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Genomic and morphological data help uncover extinction-in-progress of an unsustainably traded hill myna radiation

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The Asian songbird crisis which is currently unfolding in Southeast Asia has seen multiple bird taxa go extinct in the wild and even more slip into regional or local extinction over the span of only a few years. The hill mynas *Gracula* spp. are among its main victims, encompassing the Critically Endangered Nias Hill Myna *Gracula [religiosa] robusta* and other endangered populations across the West Sumatran Archipelago. Hill mynas are known to be present throughout this island chain but the taxonomic relationships of West Sumatran *Gracula* populations remain poorly understood. We hypothesized that the unique history of this island chain may have given rise to multiple distinct insular forms. Here we use genome-wide DNA data in concert with morphological analyses to investigate the evolutionary distinctness of these taxa. Our results identify one taxon that is surprisingly distinct despite lacking recognition in most classifications, the 'Simeulue Hill Myna' (taxon *miotera*), and a range extension of the Nias Hill Myna. Despite their lack of recognition, Simeulue Hill Mynas are genomically and morphologically as unique as their Nias counterpart, in accordance with the lack of glacial land bridges between the island of Simeulue and mainland Sumatra. Simeulue Hill Mynas went extinct in the wild sometime within the last 2–3 years, and the rescue of the last captive individuals should now be the highest priority.

Keywords: biogeography, caged, island, isolation, pet trade, population genomics, Sundaland.

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Southeast Asia is one of the richest biodiversity hotspots on Earth, home to a quarter of all avian species globally, a third of which are endemic to the region and can be found nowhere else on the planet (Clements *et al.* 2018). In the new

millennium, rampant illegal poaching of songbirds for the cage bird trade across Southeast Asia has been a primary driver of population decline and local and global extinction in the wild (Nash 1993, Sodhi *et al.*, 2004a, 2004b, Eaton *et al.* 2015), constituting the Asian songbird crisis, a situation exacerbated by some of the highest global rates of deforestation (Brooks *et al.* 1997, Brook *et al.*, 2003, Sodhi *et al.* 2004a, 2006, Symes *et al.* 2018). In Indonesia in particular, species with an elaborate vocal prowess are prized above all others (Lye 1999, Jepson & Ladle 2005, Harris *et al.* 2015).

One of the most popular and widely traded Asian cage birds is the Common Hill Myna *Gracula religiosa* (Nijman 2010) whose vocal mimicking capabilities are among the best in the avian world (Klatt & Stefanski 1974, Klingholz 1979). The Common Hill Myna complex comprises large starlings (family Sturnidae) resident throughout South and Southeast Asia (Fig. 1). Members of the complex have a glossy black plumage with yellow flaps of bare skin (i.e. wattles) on the nape, with wattle morphology often distinguishing different taxa (Feare & Craig 1998).

In spite of the Common Hill Myna's IUCN classification as of 'Least Concern' owing to its large range (BirdLife International 2018a), the species is listed on Appendix II under the Convention of International Trade in Endangered Species (CITES), and its populations have undergone widespread regional and local extinction in Southeast Asia driven by unsustainable poaching (Eaton *et al.* 2015). Consequently, they were identified as a species of high conservation priority by the Asian Songbird Trade Specialist Group under the IUCN (Lee *et al.*, 2016). Such declines have been sharpest in Indonesia: as early as two decades ago, hill mynas were virtually extirpated from Java owing to excessive trapping, with Sumatran populations plummeting sharply and demand in Indonesia now largely being met by imports from Malaysia (Shepherd *et al.* 2004). The desire for these birds throughout the region is exceedingly high, with individuals fetching hundreds to thousands of US dollars in bird markets (Chng *et al.* 2015).

Adding to the sorry conservation plight of the complex, there is taxonomic uncertainty concerning the status of a number of Indonesian taxa variably regarded as subspecies or species. Four of the nine regionally described taxa are endemic to the West Sumatran islands (Fig. 1; Craig & Feare 2019a, 2019b), also known as the Barusan Islands (Eaton *et al.* 2016), and two of these (taxa *robusta* and *enganensis*) have been flagged as distinct and worthy of species recognition by certain authorities (Eaton *et al.* 2016, Craig & Feare 2019a, 2019b). Unfortunately, all four taxa are under heavy trapping pressure. The most sought-after taxon is the Nias Hill Myna *Gracula [religiosa] robusta* (Fig. 1), variably afforded species status (Salvadori 1887, Craig & Feare 2019b) and considered Critically Endangered (Eaton *et al.* 2015) or Extinct in the Wild (Dymond 1994) until the recent rediscovery of a single pair in July 2015 by a team of conservationists from Liberec Zoo and the Indonesian Species Conservation Program, led by Rudianto Sembiring (pers. commun.). The status of other West Sumatran Island hill myna taxa is less well understood but perhaps similarly dire.

The West Sumatran/Barusan Islands are an island chain with a complex geological history. Some islands are surrounded by deep sea and have never been connected to Sumatra, whereas others were connected to Sumatra during Quaternary sea level recessions across land bridges spanning a spectrum of widths (Fig. 2). This unique geographical setting provides opportunities to assess levels of genomic divergence of individual island populations. The relative scarcity of these formerly abundant birds today only serves to underline the significance of such data for contemporary conservation efforts. To date, there has been no comprehensive molecular study on evolutionary relationships within the genus, due in no small part to the inaccessibility of the Barusan Islands and the relative rarity of many *Gracula* taxa in collections. No large-scale comparative biometric study has been conducted either, with current understanding based on a handful of museum specimens (Craig & Feare 2019b). Building a greater understanding of deeply diverged lineages and their phylogenetic relationships can better inform conservation decision-making.

The timely arrival of affordable next-generation sequencing (NGS) technologies and adaptation of their use in non-model species has facilitated the application of genome-wide molecular markers, such as single nucleotide polymorphisms (SNPs), at relatively low cost (Wagner *et al.* 2013), including in conservation-relevant research (Çilingir *et al.* 2017, Ng *et al.* 2017, Nash *et al.* 2018, Baveja *et al.* 2019, Chattopadhyay *et al.* 2019, Çilingir *et al.* 2019).

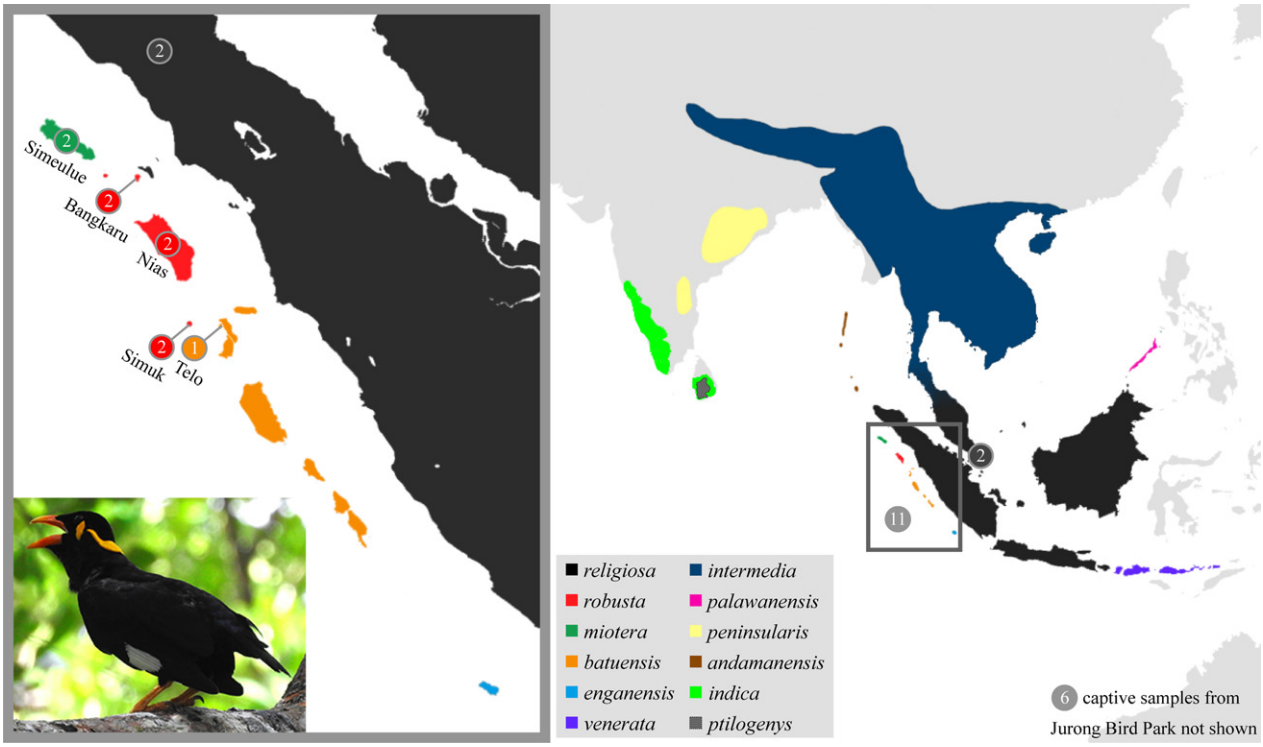

Figure 1. Left: range of *Gracula* taxa on the Barusan Islands with number of blood/tissue samples obtained from each locality shown in circles. Bottom left: a hill myna of the taxon *robusta* at an undisclosed location in the wild. Right: global range of the genus *Gracula*, according to del Hoyo *et al.* (2019), with number of blood/tissue samples obtained shown in circles. *Gracula ptilogenys* has an overlapping range with *Gracula indica*. [Colour figure can be viewed at wileyonlinelibrary.com]

This study employs an integrated approach combining morphological and biometric assessments with NGS techniques to help shed light on the identity of hill myna taxa from the Barusan Islands, with the aim of identifying appropriate conservation units for the species complex. We hypothesize that the unique geological history of the islands has given rise to multiple distinct insular *Gracula* forms, given their general subspecific and species-level endemism in birds (Eaton *et al.* 2016).

METHODS

Sample collection

Morphometric data were taken from 38 captive individuals from north Sumatra and the Barusan Islands held in private collections (Table S1). We interviewed trappers and/or owners of captive individuals and examined wattle morphology in order to ascertain the true provenance of the sampled birds (Appendix S1). Owing to intense multidirectional trade even including to/from the most remote locations, as well as insufficient knowledge about phenotypic characters of island taxa, birds for this research were carefully chosen. To avoid morphological sampling of captive individuals with a non-local origin, we primarily worked with bird owners who were themselves bird trappers. Bird markets served as the main source of individuals for the nominate *religiosa* from Sumatra, with each bird trapper and/or seller undergoing a structured interview to obtain information about the origin of each individual (Appendix S1). An important secondary factor was the price of the bird, with island taxa commanding significantly higher prices than the nominate taxon. Data collection for the Indonesian samples took place in northern Sumatra over multiple visits. Morphometric data were also collected from two individuals of taxon *intermedia* from Thailand, held at the Vogelpark Viernheim (Germany).

