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RESEARCH ARTICLE

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Description of a new species of *Hoolock* gibbon (Primates: Hylobatidae) based on integrative taxonomy

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We describe a species of *Hoolock* gibbon (Primates: Hylobatidae) that is new to science from eastern Myanmar and southwestern China. The genus of hoolock gibbons comprises two previously described living species, the western (*Hoolock hoolock*) and eastern hoolock (*H. leuconedys*) gibbons, geographically isolated by the Chindwin River. We assessed the morphological and genetic characteristics of wild animals and museum specimens, and conducted multi-disciplinary analyses using mitochondrial genomic sequences, external morphology, and craniodental characters to evaluate the taxonomic status of the hoolock population in China. The results suggest that hoolocks distributed to the east of the Irrawaddy-Nmai Hka Rivers, which were previously assigned to *H. leuconedys*, are morphologically and genetically distinct from those to the west of the river, and should be recognized as a new species, the Gaoligong hoolock gibbon or skywalker hoolock gibbon (*H. tianxing* sp. nov.). We consider that the new species should be categorized as Endangered under IUCN criteria. The discovery of the new species focuses attention on the need for improved conservation of small apes, many of which are in danger of extinction in southern China and Southeast Asia.

KEYWORDS
endangered species, gibbon, *Hoolock tianxing*, Mt. Gaoligong, new species

Peng-Fei Fan, Kai He, and Xing Chen contributed equally to this work.

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1 | INTRODUCTION

Gibbons and siamangs (Hylobatidae) are small apes inhabiting southern, eastern, and Southeast Asia. Currently, four genera (*Hoolock*, *Hylobates*, *Nomascus*, and *Symphalangus*) and up to 19 living species are recognized (Mittermeier, Rylands, & Wilson, 2013). Hoolock gibbons or hoolocks occur in the northwestern part of modern-day gibbon distribution in mainland Asia, with populations in India, Bangladesh, Myanmar, and China (Figure 1). They differ from other gibbon genera in a series of morphological (Mittermeier et al., 2013), acoustic (Geissmann, 2002), and chromosomal (Müller, Hollatz, & Wienberg, 2003) characteristics. The most evident morphological characteristic of hoolocks is their conspicuous white brow (Choudhury, 2013; Mittermeier et al., 2013), which is the source of their other common name, the white-browed gibbons.

Hoolocks were first described scientifically by Harlan (1834) under the name *Simia hoolock*. They were subsequently transferred to *Hylobates*, and then assigned to their own distinct subgenus (later elevated to genus), first *Bunopithecus* (later restricted to an extinct Quaternary gibbon from China; Groves, 2001; Prouty, Buchanan, Pollitzer, & