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The Distribution of White-Cheeked Gibbons *Nomascus siki* and *N. leucogenys* in Central Lao PDR

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Abstract

Identifying genetically unique primate populations is crucial for guiding conservation efforts. The northern white-cheeked gibbon *Nomascus leucogenys* and southern white-cheeked gibbon *Nomascus siki* have restricted global ranges and are endemic to Lao PDR and Vietnam. However, the boundary between the two species in central Lao PDR is unclear. It has been hypothesized that the Nam Theun-Nam Kading River is the major geographical barrier between the two species. To investigate the distribution of *N. siki* and *N. leucogenys*, we analyzed 90 gibbon songs, known to be species-specific, from sites in central Lao PDR and Vietnam. We also analyzed 32 recordings of gibbons in European zoological facilities that were known to represent each species, based on karyotype. Overall, the recordings included 737 male calls and 229 female calls. In the captive gibbon recordings, we found a statistically significant difference between the species in 12 acoustic parameters for males and in 10 acoustic parameters for females. We used these acoustic parameters for both males and females as training data to perform a Random Forest species classification analysis of vocal recordings of unidentified species from the wild. Our classification accuracy was 97% for males and 89% for females. We detected both *Nomascus siki* and *N. leucogenys* on both sides of the Nam Theun-Nam Kading River in Nakai-Nam Theun National Park and surrounding areas in Central Lao PDR and Vietnam. These results do not support the hypothesis that the Nam Theun-Nam Kading River is a geographical barrier between the two species in central Lao PDR, which was based on a few samples from central Lao PDR, and none from Nakai-Nam Theun National Park. These findings must be taken into consideration as part of potential reintroduction, rehabilitation or translocation programs for both species in this region.

Keywords White-cheeked gibbon · Geographic range · Vocalization · Species identification · Hybridization

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Introduction

Understanding the distribution of species facing a high risk of extinction in the wild is crucial for species-specific conservation planning. Given the rapid decline of populations of globally threatened species in the wild, an international joint approach, known as the One Plan Approach (Byers et al., 2013), implements ex situ breeding for rehabilitation programs or translocation, as well as ex situ insurance populations, for future reintroductions in the wild (Campbell et al., 2015; Carbone et al., 2023; Chai et al., 2022). However, this approach requires unambiguous knowledge of species identification and the distribution of species present (or present in the past) at the release sites to ensure the species released are of the same taxon and avoid unnatural hybridization with a different species (Diehl & Stokhaug, 2013).

Gibbons (Hylobatidae) are a family of territorial, mostly pair-living apes, composed of four generally accepted genera: *Hoolock*, *Hylobates*, *Symphalangus*, *Nomascus* (Carbone et al., 2023). Gibbons of the genus *Nomascus*, containing seven species, are the most threatened of the four genera, with five species classified as Critically Endangered and two as Endangered on the IUCN Red List of Threatened Species (Fang et al., 2020; Geissmann & Bleisch, 2020; Nguyen et al., 2020; Rawson et al., 2020a, b, c; Thinh et al., 2020). This genus occurs in the Indo-Burma Biodiversity Hotspot and is mainly restricted to the east of the Mekong River in Cambodia, Lao People's Democratic Republic (Lao PDR), Vietnam, and southern China (Thinh et al., 2010a), although a small population of *N. concolor* is found on the west bank of Mekong River in China (Fan, 2017; Fan et al., 2020). Large rivers have often been considered as geographic and genetic barriers between species. For example, this led to the identification of new populations of the Skywalker hoolock gibbon (*Hoolock tianxing*) in Myanmar, significantly increasing its distribution range (Aung et al., 2024; Fan et al., 2017).

Gibbons are well known for emitting a loud and stable pattern of vocalization, which is innate (Brockelman & Schilling, 1984; Geissmann, 1984) but can be influenced by other factors (e.g., environmental conditions, population size; Han et al., 2024) and specific for species and sex (Haimoff, 1984). These vocal signals are a valuable tool to assess taxonomic relationships (Haimoff, 1984; Konrad & Geissmann, 2006; Thinh et al., 2010a, 2011). The genus *Nomascus* has the most marked sex-specific vocalization of all gibbons. Males and females produce different sound patterns which do not overlap (Geissmann, 2002). Although statistical analyses can discriminate between crested gibbon vocalizations, using songs to identify species has led to high levels of misclassification in previous studies (Thinh et al., 2010a, 2011). However, comparing gibbon vocalizations at different levels (notes and sequences) can improve differentiation (Han et al., 2024).

The recognition of *Nomascus siki* and *N. leucogenys* as two different species has been controversial (Thinh et al., 2010b). This split is the most recent species divergence in *Nomascus*, occurring 0.55 mya, and it is difficult to distinguish between the two species based on morphology (Mootnick & Fan, 2011) and vocalizations (Thinh et al., 2011). However, studies during the past two decades, mainly based on genetics and vocalizations, have led to a consensus that the two species are distinct (Carbone et al., 2023). The two species have a restricted global range (Thinh et al., 2010a). They are endemic to Lao PDR and Vietnam. *Nomascus leucogenys* has been declared ecologically extinct in China (Fan et al., 2013). In Vietnam, both species of white-cheeked gibbons have been extirpated at several sites owing to primate-targeted hunting and habitat loss, with the remaining populations declining at a dramatic rate (Blair et al., 2011; Rawson et al., 2011; Van Tran et al., 2023; Wearn et al., 2023). Gibbon populations in Lao PDR are much larger than in Vietnam, although populations in some more accessible forested sites have decreased compared with the early 1990s (Phiapalath et al., 2012; Ministry of Agriculture and Forestry, 2011; Duckworth, 2008; Rawson et al., 2011). Given their current conservation status, site-based conservation and prioritization to protect these species is crucial. However, there are gaps in our knowledge of the distribution of *N. leucogenys* and *N. siki* (Van Tran et al., 2023). The taxonomic status of gibbons in the central provinces of Lao PDR (Bolikhamxay and Khammouan provinces; Fig. 1) remains unconfirmed. The authors of a previous study hypothesized that the Nam Theun-Nam Kading River is the major geographical barrier between *N. leucogenys* and *N. siki* (Thinh et al., 2010a, 2011). The river runs through the center of Nakai-Nam Theun National Park—one of Lao PDR's most important protected areas—because of its biodiversity conservation value and level of endemism and which is home to a large population of white-cheeked gibbon (Coudrat, 2022). If the Nam Theun-Nam Kading River is the boundary between the two species, then both species should occur in the national park (Thinh et al., 2010a). However, that study did not include any acoustic or genetic samples from Nakai-Nam Theun National Park or Khammouan province and included only two vocal and genetic samples from Bolikhamxay province in Nam Kading National Protected Area (Thinh et al., 2011). Therefore, the distribution of *N. leucogenys* and *N. siki* requires confirmation across the landscape, particularly at sites along the Nam Theun-Nam Kading River.

To clarify the distribution of *N. leucogenys* and *N. siki* in central Lao PDR, we collected gibbon song recordings across two provinces: Bolikhamxay and Khammouan and particularly in Nakai-Nam Theun National Park. We also obtained gibbon song recordings from researchers from two locations in central Vietnam. To accurately identify species, we first compared the acoustic structures and parameters of calls from individuals that were genetically confirmed to be *N. leucogenys* and *N. siki* from zoological facilities in Europe. We then used these acoustic parameters as training data to classify the recordings of unidentified species from the wild in Lao PDR and Vietnam.

Methods

Data Collection

In situ *Gibbon Recording*

We collected 90 *Nomascus* gibbon songs from seven areas in the wild (Lao PDR and Vietnam; Fig. 1; Table I). Of these songs, we collected 60 songs from various

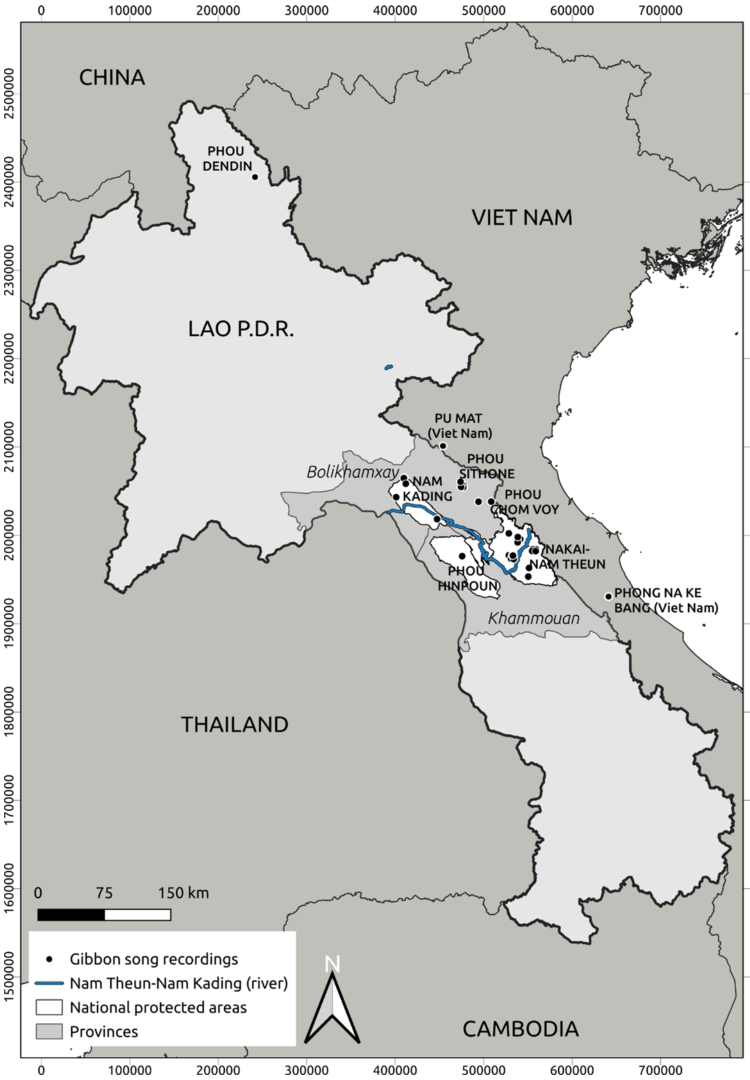

Fig. 1 Locations where we recorded 90 *Nomascus* gibbon songs in the wild at seven sites in Lao PDR and Vietnam between 2008 and 2021 (projected coordinate system UTM WGS84).