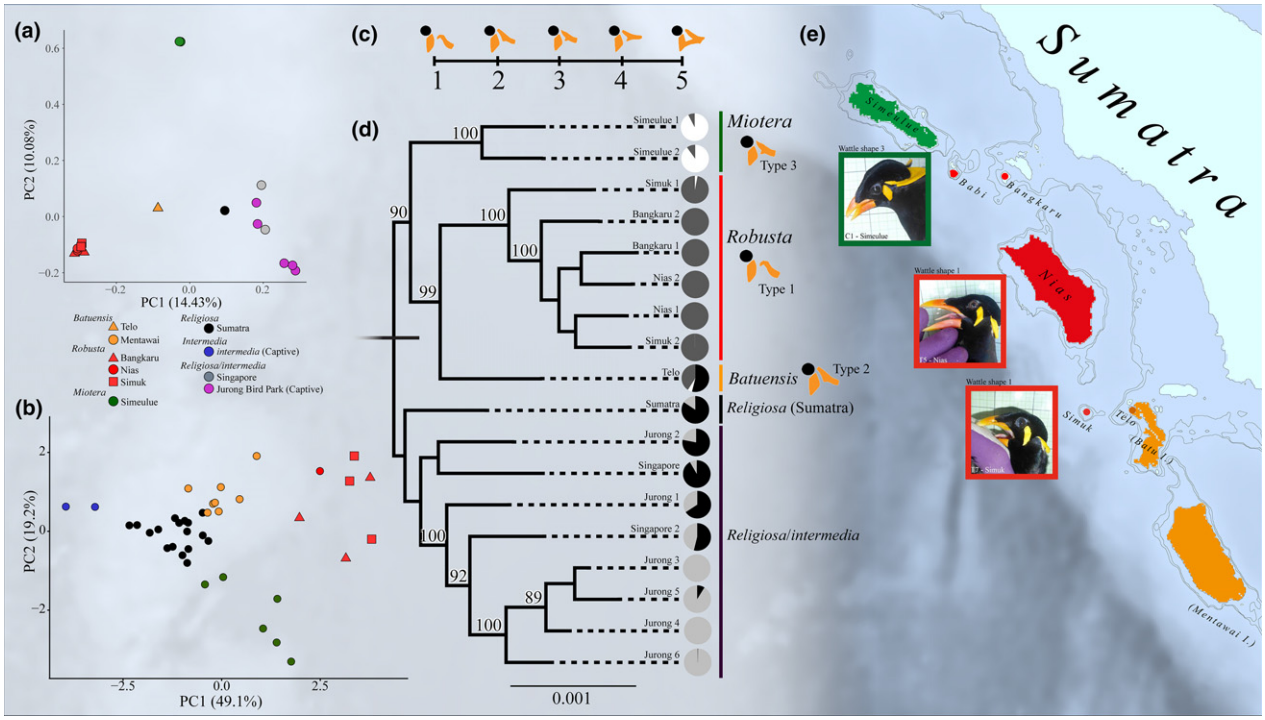

Figure 2. (a) Principal component analysis based on 6088 single nucleotide polymorphisms (SNPs) from across the genomes of 18 individuals. (b) Principal component analysis based on a subset of seven morphological characters across 38 individuals. (c) Schematic scoring system for wattle shape on an ordinal scale (0–5) based on degree of wattle connection. (d) Maximum likelihood phylogeny based on a concatenated alignment of 2 134 046 bp from across the genomes of 18 individuals. Outgroup (*Aplonis panayensis*) not shown. Only bootstrap values of well-supported clades (≥ 85) are shown. Piecharts at branch termini are based on STRUCTURE analysis of 6088 genome-wide SNPs at $K = 4$, which is the K value that was most congruent with other genomic analyses. For additional K values, see Fig. S4. (e) Bathymetric map of the West Sumatran (Barusan) Islands, with isobaths for −60 m and −120 m shown. Photos of sampled individuals are inlaid in the colour corresponding to their taxon, with *miotera* in green and *robusta* in red. The known range of each Barusan taxon is highlighted on the map (for colour, see legend on left). [Colour figure can be viewed at wileyonlinelibrary.com]

Blood samples were collected under RISTEK permit 312/FRP/E.5/Dit.KI/X/2018 and transferred to the Avian Evolution Laboratory at the National University of Singapore (NUS) under a Material Transfer Agreement between Syiah Kuala University (Banda Aceh, Indonesia) and NUS. Blood was stored in 95% ethanol and kept in a cool environment as much as possible. Blood samples were taken from eight of the aforementioned 38 captive individuals processed morphologically (Table S2). Additional samples were collected from: (a) two wild Singaporean individuals (presumably taxon *religiosa*); (b) a captive Sumatran individual (presumably taxon *religiosa*); and (c) six individuals constituting anonymous donations to Jurong Bird Park (Singapore) (Table S2). Judging from trade movement trends of songbirds in the region, the latter six donated individuals are unlikely to be of Indonesian stock and are probably sourced from range states in continental Southeast Asia. The Asian Glossy Starling *Aplonis panayensis* was used as an outgroup. Blood samples were obtained via brachial venepuncture of the left wing following the protocol detailed in Sadanandan and Rheindt (2015) under National University of Singapore IACUC permit B17-0459.

ddRADSeq library preparation

DNA extracts were prepared with a DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany) following a modified manufacturer protocol for blood and tissue. A reduced representation of the genome was prepared using a modified double-digest restriction-site-associated DNA sequencing (ddRADSeq) protocol based on Peterson *et al.* (2012). In this modified protocol, 200–500 ng of extracted genomic DNA from each sample was double-digested with

the restriction enzymes Eco*RI* and Msp*I* (New England BioLabs Inc., Ipswich, MA, USA) at 37 °C for 3.5 h followed by a clean-up. Ligation was performed with T4 ligase at 16 °C for 16 h to optimize adapter ligation, followed by ligase deactivation at 65 °C for 10 min, and dsDNA re-annealing at a decrease of 1 °C/min to 23 °C.

Ligated samples were pooled in equimolar amounts, with 30 ng of DNA per pool, and cleaned up accordingly. Fragments with a tight peak of 350 ± 31 bp were selected for with a Pippin Prep (Sage Science, Beverly, MA, USA) across all pools, within the optimal fragment size range for sequencing with Illumina HiSeq 4000. The size-selected fragments were cleaned up, then enriched and amplified for 12 cycles before performing a final bead clean-up step. All clean-ups were conducted with Agencourt® AMPure® XP beads (Beckman Coulter, Brea, CA, USA).

From the 18 samples, a total of 18 individual ddRADSeq libraries were successfully prepared and quantified using a Qubit High Sensitivity Assay kit (Thermo Fisher Scientific, Waltham, MA, USA). The fragment size of each pooled library was quantified with an Advanced Analytical Fragment Analyser (AATI), quality controlled, and spiked with 30% phiX to improve base diversity of the final sequencing library. Sequencing was carried out in combination with ~95 other samples per lane across four Illumina HiSeq 4000 lanes at the Genome Institute of Singapore and NovogeneAIT, yielding 150-bp paired-end reads. Sequenced reads have been deposited in the Sequence Read Archive (Bioproject Number: PRJN576902).

