

# Identifying A New Phylogeographic Population of the Blyth's Tragopan (*Tragopan blythii*) through Multi-locus Analyses

Jingyi Zou<sup>1</sup>, Lu Dong<sup>2</sup> , Geoffrey Davison<sup>3</sup> , Win Hlaing<sup>4</sup>, Moe Myint Aung<sup>4</sup>, Yanyun Zhang<sup>2</sup> , Zhengwang Zhang<sup>2</sup> , Nan Wang<sup>1,\*</sup> , and De Chen<sup>2,\*</sup> 

<sup>1</sup>School of Nature Conservation, Beijing Forestry University, Beijing, 100083, P.R.China.

\*Correspondence: E-mail: wangnan8854100@qq.com (Wang).

E-mail: 8854100@qq.com (Zou)

<sup>2</sup>MOE Key Laboratory for Biodiversity Science and Ecological Engineering, College of Life Sciences, Beijing Normal University, Beijing, 100875, P.R.China. \*Correspondence: E-mail: chende@bnu.edu.cn (Chen).

E-mail: donglu@bnu.edu.cn (Dong); zhangyy@bnu.edu.cn (Y. Zhang); zzw@bnu.edu.cn (Z. Zhang)

<sup>3</sup>National Biodiversity Centre, Singapore, 259569. E-mail: davisongwh53@gmail.com (Davison)

<sup>4</sup>Httamanthi Wildlife Sanctuary, Homalin Town, Sagaing Region, Myanmar. E-mail: winhlaing1441@gmail.com (Hlaing); moemyintaung1653@gmail.com (Aung)

Received 20 August 2020 / Accepted 19 April 2021 / Published 21 July 2021

Communicated by Chih-Ming Hung

Geographically and morphologically distinct populations within a species are frequently the focus of conservation efforts, especially when the populations are evolutionarily significant units. The Blyth's Tragopan (*Tragopan blythii*) is a globally-threatened species confined to South and Southeast Asia. During our field surveys in western Myanmar, we discovered a distinct group of individuals that differed in their appearance relative to all other populations. We further examined the differences in their DNA sequence using three nuclear introns and three mitochondrial genes through phylogenetic analytical methods. Our results showed the population from Mount Kennedy, Chin Hills formed reciprocal monophyletic groups with the nominate subspecies from Mount Saramati, Sagaing Division. Species delimitation analyses further confirmed this differentiation. Geographical isolation by the intervening lowlands found between high elevation habitats may have been the main cause of their differentiation. Hence, we propose that the Mount Kennedy population be viewed as a distinct evolutionarily significant unit and be given special priority for conservation.

**Key words:** Bird conservation, Edge population, Evolutionary significant units, Galliformes, *Tragopan blythii*.

## BACKGROUND

Geographically and morphologically distinct populations within a species are frequently the focus of substantial conservation effort (Frankham et al. 2010). However, geographical and morphological distinctions do not always accurately reflect the differences in the evolutionary histories of populations (e.g., Mason and Taylor 2015). Recent molecular studies have proposed

that evolutionary significant units (ESU) should command greater attention when protecting threatened species, especially ones that are facing habitat fragmentation (e.g., Bottin et al. 2007).

With recent advances in molecular technologies, molecular systematics has become one of the most vigorous disciplines for studying avian taxonomy, phylogeography, and conservation (Dong et al. 2010; Frankham et al. 2010). Although recent phylogenetic

studies are mainly based on nuclear sequences (e.g., Prum et al. 2015), adding mitochondrial DNA (mtDNA) can substantially improve the tree resolution (Corl and Ellegren 2013), especially for populations with relatively shorter periods of isolation (Zink and Barrowclough 2008). Analyses based on multi-locus sequences can provide further insights into the phylogeographic structure and evolutionary distinctness of different ESUs (Médail and Baumel 2018). Furthermore, different ESUs that are phenotypically distinct may act as potential candidates for subspecies ranking in future studies.

The adult Blyth's Tragopan (*Tragopan blythii* Jerdon, 1870) male differs from the males of the four other tragopans in having a restricted patch of red on its upper breast, an extensively grey lower breast and abdomen, and bright yellow facial skin and throat lappet (Madge and McGowan 2002). Previous investigations have shown that this species inhabits dense montane and hill slope forests, primarily at elevations between 1,800 m to 2,400 m (Madge and McGowan 2002). Blyth's Tragopans can currently be found in eastern Bhutan, northeast India, western, northern, and eastern Myanmar, as well as south Tibet and northwest Yunnan, China (McGowan and Kirwan 2020) (Fig. 1). Though the gross range exceeds 800 km from north to south and 800 km from east to west, the specific requirements of the forest type and elevation indicate that the actual area of occupancy must be much smaller. The topography suggests a range with three main prongs that extend along the mountain areas westwards to Bhutan, southwestwards along the border between Myanmar and India, and southeastwards along the border between Myanmar and China (see the possibly extant geographic range shown in BirdLife International 2020).

Two subspecies of the Blyth's Tragopan have been recognized, *T. b. blythii* and *T. b. molesworthi* (McGowan and Kirwan 2020). The male *T. b. molesworthi* differs from the nominate *T. b. blythii* in being smaller, with a narrower and more restricted band of red on its upper breast, and darker upperparts with spots that are brown rather than maroon, as well as having paler grey and less scaly underparts (McGowan and Kirwan 2020). The *T. b. molesworthi* is hypothesized to be the form that occupies the western spur of its range through Bhutan and Tibet, China (Fig. 1, McGowan and Kirwan 2020). However, a population that was found on the northern banks of the Brahmaputra, which is usually considered as within the range of *T. b. molesworthi*, has recently been re-identified as the nominate subspecies (Fig. 1, Hennache and Ottaviani 2020), indicating the risks of attributing populations to named subspecies based only on their mapped proximity. Another subspecific name has been

published, *T. b. rupchandi* (Koelz 1954), whose type locality is Blue Mountain, Lushai Hills, Mizoram in northeast India (Fig. 1). Yet, the name has not been widely accepted and is still regarded as a synonym of the nominate subspecies (Madge and McGowan 2002; Storer 1988).