locations in Nakai-Nam Theun National Park, 595–1432 m above sea level (asl) (Fig. 2). We took all these acoustic recordings of spontaneous singing (duet and solo songs) at estimated distances of 20–1500 m from non-habituated animals in the early morning, between 5:05 a.m. and 7:50 a.m. We could ascertain which side of the river the gibbon group recorded was singing from because the surveyors were either > 2 km away from large rivers (gibbon songs can be heard by human ear up to 2 km; Srikosarmatara & Brockelman, 1983) or on the riverbank of a large river. We

Table I Summary of 90 *Nomascus* gibbon songs recorded in the wild in Lao PDR and Vietnam from 2008 to 2021 and used in a Random Forest species classification analysis

Site	Year	No. recordings	Time at start of call (mean ± SD, hh:mm)	Source
Nam Kading—Lao PDR	2008	6	06:36 ± 00:41	Wildlife Conservation Society (WCS)-Lao PDR (Hallam C., unpublished)
Phou Chom Voy—Lao PDR	2012	5	06:46 ± 00:27	WCS-Lao PDR
Phou Sithone—Lao PDR	2014	13	05:29 ± 00:14	WCS-Lao PDR
Phou Dendin—Lao PDR	2015	1	missing data	Association Anoulak
Nakai-Nam Theun—Lao PDR	2017–2021	60	06:27 ± 00:26	Association Anoulak
Phou Hinpoun—Lao PDR	2021	1	05:19	IUCN-Lao PDR
Pu Mat—Vietnam	2020	1	missing data	Nguyễn Đình Duy
Phong Nha Ke Bang—Vietnam	2020	3	missing data	Nguyễn Đình Duy

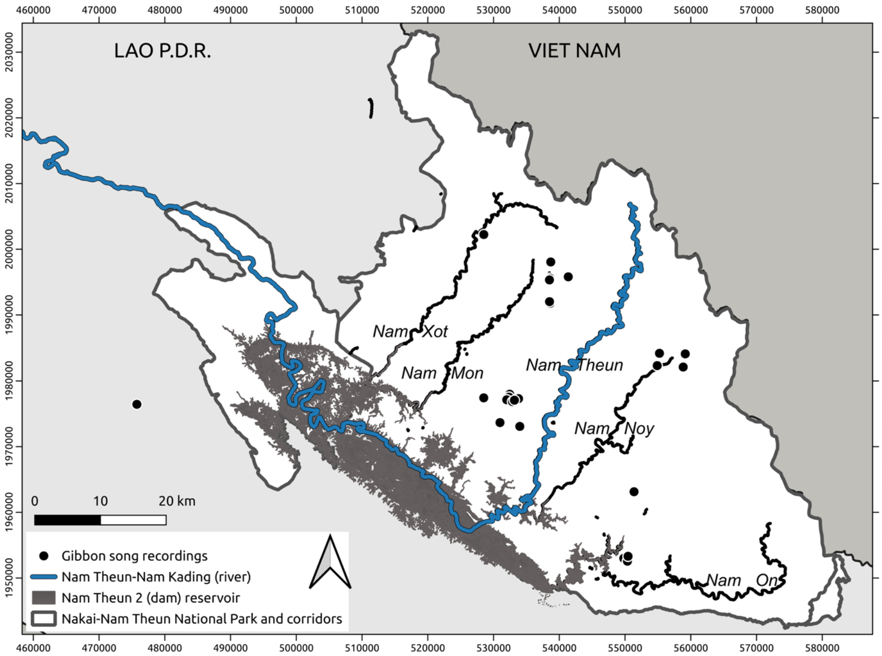

Fig. 2 Locations of 60 *Nomascus* gibbon songs we recorded in Nakai-Nam Theun National Park, Khammouan Province, Lao PDR between 2017 and 2021 (projected coordinate system UTM WGS84).

made recordings using a Fostex FR-2LE field recorder (16-bit resolution, 44.1-kHz sampling rate) with a shot-gun microphone (Rode NTG3). We obtained the other 30 gibbon song recordings from partner organizations and researchers working in sites in central Lao PDR and Vietnam (Fig. 1; Table I), who made these recordings using various types of a recording device (not specified by partners). To ensure consistency of temporal-spectral analysis resolution, we resampled all recordings to the same sampling rate and depth (44.1 kHz and 16-bit).

Ex situ *Gibbon Recording*

We obtained 32 gibbon songs from *Nomascus leucogenys* ($n = 24$) and *N. siki* ($n = 8$) from 12 European zoological facilities (Table II) where the species was based on karyotype (Species360 Zoological Information Management System (ZIMS), 2024). Partners from zoological facilities recorded vocalizations between 5:00 a.m. and 11:00 a.m. at a distance of 3 to 20 m. We resampled all recordings from captive individuals to the same sampling rate and depth as the wild recordings (44.1 kHz and 16-bit).