Sanger sequencing of mitochondrial DNA

Of the genetic samples, 16 individuals (excluding 'Singapore 2' and 'Sumatra') were included in mitochondrial analysis (Table S2). The mitochondrial gene ND2 was amplified with the primers L5219 Met (5′-CCCATACCCCGAAAATGATG-3′) and H6313 Trp (5′-ACTCTTRTT-TAAGGCTTTGAAGGC-3′) and PCR was conducted for 35 cycles at an annealing temperature of 50–53 °C (Sorenson *et al.* 1999). The PCR product was cleaned up with ExoSAP-IT (Thermo Fisher Scientific) and cycle-sequenced with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). The Applied Biosystems 96-capillary array 3730 DNA Analyzer was then used to obtain sequence reads. GenBank accession numbers are MN548122–MN548137, and are listed in detail in Table S2.

Bioinformatic analyses

Identification of SNPs from ddRADSeq data

Raw sequence read quality was assessed with FastQC (Babraham Bioinformatics, Cambridge, UK) to determine the mean Phred scores across all base positions. Mean Phred scores were ≥30 across all pools and base positions, and hence sequences were left untruncated at 150 bp. Sequence reads were then demultiplexed and filtered (-c – remove any read with an uncalled base; -q – discard reads with low-quality scores; -r – rescue barcodes and RAD-Tags) in STACKS v1.44 (Catchen *et al.* 2013). The demultiplexed sequences were aligned, using default settings, with the Burrows–Wheeler ALIGNER 0.7.15 (Li & Durbin 2009) against the genome of the confamilial Javan Myna *Acridotheres javanicus* (GenBank assembly accession: GCA_002849675.1; Low *et al.* 2017), the phylogenetically closest taxon with a reference genome available (Lovette & Rubenstein 2007). Aligned sequences were then sorted by coordinate order with SAMTOOLS 0.1.19 (Li *et al.* 2009) to facilitate subsequent data processing.

SNPs were called using the reference pipeline (ref_map.pl) in STACKS v1.44 (Catchen *et al.* 2013) with a minimum stack depth (m) of 10, and subsequently processed in the program 'populations' within STACKS to retain only loci found in at least 90% of all samples ($r = 0.9$), assigning all individuals to a single putative population (p = 1). Only the first SNP of each locus was extracted to minimize the likelihood of linkage and allow for reproducibility across iterations of SNPs called during data exploration. All remaining parameters were set to default values. During initial explorations of our dataset, we performed sensitivity analysis by calling SNPs with a stack depth of $m = 5$, but downstream analyses did not produce qualitatively different results (data not shown). Hence, we settled on $m = 10$ for final analyses.

The extracted SNPs were then filtered for linkage disequilibrium and checked for missing data in PLINK v1.9 (Chang *et al.* 2015) with a sliding window size of 25 SNPs in steps of 10 SNPs with a 0.9 r^2 threshold. Loci were checked for selection neutrality in BAYESCAN v2.1 (Foll & Gaggiotti 2008) at default settings. The maximum likelihood

estimation method in SNPRELATE (Zheng *et al.* 2012) was used to calculate pairwise relatedness among individuals to ascertain clades or groups arising from consanguinity; no individuals were found to be closely related.

Population genomic analyses

To elucidate population structure, principal component analysis (PCA) was conducted in SNPRELATE (Zheng *et al.* 2012) and population network diagrams were generated with NETVIEW (Steinig *et al.* 2015). We also ran population structure plots with the model-based STRUCTURE (Pritchard *et al.* 2000) at values of K from 2 to 5 with five iterations per K, where K represents the number of putative ancestral populations. We applied a burn-in period of 50 000 iterations and 250 000 Markov chain Monte Carlo-based simulations, and ran consensus building across all iterations in CLUMPP (Jakobsson & Rosenberg 2007).

Phylogenomic analyses

Phylogenomic analysis was performed with concatenated reads using the ipyrad workflow (https://ipyrad.readthedocs.io/), with input from sequences demultiplexed with STACKS v1.44. We allowed for a maximum of 500 aligned reads (maxdepth 500) to preclude the inclusion of paralogues, a minimum of 90% similarity between two sequences to be considered homologous (clust_threshold), and a maximum of one mismatch between the barcode files and sequenced reads (max_barcodes_mismatch), and required at least 14 of 19 samples (including the one outgroup sample) to have data present at a given locus (min_samples_locus) to be retained. All other parameters were set to default values.

Phylogenetic inference was performed with the maximum likelihood-based RAXML (Stamatakis 2014). A rapid bootstrap analysis and search for best-scoring maximum-likelihood tree (-f a) were run for 1000 iterations (-N) with the General time reversible plus Gamma model (GTRGAMMA).

Mitochondrial analyses

Mitochondrial DNA sequences were assembled in CODONCODE ALIGNER v6.0.2 and aligned with CLUSTALW (Larkin *et al.* 2007, Tamura *et al.* 2013) as implemented in MEGA7 (Kumar *et al.* 2016). All sequences were truncated to 819 bp, the shortest read fragment after accounting for reading frame. MEGA7 was then used to compute uncorrected pairwise distances and generate trees with the neighbour joining (NJ), maximum parsimony (MP) and maximum likelihood (ML) methods, each run with 1000 bootstrap replicates. NJ was run with the default setting of p-distance as the genetic distance/nucleotide substitution model; all other parameters were left at default. MP was run with all sites used for gap treatment, Tree-bisection-reconnection as the tree search method and all other parameters at default. ML was run with the Hasegawa–Kishino–Yano plus Gamma nucleotide substitution model (HKY + G), as determined by running JMODELTEST (Darriba *et al.* 2012). Using the same substitution model (HKY + G) as the ML tree, a Bayesian inference tree was reconstructed using MRBAYES v3.2.6 (Ronquist & Huelsenbeck 2003); 50 000 iterations were generated with a burn-in of 25%, leaving all other parameters at default. Two outgroup samples (*Aplonis panayensis* (GenBank accession: Q466853.1) and *Gracula ptilogenys* (GenBank accession: EF468237.1)) were included in all mitochondrial analyses.