The species is currently declining and scattered in small populations within a severely fragmented range (Fig. 1), which has led to the Blyth's Tragopan being classified as Vulnerable in the International Union for Conservation of Nature's (IUCN) Red List (BirdLife International 2020). Although the Blyth's Tragopan is thought to be locally common at a few sites in Nagaland and southern Tibet, it is rare in most of its range, including the Chin Hills in western Myanmar, which form the southern prong of its range (BirdLife International 2020). Mount Victoria, Chin Hills, located at almost the southernmost point in the Blyth's Tragopan' range (Fig. 1), is an important bird area in Myanmar. However, the number of the Blyth's Tragopan there is reportedly very low (Kim et al. 2016). It has been widely acknowledged that edge populations might harbor considerable genetic differentiation (e.g., Ratkiewicz et al. 2012, Rodríguez-Muñoz et al. 2007), and in the case of *T. blythii* the western edge population has already been considered a separate subspecies. To determine whether the southern edge population shows differentiation from other populations and requires increased conservation effort, both field surveys and molecular analyses are required.

In this study, we conducted field surveys near Mount Saramati in the Sagaing Division, Myanmar, which is close to the Indian border of Nagaland, where the nominate subspecies was first found, as well as on Mount Kennedy in the Chin Hills, Myanmar (Fig. 1). We found that the population from Mount Kennedy was morphologically and genetically distinct from the nominate subspecies. Hence, special priority for conservation should be given to the Mount Kennedy population, as we propose that it be viewed as a distinct ESU.

## MATERIALS AND METHODS

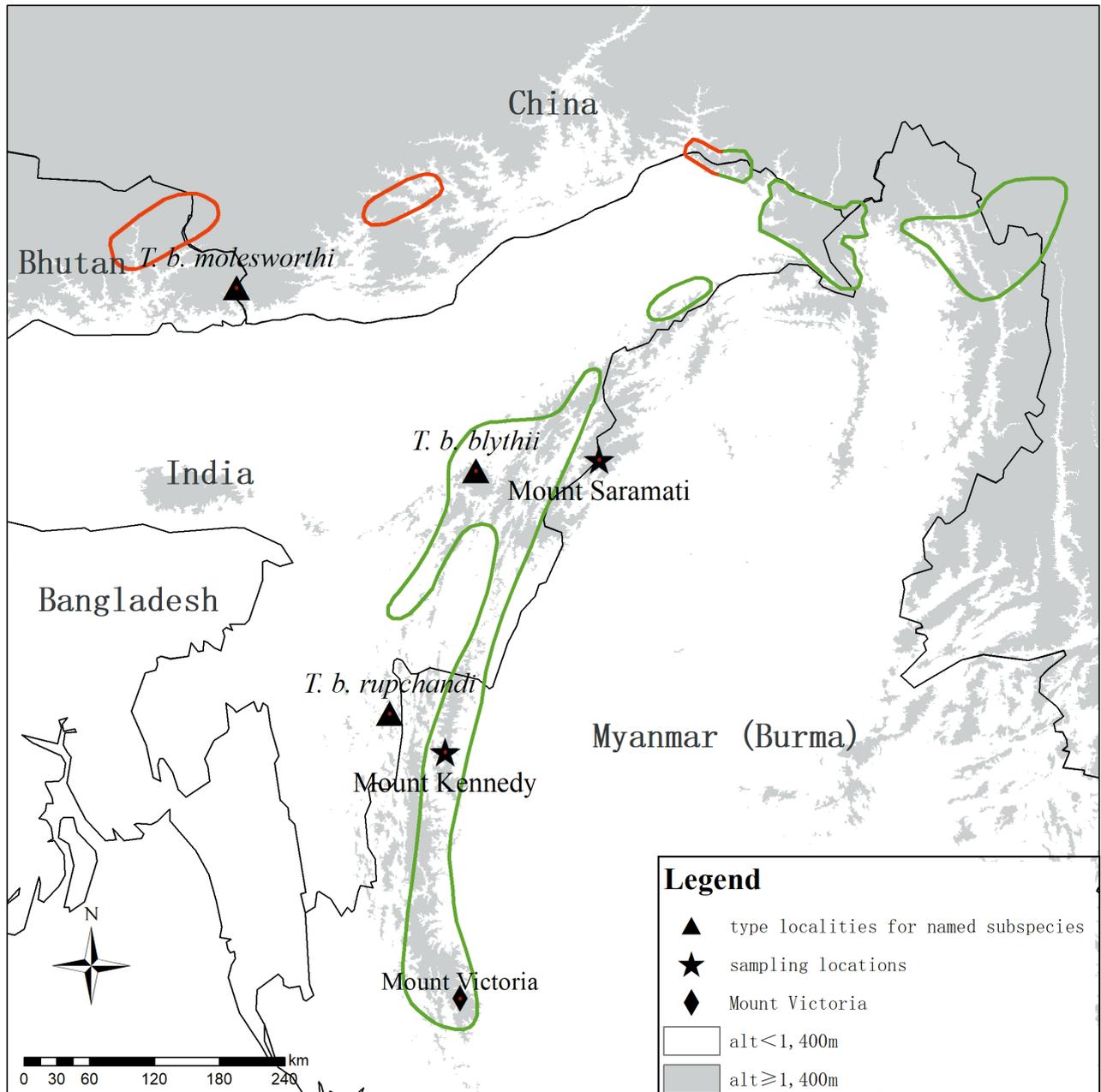
### Field Surveys

We conducted two field surveys during the breeding season of the Blyth's Tragopan from April to June 2014 and April to May 2016. The study sites were on the lower slopes of Mount Saramati in the Sagaing Division, and in the vicinity of Mount Kennedy in Chin Division, Myanmar (Fig. 1). The straight-line distance between Mount Saramati and Mount Kennedy

is approximately 340 km, with the lowest point between them reaching below 700 m in elevation.

Mount Saramati reaches an elevation of 3,840 m. In June 2014, we surveyed the northeastern slope between Makuali village and the national border. In May 2016, we conducted a second survey of the south side of Mount Saramati and the neighboring area of Chera. The forest above 1,500 m was dominated by

broad-leaved evergreen forest with scattered patches of pine trees, as well as big clumps of orchids and mosses that appeared on the tree trunks above 2,000 m. Rhododendrons appeared above 2,300 m, while oak trees were present at all elevations but became most abundant at 2,600 m elevation and above. Distinctive genera of deciduous broad-leaved trees included maples (*Acer* sp.) above 2,500 m and birches (*Betula* sp.) above



**Fig. 1.** Extant distribution of the Blyth's Tragopan *Tragopan blythii* identified by colored outlines: red represents distribution of *T. b. molesworthi*, green represents distribution of *T. b. blythii*. However, the geographical boundary between subspecies is not very clear, *T. b. blythii* was also found on the northern banks of the Brahmaputra (indicated by mixed colors), which is usually considered to be the range of *T. b. molesworthi* (Hennache and Ottaviani 2020). Geographical ranges are generated from the BirdLife's species range maps (BirdLife International and Handbook of the Birds of the World 2018).

