sargatal, D. A. Christie, & E. de Juana (Eds.), *Handbook of the Birds of the World Alive*. Lynx Edicions. https://www.hbw.com/node/59083

Clements, J. F., Schulenberg, T. S., Iliff, M. J., Roberson, D., Fredericks, T. A., Sullivan, B. L., & Wood, C. L. (2019). *The eBird/Clements checklist of birds of the world* (p. v2019). The Cornell Lab of Ornithology. http://www.birds.cornell.edu/clementschecklist/download/

del Hoyo, J., & Collar, N. J. (2016). *HBW and BirdLife International illustrated checklist of the birds of the world. Volume 2: Passerines*. Lynx Edicions.

Delacour, J. (1932). Etude systématique de quelques oiseaux nouveaux ou intéressants obtenus par la VIe expédition en Indochine. *L'Oiseau et la Revue française D'ornithologie*, *2*, 419–438. https://gallica.bnf.fr/ark:/12148/bpt6k9626316k

Durand, E. Y., Patterson, N., Reich, D., & Slatkin, M. (2011). Testing for ancient admixture between closely related populations. *Molecular Biology and Evolution*, *28*(8), 2239–2252. doi:10.1093/molbev/msr048

Eaton, D. A. (2014). PyRAD: assembly of de novo RADseq loci for phylogenetic analyses. *Bioinformatics*, *30*, 1844–1849. doi:10.1093/bioinformatics/btu121

Ellegren, H., Smeds, L., Burri, R., Olason, P. I., Backström, N., Kawakami, T., Künstner, A., Mäkinen, H., Nadachowska-Brzyska, K., Qvarnström, A., Uebbing, S., & Wolf, B. W. J. (2012). The genomic landscape of species divergence in *Ficedula* flycatchers. *Nature*, *491*, 756–760. doi:10.1038/nature11584

Enbody, E. D., Lantz, S. M., & Karubian, J. (2017). Production of plumage ornaments among males and females of two closely related tropical passerine bird species. *Ecology and Evolution*, *7*(11), 4024–4034. doi:10.1002/ece3.3000

Foll, M., & Gaggiotti, O. E. (2008). A genome scan method to identify selected loci appropriate for both dominant and codominant markers: A Bayesian perspective. *Genetics*, *180*, 977–993. doi:10.1534/genetics.108.092221

Fregin, S., Haase, M., Olsson, U., & Alström, P. (2009). Multi-locus phylogeny of the family Acrocephalidae (Aves: Passeriformes) – the traditional taxonomy overthrown. *Molecular Phylogenetics and Evolution*, *52*, 866–878. doi:10.1016/j.ympev.2009.04.006

Fuchs, J., Alström, P., Yosef, R., & Olsson, U. (2019). Miocene diversification of an open-habitat predatorial passerine radiation, the shrikes (Aves: Passeriformes: Laniidae). *Zoologica Scripta*, *48*, 571–588. doi:10.1111/zsc.12363

Fuchs, J., Douno, M., Bowie, R. C. K., & Fjeldså, J. (2018). Taxonomic revision of the Square-tailed Drongo species complex (Passeriformes: Dicruridae) with description of a new species from western Africa. *Zootaxa*, *4438*, 105–127. doi:10.11646/zootaxa.4438.1.4

Garg, K. M., Chattopadhyay, B., Wilton, P. R., Prawiradilaga, D. M., & Rheindt, F. E. (2018). Pleistocene land bridges act as semipermeable agents of avian gene flow in Wallacea. *Molecular Phylogenetics and Evolution*, *125*, 196–203. doi:10.1016/j.ympev.2018.03.032

Garg, K. M., Sam, K., Chattopadhyay, B., Sadanandan, K. R., Koane, B., Ericson, P. G., & Rheindt, F. E. (2019). Gene flow in the Müllerian mimicry ring of a poisonous Papuan songbird clade (*Pitohui*; Aves). *Genome Biology and Evolution*, *11*(8), 2332–2343. doi:10.1093/gbe/evz168

Garg, K. M., Tizard, R., Ng, N. S., Cros, E., Dejtaradol, A., Chattopadhyay, B., Pwint, N., Päckert, M., & Rheindt, F. E. (2016). Genome-wide data help identify an avian species-level lineage that is morphologically and vocally cryptic. *Molecular Phylogenetics and Evolution*, *102*, 97–103. doi:10.1016/j.ympev.2016.05.028