Morphometrics

Eighteen morphological characters were identified (Table S1), 14 of which were quantitative and four qualitative. We used an Extol 0–150 mm digital calliper with a resolution of 0.01 mm for various measurements, an ornithological ruler for wing length (25 cm) and a 30-cm ornithological ruler for tail length (Appendix S2). Individuals were assigned a unique identification code. The estimated age of each individual was expressed in terms of the number of months and/or years spent with its owner (Appendix S1). Each individual was photographed against graph paper (1 mm). Wattles were photographed dorsally and laterally, the open wing dorsally for all individuals, ventrally for some others, and laterally for closed wings; the percentage of white patch extent was computed using the imaging software NIS ELEMENTS (Nikon, Tokyo, Japan), which has been designed to work with irregular settings (Appendix S3). Qualitative features were evaluated visually with the bird in hand (e.g. iris colour) or with photos. All measurements and scoring were performed by T.Š. To preclude subjectivity, repeated scoring of 'Wattle shape, classification 1' and 'Wattle shape, classification 2' (measurements 16 and 17 in Appendix S2) was carried out by four independent examiners (including T.O.), with majority

assignments accepted in the absence of a unanimous decision. Repeatability was tested with function 'rpt' in the R package rptR and a mixed-effect modelling approach (Nakagawa & Schielzeth 2010, Dingemanse & Dochtermann 2013).

PCA and canonical discriminant analysis (CDA) were performed using R with the prcomp function (in the R package 'stats') and candisc function (in the R package 'candisc'), respectively. Both PCA and CDA were conducted using the following measurements (in mm): tarsus length, tarsus width, sternum length, flattened wing length, skull width, white patch on secondaries and wattle shape classification 1 (Table S1, Appendix 2); binary parameters or those with missing data were excluded. Based on morphological data from this study, we have developed a field identification key to aid in distinguishing among the different taxa (Fig. 3).

RESULTS

Genomic analyses

A total of 78 329 782 individual 150-bp paired-end sequence reads were obtained from all 18 individuals; after filtering, 75 044 121 reads (95.8%) were retained, with a mean of 4 169 117 reads per individual and a mean read coverage of 89.814× per locus and per individual. After filtering for linkage disequilibrium we obtained 6088 SNPs; no SNP was removed on the basis of our selection test. A concatenated dataset averaging 7911 loci comprising some 2 134 046 bp was prepared in IPYRAD and used in downstream phylogenomic analyses.

A PCA across all 18 individuals revealed three clusters:

- a *religiosa/intermedia* cluster, comprising the Singaporean samples, a Sumatran individual and the captive birds from Jurong Bird Park;
- a *robusta* cluster, comprising the individuals from Bangkaru, Nias and Simuk;
- a *miotera* cluster, comprising the individuals from Simeulue (Fig. 2a).

The Telo individual, *batuensis*, emerged as intermediate between the *robusta* and *religiosa* clusters. A population network analysis corroborated the same clustering pattern at a maximum nearest neighbour threshold (k) of 5 (Fig. S4).

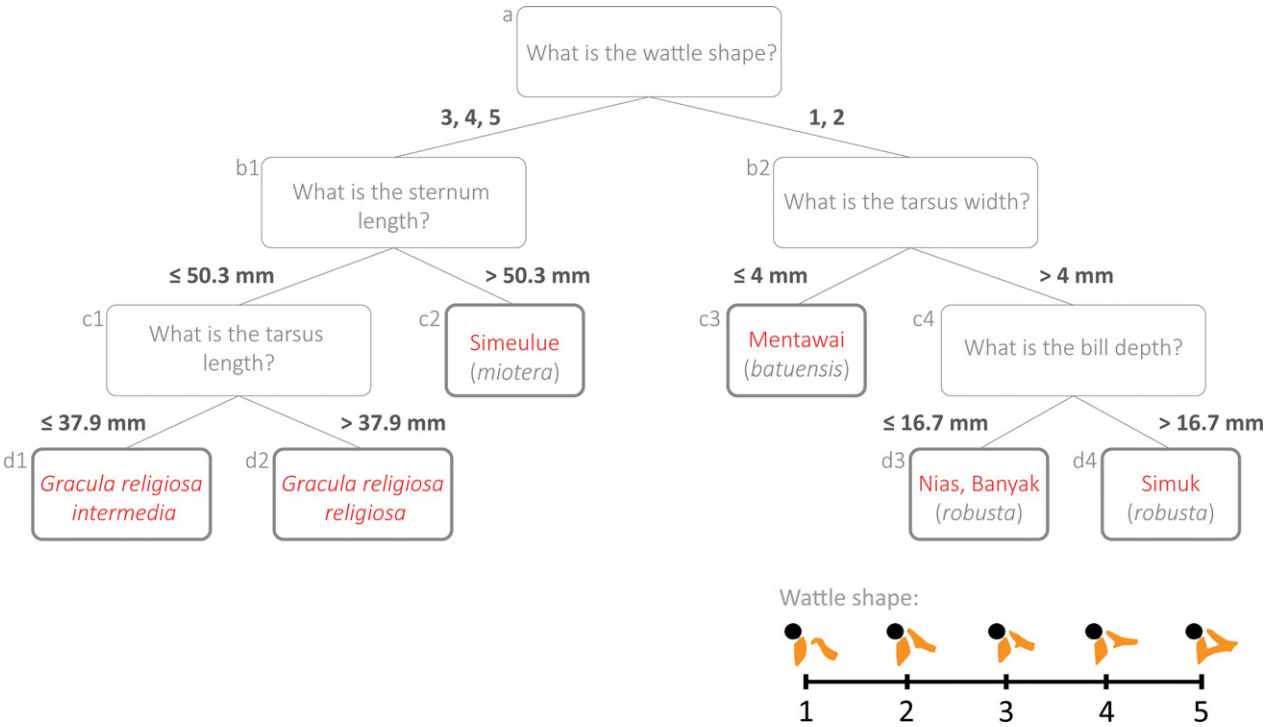

Figure 3. Field identification key for hill mynas based on empirical morphological data of the individuals in this study (i.e. west Sumatran islands and Sumatra). Further information on determining wattle type can be found in Appendix S2. [Colour figure can be viewed at wileyonlinelibrary.com]

We performed cluster-based population structure analysis across all relevant K values up to $K = 5$. These analyses supported a distinct cluster of samples from Bangkaru, Nias and Simuk (*robusta*) (dark grey in pie charts in Fig. 2d). Simeulue samples (*miotera*) emerged as a second cluster distinct from all other sampled taxa (white in Fig. 2d). The wild Singaporean and Sumatran samples (nominate *religiosa*) formed a third cluster (black in Fig. 2d), and the Telo sample (*batuensis*) displayed a genomically admixed pattern, with contributions from *robusta* to a genotype dominated by nominate *religiosa* (Fig. 2d). Finally, four of the six captive samples from Jurong Bird Park formed a fourth cluster (light grey in Fig. 2d), with a speculated origin somewhere in mainland Southeast Asia (perhaps representing the range of *intermedia*; Fig. 1), whereas the other two captive samples displayed a similar genotype but with some admixture from *religiosa*.