Table II Summary of 32 *Nomascus* gibbon song recordings obtained from European zoological parks between 2015 and 2023, identified to species (*N. leucogenys* and *N. siki*) based on karyotype, and used in a Random Forest species classification analysis as training data to classify gibbon vocalizations recorded in the wild

Name of institution	Country	Distance from recorder to animal (m)	Date	*Nomascus* species	No. individuals
Parc Zoologique et Botanique de Mulhouse	France	5	10-May-22	*N. leucogenys*	3
Tregomeur Zoo	France	4	12-Dec-21	*N. leucogenys*	2
Tregomeur Zoo	France	4	14-Dec-21	*N. leucogenys*	2
Tregomeur Zoo	France	4	19-Dec-21	*N. leucogenys*	2
Parc de Clères	France	10	21-dec-21	*N. leucogenys*	3
Parc de Clères	France	20	4-Jan-22	*N. leucogenys*	3
Parken Zoo	Sweden	5 to 15	21-Jan-22	*N. leucogenys*	2
Parken Zoo	Sweden	5 to 15	20-Jan-22	*N. leucogenys*	2
Lyon Zoo	France	5	7-Jun-22	*N. leucogenys*	2
Lyon Zoo	France	5	29-Jun-22	*N. leucogenys*	2
Lyon Zoo	France	5	29-Jun-22	*N. leucogenys*	2
Lyon Zoo	France	5	29-Jun-22	*N. leucogenys*	2
Lyon Zoo	France	5	29-Jun-22	*N. leucogenys*	2
Lyon Zoo	France	5	29-Jun-22	*N. leucogenys*	2
Lyon Zoo	France	5	29-Jun-22	*N. leucogenys*	2
Lyon Zoo	France	5	29-Jun-22	*N. leucogenys*	2
Lyon Zoo	France	5	29-Jun-22	*N. leucogenys*	2
Lyon Zoo	France	5	29-Jun-22	*N. leucogenys*	2
Lyon Zoo	France	5	4-Jul-22	*N. leucogenys*	2
Liberec Zoo	Czech Republic	4	8-Aug-17	*N. leucogenys*	4
Ústí n. Labem Zoo	Czech Republic	4	13-Aug-19	*N. leucogenys*	4
Plzeň Zoo	Czech Republic	6	5-Dec-23	*N. leucogenys*	2
Apenheul Zoo	Netherland	8	30-Aug-19	*N. leucogenys*	4
Warshaw Zoo	Poland	9	26-Aug-19	*N. leucogenys*	3

Table II (continued)

Name of institution	Country	Distance from recorder to animal (m)	Date	*Nomascus* species	No. individuals
Parc Zoologique et Botanique de Mulhouse	France	5	4-May-22	*N. siki*	2
Vallee des Singes	France	10	11-Mar-23	*N. siki*	2
Vallee des Singes	France	10	11-Mar-23	*N. siki*	2
Vallee des Singes	France	10	11-Mar-23	*N. siki*	2
Parc Zoologique et Botanique de Mulhouse	France	8	22-Jul-15	*N. siki*	4
Parc Zoologique et Botanique de Mulhouse	France	Missing data	Missing data	*N. siki*	4
Parc Zoologique et Botanique de Mulhouse	France	Missing data	Missing data	*N. siki*	4
Parc Zoologique et Botanique de Mulhouse	France	Missing data	Missing data	*N. siki*	4

Acoustic Analysis

We use the acoustic terminology (Online Resource Appendix S1) proposed by Konrad and Geissmann (2006), Ruppell (2010), and Thinh et al. (2011). *N. leucogenys* and *N. siki* gibbon songs have sex and species-specific vocal patterns (Ruppell, 2010; Thinh et al., 2011). Male calls are characterized by boom notes and staccato notes that precede a multi-modulation phrase (a series of highly frequency modulated). The boom note is usually emitted as a single, intense note of constant frequency and is produced when the throat sac inflates. The staccato notes are short, relatively monotonally repeated sounds. The multimodulation phrase is the most conspicuous acoustic structure and is divided into several notes, i.e., Note 1, Note 2, Note 3, and sometimes Last notes (Fig. 3). Last notes consist of one or several notes. The first note of the multimodulated phrase is of ascending frequency only; rapid changes in frequency modulation (a steep up-and-down sweep) occur on the second and sometimes on the third note (Haimoff, 1984; Ruppell, 2010; Schilling, 1984; Thinh et al., 2011). Females produce the great call only, which consists of oo notes, bark notes, and a twitter sound (Haimoff, 1984; Konrad & Geissmann, 2006; Ruppell, 2010; Thinh et al., 2011; Fig. 4).

First, we selected calls suitable for acoustic analysis via visual inspection of spectrograms. These were calls where we could clearly identify all parts of male and female calls (we could identify each note visually in the spectogram). In the 122 song recordings obtained in situ ($n = 90$) and ex situ ($n = 32$), we analyzed 229 female great calls and 737 multimodulation phrases of the male calls. We performed acoustic analysis by using Avisoft SASLab Pro version 5.2 software (Avisoft Bioacoustics, Berlin, Germany). We generated spectrograms using the following settings: FFT length = 1024; frequency resolution = 12 Hz; temporal resolution = 21.3 ms; overlap = 75%; window type = Hamming. We calculated 57 acoustic parameters