Gibson, R., & Baker, A. (2012). Multiple gene sequences resolve phylogenetic relationships in the shorebird suborder Scolopaci (Aves: Charadriiformes). *Molecular Phylogenetics and Evolution*, *64*, 66–72. doi:10.1016/j.ympev.2012.03.008

Gill, F., & Donsker, D. (2018). *IOC World Bird List (v 8.1)*. http://www.worldbirdnames.org/

Gill, F., Donsker, D. & Rasmussen, P. (2021). *IOC World Bird List (v11.2)*. doi:10.14344/IOC.ML.11.2

Green, R. E., Krause, J., Briggs, A. W., Maricic, T., Stenzel, U., Kircher, M., Patterson, N., Li, H., Zhai, W., Fritz, M. H. Y., & Hansen, N. F. (2010). A draft sequence of the Neandertal genome. *Science*, *328*(5979), 710–722. doi:10.1126/science.1188021

Guoy, M., Guindon, S., & Gascuel, O. (2009). SeaView Version 4: A multiplatform graphical user interface for sequence

14636409, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/zsc.12568, Wiley Online Library on [31/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

alignment and phylogenetic tree building. *Molecular Biology and Evolution*, *27*, 221–224. doi:10.1093/molbev/msp259

Gwee, C. Y., Christidis, L., Eaton, J. A., Norman, J. A., Trainor, C. R., Verbelen, P., & Rheindt, F. E. (2017). Bioacoustic and multi-locus DNA data of *Ninox* owls support high incidence of extinction and recolonisation on small, low-lying islands across Wallacea. *Molecular Phylogenetics and Evolution*, *109*, 246–258. doi:10.1016/j.ympev.2016.12.024

Gwee, C. Y., Eaton, J. A., Garg, K. M., Alström, P., van Balen, B., Hutchinson, R. O., Prawiradilaga, D. M., & Rheindt, F. E. (2019). Cryptic diversity in *Cyornis* (Aves: Muscicapidae) jungle-flycatchers flagged by simple bioacoustic approaches. *Zoological Journal of the Linnean Society*, *186*, 725–741. doi:10.1093/zoolinnean/zlz003

Gwee, C. Y., Garg, K. M., Chattopadhyay, B., Sadanandan, K. R., Prawiradilaga, D. M., Irestedt, M., Lei, F. M., Bloch, L. M., Lee, J. G. H., Irham, M., Haryoko, T., Soh, M. C. K., Peh, K. S.-H., Rowe, K. M. C., Ferasyi, T. R., Wu, S., Wogan, G. O. U., Bowie, R. C. K., & Rheindt, F. E. (2020). Phylogenomics of white-eyes, a 'great speciator', reveals Indonesian archipelago as the center of lineage diversity. *eLife*, *9*, e62765. doi:10.7554/eLife.62765

Gwee, C. Y., Lee, Q. L., Mahood, S. P., Le Manh, H., Tizard, R., Eiamampai, K., Round, P. D., & Rheindt, F. E. (2021). The interplay of colour and bioacoustic traits in the differentiation of a Southeast Asian songbird complex. *Molecular Ecology*, *30*, 297–309. doi:10.1111/mec.15718

Harris, R. B., Alström, P., Ödeen, A., & Leache, A. D. (2018). Discordance between genomic divergence and phenotypic variation in a rapidly evolving avian genus (*Motacilla*). *Molecular Phylogenetics and Evolution*, *120*, 183–195. doi:10.1016/j.ympev.2017.11.020

Hess, J. E., Matala, A. P., & Narum, S. R. (2011). Comparison of SNPs and microsatellites for fine-scale application of genetic stock identification of Chinook salmon in the Columbia River Basin. *Molecular Ecology Resources*, *11*(Suppl. 1), 137–149. doi:10.1111/j.1755-0998.2010.02958.x

Jacobsen, F., & Omland, K. E. (2012). Extensive introgressive hybridization within the northern oriole group (Genus *Icterus*) revealed by three-species isolation with migration analysis. *Ecology and Evolution*, *2*, 2413–2429. doi:10.1002/ece3.3

Jønsson, K. A., Fabre, P. H., Fritz, S. A., Etienne, R. S., Ricklefs, R. E., Jørgensen, T. B., Fjeldså, J., Rahbek, C., Ericson, P. G. P., Woog, F., Pasquet, E., & Irestedt, M. (2012). Ecological and evolutionary determinants for the adaptive radiation of the Madagascan vangas. *Proceedings of the National Academy of Sciences of the United States of America*, *109*, 6620–6625. doi:10.1073/pnas.1115835109

