Cryptic diversity in *Cyornis* (Aves: Muscicapidae) jungle-flycatchers flagged by simple bioacoustic approaches

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Despite the ongoing taxonomic revolution incorporating multiple species delimitation methods, knowledge gaps persist in the taxonomy of comparatively well-studied animal groups such as birds. Morphologically cryptic species risk slipping under the conservation radar, as they get mistakenly united with other species. Here, we employed six to 11 vocal parameters of each population to examine the species delimitation of nine *Cyornis* jungle-flycatcher species complexes distributed across Asia. We found moderate to strong vocal evidence for the taxonomic elevation of ten cryptic *Cyornis* species. Additionally, we conducted mitochondrial and genome-wide SNP analyses for two of the *Cyornis* complexes to examine the effectiveness of bioacoustics as a tool for avian species delineation and found congruent results between vocal and molecular data. Therefore, we propose a taxonomic reclassification of the complicated *Cyornis* species complexes and recommend routine application of bioacoustics in avian taxonomic classification.

ADDITIONAL KEYWORDS: species delineation – biodiversity – revision.

INTRODUCTION

DNA-based techniques, including DNA barcoding and Next Generation Sequencing, have helped uncover cryptic species-level diversity across many animal groups (Hebert *et al.*, 2004; Hajibabaei *et al.*, 2007; Lohman *et al.*, 2010; Dincă *et al.*, 2011; Kress *et al.*, 2015; Chattopadhyay *et al.*, 2016; Grabowski *et al.*, 2017; Ng *et al.*, 2017; Garg *et al.*, 2018). Even so, considerable taxonomic discrepancies remain, even in comparatively well-studied groups such as birds (Saitoh *et al.*, 2015; Garg *et al.*, 2016; Olsson *et al.*, 2016; Stervander *et al.*, 2016; Pulido-Santacruz *et al.*, 2018). Molecular research on widely distributed species complexes that are challenging to delimit, based on morphology, may take a long time to complete pending comprehensive

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sample acquisition. With the sixth mass extinction underway (Barnosky *et al.*, 2011; Ceballos *et al.*, 2015; McCallum, 2015), cataloguing the species on Earth becomes a race against time. Supporting tools to scan for cryptic species are required when genetic data are unavailable (Dincă *et al.*, 2011; Alström *et al.*, 2015; Ceballos *et al.*, 2017). Although traditional taxonomy primarily relies on morphological comparisons to delimit species, this practice can be inadequate for species complexes that are morphologically conserved. Therefore, other supporting tools for taxonomic classification should be taken into consideration.

Here, we aim to investigate the reliability of a simple set of bioacoustic parameters for routine application in the delineation of songbird species limits and detection of cryptic passerine species. Vocalizations are an important form of communication in songbirds for territory establishment, mate attraction and pair bond maintenance (Kroodsma & Miller, 1982; Kroodsma & Byers, 1991; Catchpole & Slater, 2003) and have been shown to be effective in multiple cryptic species discoveries (Lambert & Rasmussen, 1998; King, 2002a, b; King & Robson, 2008; Alström *et al.*, 2011; Rheindt *et al.*, 2011; Rasmussen *et al.*, 2012; Alström *et al.*, 2015; Ng *et al.*, 2016; Ng & Rheindt, 2016; Cros & Rheindt, 2017; Gwee *et al.*, 2017; Prawiradilaga *et al.*, 2017). In addition, sound recordings of most birds, with the exception of some protected species that are threatened by the songbird trade, are readily accessible online and comparatively easy to obtain, thus greatly shortening the time required to detect potential cryptic species. Acoustic parameter sets for species delimitation similar to ours have previously been proposed (e.g. Tobias *et al.*, 2010), but have rarely been applied across multiple species complexes in oscine songbird radiations for flagging cryptic species-level diversity.

In the present study, we measured and compared six to 11 bioacoustic parameters across the vocalizations of nine *Cyornis* species complexes distributed across Asia. The genus *Cyornis* is notorious for its confusing and controversial taxonomy, partly because many *Cyornis* species share a very similar plumage, especially among females, which are almost invariably brown in colour (Stresemann & de Schauensee, 1936; Renner *et al.*, 2009; Sangster *et al.*, 2010; Zhang *et al.*, 2016; Eaton & Rheindt, 2017; Garg *et al.*, 2018). In most previous treatments, the genus *Cyornis* consisted exclusively of flycatchers with a blue-backed male plumage, but Sangster *et al.* (2010) found two species of brown-backed flycatchers, *Rhinomyias umbratilis* and *R. olivaceus* (*sensu* Dickinson, 2003), genetically nested in *Cyornis*. The placement of these brown-backed flycatchers in *Cyornis* underscores the pitfalls of relying solely on plumage variation for classification. Based on the distinct melodious, tinkling quality of *Cyornis* songs, Eaton *et al.* (2016b) attributed an additional three former *Rhinomyias* flycatchers with male brown backs to *Cyornis* jungle-flycatchers, emphasizing the importance of bioacoustic traits in flycatcher systematics. To accommodate the fact that the new genus-level classification includes multiple all-brown species in *Cyornis*, we discard the previous use of 'blue-flycatcher' and follow Eaton *et al.* (2016b) in using 'jungle-flycatcher' as the standardized common name for all *Cyornis* species.

However, while bioacoustics have helped reveal the true genus affinity of multiple former *Rhinomyias* flycatchers, species-level taxonomy in *Cyornis* remains in its infancy. In this study, we analyzed the bioacoustics of 12 out of the 25 *Cyornis* species recognized by Dickinson & Christidis (2014) and address the taxonomic discrepancies observed across the nine *Cyornis* species complexes to which they belong. In a number of cases where bioacoustic results did not easily lend themselves to interpreting whether taxa are clinal or discrete in nature, we additionally compared them with mitochondrial sequences and genome-wide single nucleotide polymorphism (SNP) data, some of it specifically generated for this study, to ascertain the extent of congruence and to evaluate the effectiveness of the set of vocal parameters applied in our study. Furthermore, genome-wide SNPs have been shown to overcome the limitations of low individual sample sizes (McCormack *et al.*, 2013).

MATERIAL AND METHODS

BIOACOUSTIC SAMPLING

We collected and measured a total of 283 sound recordings from online sound libraries xeno-canto (https://www.xeno-canto.org), Macaulay Library (https://www.macaulaylibrary.org), and Avian Vocalizations Center (http://www.avocet.zoology.msu.edu), in addition to our personal field sound collections (Supporting Information, Appendix S1). A total of 72 recordists have contributed to the set of sound recordings used in our study. Although different recording equipment was used among recordists, spectrogram inspection revealed that there was negligible equipment bias. Altogether, we collected sound recordings of 37 *Cyornis* taxa (*sensu* Dickinson & Christidis, 2014) and two undescribed taxa from the Togian Islands and Meratus Mountains (see Table 1). Dickinson & Christidis's (2014) treatment was applied as our baseline taxonomy, with a nomenclatural correction of *C. unicolor infuscatus* Hartert, 1902 in Dickinson & Christidis (2014) to *C. unicolor cyanopolia* (Mlíkovský, 2011).