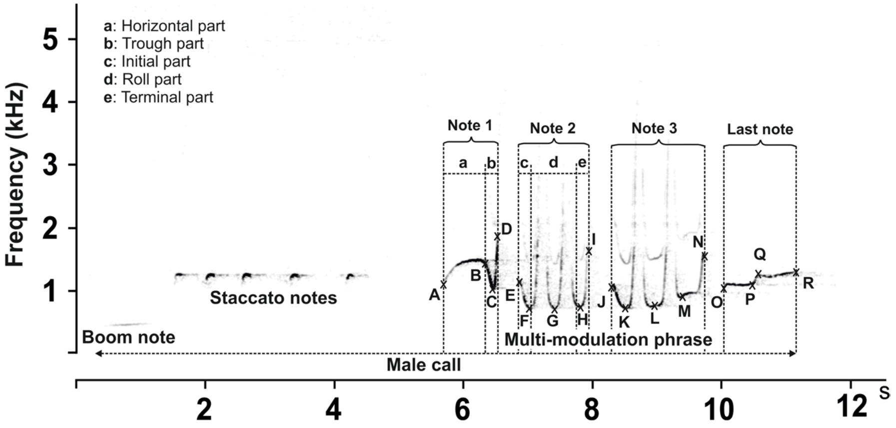

Fig. 3 Representative spectrogram, indicating parameters measured (A–R) for the multimodulation phrase in a *Nomascus* male call.

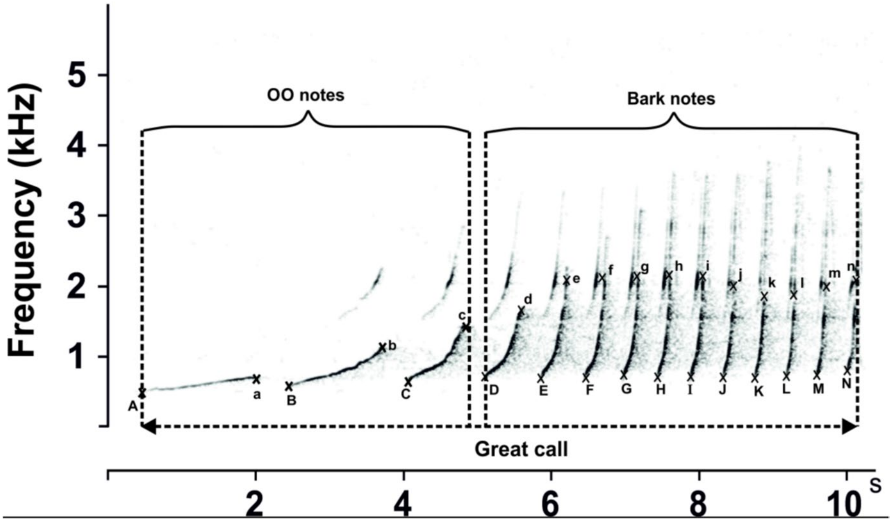

Fig. 4 Representative spectrogram indicating parameters we measured (A–N) for the *Nomascus* female great call.

describing the temporal and frequency structure of male and female gibbon call patterns (Online Resource Appendix S2).

Statistical Analysis

We performed all statistical analyses by using R software version 4.3.1 (R Core Team, 2024). To investigate which acoustic parameters differentiate between captive males of *N. leucogenys* and *N. siki*, and captive females of *N. leucogenys* and *N. siki*, we used a Mann–Whitney test to compare a total of 57 acoustic parameters (36 for males and 23 for females) between males of *N. leucogenys* and *N. siki* and between females of *N. leucogenys* and *N. siki*. We then removed autocorrelated (correlation value of more than 0.7) acoustic parameters from the analysis. We used the remaining acoustic parameters as training data to classify species in vocal recordings of unidentified individuals from the wild using Random Forest (RF) analysis (Breiman, 2001). We used the “Random Forest” package in R to classify recordings of unidentified wild individuals using the model with the most accurate species and sex classification. We configured the RF algorithm with 1000 trees and 5 nodes (Lehmann et al., 2022).

Data Availability Statement

All raw data analyzed during this study are included in this published article and its supplementary information files (Online Resources Appendix S8, Appendix S9).

Results

Vocal Differences Between N. Leucogenys and N. Siki in Captivity

We found significant species differences in acoustic parameters between captive *N. leucogenys* and *N. siki* of both sexes (Online Resource Appendix S3).

Difference in Vocal Structure Between Captive Male Nomascus Leucogenys and N. Siki

The structure of the male call was very similar in both species and increased in complexity during vocalizations, including a boom note, staccato notes, and a multimodulation phrase (Figs. 5a, c). The boom note of both species was produced at around 0.4 kHz. The boom note of *N. leucogenys* was longer and more pronounced than that of *N. siki*. Staccato notes in *N. leucogenys* were larger and have a clearly recognizable inverted triangle structure and are uttered in a monotonous series where one note closely follows another. Male *N. leucogenys* included staccato notes in 100% (114 of 114) of vocalizations. In contrast, male *N. siki*