Kalinowski, S. T., Taper, L. M., & Marshall, T. C. (2007). Revising how the computer program CERVUS accommodates genotyping error increases success in paternity assignment. *Molecular Ecology*, *15*, 1099–1106. doi:10.1111/j.1365-294x.2007.03089.x

Kearns, A. M., Restani, M., Szabo, I., Schrøder-Nielsen, A., Kim, J. A., Richardson, H. M., Marzluff, J. M., Fleischer, R. C., Johnsen, A., & Omland, K. E. (2018). Genomic evidence of speciation reversal in ravens. *Nature Communications*, *9*, 906. doi:10.1038/s41467-018-03294-w

Kjeldsen, S. R., Zenger, K. R., Leigh, K., Ellis, W., Tobey, J., Phalen, D., Melzer, A., FitzGibbon, S., & Raadsma, H. W. (2016). Genome-wide SNP loci reveal novel insights into koala (*Phascolarctos cinereus*) population variability across its range. *Conservation Genetics*, *17*, 337–353. doi:10.1007/s10592-015-0784-3

Krosby, M., & Rohwer, S. (2009). A 2000km genetic wake yields evidence for northern glacial refugia and hybrid zone movement in a pair of songbirds. *Proceedings of the Royal Society B*, *276*, 615–621. doi:10.1098/rspb.2008.1310

Lamichhaney, S., Berglund, J., Almén, S. A., Maqbool, K., Grabherr, M., Martinez-Barrio, A., Promerová, M., Rubin, C. J., Wang, C., Zamani, N., Grant, B. R., Grant, P. R., Webster, M. T., & Andersson, L. (2015). Evolution of Darwin's finches and their beaks revealed by genome sequencing. *Nature*, *518*, 371–375. doi:10.1038/nature14181

Lerner, H. R. L., Meyer, M., James, H. F., Hofreiter, M., & Fleischer, R. C. (2011). Multilocus resolution of phylogeny and timescale in the extant adaptive radiation of Hawaiian honeycreepers. *Current Biology*, *21*, 1838–1844. doi:10.1016/j.cub.2011.09.039

Li, H., & Durbin, R. (2009). Fast and accurate short read alignment with burrows-wheeler transform. *Bioinformatics*, *25*, 1754–1760. doi:10.1093/bioinformatics/btp324

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., & 1000 Genome Project Data Processing Subgroup. (2009). The sequence alignment/map (SAM) format and SAMtools. *Bioinformatics*, *25*, 2078–2079. doi:10.1093/bioinformatics/btp352

Lopes, R. J., Johnson, J. D., Toomey, M. B., Ferreira, M. S., Araujo, P. M., Melo-Ferreira, J., Andersson, L., Hill, G. E., Corbo, J. C., & Carneiro, M. (2016). Genetic basis for red coloration in birds. *Current Biology*, *26*, 1427–1434. doi:10.1016/j.cub.2016.03.076

Malinsky, M., Matschiner, M., & Svardal, H. (2020). Dsuite – Fast D-statistics and related admixture evidence from VCF files. *Molecular Ecology Resources*, *21*, 584–595. doi:10.1111/1755-0998.13265

McCoy, D. E., & Prum, R. O. (2019). Convergent evolution of super black plumage near bright color in 15 bird families. *Journal of Experimental Biology*, *222*, jeb208140. doi:10.1242/jeb.208140

Morin, P. A., Luikart, G., Wayne, R. K., & the SNP workshop group. (2004). SNPs in ecology, evolution and conservation. *Trends in Ecology and Evolution*, *19*, 208–216. doi:10.1016/j.tree.2004.01.009

Nater, A., Burri, R., Kawakami, T., Smeds, L., & Ellegren, H. (2015). Resolving evolutionary relationships in closely related species with whole-genome sequencing data. *Systematic Biology*, *64*, 1000–1017. doi:10.1093/sysbio/syv045

Neuditschko, M., Khatkar, M. S., & Raadsma, H. W. (2012). NetView: A high-definition network-visualization approach to detect fine-scale population structures from genomewide patterns of variation. *PLoS One*, *7*, e48375. doi:10.1371/journal.pone.0048375

Ng, E. Y. X., Eaton, J. A., Verbelen, P., Hutchinson, R. O., & Rheindt, F. E. (2016). Using bioacoustic data to test species limits in an Indo-Pacific Island radiation of *Macropygia* cuckoo doves. *Biological Journal of the Linnean Society*, *118*, 786–812. doi:10.1111/bij.12768