Table 1. List of *Cyornis* taxa (*sensu* Dickinson & Christidis, 2014) and the corresponding number of sound recordings used in the present study

No.	Taxon	Author and year	No. of sound recordings
1	*C. colonus colonus*	(Hartert, 1898)	4
2	*C. colonus pelingensis*	(Vaurie, 1952)	5
3	*C. rubeculoides rubeculoides*	(Vigors, 1831)	6
4	*C. rubeculoides glaucicomans*	Thayer & Bangs, 1909	15
5	*C. rubeculoides dialilaemus*	Salvadori, 1889	3
6	*C. rubeculoides rogersi*	Robinson & Kinnear, 1928	5
7	*C. rubeculoides klossi*	Robinson, 1921	6
8	*C. hainanus*	(Ogilvie-Grant, 1900)	19
9	*C. banyumas banyumas*	(Horsfield, 1821)	2
10	*C. banyumas ligus*	(Deignan, 1947)	8*
11	*C. banyumas montanus*	Robinson & Kinnear, 1928	6
12	*C. banyumas whitei*	Harington, 1908	2
13	*C. banyumas coerulifrons*	Baker, E.C.S., 1918	3
14	*C. banyumas lekhakuni*	(Deignan, 1956)	5
15	*C. magnirostris*	Blyth, 1849	2
16	*C. lemprieri*	(Sharpe, 1884)	5
17	*C. ruficauda boholensis*	(Rand & Rabor, 1957)	2
18	*C. ruficauda isola*	(Hachisuka, 1932)	1
19	*C. ruficauda ocularis*	(Bourns & Worcester, 1894)	1
20	*C. ruficauda samarensis*	(Steere, 1890)	3
21	*C. olivaceus olivaceus*	(Hume, 1877)	14
22	*C. caerulatus caerulatus*	(Bonaparte, 1857)	14
23	*C. caerulatus albiventer*	Junge, 1933	6
24	*C. tickelliae tickelliae*	Blyth, 1843	10
25	*C. tickelliae jerdoni*	Holdsworth, 1872	15
26	*C. tickelliae indochina*	Chasen & Kloss, 1928	12
27	*C. tickelliae sumatrensis*	(Sharpe, 1879)	5
28	*C. unicolor unicolor*	(Blyth, 1843)	22
29	*C. unicolor cyanopolia*	Blyth, 1870	22
30	*C. unicolor diaoluoensis*	(Cheng, Yang & Lu, 1981)	16
31	*C. rufigastra rufigastra*	(Raffles, 1822)	7
32	*C. rufigastra djampeanus*	(Hartert, 1896)	6
33	*C. rufigastra omissus*	(Hartert, 1896)	7
34	*C. rufigastra peromissus*	Hartert, 1920	2
35	*C. rufigastra kalaoensis*	(Hartert, 1896)	3
36	*C. rufigastra simplex*	Blyth, 1870	2
37	*C. rufigastra philippinensis*	Sharpe, 1877	5

*Four of the recordings were from Panaitan Island population, which is recognized as a separate subspecies, *C. banyumas mardii*, in other taxonomic classifications.

VOCAL ANALYSES

We used Raven Pro 1.5 (Bioacoustics Research Program, Cornell Laboratory of Ornithology, Ithaca, NY, USA) to inspect and measure parameters of each recording. Default settings of Raven Pro were used except window size, which was adjusted to 1024 to obtain the best resolution across all recordings. We inspected all recordings to remove duplicates and recordings of obviously misidentified species that clearly referred to the known vocalizations of other species. Due to the song-learning abilities of oscine songbirds (Kroodsma & Miller, 1982), intraspecific variation and heterospecific songs learned from the external environment may be present in some recordings. Therefore, the most ubiquitous song structure, defined as a motif herein, across recordings of each taxon was chosen for homologous comparison among members of the same species complex. We measured a total of six vocal parameters across all species complexes: (1) number of elements per motif

(i.e. number of syllables in a song), (2) duration of a motif, (3) minimum frequency of a motif, (4) maximum frequency of a motif, (5) peak frequency (i.e. the frequency with the highest amplitude) of a motif and (6) bandwidth (i.e. maximum minus minimum frequency) of a motif. Members of the *C. banyumas* and *C. tickelliae* species complexes are characterized by songs with a large degree of frequency modulation between the first and second half of a song rendition. Thus, we added five more parameters into the analyses of these two species complexes: (1) average centre frequency (i.e. the frequency that divides the selection into two frequency intervals of equal energy), (2) average centre frequency of the first half of the motif, (3) average centre frequency of the second half of the motif, (4) average difference in the centre frequencies of the first and second halves of the motifs and (5) average proportion of time to reach minimum frequency in a motif. We measured at least five motifs for each recording, or we measured all motifs when the recording contained less than five motifs.

Rstudio v.1.0.143 (https://www.rstudio.com) was used to conduct principal component analysis (PCA) on the vocal dataset to distinguish clinal bioacoustic variation from discrete variation. Additionally, we assessed the vocal diagnosability of variables using the criterion outlined by Isler *et al.* (1998), henceforth referred to as the 'Isler Criterion'. The Isler Criterion is based on two conditions: (1) there must be no overlap between the ranges of measurements between the two taxa being compared and (2) the means ($\bar{x}$) and standard deviations (SD) of the taxon (t) with the smaller set of measurements (a) and the taxon with the larger set of measurements (b) have to meet the following requirement: $\bar{x}_a + t_a \text{SD}_a \leq \bar{x}_b - t_b \text{SD}_b$, where t_i refers to the t-score at the 95th percentile of the t distribution for $n-1$ degrees of freedom. Since there must be no overlap between the two sets of measurements, measurements of one taxon are uniformly higher than the other and a one-tailed test with a significance level of 5% was used. This criterion has been used as a bioacoustic species delimitation method across a wide variety of Asian birds, including pigeons (Rheindt *et al.*, 2011; Ng *et al.*, 2016; Ng & Rheindt, 2016), nightjars (Sangster & Rozendaal, 2004), owls (Gwee *et al.*, 2017) and oscine songbirds (Cros & Rheindt, 2017; Prawiradilaga *et al.*, 2017).

Linear discriminant analysis (LDA) was applied to the vocal dataset of species complexes that displayed strong vocal divergence in PCA and under the Isler Criterion, to further assess the accuracy of vocal identification among taxa. For each of these complexes, indistinct taxa were combined so that each group represents a vocally distinct cluster identified by PCA and/or Isler Criterion. We used the R package 'Mass' to conduct LDA and set equal prior probabilities for each cluster. A scatterplot of LD1 and LD2 were plotted on

the x-axis and y-axis, respectively, except for complexes with two distinct clusters (e.g. *C. tickelliae* species complex) as only one LD is generated for discriminant function analysis of dataset with two input groups.

DNA EXTRACTIONS

In order to examine the congruence between bioacoustic and molecular data, we examined mitochondrial DNA sequences and genome-wide SNP data across 11 samples of the *C. unicolor* and *C. rufigastra* species complexes (Table 2). DNA extractions were conducted using DNeasy Blood & Tissue Kits (Qiagen, Hilden, Germany) using the manufacturer's recommended protocol.

MITOCHONDRIAL DNA SEQUENCING

Cytochrome *b* (Cyt*b*) was amplified with primers L14995 and H16065 (Helbig *et al.*, 1995). Polymerase chain reactions (PCR) were conducted in 25 μL reaction volumes, which comprised 2.5 μL 10X *Taq* PCR buffer, 2 μL MgCl$_2$, 0.5 μL dNTPs, 0.5μL of each primer (10 μm), 0.125 μL *Taq* DNA polymerase, 1 μL mtDNA template and 17.9 μL molecular grade water. After initial denaturation (5 min at 95 °C), 40 cycles of 40 s at 95 °C, 1 min at 45 °C and 2 min at 72 °C were run on a SuperCycler Trinity thermocycler. PCR product clean-up and sequencing followed Sadanandan & Rheindt (2015). DNA sequences were assembled with CodonCode Aligner v.8.0.1 and aligned with MEGA v.7 using ClustalW into an alignment of 1046 base pairs (bp) length (Larkin *et al.*, 2007; Kumar *et al.*, 2016).