2,800 m.

We visited Mount Kennedy in April–May 2014 between Falam and Tedim villages, in the north of Chin State. The forest above 2,300 m was dominated by rhododendrons and oaks, especially on the east facing slopes. Below this elevation, it was dominated by other evergreen broadleaf and pine trees. Old forests mainly appeared above 2,000 m on the steep slopes. Apart from small areas of apparently undisturbed forest patches at high altitudes, most other forests had been burnt and were dominated by shrubs and young trees. We surveyed two patches, Fort White and Mount Kennedy, close to Sozang village. Fort White is an old forest patch with tree heights between 13–25 m, less than 2 km<sup>2</sup> in extent, at 2,000–2,500 m elevation on steep east facing slopes. It has been isolated from other forest fragments by young regrowth after recent fires. Mount Kennedy, just north of Fort White, is approximately 2,715 m high, 5–6 km<sup>2</sup> of old forest lies on the north and west slopes of Mount Kennedy between 2,100–2,700 m, surrounded by second growth forest patches with tree heights of 8–15 m that were formed as a result of a recent fire. Shrubs and young forest patches mainly appeared below 2,100 m, while some grassland occurred at all elevations due to recent carelessly-tended fires.

Transect walks of varying lengths were conducted along existing forest trails and also by walking through the habitat. We camped and walked in the forest along the transects in the morning (from 0430 to 0630 h) to record the distinctive calls of male Blyth's Tragopans. When encountering tragopans along the transects, we kept static, and the birds stayed concealed in the undergrowth, then moved away slowly, which sometimes allowed us to photograph (Camera: Canon 400D, Lens: Canon 70–300 mm f/4-5.6 ISII USM) and

observe (binocular: 10 × 40) the birds from a distance as short as 15 m. We also collected pictures of local Blyth's Tragopans when we visited the villages near Mount Kennedy, and pictures of the Blyth's Tragopan from the area neighboring Mount Saramati were collected through World Pheasant Association (WPA) for comparing the morphological differences.

### Sampling, DNA extraction, PCR, and sequencing

Local people hunt Blyth's Tragopans for food and sometimes keep attractive feathers, body parts or even whole skins around their home. We visited villages at Mount Kennedy and Mount Saramati to collect information and samples of the Blyth's Tragopan. We obtained five samples—including blood, feathers and toe pads—from individuals in Mount Kennedy (K1–K5, Table 1). K1 was from a hunted subadult male. K2 and K3 were from the feathers of Blyth's Tragopans hunted by local people, discarded in their home range. K4 was from a hunted female. K5 was from a male skin kept by a local family. In Mount Saramati, we obtained two samples from two male skins kept by local families (Figs. S1, S2, Table 1). Due to the difficulty in capturing live bird, we did not measure the Blyth's Tragopan in the field, but we measured the dead female (K4) and the subadult male (K1) using a tape with mm scale (Table S1). Because the skins were kept in poor condition and had deteriorated, we did not use them to compare facial color or morphological differences. No specimens were deliberately hunted for the purposes of our surveys.

To quantify the genetic differentiation between the Mount Kennedy population and the nominate subspecies, we compared their differences to those found between *Tragopan caboti caboti* (N = 4) and *T. c.*

**Table 1.** Sampling information used in this study

Taxon	Sample numbers	Type	Sampling sites
<i>Tragopan caboti caboti</i>	Tcc1	Blood	Zhejiang, China
	Tcc2	Blood	Zhejiang, China
	Tcc3	Blood	Zhejiang, China
	Tcc4	Blood	Zhejiang, China
<i>Tragopan caboti guangxiensis</i>	Tcg1	Hepar	Jiangxi, China
	Tcg2	Hepar	Jiangxi, China
	Tcg3	Hepar	Jiangxi, China
<i>Tragopan blythii</i>	Fig. S1	Skin	Saramati, Myanmar
	Fig. S2	Skin	Saramati, Myanmar
	K1	Blood	Kennedy, Myanmar
	K2	Feather	Kennedy, Myanmar
	K3	Feather	Kennedy, Myanmar
	K4	Toe pad	Kennedy, Myanmar
	K5	Toe pad	Kennedy, Myanmar

*guangxiensis* (N = 3). The detailed sampling information is shown in table 1. The sampling was conducted under the supervision of the Forest Department's Nature and Wildlife Conservation Division of the Myanmar Ministry of Natural Resources and Environmental Conservation. Genomic DNA was extracted using a DNeasy Blood & Tissue Kit (Qiagen, Germany).

We amplified three mtDNA genes—cytochrome oxidase subunit 1 (CO1), cytochrome *b* (CYTB), and NADH dehydrogenase subunit 2 (ND2)—and three nuclear introns—clathrin heavy chain-like 1 (CLTCL1) intron 7, fibrinogen beta chain (FGB) intron 5, and ovalbumin (SERPINB14) intron 3 (Table S2). The PCR conditions were as follows: 95°C for 5 min, 35 cycles of denaturation at 95°C for 30 s, annealing for 30 s using the temperature described in table S2, and elongation at 72°C for 60–90 s, followed by a final elongation at 72°C for 10 min using a Veriti Thermal Cycler (Applied Biosystems, Paisley, UK). Both strands of each PCR product were sequenced.

### Phylogenetic analysis

Sequences were assembled by DNAMAN v8 (Lynnon Biosoft Bioinformatic Solutions, USA) and visually inspected to ensure the accuracy of the variable sites. In addition, we downloaded the sequences of *T. blythii* from GenBank for all the studied loci except for CO1. The closely related *T. temminckii* was designated as the outgroup (GenBank accession numbers are shown in Table S3). We used MUSCLE (Edgar 2004) implemented in MEGA X (Kumar et al. 2018) to obtain six partitions.