Ng, N. S., Wilton, P. R., Prawiradilaga, D. M., Tay, Y. C., Indrawan, M., Garg, K. M., & Rheindt, F. E. (2017). The effects of Pleistocene climate change on biotic differentiation in a montane songbird clade from Wallacea. *Molecular Phylogenetics and Evolution*, *114*, 353–366. doi:10.1016/j.ympev.2017.05.007

Oliver, P. M., Adams, M., Lee, M. S. Y., Hutchinson, M. N., & Doughty, P. (2009). Cryptic diversity in vertebrates: Molecular

14636409, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/zsc.12568, Wiley Online Library on [31/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

data double estimates of species diversity in a radiation of Australian lizards (*Diplodactylus*, Gekkota). *Proceedings of the Royal Society B*, *276*, 2001–2007. doi:10.1098/rspb.2008.1881

Peterson, B. K., Weber, J. N., Kay, E. H., Fisher, H. S., & Hoekstra, H. E. (2012). Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS One*, *7*, e37135. doi:10.1371/journal.pone.0037135

Poelstra, J. W., Vijay, N., Bossu, C. M., Lantz, H., Ryll, B., Müller, I., Baglione, V., Unneberg, P., Wikelski, M., Grabherr, M. G., & Wolf, J. B. W. (2014). The genomic landscape underlying phenotypic integrity in the face of gene flow in crows. *Science*, *344*, 1410–1414. doi:10.1126/science.1253226

Prost, S., Armstrong, E. E., Nylander, J., Thomas, G. W. C., Suh, A., Petersen, B., Dalen, L., Benz, B. W., Blom, M. P. K., Palkopoulou, E., Ericson, P. G. P., & Irestedt, M. (2019). Comparative analyses identify genomic features potentially involved in the evolution of birds-of-paradise. *GigaScience*, *8*, 1–12. doi:10.1093/gigascience/giz003

R Core Team. (2014). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. www.R-project.org

Raj, A., Stephens, M., & Pritchard, J. K. (2014). fastSTRUCTURE: Variational inference of population structure in large SNP data sets. *Genetics*, *197*, 573–589. doi:10.1534/genetics.114.164350

Rambaut, A., Suchard, M. A., Xie, D., & Drummond, A. J. (2014). *Tracer v1. 6*. http://beast.bio.ed.ac.uk/Tracer

Reddy, S., Driskell, A., Rabosky, D. L., Hackett, S. J., & Schulenberg, T. S. (2012). Diversification and the adaptive radiation of the vangas of Madagascar. *Proceedings of the Royal Society B*, *279*, 2062–2071. doi:10.1098/rspb.2011.2380

Rheindt, F. E., & Edwards, S. V. (2011). Genetic introgression: An integral but neglected component of speciation in birds. *Auk*, *128*, 620–632. doi:10.1525/auk.2011.128.4.620

Rheindt, F. E., Fujita, M. K., Wilton, P. R., & Edwards, S. V. (2014). Introgression and phenotypic assimilation in *Zimmerius* flycatchers (Tyrannidae): Population genetic and phylogenetic inferences from genome-wide SNPs. *Systematic Biology*, *63*, 134–152. doi:10.1093/sysbio/syt070

Rheindt, F. E., Norman, J. A., & Christidis, L. (2008). DNA evidence shows vocalizations to be a better indicator of taxonomic limits than plumage patterns in *Zimmerius* tyrant-flycatchers. *Molecular Phylogenetics and Evolution*, *48*, 150–156. doi:10.1071/MU08020

Sangster, G., Alström, P., Forsmark, E., & Olsson, U. (2010). Multi-locus phylogenetic analysis of Old World chats and flycatchers reveals extensive paraphyly at family, subfamily and genus level (Aves: Muscicapidae). *Molecular Phylogenetics and Evolution*, *57*, 380–392. doi:10.1016/j.ympev.2010.07.008

Sangster, G., Alström, P., Gaudin, J., & Olsson, U. (2021). A new genus for the White-tailed Flycatcher *Cyornis concretus* (Aves: Muscicapidae). *Zootaxa*, *5072*, 599–600. doi:10.11646/zootaxa.5072.6.7

Shakya, S. B., Lim, H. C., Moyle, R. G., Rahman, M. A., Maklarin, L., & Sheldon, F. H. (2019). A cryptic new species of bulbul from Borneo. *Bulletin of the British Ornithologists' Club*, *139*, 46–55. doi:10.25226/bboc.v139i1.2019.a3