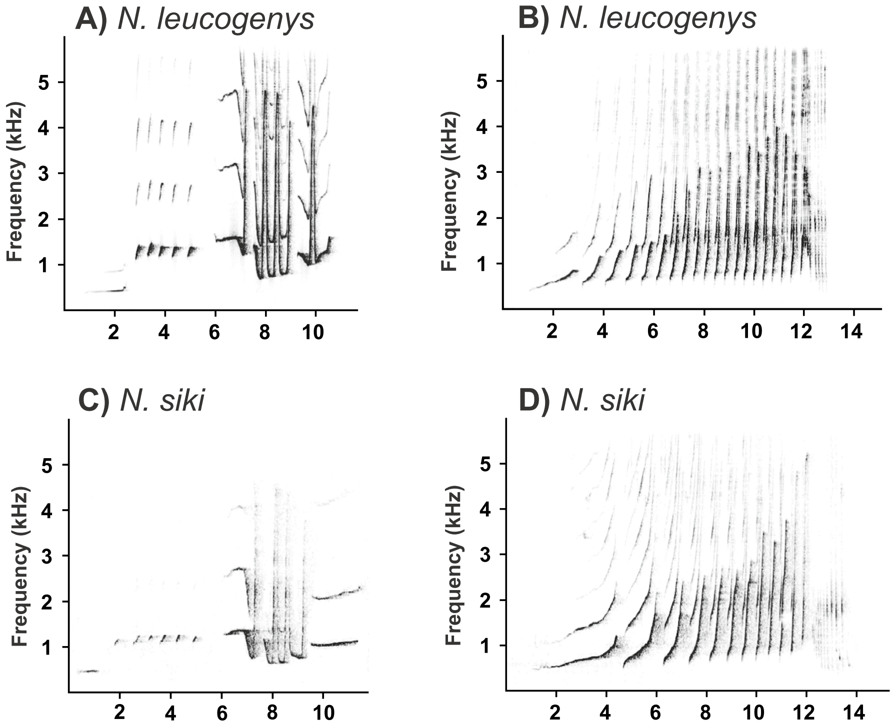

Fig. 5 Representative spectrograms showing a typical vocal pattern in genetically identified male (**a**, **c**) and female (**b**, **d**) northern white-cheeked gibbon (*N. leucogenys*) and southern white-cheeked gibbon (*N. siki*). Recorded in zoological facilities in Europe between 2015 and 2022.

included staccato notes in 75% (26 of 34) of vocalizations. The staccato notes of *N. siki* had either a less pronounced structure and were not the same shape as *N. leucogenys* with a greater distance between notes and a tendency to a monotonal series (42%, 15 of 26) or with a larger and clearly recognizable inverted triangle structure that was typical of the males in *N. leucogenys* (33%, 11 of 26). The mean number of staccato notes was numerically higher in male *N. leucogenys* (4.7 ± 3.7, range 2–14) than in male *N. siki* (3.2 ± 5.1, range 1–15).

For both species, the multi-modulation phrase consisted of three or sometimes four notes. In male *N. leucogenys* (81%, 92 of 114), four notes were used more often to form a multimodulation phrase than in male *N. siki* (34%, 12 of 34). The multimodulation phrase in male *N. leucogenys* started at a lower frequency but reached a higher frequency than that in male *N. siki* (Table III). The duration of the multi-modulation phrase was similar in both species, but the duration of the individual notes varied (Table III). In male *N. leucogenys*, the first (horizontal) part of the first note had a long section with a relatively stable shape. In contrast, in male *N. siki* the first (horizontal) part of the first note had a shorter section with a slightly different shape (Table III). In both species, there was a rapid down-up sweep of frequency in the second part (trough part) of the first note, which was deeper in males *N. siki* than in males *N. leucogenys* (Figs. 5 a, c). In male *N. leucogenys*, there were repeated rapid changes in frequency modulations (steep up-and-down sweep) on the second note involving two frequency modulations and the third note involving one frequency modulation, whereas in male *N. siki*, frequency modulation was limited to the second note and involved two frequency modulations. The rapid frequency modulation changes in male *N. leucogenys* were stereotyped and balanced in shape. In male *N. siki*, the final frequency

Table III Acoustic characteristics of the male vocal pattern in captive *Nomascus leucogenys* and *N. siki*, recorded in European zoos recorded between 2015 and 2023

Acoustic parameter	Northern white-cheeked gibbon (*Nomascus leucogenys*) *N* = 114 Mean ± SD	Southern white-cheeked gibbon (*Nomascus siki*) *N* = 34 Mean ± SD
Total duration of multi-modulation phrase (s)	4.78 ± 0.49	4.89 ± 0.77
Duration of first note (s)	1.35 ± 0.24	1.23 ± 0.16
Duration of horizontal part (s)	1.03 ± 0.25	0.91 ± 1.47
Duration of trough part (s)	0.33 ± 0.11	0.35 ± 0.11
Duration of second note (s)	1.39 ± 0.29	1.42 ± 0.19
Duration of initial part (s)	0.23 ± 0.08	0.23 ± 0.09
Duration of roll part (s)	0.97 ± 0.20	1.05 ± 0.17
Duration of terminal part (s)	0.19 ± 0.18	0.14 ± 0.09
Duration of third note	1.03 ± 0.49	1.63 ± 0.78
Lowest frequency (kHz)	1262.30 ± 141.48	1286.70 ± 139.37
Highest frequency (kHz)	4368.67 ± 660.52	4196.74 ± 461.48
No. staccato notes	4.7 ± 3.7 (range 2–14)	3.2 ± 5.1 (range 1–15)

modulation showed a different pattern to the preceding frequency modulations (Figs. 5 a, c).