Next, we combined the six partitions into three combined datasets in the phylogenetic analyses: mtDNA, nuclear, and combined sequences. Maximum likelihood (ML) analyses were performed in RAxML v8.2.12 (Stamatakis 2014) using the GTRGAMMA model in each partition with the '-f a' option, which generates the optimal tree and conducts 100 rapid bootstrap searches. Then, Bayesian inference (BI) was performed in BEAST v1.10.4 (Suchard et al. 2018) using the best-fitting nucleotide substitution model in each partition obtained from jModelTest v2.1.7 (Darriba et al. 2012). BI were run for 10,000,000 generations and the chains were sampled every 1,000 generations. The posterior probabilities were assessed using Tracer v1.7.1 (Rambaut et al. 2018) to ensure that the effective sample sizes for all parameters were larger than 200. The first 25% of the total number of generated trees were discarded as burn-in, while the remaining trees were used to calculate the consensus tree using TreeAnnotator v1.10.4 (Suchard et al. 2018).

### Genetic distances

We used the PHASE algorithm (Stephens and Donnelly 2003; Stephens et al. 2001) that was implemented in the DNASP v6.12.03 (Rozas et al. 2017) to reconstruct the nuclear haplotypes of each individual of the nominate subspecies (Figs. S1 and S2, attribution based on their geographic range, Fig. 1) and the Mount Kennedy population (K1–K5) of the Blyth's Tragopan, as well as *T. c. caboti* and *T. c. guangxiensis* of the Cabot's Tragopan. We conducted 5,000 iterations with thinning at every 100 steps and discarded the first 5,000 samples as burn-in. We excluded the haplotypes for which the phases were determined at probability values less than 60% (Sotka et al. 2004). Next, we calculated the mean genetic distances of the phased nuclear sequences, mtDNA, and the combined sequences between and within the nominate subspecies and the Mount Kennedy population in MEGA X (Kumar et al. 2018) using the Kimura two-parameter (K2P) model, in which the standard error estimates were obtained using a bootstrap procedure of 500 replications. Then, we compared the genetic distances of the Blyth's Tragopan to those found between populations of the Cabot's Tragopan, *i.e.*, *T. c. caboti* and *T. c. guangxiensis*.

### Species delimitation analyses

The species delimitation analyses (A10) were implemented in BPP v4.3 (Flouri et al. 2018), in which we used six partitions for the nominate subspecies (Figs. S1 and S2) and the Mount Kennedy populations (K1–K5) of the Blyth's Tragopan, as well as one individual of *T. c. caboti* and one of *T. temminckii*. The guide tree was collected from the aforementioned phylogenetic analyses. We set  $\theta$  (2:1000) and  $\tau$  (2:2000) to be the prior distributions. Three independent runs were performed using different random seeds and starting trees to confirm the consistency between runs. Each run took samples every 100 iterations and after 8,000 burn-in iterations, resulting in a total of 100,000 samples.

## RESULTS

In the vicinity of Mount Kennedy, calls of male Blyth's Tragopans were recorded at 1 site in Fort White and 6 sites in Mount Kennedy in the field, and 6 wild birds (1 male, 1 female and 3 subadults in Mount Kennedy; 1 male in Fort White) were seen. In the vicinity of Mount Saramati, calls of male Blyth's Tragopans were recorded at 4 sites in the neighboring area of Chera and 10 sites in the south side of Saramati in the field, and 2 wild birds were seen in the south side

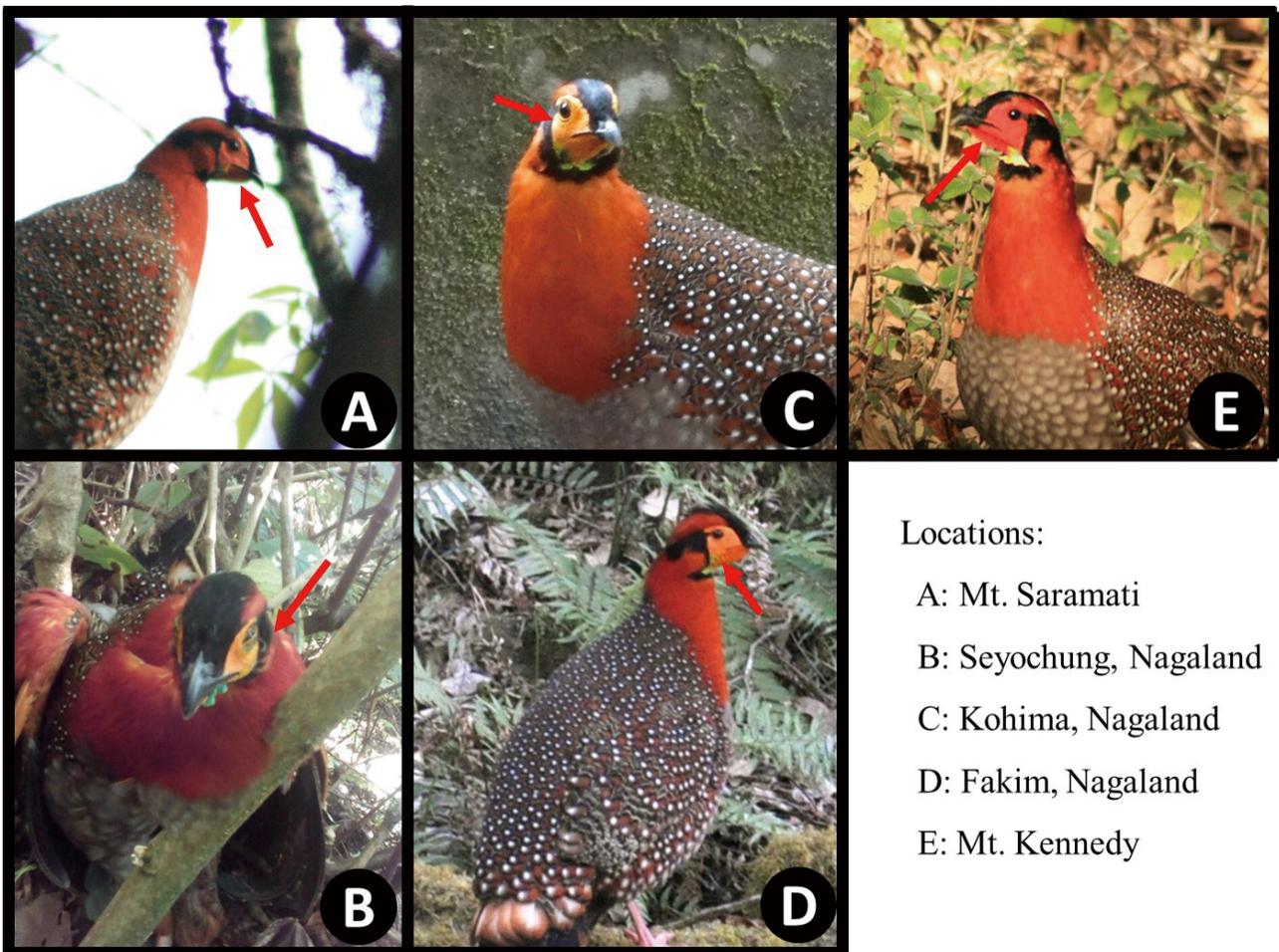
of Saramati. During the period from April to June, males were heard calling exclusively between 2,100 (Mount Kennedy) or 2,400 m (Mount Saramati) upwards to 2,900 m elevation in tall mixed broadleaf forest dominated by oaks and chestnuts up to 25 m high. At positions where tragopans were recorded, undergrowth density was highly variable beneath the continuous canopy, some areas very open and others dominated by small bamboos, ferns and mixed dicot herbage.