LIBRARY PREPARATION OF GENOME-WIDE SNPS

The double digest restriction enzyme associated DNA sequence (ddRADseq) libraries were prepared following Peterson *et al.* (2012) and Chattopadhyay *et al.* (2016). We used 6 bp EcoRI-HF and 4 bp MspI restriction enzymes to digest the DNA and used a tight size selection with Pippin Prep (Sage Science) to select fragments with a mean size of 330 bp. The final ddRADseq library was prepared with 12 PCR cycles of pooled samples in equimolar concentration. We conducted a 150 bp paired-end run on an Illumina HiSeq 4000 with a 40% spike using whole genome libraries to avoid low sequence diversity issues.

ddRADseq DATA FILTERING AND DATA MATRIX GENERATION

We used the ipyrad 0.7.23 (Eaton, 2014) pipeline to generate the sequence data matrix for the genome-wide SNP dataset. We performed a visual quality check for the raw data using FASTQC (Andrews, 2010). We demultiplexed and trimmed the raw reads to 140 bp

Table 2. Information about the molecular samples used in this study. Italicized Genbank accession numbers refer to samples sourced from previous studies. Abbreviations: Cyt*b*, cytochrome *b* (mitochondrial gene); AMNH, American Museum of Natural History, New York; IOZ, Institute of Zoology, Chinese Academy of Sciences, Beijing; MZB, Museum Zoologicum Bogoriense, Cibinong, West Java; NRM, Swedish Museum of Natural History, Stockholm; VAST, Vietnam Academy of Science and Technology, Hanoi

Taxon	Sample voucher number and institution	Collecting locality	Genbank accession no. for cyt*b*
Cyornis unicolor unicolor	AMNH PRS2252	Vietnam	*HM633291* (Sangster *et al.*, 2010) -
Cyornis unicolor unicolor	IOZ JF467	Viengthong, Laos	MK351778
Cyornis unicolor unicolor	VAST 25414	Muong Nhe Nature Reserve, Vietnam	MK351779
Cyornis unicolor diaoluoensis	IOZ 6268	Wuzhi Shan, Hainan, China	*KP337022* (Zhang *et al.* 2016) -
Cyornis unicolor diaoluoensis	IOZ HND131	Hainan, China	MK351777
Cyornis rufigastra rufigastra	AMNH 15113	Sabah, Malaysia	MK351770
Cyornis rufigastra rufigastra	AMNH 14999	Quezon, Philippines	MK351769
Cyornis rufigastra omissus	MZB LUW10	Luwuk, Sulawesi, Indonesia	MK351771
Cyornis rufigastra ssp. nov.	MZB TOG07	Togian, Indonesia	MK351772
Cyornis rufigastra ssp. nov.	MZB TOG08	Togian, Indonesia	MK351773
Cyornis rufigastra ssp. nov.	MZB TOG09	Togian, Indonesia	MK351774
Cyornis rufigastra ssp. nov.	MZB TOG14	Togian, Indonesia	MK351775
Cyornis rufigastra ssp. nov.	MZB TOG20	Togian, Indonesia	MK351776
Cyornis concretus cyaneus	NRM 2004.345	Vietnam	*HM633288* (Sangster *et al.*, 2010) -
Cyornis colonus colonus	MZB TBU14	Taliabu, Indonesia	-

in ipyrad and discarded any read in which more than five bases were below a PHREAD score of 20. Then we set the minimum depth for statistical base calling to 10 and used a clustering threshold of 0.85 for *de novo* assembly. For both species complexes we used an individual of *C. colonus colonus* as the outgroup and allowed for no missing data in the final sequence matrix. The total length of sequence matrix used for phylogenetic analyses were 1 321 570 bp for *C. unicolor* and 1 163 389 bp for *C. rufigastra* species complexes.

PHYLOGENETIC ANALYSES

We generated independent maximum likelihood trees for the *C. unicolor* and *C. rufigastra* species complexes using raxmlGUI 1.5 (Silvestro & Michalak, 2012) for mitochondrial Cyt*b* and the concatenated SNP data separately. In both cases, we used the GTR + Gamma model of sequence evolution and performed a single full maximum likelihood tree search, employing the rapid bootstrap algorithm with 1000 replicates for

the concatenated genome-wide data and with 10 000 replicates for the Cyt*b* sequences. The final trees were visualized in FigTree v.1.4.2 (Rambaut, 2015).

RESULTS

BIOACOUSTIC DIVERGENCES

Bioacoustic PCA of members of the *C. colonus* species complex indicates pronounced vocal divergence between the taxa from Peleng and Taliabu, further supported by two vocal parameters that emerge diagnosable under the conservative Isler Criterion (Fig. 1A; Table 3). Similarly, LDA identifies two distinct vocal clusters (Supporting Information, Appendix S2).

PCA and LDA of vocal parameters in the *C. rubeculoides* species complex corroborate that *C. r. glaucicomans* is vocally distinct from *C. r. rubeculoides*, *C. r. dialilaemus*, *C. r. rogersi*, *C. r. klossi* and *C. hainanus*, whereas the latter five share generally similar vocalizations with
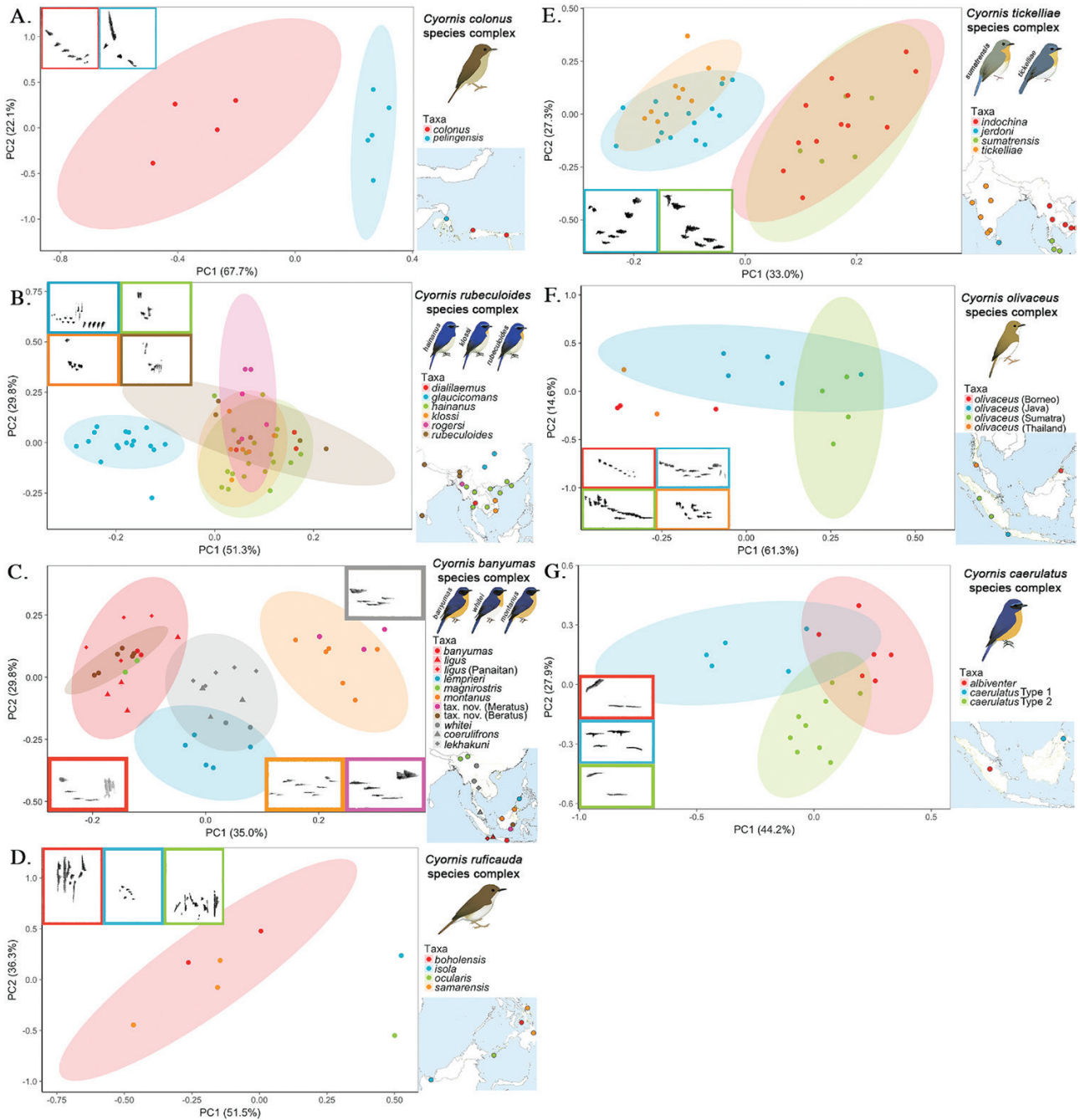
Figure 1. Principal component analysis (PCA) based on six vocal parameters (11 for the *C. banyumas* complex in panel C and the *C. tickelliae* complex in panel E) for seven species complexes, with ellipses representing 95% confidence intervals of the principal component (PC) scores for each taxon represented by ≥4 individuals. Sample localities of recordings for each species complex are indicated on the map and a colour-coded spectrogram of each selected taxon is shown inside the PCA plot.