Difference in Vocal Structure Between Captive Female Nomascus Leucogenys and N. Siki

The structure of the female great call was similar in the two species (Figs. 5b, d). The notes of the great call follow the previous notes closely. After the climax of the acceleration, the last notes fade into twitter. The interval between the notes was shorter in female *N. leucogenys* than in female *N. siki*. The total duration of the great call was numerically slightly shorter in female *N. leucogenys* than in female *N. siki* (Table IV). The total number of notes in the great calls was slightly higher in female *N. siki* than in female *N. leucogenys* (Table 4). There was no difference in the number of oo notes between species, but female *N. leucogenys* had fewer bark notes than females of *N. siki* (Table IV). The first note was longer than the following notes, which became progressively shorter (Figs. 5b, d). Female *N. leucogenys* had a lower frequency at the beginning of the great call than female *N. siki* (Table IV). The frequency of notes increased until it reached the highest frequency towards the end of a great call, and then the twitter sounds were produced (Figs. 5b, d).

Species Classification of Wild Gibbons

We selected 148 male calls and 92 female calls from the captive gibbons to cross-validate the RF classification, following the 80–20 rule (Lehmann et al., 2022). We included 12 of the 36 acoustic parameters in males (Online Resource Appendix S4) and 10 of the 23 acoustic parameters in females (Online Resource Appendix S5) in the RF analysis. We could classify species using RF based on these acoustic

Table IV Acoustic characteristics of the female great call in captive *Nomascus leucogenys* and *N. siki* in European zoos recorded between 2015 and 2023

	Northern white-cheeked gibbon (*Nomascus leucogenys*) *N* = 68	Southern white-cheeked gibbon (*Nomascus siki*) *N* = 24
	Mean ± SD	Mean ± SD
Total duration of great call (s)	11.05 ± 1.17	11.93 ± 5.31
Duration of first note (s)	1.94 ± 0.55	2.07 ± 0.88
Lowest frequency (kHz)	436.62 ± 50.45	482.92 ± 64.77
Highest frequency (kHz)	2635.82 ± 751.93	2361.25 ± 634.71
Total notes	14.38 ± 2.29	14.88 ± 3.92
No. oo notes	5.65 ± 2.06	5.67 ± 3.53
No. bark notes	8.51 ± 2.68	9.21 ± 2.34

parameters with an accuracy of 97% for males (Online Resource Appendix S6) and 89% for females (Online Resource Appendix S7).

We found both male and *N. leucogenys* and *N. siki* on both sides (north and south) of the Nam Theun-Nam Kading River (Fig. 6a). In males, *N. siki* was more prevalent south than north of the river (Fig. 6a). In females, *N. leucogenys* were more prevalent north of the river, and *N. siki* was slightly more prevalent in the south (Fig. 6b).

Discussion

We were able to describe the difference in vocal structure between captive *N. leucogenys* and *N. siki* in both sexes, which allowed us to classify the vocalizations recorded in the wild of unknown species with an accuracy of 97% for males and 89% for females. We detected both *Nomascus siki* and *N. leucogenys* on both sides of the Nam Theun-Nam Kading River in Nakai-Nam Theun National Park and surrounding areas in Central Lao PDR and Vietnam. These results do not support the hypothesis that the Nam Theun-Nam Kading River is a geographical barrier between the two species in central Lao PDR, which was based on a few samples

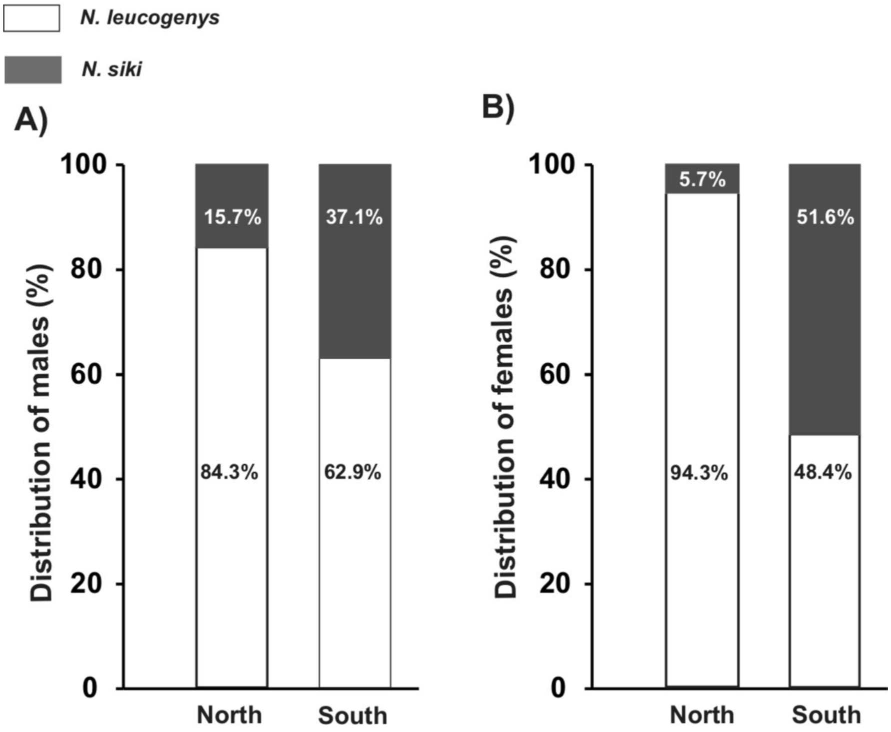

Fig. 6 Results of Random Forest analysis showing the classification of male (**A**) and female (**B**) calls of *Nomascus siki* and *N. leucogenys* to the north and south of the Nam Theun-Nam Kading River in central Lao PDR and Vietnam recorded between 2008 and 2021.