Silvestro, D., & Michalak, I. (2012). raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution*, *12*, 335–337. doi:10.1007/s13127-011-0056-0

Singh, A., Gupta, S. K., Alström, P., Mohan, D., Hooper, D. H., Kumar, R. S., Bhatt, D., Singh, P., & Price, T. D. (2019). Taxonomy of cryptic species in the *Cyornis rubeculoides* complex in the Indian subcontinent. *Ibis*, *162*, 924–935. doi:10.1111/ibi.12735

Stervander, M., Illera, J. C., Kvist, L., Barbosa, P., Keehnen, N. P., Pruisscher, P., Bensch, S., & Hansson, B. (2015). Disentangling the complex evolutionary history of theWestern Palearctic blue tits (Cyanistesspp.) – phylogenomic analyses suggestradiation by multiple colonisation events and subsequent isolation. *Molecular Ecology*, *24*, 2477–2494.

Stresemann, E., & de Schauensee, R. M. (1936). Notes on some South Asiatic species of the genus *Cyornis*. *Proceedings of the Academy of Natural Sciences of Philadelphia*, *88*, 337–352.

Taylor, R. S., Bolton, M., Beard, A., Birt, T., Deane-Coe, P., Raine, A. F., Gonzalez-Solis, J., Lougheed, S. C., & Friesen, V. L. (2019). Cryptic species and independent origins of allochronic populations within a seabird species complex (*Hydrobates* spp.). *Molecular Phylogenetics and Evolution*, *139*, 106552. doi:10.1016/j.ympev.2019.106552

Toews, D. P. L., Taylor, S. A., Vallender, R., Brelsford, A., Butcher, B. G., Messer, P. W., & Lovette, I. J. (2016). Plumage genes and little else distinguish the genomes of hybridizing warblers. *Current Biology*, *26*(17), 2313–2318. doi:10.1016/j.cub.2016.06.034

Toon, A., Hughes, J., Baker, A., & Mather, P. (2003). Discordance between morphology and genetic structure among three plumage forms of the Australian Magpie. *Emu*, *103*, 337–343. doi:10.1071/MU02032

Wagner, C. E., Keller, I., Wittwer, S., Selz, O. M., Mwaiko, S., Greuter, L., Sivasundar, A., & Seehausen, O. (2012). Genome-wide RAD sequence data provide unprecedented resolution of species boundaries and relationships in the Lake Victoria cichlid adaptive radiation. *Molecular Ecology*, *22*, 787–798. doi:10.1111/mec.12023

Weir, J. T., & Schluter, D. (2008). Calibrating the avian molecular clock. *Molecular Ecology*, *17*, 2321–2328. doi:10.1111/j.1365-294X.2008.03742.x

Zhang, D., Tang, L., Cheng, Y., Hao, Y., Xiong, Y., Song, G., Qu, Y., Rheindt, F. E., Alström, P., Jia, C., & Lei, F. M. (2019). "Ghost Introgression" as a cause of deep mitochondrial divergence in a bird species complex. *Molecular Biology and Evolution*, *36*, 2375–2386. doi:10.1093/molbev/msz170

Zhang, Z., Wang, X., Huang, Y., Olsson, U., Martinez, J., Alström, P., & Lei, F. M. (2016). Unexpected divergence and lack of divergence revealed in continental Asian *Cyornis* flycatchers (Aves: Muscicapidae). *Molecular Phylogenetics and Evolution*, *94*, 232–241. doi:10.1016/j.ympev.2015.08.024

Zheng, X., Levine, D., Shen, J., Gogarten, S. M., Laurie, C., & Weir, B. S. (2012). A high-performance computing toolset for relatedness and principal component analysis of SNP data. *Bioinformatics*, *28*, 3326–3328. doi:10.1093/bioinformatics/bts606

Zuccon, D., & Ericson, P. G. P. (2010). A multi-gene phylogeny disentangles the chat-flycatcher complex (Aves: Muscicapidae). *Zoologica Scripta*, *39*, 213–224. doi:10.1111/j.1463-6409.2010.00423.x

14636409, 2023, 1, Downloaded from https://onlinelibrary.wiley.com/doi/10.1111/zsc.12568, Wiley Online Library on [31/01/2023]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Zuccon, D., Prŷs-Jones, R., Rasmussen, P. C., & Ericson, P. G. P. (2012). The phylogenetic relationships and generic limits of finches (Fringillidae). *Molecular Phylogenetics and Evolution*, *62*, 581–596. doi:10.1016/j.ympev.2011.10.002

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