We collected pictures of 7 male Blyth's Tragopans, including two around Mount Kennedy (1 from our field photography, Fig. 2E; 1 from villager taken in 2011, Fig. S1), and five around Mount Saramati (2 from our field photography, Fig. 2A and Fig. S2; 3 from WPA, Fig. 2B–2D). By direct observation of the color of the bare facial skin of male Blyth's Tragopans around Mount Kennedy, we found it to be red, very similar to the upper breast plumage (Fig. 2E and Fig. S1), while in the individuals from Mount Saramati and its surroundings, the face is bright yellowish or orange, making a distinct

color contrast with the breast (Fig. 2A to 2D and Fig. S2).

We obtained a total of 3,949 bp of combined sequences, including 2,429 mtDNA and 1,520 nuclear sequences. The recovered topologies from the ML and BI of the combined sequences as well as the mtDNA showed that the Mount Kennedy population (K1K5) formed a monophyletic clade with high bootstrap values (Fig. 3A, 3B), while the representatives of the nominate subspecies (Figs. S1, S2) and *T. blythii* taken from GenBank formed another monophyletic clade. However, neither the ML or BI calculated from the nuclear sequences revealed this monophyly nor the monophyly between the subspecies of *T. caboti* (Fig. 3C).

The genetic distances between the Mount Kennedy population and the nominate subspecies were 0.003 and 0.004 in terms of combined sequence and mtDNA, which were higher than the distances within each population (approximately 0.001) (Table 2), while the genetic distances of nuclear sequences were similar



**Fig. 2.** The color of bare facial skin of male Blyth's Tragopans in Mount Kennedy, Mount Saramati and areas nearby. The geographical locations indicate where the images were taken.

within and between populations (Table 2). Similarly, the genetic distances between the subspecies of *T. caboti* were distinct in terms of combined sequence and mtDNA, but not for nuclear sequences (Table 2).

The species delimitation analyses showed that the probability values of defining four potential taxa (including *T. caboti* and *T. temminckii*) were about 0.94, which further demonstrated the presence of genetic differentiation between the Mount Kennedy population and the nominate subspecies of *T. blythii*.

### DISCUSSION

In this study, we found that the Blyth’s Tragopan from Mount Kennedy differs in face color from that of Mount Saramati, which borders Nagaland and should represent the nominate subspecies (Fig. 1). Furthermore, phylogenetic and species delimitation analyses showed that the Mount Kennedy population and the nominate subspecies were genetically differentiated (Fig. 3). Taken together, the population from Mount Kennedy is genetically isolated and might represent a new ESU. It is likely that the Blyth’s Tragopan from Mount Victoria, which is 220 km further south, should be included in the Mount Kennedy population as the montane forest habitat is connected across the two sites at elevations above 1400 m (Fig. 1), and this area of the Chin Hills has been under limited threat except for forest fires (Rao et al. 2013).