one another (Fig. 1B; Supporting Information, Appendix S2). Similarly, *C. r. glaucicomans* is vocally diagnosable from all other members of the complex by one to two parameters under the Isler Criterion, while the other members of the complex do not

exhibit any diagnosable parameters among one another (Table 3).

PCA based on 11 vocal parameters reveals that the *C. banyumas* species complex consists of four vocally distinct clusters that separate *C. lemprieri*, as well as

Table 3. A summary of parameters determined to be diagnosable under the Isler Criterion (denoted by 'X') as identified in each pairwise comparison across eight species complexes. The following vocal parameters were assessed: (a) average number of elements per motif; (b) average duration of a motif; (c) average minimum frequency of a motif; (d) average maximum frequency of a motif; (e) average peak frequency of a motif; (f) average bandwidth of a motif; (g) average centre frequency; (h) average centre frequency of the first half of a motif; (i) average centre frequency of the second half of am otif; (j) average difference in the centre frequencies of first and second halves of motifs; and (k) average proportion of time to reach minimum frequency in a motif. Parameters (g) to (k) were only applied to the *Cyornis banyumas* and *Cyornis tickelliae* species complexes

Pairwise comparisons	a	b	c	d	e	f	g	h	i	j	k
C. colonus species complex											
colonus vs. **pelingensis**			X			X					
C. rubeculoides species complex											
glaucicomans vs. **rubeculoides**	X	X									
glaucicomans vs. **dialilaemus**		X		X							
glaucicomans vs. **rogersi**		X									
glaucicomans vs. **hainanus**	X	X									
glaucicomans vs. **klossi**	X	X									
hainanus vs. **rubeculoides**											
hainanus vs. **dialilaemus**											
hainanus vs. **rogersi**											
hainanus vs. **klossi**											
klossi vs. **rubeculoides**											
klossi vs. **dialialemus**											
klossi vs. **rogersi**											
C. banyumas species complex											
banyumas + ligus vs. **whitei + coerulifrons + lekhakuni**							X				
banyumas + ligus vs. **montanus**							X	X	X		
banyumas + ligus vs. **tax. nov. (Meratus)**							X	X	X		
banyumas + ligus vs. **tax. nov. (Beratus)**											
whitei + coerulifrons + lekhakuni vs. **montanus**								X			
whitei + coerulifrons + lekhakuni vs. **tax. nov. (Meratus)**									X		
whitei + coerulifrons + lekhakuni vs. **tax. nov. (Beratus)**							X		X		
montanus vs. **tax. nov. (Meratus)**											
montanus vs. **tax. nov. (Beratus)**					X		X	X	X		X
magnirostris vs. **banyumas + ligus**											
magnirostris vs. **whitei + coerulifrons + lekhakuni**							X				
magnirostris vs. **montanus**							X	X	X		
magnirostris vs. **tax. nov. (Meratus)**							X		X		
magnirostris vs. **tax. nov. (Beratus)**											
lemprieri vs. **banyumas + ligus**							X				
lemprieri vs. **whitei + coerulifrons + lekhakuni**	X			X		X					
lemprieri vs. **montanus**	X			X		X	X				
lemprieri vs. **tax. nov. (Meratus)**		X		X		X			X		
lemprieri vs. **magnirostris**							X	X	X		
lemprieri vs. **tax. nov. (Beratus)**	X		X	X	X	X	X		X		
C. tickelliae species complex											
tickelliae vs. **sumatrensis**										X	X
tickelliae vs. **indochina**										X	X
tickelliae vs. **jerdoni**											
jerdoni vs. **sumatrensis**										X	X
jerdoni vs. **indochina**										X	
indochina vs. **sumatrensis**											
C. olivaceus species complex											

Table 3. Continued

Pairwise comparisons	a	b	c	d	e	f	g	h	i	j	k
Borneo vs. **Java**					X						
Borneo vs. **Sumatra**											
Borneo vs. **Thailand**											
Java vs. **Sumatra**											
Java vs. **Thailand**											
Sumatra vs. **Thailand**	X										
C. caerulatus species complex											
caerulatus **Type 1** vs. *albiventer*											
caerulatus **Type 2** vs. *albiventer*											
C. unicolor species complex											
unicolor vs. *diaoluoensis*											
unicolor vs. *cyanopolia*											
cyanopolia vs. *diaoluoensis*			X								
C. rufigastra species complex											
rufigastra vs. *djampeanus*				X		X					
rufigastra vs. *kalaoensis*			X			X					
rufigastra vs. *omissus*						X					
rufigastra vs. **tax. nov. (Togian)**				X		X					
rufigastra vs. *philippinensis*											
philippinensis vs. *djampeanus*				X		X					
philippinensis vs. *kalaoensis*			X	X		X					
philippinensis vs. *omissus*				X		X					
philippinensis vs. **Togian**				X		X					
djampeanus vs. *kalaoensis*	X										
djampeanus vs. *omissus*	X										
djampeanus vs. **tax. nov. (Togian)**											
kalaoensis vs. *omissus*	X										
kalaoensis vs. **tax. nov. (Togian)**			X								
omissus vs. **tax. nov. (Togian)**											

The taxa are bolded to distinguish them from each species complex.

Javan, Indochinese and Bornean populations (except from Mt. Beratus) from one another. This is further supported by one to three diagnosable parameters under the Isler Criterion (Fig. 1C; Table 3). The monotypic *C. lemprieri* is vocally distinct from all members of the *C. banyumas* species complex, but overlaps slightly in PCA with the Indochinese population, with at least three vocal parameters emerging as Isler-diagnosable (Fig. 1C; Table 3). The monotypic *C. magnirostris* is vocally distinct from all members of the *C. banyumas* species complex except from the Javan and Mt. Beratus (Borneo) populations (Fig 1C; Table 3). The Meratus Mts. population is vocally distinct from all other members of the *C. banyumas* species complex except from the Bornean *C. b. montanus* population, which overlaps slightly in PCA, but emerges as a distinct cluster from *C. b. montanus* in LDA (Supporting Information, Appendix S2).

Cyornis ruficauda isola from Borneo and *C. r. ocularis* from the Sulu Islands appear to be vocally distinct from other taxa in the *C. ruficauda* complex occurring in the main Philippines Islands (Fig. 1D; Supporting Information, Appendix S2). However, only one sample each of *C. r. isola* and *C. r. ocularis* was available, rendering the Isler Criterion inapplicable.