from central Lao PDR, and none from Nakai-Nam Theun National Park (Thinh et al., 2010a, 2011). The latter study was based on genetic and vocalization samples from the wild, where four crested gibbon species could be discriminated statistically but with a test accuracy of only 55.6% leading to a high rate of misclassifications to species of the vocalizations of unknown species from the wild (Thinh et al., 2011). In contrast, our classification using vocalizations from genetically identified gibbons in captivity had high accuracy.

Habitat types and climate characteristics are the main determinants of species distribution in Lao PDR. The Nam Theun-Nam Kading River is not a biogeographic barrier to other species of primates or other large arboreal mammal species (Duckworth et al., 1999). Our findings of both *N. siki* and *N. leucogenys* on both sides of the river match these patterns. Furthermore, the upstream section of the river in Nakai-Nam Theun National Park is narrow enough for gibbons to cross and disperse on either side (C. Coudrat, personal communication). This situation could have resulted in a transition zone between the two species where they occur in sympatry or hybridize. Hybridization between *Nomascus siki* and *N. leucogenys* has been recorded several times ex situ (Nie et al., 2018), suggesting that it may occur in the wild. Gibbon demographic history has included gene flow between species and natural hybridization continues to occur episodically between gibbon species (Arnold & Meyer, 2006; Kim et al., 2011). Hybridization occurs naturally in narrow zones of species overlap in other gibbon species (Arnold & Meyer, 2006), for example, in *Hylobates lar* and *H. pileatus* in Thailand, where a contiguous population led to introgressive hybridization (Asensio et al., 2017; Geissmann, 1984; Matsudaira et al., 2022). These observations highlight the potential for natural hybridization between *N. siki* and *N. leucogenys* in Nakai-Nam Theun National Park, underscoring the need for further research to assess its frequency, genetic implications, and conservation significance.

Although the percentage of classification for females was high (89%), the small sample size—particularly for captive *Nomascus siki*—leaves room for uncertainty in our classification results for females. The finer differences in the acoustic structure of potential wild hybrid females could theoretically be attributed to either *Nomascus leucogenys* or *Nomascus siki*. This is supported by the fact that the vocal patterns of females from both species are very similar. In addition, the acoustic signature of the two species may be more similar at closer geographic distances, preventing the assignation to the correct taxa. This was suggested in the previous study (Thinh et al., 2011) by misclassifications between neighboring groups of *N. siki* and *N. leucogenys*, but not for groups of both species occurring at a larger geographic distance. A recent study compared the vocalization patterns (note, sequence, and great call sequence) of three *Nomascus* species in three different regions of China (*N. nasutus* from Gangxi Province, *N. concolor* from Yunnan Province, *N. hainanus* from Hainan Island) and found significant differences in note, sequence and great call sequence but not in other vocalization parameters (boom and premodulated notes), suggesting that factors (e.g., environmental conditions, population size) other than genetic inheritance (Brockelman & Schilling, 1984; Geissmann, 1984) are involved in species differences or similarities in vocal structures (Han et al., 2024). This suggests that closely related allopatric species occurring in similar ecological systems, such as our two study species, may show more similar vocalizations than gibbon species associated with different habitats and climates. Further research integrating both genetic and ecological factors is needed to determine the extent to which geographic proximity influences vocal similarity in our two study species.

Implications for Gibbon Conservation

Our study found no clear geographic barrier between *N. siki* and *N. leucogenys* in central Lao PDR, which must be taken into consideration as part of potential reintroduction, rehabilitation, or translocation programs for both species. Accurate gibbon species identification is crucial in such programs to ensure the selection of appropriate release sites within the species' historical range, thereby preventing ecological mismatches and potential negative impacts on local ecosystems. The IUCN's Best Practice Guidelines for the Rehabilitation and Translocation of Gibbons (Campbell et al., 2015) emphasize that misidentification can lead to unsuccessful reintroductions and unintended ecological consequences. Human disturbance has also played a role in the current distribution of species owing to human-mediated dispersal of species (Capinha et al., 2015). Therefore, thorough taxonomic assessments are essential before any translocation or reintroduction efforts.

Regardless of species identification in central Lao PDR, including sites where there may be stable natural hybrid systems (Allendorf et al., 2001; Bohling, 2016), in situ conservation interventions at all sites holding gibbon populations are critical for the long-term survival of both species, for which any population should be considered of high conservation value. The development of diagnostic tools to identify the species of gibbon individuals and to establish whether they are hybrids will contribute to species management and conservation plans (Baicharoen et al., 2014; Carbone et al., 2009). Our findings clarify the distribution of *N. siki* and *N. leucogenys* in central Lao PDR, while emphasizing the need for further research on the vocal, genetic, and ecological interactions between *N. siki* and *N. leucogenys* in central Lao PDR, and highlighting the importance of accurate species identification for effective conservation and management strategies.

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Declarations

Conflict of Interest The authors declare that they have no conflict of interest.

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