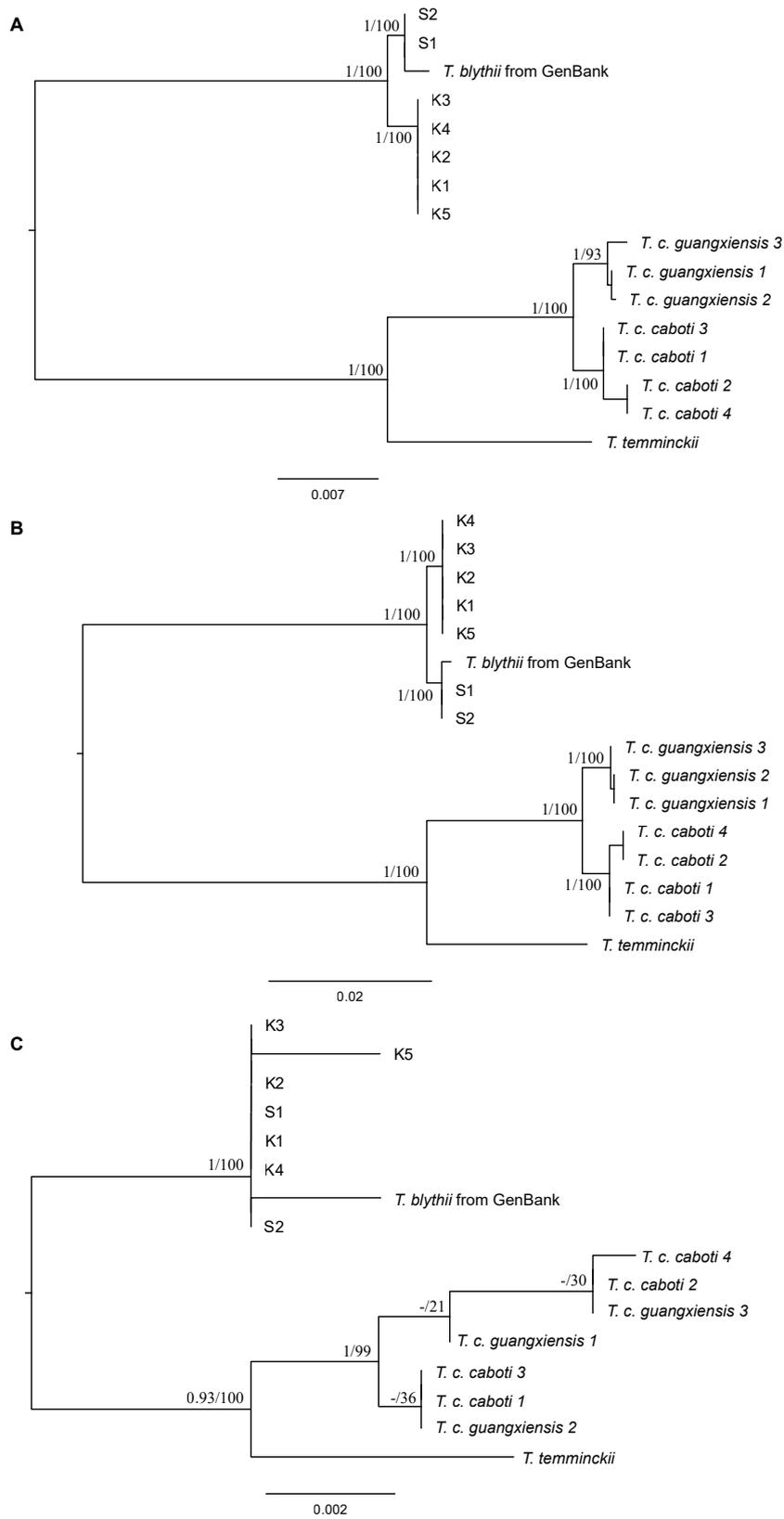
Due to the sparse population and the difficulties in collecting specimens in the wild, quantitative data was limited with regard to Blyth’s Tragopans from Mount Kennedy and Mount Saramati. The color of bare facial skin in males is typically yellow in both recognized subspecies of the Blyth’s Tragopan (McGowan and Kirwan 2020). The photographs of both subspecies available on IBC (<https://www.hbw.com/ibc>) show either yellow or orange color on their bare facial skin, and males near Putao in extreme northern Myanmar are confirmed to have yellow bare facial skin (King et

al. 2001). We are aware that facial color might exhibit individual and seasonal variation, especially for these yellow/orange/red colors which may be influenced by food sources and changes in reproductive status (Hill and McGraw 2006). Our observations were consistent over the years (e.g., the bare facial skin in male Tragopans are red in 2011 and 2014, Fig. 2E and Fig. S1), and confined to the same short period of the breeding season, which means that our observations are not merely of individual or seasonal variation, and might truly reflect morphological distinctness of the Mount Kennedy population. Another possibility might be that some food sources in Mount Kennedy are rich in carotenoid pigments, leading to the consistent red bare facial skin in this population, which needs to be further investigated. Overall, the distinct facial colorations suggest that the Mount Kennedy population might be reproductively isolated from other populations because facial color is an important trait in the genus *Tragopan*, perhaps under sexual selection (Islam 1991).

The genetic distances in the combined sequences and mtDNA between populations of *T. caboti* and *T. blythii* were larger than those within each population, whereas the genetic distances in the nuclear sequences were similar across the populations of both species (Table 2). Such differences were reflected in the phylogenetic trees, the combined sequences and mtDNA separated populations of both species with high support (Fig. 3A and 3B), while the nuclear sequences failed to divide populations of either species (Fig. 3C). Although this “mtDNA structured, nuclear loci uninformative” situation can be attributed to the higher mutation rate and shorter coalescent time in mtDNA (Zink and Barrowclough 2008), it could also have been caused by gene flow through males (e.g., Caparroz et al. 2009). However, most Blyth’s Tragopans are found between elevations of 1,800 and 2,400 m, and rarely below 1,400 m (McGowan and Kirwan 2020). Mount Kennedy and Mount Saramati are separated by a long stretch of lower elevations, i.e., approximately 160 km of land below 1,400 m (Fig. 1). Such a distance is likely

**Table 2.** Mean genetic distances between and within the Mount Kennedy population and the nominate subspecies of the Blyth’s Tragopan, and the two subspecies of the Cabot’s Tragopan

Mean genetic distance	Phased nuclear sequences	MtDNA	Combined sequence
within Mount Kennedy population	0.002 ± 0.001	0	0.001 ± 0.000
within nominate subspecies	0.002 ± 0.001	0	0.001 ± 0.000
between populations of <i>T. blythii</i>	0.002 ± 0.001	0.004 ± 0.001	0.003 ± 0.001
within <i>T. c. caboti</i>	0.003 ± 0.001	0.001 ± 0.001	0.002 ± 0.000
within <i>T. c. guangxiensis</i>	0.003 ± 0.001	0	0.001 ± 0.000
between subspecies of <i>T. caboti</i>	0.003 ± 0.001	0.008 ± 0.002	0.006 ± 0.001



**Fig. 3.** Phylogenetic trees of the *Tragopan blythii* and outgroups. (A) Tree based on combined sequences; (B) Tree based on mtDNA; (C) Tree based on nuclear sequence. Values on the labels indicate posterior probabilities/bootstrap support calculated by Bayesian inference/ML inference. “-” means the topology estimated from Bayesian inference is different from ML inference.

to inhibit gene flow between the two populations due to niche conservatism that retains ancestral ecological characteristics such as altitude ranges (Wiens and Graham 2005). Besides, the nuclear sequences failed to separate subspecies of *T. caboti* as well (Fig. 3C), which means that a few nuclear genes are not sufficient to detect a recent split in this genus.

Our results refer to genetic differentiation along the southerly prong of the Blyth's Tragopan's known distribution formed by the Chin Hills. The recognition of the subspecies *T. b. molesworthi* on the westerly prong, formed by the eastern Himalayas, implies the presence of genetic differentiation there as well. The shallow genetic differentiation between the Mount Kennedy population and the nominate subspecies (Table 2) imply recent divergence, which is likely due to fragmentation of populations by recent fluctuations such as Pleistocene glacial and interglacial cycles that affected the altitudinal zonation of forest vegetation (Renner and Rappole 2011; Basumatary et al. 2015). Such glaciation-based isolation may also have caused the *T. b. molesworthi* subspecies to diverge, and there is likely more extensive phylogeographic structuring across the range of the Blyth's Tragopan.

Based on the morphological and genetic distinctness of the Mount Kennedy population, it is possible that the southern edge population is a distinct subspecies. Although the genetic distances for the combined sequences and mtDNA between subspecies of *T. caboti* were slightly larger than those between the Mount Kennedy population and the nominate subspecies of *T. blythii* (Table 2), their genetic differentiation was significant and highly supported by phylogenetic and species delimitation analyses. However, the limited number of samples and the lack of skin samples in this study hampered this distinction. Furthermore, whether the Mount Kennedy population belongs to the already described race *T. b. rupchandi* from Blue Mountain, Lushai Hills is unclear, because the bare facial skin color was not reported in that taxon (Koelz 1954). Blue Mountain is on a nearby hill range (< 50 km) that is parallel to the Chin Hills, but it is separated by land at an elevation that is as low as 305 m in some places (Fig. 1), which might inhibit gene flow between these two populations. Thus, more samples are required to confirm the subspecies status of the Mount Kennedy population and to confirm whether or not *T. b. rupchandi* is a valid and applicable name.

## CONCLUSIONS

Our study demonstrated that the Blyth's Tragopan from the southern edge of its range in Mount Kennedy,

Chin Hills, formed a distinct ESU, and the very small areas of remaining habitat in combination with being morphologically distinct suggest that this population should be given special priority for conservation. Although the NatmaTaung National Park (*i.e.*, Mount Victoria) in Chin Hills has been designated an important bird conservation area, the Blyth's Tragopan is very rare in this region. In addition, the Blyth's Tragopan is declining in most of its range: it has not been recorded since the early 1970s in Bhutan, is rare in most of India, and is locally uncommon on Mt Majed and Mt Emawbon in North Myanmar (BirdLife International 2020). Therefore, more ecological, genetic, and genomic studies are required to better understand and conserve this endangered species.

**Acknowledgment:** Our fieldwork in Myanmar was supported by the World Pheasant Association and carried out in collaboration with the Nature and Wildlife Conservation Division, Forest Department, Myanmar Ministry of Natural Resources and Environmental Conservation. The genetic work was supported by the National Key Program of Research and Development, Ministry of Science and Technology (2016YFC0503200), and the Biodiversity Survey, Monitoring and Assessment Project (2019–2023) of the Ministry of Ecology and Environment, China. We thank Mr. James Goodhart for his financial support for the project and Mr. John Corder for project assistance. Mr. Win Aung (Myanmar) and the NGO Woodland Travel helped to conduct the field work. Alexander Pack-Blumenau helped to provide contacts for administration and field work and shared his experiences of field work in Myanmar. We thank KhynMaungGyi for guiding us to the survey sites and helping to collect information on the Blyth's Tragopan. For figures and photographs, we thank Zhen Wang (Fig. 1), Longtili (Fig. 2B), Vilhoutuonuo Theunuo (Fig. 2C) and Satem Longchar (Fig. 2D). We also thank our associate editor Dr. Chih-Ming Hung, as well as Professor Rebecca Kimball and another anonymous reviewer whose feedbacks helped to improve the manuscript substantially.

**Authors' contributions:** JZ, LD, YZ, ZZ, and DC did the experiment and phylogenetic analyses. GD, WH, MMA, and NW did the field surveys, collected the samples and phenotype data. JZ, DC and NW wrote the manuscript. All authors read and revised the manuscript.

**Competing interests:** The authors declare that they have no conflict of interests.

**Availability of data and materials:** All sequences, alignments and tree files were deposited in Mendeley

Data: doi:10.17632/jcs246skcn.1.