The South-East Asian *C. tickelliae sumatrensis* and *C. t. indochina* are vocally distinct from the South Asian *C. t. tickelliae* and *C. t. jerdoni* in PCA and LDA, based on 11 vocal parameters, two of which emerge as diagnosable under the Isler Criterion (Fig. 1E; Table 3; Supporting Information, Appendix S2).

Bioacoustic PCA does not reveal differentiation among populations of *C. olivaceus*, despite Isler-diagnosability in two pairwise population comparisons (Fig. 1F; Table 3). Similarly, two song types are detected in the Bornean *C. c. caerulatus*, but neither was distinctly different from the Sumatran *C. c. albiventer* in PCA and under the Isler Criterion (Fig. 1G; Table 3).

BIOACOUSTIC AND GENETIC DIVERGENCES

PCA reveals a vocal cline among the three subspecies of *C. unicolor*, corroborated by the geographically

intermediate taxon *C. u. unicolor* not being diagnosably differentiated from the two terminal taxa under the Isler Criterion (Fig. 2A; Table 3). Both mtDNA and genome-wide trees reveal a shallow genetic divergence (e.g. 0.2%–0.4% in mtDNA) between *C. u. unicolor* and *C. u. diaoluoensis* (Fig. 2A; Supporting Information, Appendix S3), supporting the lack of vocal divergence observed between the two taxa.

PCA and bioacoustic analysis under the Isler Criterion of the *C. rufigastra* species complex shows that the Philippine and Sundaic taxa (*C. r. rufigastra*, *C. r. simplex* and *C. r. philippinensis*) are vocally distinct from Wallacean taxa (*C. r. omissus*, *C. r. peromissus*, *C. r. djampeanus*, *C. r. kalaoensis* and an undescribed Togian population; Fig. 2B; Table 3). Our mtDNA and genome-wide trees show congruent results with well-supported bootstrap

(>90%), revealing deep genetic divergence (e.g. ~3.5% in mtDNA) between *C. r. rufigastra* and *C. r. omissus* (Fig. 2B; Supporting Information, Appendix S4), further supporting the division into a Wallacean and a Sundaic cluster. In comparison, both mtDNA and genome-wide trees support little genetic divergence between the Sundaic and Philippine populations (e.g. 1.1% in mtDNA). Among the Wallacean taxa, we find only little vocal differentiation among the undescribed Togian population, *C. r. omissus* from the main island of Sulawesi and the Selayar population, *C. r. peromissus* (Fig. 2B; Table 3). The lack of vocal divergence between the Togian population and *C. o. omissus* from Sulawesi is corroborated by shallow genetic divergence in both mtDNA and genome-wide data (~0.3% in mtDNA; Fig. 2B; Supporting Information, Appendix S4). Although
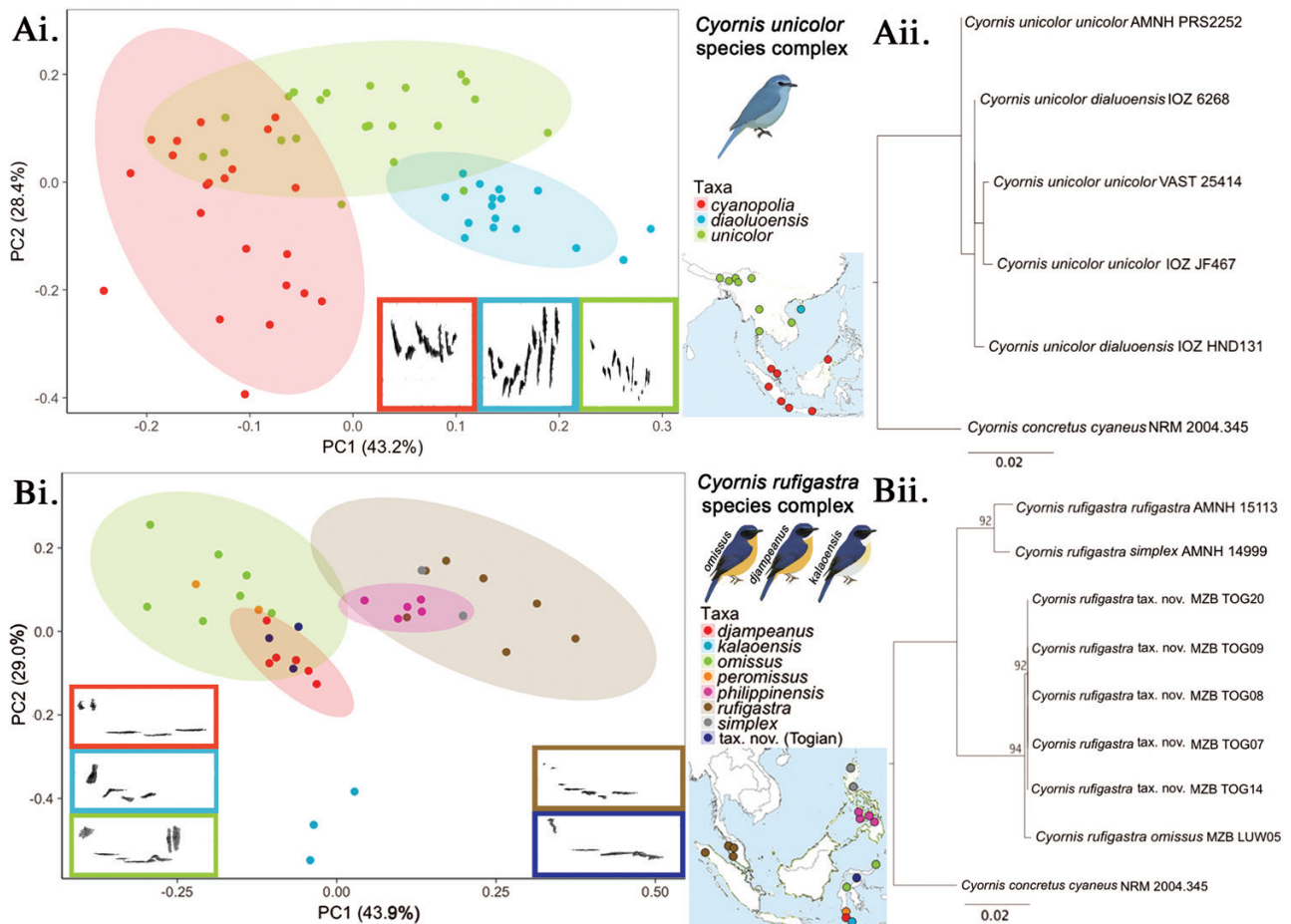

Figure 2. A, *Cyornis unicolor* species complex; B, *C. rufigastra* species complex. Panel (i) principal component analysis (PCA) based on six vocal parameters, with ellipses representing 95% confidence intervals of the principal component (PC) scores for each taxon represented by ≥4 individuals. Sample localities of recordings for each species complex are indicated on the map and a colour-coded spectrogram of each selected taxon is shown inside the PCA plot. Panel (ii) maximum likelihood phylogeny based on the mitochondrial cytochrome b gene constructed with RAxML, with bootstrap support ≥90% shown beside node.

the Tanahjampea population, *C. r. djampeanus*, overlaps with *C. r. omissus* in PCA, it is found to be diagnosable by one vocal parameter under the Isler Criterion (Fig. 2B; Table 3). Our bioacoustic results also demonstrated that the Kalao population, *C. r. kalaoensis*, is vocally highly distinct from all other members of the species complex (Fig. 2B; Table 3; Supporting Information, Appendix S2).