The phylogeny based on genomic data (Fig. 2d) supported population-genomic analyses by showing a highly distinct Simeulue clade (*miotera*) and a clade including Bangkaru, Nias and Simuk (*robusta*), both with high bootstrap support. The Telo sample grouped basal to the *robusta* samples with high branch support. The Barusan taxa distributed across the islands off West Sumatra formed a well-supported clade to the exclusion of all other populations (Fig. 2d).

Mitochondrial analysis

The ML phylogeny based on mitochondrial data supported a distinct Simeulue *miotera* lineage within a highly supported Sundaic clade that also included the *robusta* cluster (Nias, Simuk, Bangkaru), *batuensis* (Telo) and the wild Singaporean sample (Fig. S5). The Jurong Bird Park samples formed a well-supported sister clade. The NJ, MP and Bayesian phylogenies (Figs S6–S8) corroborated the distinctness of the *miotera* and *robusta* clades. General uncorrected pairwise divergences within the Barusan (=West Sumatran Island) clade range up to 0.98%, whereas the sole Singaporean sample (*religiosa*) that emerged in the Sundaic mitochondrial clade along with all Barusan samples (Figs S5–S8) was characterized by mitochondrial divergences ranging between 0.37 and 0.61 (data not shown). In contrast, individuals belonging to the distinct sister clade (Figs S5–S8), presumably attributable to subspecies *intermedia*, diverged from the Sundaic clade (including all Barusan samples) by 1.59–1.83%.

Morphological analysis

Among the individuals sampled, both the nominate *religiosa* and the *intermedia* individuals exhibited smaller biometric measurements than the *robusta* and *miotera* groups; the *batuensis* individuals were intermediate between the widespread forms (*religiosa*, *intermedia*) and the other West Sumatran small-island forms (*robusta*, *miotera*) (Table S1). The scores of the five independent examiners evaluating wattle shape were highly repeatable for both measurements 16 and 17 (Appendix S2) at 84% (95% confidence interval (CI) 75–89%) and 90% (95% CI: 84–94%), respectively. Both PCA (Fig. 2b) and CDA (Fig. S9) grouped the measured individuals into five clusters corresponding to genomic divisions (Fig. 2) and previously accepted taxonomic arrangements.

DISCUSSION

Distinct hill myna lineages reflect historical isolation of West Sumatran islands

Sundaland has a complex biogeographical history. Repeated glaciations and deglaciations predominantly at higher northern latitudes across the Quaternary (Matthews 1990) led to a cyclical rise and fall of global sea levels (Voris 2000). The West Sumatran islands have been affected to different degrees by such sea level change, with some islands variably connected to the main Sumatran landmass and others not (Voris 2000; Fig. 2e), alternately facilitating and impeding gene flow. The isolation of some of these 'satellite' islands may have given rise to a number of endemic hill myna forms.

Simeulue, the northernmost large island within the Barusan group, has never been connected to the main Sumatran landmass, even during past glacial maxima when sea levels were at their lowest (Fig. 2e). Hill mynas on this island were described as *miotera* by Oberholser (1912), and although widely synonymized with *religiosa* in the literature (Table 1), they emerge as distinct from all other sampled taxa based on both biometric and genomic analysis (Figs 2 and S4, Table S1). Their level of distinctness is at least as pronounced as that of

robusta from Nias (Fig. 2a,b), justifying equal treatment. Given Simeulue's lack of Quaternary land bridges, colonization by *miotera* must have occurred through overwater dispersal. Indeed, Simeulue is characterized by pronounced avian endemism for an island of its size (2310 km^2), with endemics including the Simeulue Scops Owl *Otus umbra*, the Simeulue Parrot *Psittinus abbottii* and a distinct subspecies of the White-bellied Woodpecker *Dryocopus javensis parvus* (Eaton *et al.* 2016).

Nias, the largest West Sumatran island, is mainly surrounded by deep sea, although a narrow overwater ridge may have connected it to the Sumatran mainland during the lowest sea levels of the Quaternary (see bathymetric lines at the northern tip of Nias in Fig. 2e). The island has long been known to harbour a distinct *Gracula* taxon (Salvadori 1887, Finsch 1899), *robusta*, which has variously been elevated to species status by multiple authorities (Table 1). Its described range extends to the deep-sea island of Babi and some shelf islands of the Banyak Archipelago, including Bangkaru (Salvadori 1887; Fig. 2e), and our data confirm the identity of the Bangkaru population as *robusta*. Unexpectedly, genomic and morphological data now also support an expansion of *robusta* to include the population on the tiny deep-sea island of Simuk off the Batu Archipelago (Fig. 2e).

Table 1. A comparison of classifications of Nias (*robusta*) and Simeulue Hill Mynas (*miotera*) among avian taxonomic authorities, as well as the Indonesian government's list of protected species.