**Consent for publication:** All authors have approved the manuscript and agree with submission to Zoological Studies.

**Ethics approval consent to participate:** Not applicable.

## REFERENCES

- Basumatary SK, Tripathi S, Bera SK, Nautiyal CM, Devi N, Sarma GC. 2015. Late Pleistocene palaeoclimate based on vegetation of the Eastern Himalayan foothills in the Indo-Burma Range, India. *Palynology* **39**:220–233. doi:10.1080/01916122.2014.945665.
- BirdLife International. 2020. *Tragopan blythii*. The IUCN Red List of Threatened Species 2020:e.T22679163A177682428. doi:10.2305/IUCN.UK.2020-3.RLTS.T22679163A177682428.en. Downloaded on 19 March 2021.
- BirdLife International and Handbook of the Birds of the World. 2018. Bird species distribution maps of the world. Version 2018.1. Available at <http://datazone.birdlife.org/species/requestdis>.
- Bottin L, Tassin J, Nasi R, Bouvet J-M. 2007. Molecular, quantitative and abiotic variables for the delineation of evolutionary significant units: Case of sandalwood (*Santalum austrocaledonicum* Vieillard) in New Caledonia. *Conserv Genet* **8**:99–109. doi:10.1007/s10592-006-9152-7.
- Caparroz R, Miyaki CY, Baker AJ. 2009. Contrasting phylogeographic patterns in mitochondrial DNA and microsatellites: evidence of female philopatry and male-biased gene flow among regional populations of the blue-and-yellow acaw (Psittaciformes: *Ara ararauna*) in Brazil. *The Auk* **126**:359–370. doi:10.1525/auk.2009.07183.
- Corl A, Ellegren H. 2013. Sampling strategies for species trees: The effects on phylogenetic inference of the number of genes, number of individuals, and whether loci are mitochondrial, sex-linked, or autosomal. *Mol Phylogenet Evol* **67**:358–366. doi:10.1016/j.ympev.2013.02.002.
- Darriba D, Taboada GL, Doallo R, Posada D. 2012. jModelTest 2: More models, new heuristics and parallel computing. *Nat Methods* **9**:772. doi:10.1038/nmeth.2109.
- Dong L, Zhang J, Sun Y, Liu Y, Zhang Y, Zheng G. 2010. Phylogeographic patterns and conservation units of a vulnerable species, Cabot's Tragopan (*Tragopan caboti*), endemic to southeast China. *Conserv Genet* **11**:2231–2242.
- Edgar RC. 2004. MUSCLE: Multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res* **32**:1792–1797. doi:10.1093/nar/gkh340.
- Flouri T, Jiao X, Rannala B, Yang Z. 2018. Species tree inference with bpp using genomic sequences and the multispecies coalescent. *Mol Biol Evol* **35**:2585–2593. doi:10.1093/molbev/msy147.
- Frankham R, JD Ballou, DA Briscoe. 2010. Introduction to conservation genetics. (2nd ed.). Cambridge University Press, Cambridge, UK.
- Hennache A, Ottaviani M. 2020. Monograph of the pheasants. World Pheasant Association.
- Hill GE, McGraw KJ. 2006. Bird coloration, volume 1: Mechanisms and measurements. Harvard University Press, Cambridge, MA, USA.
- Islam K. 1991. Evolutionary history and speciation of the genus *Tragopan*. Dissertation, Oregon State University.
- Kim H-k, Jeong M-s, Lee W-s, Choi J-i. 2016. A rapid assessment of avifauna at high-altitude habitats in natmataung national park, myanmar. *Korean Journal of Ornithology* **23**:49–59.
- King B, Buck H, Ferguson R, Fisher T, Goblet C, Nickel H et al. 2001. Birds recorded during two expeditions to north myanmar (burma). *Forktail* **17**:29–40.
- Koelz WN. 1954. 'Ornithological studies'. I. New birds from iran, afghanistan, and India. *Contrib Inst Reg Expl 1*, ann arbor, michigan, pp. 1–33.
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. Mega x: Molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol* **35**:1547–1549. doi:10.1093/molbev/msy096.
- Madge S, McGowan P. 2002. Pheasants, partridges and grouse. Christopher Helm, London, UK.
- Mason NA, Taylor SA. 2015. Differentially expressed genes match bill morphology and plumage despite largely undifferentiated genomes in a Holarctic songbird. *Mol Ecol* **24**:3009–3025. doi:10.1111/mec.13140.
- McGowan PJK, Kirwan GM. 2020. Blyth's tragopan (*Tragopan blythii*), version 1.0. In birds of the world (j. Del hoyo, a. Elliott, j. Sargatal, d. A. Christie, and e. De juana, editors). Cornell Lab of Ornithology, Ithaca, NY, USA.
- Médail F, Baumel A. 2018. Using phylogeography to define conservation priorities: the case of narrow endemic plants in the mediterranean basin hotspot. *Biol Conserv* **224**:258–266. doi:10.1016/j.biocon.2018.05.028.
- Prum RO, Berv JS, Dornburg A, Field DJ, Townsend JP, Lemmon EM et al. 2015. A comprehensive phylogeny of birds (Aves) using targeted next-generation DNA sequencing. *Nature* **526**:569–573. doi:10.1038/nature15697.
- Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. 2018. Posterior summarization in bayesian phylogenetics using tracer 1.7. *Syst Biol* **67**:901–904. doi:10.1093/sysbio/syy032.
- Rao M, Saw H, Platt SG, Tizard R, Poole C, Than M et al. 2013. Biodiversity conservation in a changing climate: a review of threats and implications for conservation planning in myanmar. *AMBIO* **42**:789–804. doi:10.1007/s13280-013-0423-5.
- Ratkiewicz M, Matusiuk M, Kowalczyk R, Konopiński MK, Okarma H, Ozolins J et al. 2012. High levels of population differentiation in eurAsian lynx at the edge of the species' western range in europe revealed by mitochondrial DNA analyses. *Ani Conserv* **15**:603–612. doi:10.1111/j.1469-1795.2012.00556.x.
- Renner SC, Rappole JH. 2011. Bird diversity, biogeographic patterns, and endemism of the eastern Himalayas and southeastern sub-Himalayan mountains. *Ornithological Monographs* **70**:153–166. doi:10.1525/om.2011.70.1.153.
- Rodríguez-Muñoz R, Mirol PM, Segelbacher G, Fernández A, Tregenza T. 2007. Genetic differentiation of an endangered capercaillie (*Tetrao urogallus*) population at the southern edge of the species range. *Conserv Genet* **8**:659–670. doi:10.1007/s10592-006-9212-z.
- Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE et al. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Mol Biol Evol* **34**:3299–3302. doi:10.1093/molbev/msx248.
- Sotka EE, Wares JP, Barth JA, Grosberg RK, Palumbi SR. 2004. Strong genetic clines and geographical variation in gene flow in the rocky intertidal barnacle *Balanus glandula*. *Mol Ecol* **13**:2143–2156. doi:10.1111/j.1365-294X.2004.02225.x.
- Stamatakis A. 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics* **30**:1312–1313. doi:10.1093/bioinformatics/btu033.
- Stephens M, Donnelly P. 2003. A comparison of bayesian methods

- for haplotype reconstruction from population genotype data. *The American Journal of Human Genetics* **73**:1162–1169.
- Stephens M, Smith NJ, Donnelly P. 2001. A new statistical method for haplotype reconstruction from population data. *Am J Hum Genet* **68**:978–989. doi:10.1086/379378.
- Storer RW. 1988. Type specimens of birds in the collections of the University of Michigan Museum of Zoology. Museum of Zoology, University Of Michigan, USA.
- Suchard MA, Lemey P, Baele G, Ayres DL, Drummond AJ, Rambaut A. 2018. Bayesian phylogenetic and phylodynamic data integration using Beast 1.10. *Virus Evol* **4**(1):vey016. doi:10.1093/ve/vey016.
- Wiens JJ, Graham CH. 2005. Niche conservatism: integrating evolution, ecology, and conservation biology. *Annu Rev Ecol Evol S* **36**:519–539. doi:10.1146/annurev.ecolsys.36.102803.095431.
- Zink RM, Barrowclough GF. 2008. Mitochondrial DNA under siege in avian phylogeography. *Mol Ecol* **17**:2107–2121. doi:10.1111/j.1365-294X.2008.03737.x.

## Supplementary materials

**Fig. S1.** Picture of the Blyth's Tragopan around Mount Kennedy taken in 2011 by a local villager. (download)

**Fig. S2.** Picture of the Blyth's Tragopan taken from Mount Saramati in 2016. (download)

**Table S1.** Measurements (mm) of Blyth's Tragopans hunted by villagers in Mount Kennedy. (download)

**Table S2.** Amplification and sequencing primers. (download)

**Table S3.** GenBank numbers for *T. blythii* and *T. temminckii*. (download)