DISCUSSION

Bioacoustics plays a significant role in the maintenance of reproductive isolation in birds in general (Clayton, 1990; Brambilla *et al.*, 2008) and has been hailed as a valuable tool for species delimitation in non-oscines (King, 2002b; Rheindt *et al.*, 2011; Rasmussen *et al.*, 2012; Ng & Rheindt, 2016; Gwee *et al.*, 2017). In oscine songbirds, such as *Cyornis* flycatchers, juveniles are capable of learning acoustic motifs from adult conspecifics and from the external environment (Clayton, 1990; Beecher & Brenowitz, 2005), rendering their songs among the most vocally complex bioacoustic signals in nature. Nevertheless, the need for successful communication generates selective pressure to keep vocalizations sufficiently homogeneous within oscine species so as to render them excellent tools for species delimitation (van Balen *et al.*, 2013; Cros & Rheindt, 2017; Prawiradilaga *et al.*, 2017; Alström *et al.*, 2018). To deal with the extensive bioacoustic flexibility of oscines, recordings containing heterospecific song structures were removed to ensure homologous comparisons among taxa. Moreover, vocal variation in a taxon is penalized under the conservative Isler Criterion (see Methods), thus interspecific vocal variation would have to be significantly greater to pass this stringent test. Therefore, we are confident that our approach is adept at taking oscine vocal variability into account.

Our bioacoustic approach across multiple *Cyornis* jungle-flycatcher species complexes provided a variety of insights into flycatcher species-level differentiation. Some of these insights reinforce previously published molecular findings. However, more importantly, some of them point to deep differentiation in *Cyornis* species complexes that has either been previously overlooked or insufficiently documented.

BIOACOUSTIC RESULTS REINFORCE PUBLISHED GENETIC FINDINGS

The deep vocal divergence observed between the taxa on Peleng and Taliabu in the *C. colonus* species complex (Fig. 1A; Table 3; Supporting Information, Appendix S2) is congruent with a recent genomic study in which Garg *et al.* (2018) found the Sula

Island taxon *C. c. colonus* to be deeply diverged from the Peleng Island taxon *C. c. pelingensis* and proposed a species-level division between them, which we here support. In summary, we propose the following taxonomic arrangement for the *C. colonus* species complex. The distribution range of each taxon is based on del Hoyo & Collar's (2016) classification.

- *C. colonus* (E. J. O. Hartert, 1898). Sula jungle-flycatcher – Sula Islands.
- *C. pelingensis* (Vaurie, 1952). Banggai jungle-flycatcher – Banggai Islands.

Similarly, vocal results in the *C. rubeculoides* species complex were at least partially congruent with previously published genetic findings (Zhang *et al.*, 2016). Both vocal and genetic data support a deep divergence between *C. r. glaucicomans* and other members of the species complex, as well as a lack of divergence between *C. hainanus* and *C. r. klossi* (Zhang *et al.*, 2016; Fig. 1B; Table 3; Supporting Information, Appendix S2). *Cyornis r. rubeculoides* and *C. r. rogersi* were found to be genetically differentiated from *C. hainanus* and *C. r. klossi* (Zhang *et al.*, 2016). However, our bioacoustic analyses were unable to detect deep vocal differences among *C. hainanus*, *C. r. rubeculoides*, *C. r. rogersi*, *C. r. dialilaemus* and *C. r. klossi* (Fig. 1B; Table 3; Supporting Information, Appendix S2). Further research, including genome-wide DNA data, is needed to shed light on the complicated relationships among these taxa, given that subspecies *klossi* has traditionally been attributed to *C. rubeculoides*, but was recently re-assigned to *C. hainanus* on account of being indistinguishable based on analyses of three mtDNA markers and five nuclear introns (Zhang *et al.*, 2016). In the meantime, we advocate a taxonomic treatment in which *klossi* is subsumed under *C. hainanus* (following Zhang *et al.*, 2016), *C. glaucicomans* is separated as a species (again following Zhang *et al.*, 2016), while *C. rubeculoides* continues to be recognized as distinct from *C. hainanus* according to traditional taxonomy. In summary, we propose the following taxonomic arrangement for the *C. glaucicomans* species complex:

- *C. glaucicomans* Thayer and Bangs, 1909. Chinese jungle-flycatcher – SC & S China.
- *C. rubeculoides*. Blue-throated jungle-flycatcher:
  ○ *C. rubeculoides rubeculoides* (Vigors, 1831) – Himalayas from NE Pakistan, E to NE India, S China and W, N & NE Myanmar.
  ○ C. *rubeculoides rogersi* Robinson & Kinnear, 1928 – SW Myanmar.

- ○ *C. rubeculoides dialilaemus* Salvadori, 1889 – E & SE Myanmar and N & W Thailand.

- • *C. hainanus*. Hainan jungle-flycatcher:

- ○ *C. hainanus hainanus* (Ogilvie-Grant, 1900) – C, E & S Myanmar, S China, Thailand, Laos, Cambodia and Vietnam.
- ○ *C. hainanus klossi* Robinson, 1921 – E Thailand, S Laos, E Cambodia and Vietnam.

Our bioacoustic results revealed that the taxonomically challenging *C. banyumas* species complex consists of at least four vocally distinct species (Fig. 1C; Table 3; Supporting Information, Appendix S2). Unsurprisingly, the monotypic *C. lemprieri* from Palawan, which has sometimes been treated as part of the *C. banyumas* complex (Renner *et al.*, 2009), was found to be vocally distinct from all other members of the *C. banyumas* complex under the Isler Criterion (Table 3). Among the remainder, our vocal data were unable to differentiate between *C. magnirostris* and the Javan nominate group (*C. b. banyumas* and *C. b. ligus*), as well as the undescribed Bornean population from Mt. Beratus (Fig. 1C; Table 3; Supporting Information, Appendix S2), despite *C. magnirostris* being widely accepted as a monotypic species based on its disjunct range and significant morphometric and plumage differences (Renner *et al.*, 2009). However, this vocal similarity could be a plesiomorphic trait retained by these widely allopatric populations (*C. magnirostris*, the Javan group and the Mt. Beratus population), which never come into contact, but are geographically separated by multiple intervening taxa that are vocally distinct. In contrast, the Indochinese *whitei* group (*C. b. whitei*, *C. b. coerulifrons* and *C. b. lekhakuni*), which overlaps with wintering populations of *C. magnirostris*, has evolved a distinct vocalization (Fig. 1C; Table 3; Supporting Information, Appendix S2), perhaps aided by character displacement as suggested by Renner *et al.*'s (2009) morphometric comparisons, which attested to greater differences between *C. magnirostris* and sympatric *C. b. whitei* vs. lesser differences between *C. magnirostris* and the allopatric Javan *C. b. banyumas*. This discrete vocal and morphometric differentiation between *C. magnirostris* and the neighbouring *whitei* group would support continued species status of *C. magnirostris*.

Both vocal and genetic data support deep divergence between the Javan nominate (*C. b. banyumas* and *C. b. ligus*) and the Indochinese *whitei* (*C. b. whitei*, *C. b. coerulifrons* and *C. b. lekhakuni*) groups (Zhang *et al.*, 2016). The Bornean *C. b. montanus* is vocally distinct from both the Javan nominate and Indochinese *whitei* groups (Fig. 1C; Table 3; Supporting Information, Appendix S2), corroborated by strong plumage differences between *C. b. montanus* and

other members of the *C. banyumas* species complex (Renner *et al.*, 2009). Based on our set of parameters, the undescribed Meratus Mts. taxon was found to be vocally distinct from all other members of the *C. banyumas* species complex, including from fellow Bornean *C. b. montanus* based on LDA (Supporting Information, Appendix S2), confirming Eaton *et al.*'s (2016a) qualitative vocal impressions of pronounced bioacoustic differences from *montanus* and a recent demonstration of deep mtDNA divergence (3.27%) between the two (Shakya *et al.*, 2018). A more comprehensive study, including genome-wide DNA data and a more extensive bioacoustic analysis, is required to elucidate the taxonomic status of all three Bornean populations of the *C. banyumas* species complex (i.e. *montanus* and the undescribed populations from Mt. Beratus and the Meratus Mts.). In the meantime, we propose a provisional taxonomic treatment recognizing a total of six species on the basis of available vocal, genetic and morphological data:

- • *C. magnirostris* Blyth, 1849. Large jungle-flycatcher – C & E Himalayas, India and N Myanmar.
- • *C. lemprieri* (Sharpe, 1884). Palawan jungle-flycatcher – Palawan.
- • *C. banyumas*. Javan jungle-flycatcher:
  - ○ *C. banyumas banyumas* (Horsfield, 1821) – C & E Java.
  - ○ *C. banyumas ligus* (Deignan, 1947) – W Java.
  - ○ *C. banyumas mardii* (Hoogerwerf, 1962) – Panaitan Island (off W Java).
- • *C. whitei*. Hill jungle-flycatcher:
  - ○ *C. whitei whitei* Harington, 1908 – N & E Myanmar, SC China, N Thailand, N & C Laos and N Vietnam.
  - ○ *C. whitei coerulifrons* E. C. S. Baker, 1918 – S Thailand and N & C Peninsular Malaysia.
  - ○ *C. whitei lekhakuni* (Deignan, 1956) – hills of S Thailand.
  - ○ *C. whitei deignani* Meyer de Schauensee, 1939 – SE Thailand.
- • *C. montanus* Robinson & Kinnear, 1928. Dayak jungle-flycatcher – Borneo (except Meratus Mts.).
- • *Cyornis* sp. nov. Meratus jungle-flycatcher – Meratus Mts. (SE Kalimantan).

BIOACOUSTIC RESULTS SUPPORTED BY NOVEL MITOCHONDRIAL AND GENOMIC DATA

Our bioacoustic data hints at a vocal cline among the three subspecies of *C. unicolor* (Fig. 2A), which is corroborated by shallow divergences in both mitochondrial and genomic data. The acoustic impression of songs is one of almost identical song motifs at gradually increasing pitch from northern

populations towards *cyanopolia* in the south. Although abrupt avifaunal transitions along the Isthmus of Kra are observed in multiple bird species ([Hughes *et al.*, 2003](); [Dejtaradol *et al.*, 2016]()), the nominate population *C. u. unicolor* from the drier monsoonal parts of South-East Asia is vocally and genetically undifferentiated from the Sundaic *C. c. cyanopolia* ([Fig. 2A](); [Supporting Information, Appendix S3]()). The most likely interpretation is that regular gene flow may occur between *C. u. unicolor* in the central part of the geographical distribution and the two terminal subspecies on either side ([Fig. 2A]()). Under this scenario, it is unsurprising that the two terminal taxa would be differentiated by one diagnosable vocal character ([Table 3]()), while being undifferentiated towards the central nominate taxon. In summary, we propose the following taxonomic arrangement for the *C. unicolor* species complex:

- *C. unicolor unicolor* (Blyth, 1843) – Garhwal (W Uttarakhand) and from C Nepal E in Himalayas to NE India, S China, Myanmar, Thailand (except C & S), N & C Laos and Vietnam.
- *C. unicolor diaoluoensis* (Cheng, Yang & Lu, 1981) – Hainan Island.
- *C. unicolor cyanopolia* Blyth, 1870 – Malay Peninsula, Sumatra, Java and Borneo.

According to our bioacoustic results, the highly complicated *C. rufigastra* species complex may consist of up to four vocally distinct populations ([Fig. 2B](); [Table 3]()): *rufigastra*, *omissus*, *kalaoensis* and possibly *djampeanus*. Our genetic and vocal data support previous suggestions to separate the forms from Sulawesi and satellite islands, leaving *C. rufigastra* mainly as a Greater Sundanese and Philippine species ([Fig 2B](); [Table 3](); [Supporting Information, Appendix S2]()). Sulawesi and its satellite islands lie in the Wallacean region, which has never been connected to the Sunda Shelf, thus high avian endemism is observed: more than 90 bird species on Sulawesi and its satellite islands are endemic, such as the maleo (*Macrocephalon maleo*), Sulawesi myna (*Basilornis celebensis*) and streak-headed white-eye (*Heleia squamiceps*). A recently discovered population on the Togian Islands ([Rheindt *et al.*, 2014]()) showed little genetic and vocal divergence from *omissus* from the main island of Sulawesi, despite differences in habitat and – less so – phenotype ([Eaton *et al.*, 2016]()b). In contrast, strong vocal divergence between *kalaoensis* and all other members of the *C. rufigastra* species complex support the stark plumage differences of this uniquely coloured taxon, thus supporting taxonomic elevation to *C. kalaoensis* ([Fig 2B](); [Table 3](); [Supporting Information, Appendix S2]()). Inconclusive vocal patterns were observed in the Tanahjampea Island race *djampeanus*, which was vocally more similar to *omissus*, but distinguishable in one diagnosable parameter under the conservative Isler Criterion ([Fig. 2B](); [Table 3](); [Supporting Information, Appendix S2]()). The form *djampeanus* has recently been upgraded to species level, with *kalaoensis* as a subspecies ([Eaton *et al.*, 2016](); [del Hoyo & Collar, 2018]()). However, pending more comprehensive results of genomic inquiries, our vocal data and the unusually bleached plumage of *kalaoensis* are more supportive of a treatment of *kalaoensis* as a separate and independent species, while *djampeanus* could conservatively be retained under *C. omissus* pending genetic data collection. In summary, we propose the following taxonomic arrangement for the *C. rufigastra* species complex:

- *C. rufigastra*. Mangrove jungle-flycatcher:
  - *C. rufigastra rufigastra* (Raffles, 1822) – Malay Peninsula, Sumatra and Borneo.
  - *C. rufigastra karimatensis* Oberholser, 1924 – Karimata Island (off SW Borneo).
  - *C. rufigastra rhizophorae* Stresemann, 1925 – Sebesi Island (extreme S Sumatra), Bangka, Belitung and Java.
  - *C. rufigastra longipennis* Chasen & Kloss, 1930 – Karimunjawa Islands (N of C Java).
  - *C. rufigastra simplex* Blyth, 1870 – N Philippines.
  - *C. rufigastra mindorensis* Mearns, 1907 – Mindoro (NC Philippines).
  - *C. rufigastra marinduquensis* DuPont, 1972 – Marinduque (NC Philippines).
  - *C. rufigastra philippinensis* Sharpe, 1877 – C, W & S Philippines, including Palawan and Sulu Archipelago.
- *C. omissus*. Sulawesi jungle-flycatcher:
  - *C. omissus omissus* (E. J. O. Hartert, 1896) – Sulawesi.
  - *C. omissus peromissus* E. J. O. Hartert, 1920 – Selayar Island.
  - *C. omissus* subsp. nov. – Togian Islands.
  - *C. omissus djampeanus* (E. J. O. Hartert, 1896) – Tanahjampea Island.
- *C. kalaoensis* (E. J. O. Hartert, 1896). Kalao jungle-flycatcher – Kalao Island.