	Nias Hill Myna (*robusta*)	Simeulue Hill Myna (*miotera*)
Howard and Moore Checklist of the Birds of the World (Christidis *et al.*, 2018)	Potential split	Synonymized
Clements Checklist of Birds of the World (Clements *et al.* 2018)	Species split	Synonymized
Handbook of the Birds of the World (del Hoyo *et al.*, 2019)	Species split	Subspecies status
Birds of the Indonesian Archipelago (Eaton *et al.* 2016)	Potential split	Synonymized
International Ornithological Congress World Bird List (Gill & Donsker 2016)	Species split	Synonymized
Indonesian List of Protected Species (Ministry of Environment & Forestry 2018)	Species split	Not applicable

A 'potential split' represents an explicit acknowledgement of a taxon's potential species status without officially recognizing it as such; the status 'synonymized' signifies that the taxon in question is not recognized as a separate taxonomic entity. Cells in green indicate the taxon in question has been synonymised with the nominate; cells in red indicate that the taxon in question has been elevated to species status, and cells in yellow indicate intermediate and ambiguous situations. [Colour version of this Table can be viewed at wileyonlinelibrary.com]

The Nias Hill Myna is highly dispersive

The close genetic affinity of populations on Simuk, Bangkaru and Nias defies an easy historical explanation for the evolution of *robusta*. The taxon was known to be present in the West Sumatran islands as far back as the early 20th century (Richmond 1903, Ripley 1944), prior to the precipitous rise of songbird trade activity in Indonesia. Two overwater dispersal events would have to be invoked to explain the dispersal from Nias (the largest island) to Simuk, a tiny deep-sea island, and Bangkaru, a small shelf island unconnected to Nias (Fig. 2e). A third overwater dispersal event needs to be invoked to explain its occurrence on the unsampled deep-sea island of Babi (Fig. 2e). This occurrence pattern suggests that *robusta* may be a hyper-mobile taxon, more dispersive than 'mainland forms' and adept at colonizing small islands while failing to establish a bridgehead on larger landmasses due to competitive exclusion by resident forms (Diamond 1974, 1975). The larger body size of *robusta* supports this hypothesis, making for a sturdier bird that is more capable of overcoming overwater distances of tens of kilometres. The existence of such highly dispersive bird lineages is well known in other Indonesian and Australasian bird groups, such as *Zosterops* white-eyes (Moyle *et al.* 2009).

The large island of Nias is likely to be a source area for *robusta*; at least, the population on tiny Bangkaru probably represents a recent colonization. This colonization could have proceeded from the more distant Nias around 12 000 years ago when rising sea levels cut off Bangkaru from the main landmass of Sumatra, perhaps in the wake of a stochastic extinction event of a potential original *religiosa* population that is certain to have lived on Bangkaru during times of connection (MacArthur & Wilson 1967, Gwee *et al.* 2017), allowing for recolonization by the more dispersive *robusta*, which is more adequately equipped for a small-island lifestyle.

The admixed nature of *batuensis*

Telo, the type locality of the taxon *batuensis* (Finsch 1899), is a member of the shelf islands collectively known as the Batu Archipelago. Little is known about the taxonomic status of *batuensis*, although populations on the nearby deep-sea islands of the Mentawai Archipelago (e.g. Siberut) are generally subsumed under it (Eaton *et al.* 2015; Figs 1 and 2); the conclusions drawn regarding the genomic identity of *batuensis* in the study may not be representative of the Mentawai population, owing to the lack of genetic sampling on the aforementioned islands in this study. Genome-wide SNPs and morphometry indicate an admixed phenotype and genotype of *batuensis*, roughly intermediate between *robusta* from Nias/Simuk and *religiosa* from the main Sundaic landmass of Sumatra and Singapore (Fig. 2). The shallow separation and frequent land connection between the Batu Islands and Sumatra would have allowed for gene flow during recent glacial maxima. Telo's original *religiosa* population from the mainland may have become admixed with invading individuals of *robusta* upon island formation, explaining their intermediate genomic signature. Alternatively, if Telo's original birds belonged to *robusta*, land bridge formation during the last glacial maximum would have allowed for incursion of nominate *religiosa* from Sumatra for subsequent admixture.

Extinction in progress of a hill myna island radiation

The Nias Hill Myna *G. [religiosa] robusta* is considered Critically Endangered by the IUCN (BirdLife International 2018b). Following heavy trapping pressure in the 1990s and early 2000s, it all but disappeared on the markets in Medan and other major Sumatran cities, with only a pair found in the wild on Nias in 2015 (Rudianto Sembiring pers. commun.). A recent excursion to Babi Island (24 July 2018) off the southeastern coast of Simeulue yielded a sighting of a small wild flock at an undisclosed location over good forest (F. E. Rheindt unpubl. data), and Bangkaru is currently known to harbour a small and heavily protected population of *robusta* at an undisclosed location. The two Simuk individuals sampled for genomic studies in this study were said to have been acquired from the wild in recent times (T. Švejcarová unpubl. data), extending hope that this small, outlying island may also hold a remnant population thus far undetected. Our study not only confirms the genomically and morphologically distinct status of *robusta*, but also suggests that this form may have acquired unique adaptations (particularly its stronger build and greater size), equipping it for a dispersive lifestyle, thereby allowing it to colonize smaller deep-sea islands unsuitable to mainland populations. These insights only heighten the sense of urgency for its protection from extinction, regardless of taxonomic status afforded.

The greatest surprise of this study was the pronounced genomic and morphological distinctness of *miotera* from Simeulue, a taxon widely synonymized in previous treatises (Table 1). Approaching *robusta* in size, it is different from the latter in wattle morphology (Fig. 2, Table S1) and has emerged as a taxon as genomically distinct as *robusta*. On multiple recent excursions to Simeulue, most recently in July 2018, we were unable to find the bird and learned from locals that there had been a great drive to catch the last survivors on the island in response to a wealthy person's bounty on these birds.

Species delimitation of allopatric forms can be highly contentious (Mayr 1942). Taxonomic inflation, a phenomenon in which subspecies are elevated to full species resulting from a change in the species concept used, has been named as a culprit for artificially increasing the number of conservation targets by critics, although a recent survey found no support for taxonomic inflation in bird taxonomy (Sangster 2009). Nevertheless, the taxonomic rank of the endemic hill myna taxa on the West Sumatran islands has large consequences for their conservation, with full species receiving more attention than subspecies in a variety of ways, from funding to breeding programmes (Margules & Pressey 2000).

Of five major recent authorities on avian taxonomy in Indonesia, three have officially elevated the Nias Hill Myna (taxon *robusta*) to full species status, with the remaining two acknowledging its elevated status without officially adopting the same treatment, i.e. a potential split (Table 1). Both taxa *religiosa* and *robusta* are officially protected species in Indonesia (Ministry of Environment and Forestry (Indonesia) 2018) under MoEF RI Regulations (P.106/MENLHK/SETJEN/KUM.1/12/2018). In stark contrast, the Simeulue Hill Myna (taxon *miotera*) has been synonymized with the nominate *religiosa* by all but one authority, with

Table 2. A list of taxa within the hill myna *G. religiosa* complex with known distribution from del Hoyo *et al.* (2019) and diagnostic characters for sampled taxa.

Taxon	Distribution	Diagnostic characters
Gracula religiosa (Common Hill Myna)		
Gracula religiosa religiosa	S Thailand, Peninsular Malaysia, Sumatra, Bangka, Belitung, N Natuna, Borneo, Java and Bali	Tarsus length >37.9 mm and wattle shape 4 (Fig. 3): Hind wattle reaches all the way to the eye just short of the front wattle, forming a significant sharp, pointed spur extending out near the posterior midpoint of the front wattle
Gracula [religiosa] robusta	Banyak Is (Bangkaru and Tuangku), Babi and Nias, off NW Sumatra	Wattle shape 1 (Fig. 3): Hind wattle is considerably disconnected from front wattle but extends anteriorly to about the same height as the mid-point of the front wattle; the tip of the hind wattle points downwards
Gracula [religiosa] miotera	Simeulue	Sternum length >57 mm and wattle shape 3 (Fig. 3): Hind wattle reaches to the eye just short of the front wattle, and exhibits a significant spur-like extension extending out in a right angle near the posterior midpoint of the front wattle
Gracula religiosa batuensis	Batu Is and Mentawai Is, off W Sumatra	Wattle shape 2 (Fig. 3): Hind wattle reaches all the way to the eye just short of the front wattle, and exhibits a small flat spur extending out near the posterior mid-point of the front wattle
Gracula religiosa intermedia	N & NE Indian Subcontinent E to Myanmar and S China (including Hainan), S to N peninsular Thailand, Cambodia and Indochina	Tarsus length <34 mm and wattle shape 5 (Fig. 3): Hind wattles are connected with the front wattles via a slim stripe
Gracula religiosa peninsularis	EC India (Odisha and SE Madhya Pradesh)	Not sampled
Gracula religiosa andamanensis	Coco Is (S of W Myanmar), Andaman Is and Nicobar Is	Not sampled
Gracula religiosa palawanensis	Palawan	Not sampled
Gracula religiosa enganensis	Enggano, W of S tip of Sumatra	Not sampled
Gracula [religiosa] venerata	Lesser Sundas from Lombok and Sumbawa E to Pantar and Alor	Not sampled

Taxa with possible species status are indicated with species name in square brackets; a final taxonomic verdict is contingent on an analysis of all taxa within the complex.

the latter only mentioning its status as a subspecies of *Gracula religiosa* (Table 1); the Indonesian list of protected species (Ministry of Environment and Forestry (Indonesia) 2018) does not include subspecies as candidates for protection – *miotera* is subsumed under the taxon *religiosa* and not given special attention. In light of our findings, if *miotera* is held to the same standards, it ought to be recognized at the same taxonomic level as *robusta*, owing to its pronounced level of genomic and morphological distinction (Table 2).

Simeulue Hill Mynas have recently become extinct in the wild (F. E. Rheindt, T. Švejcarová & T. Ouhel unpubl. data) but may still be found in captivity, albeit in greatly diminished numbers. Lamentably, these few surviving captive individuals are probably either being hybridized with other taxa by breeders and owners or may die without producing offspring. The conservation situation of the Simeulue Hill Myna is extremely urgent. Immediate procurement of remaining captive individuals, together with a carefully considered breeding programme, is thus of paramount importance. The need for further field research across the Barusan Islands, especially on Simeulue, is crucial to building a more comprehensive understanding of the species complex. Only by taking such action can we attempt to prevent the imminent, irreversible loss of this unique lineage.

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AUTHOR CONTRIBUTIONS

D.Y.J.N. performed all genomic and mitochondrial analyses, wrote the main text, and prepared Figs 1, 2 and S4–S8, and Tables 1, 2 and S2. T.Š. performed the field sample collection and the morphological analyses, wrote the text for all morphological components, and prepared the base material for Figures 3 and S1–S3, Table S1, and Appendices S1–S3. K.R.S. provided instruction and input for mitochondrial analyses, and input and critique for the main manuscript text. T.R.F., D.M.P. and T.O. facilitated and oversaw field sample collection. J.G.H.L. supervised the project and provided critique and review for the text. E.Y.X.N. performed and assisted in subsequent genomic analyses, provided input and critique for all components of the project, and aided in preparation and review of all figures and tables. F.E.R. conceptualized and supervised the project, and provided input and review for all components, from analyses to text.

DATA AVAILABILITY STATEMENT

All scripts and files needed to reproduce our analyses are available on GitHub (https://github.com/dominicnyj/barusan-hill-mynas-2020/) and sequenced reads for ddRADSeq are available from the Sequenced Read Archive (accession no. PRJNA576902).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. List of individuals with measured morphological characteristics.

Table S2. Sample name, tissue type, locality, country, source and mean DNA sequence coverage for all genetic samples.

Figure S1. Bill measurements.

Figure S2. Wattle shape, classification 1 (on an ordinal scale from 1 to 5).

Figure S3. Wattle shape, classification 2 (on an ordinal scale from 0 to 10).

Figure S4. Left: Population structure analysis based on genome-wide SNP data using STRUCTURE (Pritchard et al. 2000) for K = 3–5. Right: Population network of 18 sampled individuals based on genome-wide SNP data using NetView (Steinig et al. 2015) with a maximum nearest neighbour threshold of 5; colours correspond to populations.

Figure S5. Best maximum likelihood mitochondrial tree based on an 819-bp alignment of ND2. Bootstrap values shown.

Figure S6. Best neighbour joining mitochondrial tree based on an 819-bp alignment of ND2.

Figure S7. Best maximum parsimony mitochondrial tree based on an 819-bp alignment of ND2.

Figure S8. Bayesian tree based on an 819-bp alignment of ND2.

Figure S9. Canonical discriminant analysis of a subset of seven morphological characters across 38 individuals.

Appendix S1. Interview protocol.

Appendix S2. Protocol for biometrics.

Appendix S3. Protocol for measurement of wing patches.