BIOACOUSTIC SCANS FOR CRYPTIC SPECIES IN WIDESPREAD AND UNDERSTUDIED COMPLEXES

The geographical distribution of *C. ruficauda* encompasses Borneo, the Sulu Archipelago and the main Philippine island groups (Visayas and Mindanao), henceforth referred to as the main Philippine islands. Despite being lumped into a single species based on morphology ([Kennedy *et al.*, 2000](); [Dickinson & Christidis, 2014](); [Clement, 2018]()), we found distinct vocal differences between the Bornean race *isola*,

the Sulu race *ocularis* and the other races ([Fig. 1D]; Supporting Information, Appendix S2), suggesting that the deep sea channels between Borneo and Sulu and between Sulu and the main Philippine islands have prevented gene flow among these populations during periods of global sea-level recession when more shallow neighbouring seas were exposed as land (Bintanja *et al.*, 2005). On the other hand, little vocal differentiation was observed between *boholensis* and *samarensis*, reflecting the proximity of Mindanao to the Visayas during global ice ages allowing for gene flow. In conjunction with the distinct phenotypic differences among these three groups, e.g. a rufous orbital ring in *ocularis* (Kennedy *et al.*, 2000; Clement, 2018), and despite our somewhat low vocal sample size, we suggest that the *C. ruficauda* species complex consists of three species, supporting a previous study that found Philippine avian endemism to be severely underestimated (Lohman *et al.*, 2010). More sound recordings are required to include other races not analyzed in the present study and to ascertain the vocal differences observed. In addition, future research should include genetic characters to unravel the divergence dynamics and speciation mechanisms within the *C. ruficauda* complex. In summary, we propose the following taxonomic arrangement for the *C. ruficauda* species complex:

- *C. ruficauda*. Philippine jungle-flycatcher:
  - *C. ruficauda ruficauda* (Sharpe, 1877) – Basilan.
  - *C. ruficauda samarensis* (Steere, 1890) – Samar, Biliran, Leyte, Dinagat, E & C Mindanao.
  - *C. ruficauda boholensis* (Rand & Rabor, 1957) – Bohol.
  - *C. ruficauda zamboanga* (Rand & Rabor, 1957) – W Mindanao.
- *C. ocularis* (Bourns & Worcester, 1894). Sulu jungle-flycatcher – Sulu Archipelago.
- *C. ruficrissa*. Crocker jungle-flycatcher:
  - *C. ruficrissa ruficrissa* (Sharpe, 1887) – Mt. Kinabalu (N Borneo).
  - *C. ruficrissa isola* (Hachisuka, 1932) – Mountains of Borneo (except Mt. Kinabalu).

We found evidence of vocal divergence between South Asian *C. t. tickelliae* and *C. t. jerdoni* vs. South-East Asian *C. t. sumatrensis* and *C. t. indochina* ([Fig. 1E]; Table 3; Supporting Information, Appendix S2; del Hoyo *et al.*, 2018), which are usually considered conspecific on the basis of similar male plumages. Our findings concur with Rasmussen & Anderton (2005), who documented vocal and plumage differences, and with bioacoustic results independently obtained by Boesman (2016). It also concurs with the considerable plumage differences observed between the females of each taxon (Rasmussen & Anderton, 2005; del Hoyo *et al.*, 2018): females of the South-East Asian taxa have

an olive head, neck and upperparts, whereas females of the South Asian taxa are pale blue, thus supporting the taxonomic split of the South-East Asian taxa (*C. t. sumatrensis* and *C. t. indochina*) from the South Asian taxa (*C. t. tickelliae* and *C. t. jerdoni*). In summary, we propose the following taxonomic arrangement for the *C. tickelliae* species complex:

- *C. tickelliae*. Tickell's jungle-flycatcher:
  - *C. tickelliae tickelliae* Blyth, 1843 – S Nepal and N, C & S India.
  - *C. tickelliae jerdoni* Holdsworth, 1872 – Sri Lanka.
- *C. sumatrensis*. Indochinese jungle-flycatcher:
  - *C. sumatrensis sumatrensis* (Sharpe, 1879) – S Myanmar, S Thailand, Peninsular Malaysia and NE Sumatra.
  - *C. sumatrensis indochina* Chasen & Kloss, 1928 – SE Myanmar, Thailand (except C & S), Cambodia, S Laos and C Vietnam.
  - *C. sumatrensis lamprus* Oberholser, 1917 – Anamba Island (off E Peninsular Malaysia).

Although the Sundaic *C. olivaceus* is a widespread species and relatively common in Sumatra and Java, limited studies have been carried out on this jungle-flycatcher. Until recently, it was mistakenly treated as a *Rhinomyias* due to its seemingly different plumage in comparison to most other *Cyornis* species. Our bioacoustic analyses detected limited geographical variation between the Bornean and Javan populations and between the Sumatran and Thai populations (Table 3). However, strong vocal differentiation is absent among the four populations ([Fig. 1F]), thus supporting the taxonomic treatment of a single species. Similarly, the greater Sundanese *C. caerulatus* is very poorly known in life, especially the Sumatran *C. c. albiventer*. Although our bioacoustic analyses did not detect strong vocal distinction between the Sumatra and Sabah populations, we encountered two different song types of *C. c. caerulatus*: one of which is a consistent four-element phrase, while the other consists of two to three elements in a descending phrase (see [Fig. 1G]).

CONSERVATION IMPLICATIONS

Overall, we propose taxonomic revisions to six out of the nine *Cyornis* species complexes in the present study, resulting in the elevation of ten taxa to species status. Our recommendation for a taxonomic elevation of *C. kalaoensis* generates an additional small-island endemic, but we do not believe that it would currently classify as threatened given that it is common in secondary habitat (Eaton & Rheindt, 2017). However, limiting the distribution range of *C. banyumas* to Java calls for an urgent need to reconsider the conservation

status of this endemic species. The Javan *C. banyumas* population was found to be declining as a result of the bird trade (Eaton *et al.*, 2015). The species is now very rarely encountered in the wild, and a network of Javan birders and professional bird guides only recorded the taxon a handful of times in 2018 (pers. obs.), thus the species may warrant elevation in conservation status to Critically Endangered according to criterion A2 (IUCN, 2018).

Our study demonstrates that a relatively small panel of vocal parameters can be employed as a useful tool in cryptic species detection in *Cyornis* flycatchers, which are characterized by great levels of hidden species diversity (Eaton *et al.*, 2016; del Hoyo *et al.*, 2018) and that their application was in agreement with genetic data (present data; Zhang *et al.*, 2016; Garg *et al.*, 2018). Using recordings collected by both professional and recreational ornithologists, and vetted by us for correct species identification (see Methods), we were able to detect vocal divergences, or a lack thereof, in various species complexes, thereby providing important taxonomic and conservation insights by flagging cryptic conservation units that may otherwise slip under the radar and face extinction threats. Despite great progress in the ongoing taxonomic revolution that incorporates bioacoustic and genomic methodologies, our study suggest knowledge gaps remain even in bird taxonomy, the best-studied animal group on Earth. Given that vocal traits are of critical importance in the maintenance of species integrity in songbirds, and can be even more appropriate for species delimitation than plumage in some groups (Rheindt *et al.*, 2008), we propose a routine application of simple vocal parameter sets – adjusted to taxonomic grouping – to scan for additional cryptic species-level diversity across songbirds. However, we emphasize that the application of such simple standard parameter sets, whether bioacoustic or morphological (e.g. Tobias *et al.*, 2010), can only ever serve to flag units of interest and produce provisional new treatments, but not to override well-established treatments based on integrative genomic, bioacoustic and morphological data.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site.

Appendix S1. Details of all recordings analyzed in the present study. Abbreviations used for accession no.: XC, xeno-canto; ML, Macaulay Library; AV, Avian Vocalization Center; BAS,Bas Van Balen's personal collection; JAE, James A. Eaton's personal collection; PA, Per Alström's personal collection; RH, Rob O. Hutchison's personal collection; FR, Frank E. Rheindt's personal collection.

Appendix S2. Linear discriminant analysis (LDA) of six species complexes consisting of distinct vocal groups.

Appendix S3. Maximum likelihood phylogeny of *Cyornis unicolor* species complex using concatenated genome wide SNPs constructed with RAxML, with bootstrap support ≥90% shown beside node.

Appendix S4. Maximum likelihood phylogeny of *Cyornis rufigastra* species complex using concatenated genome wide SNPs constructed with RAxML, with bootstrap support ≥90% shown beside node.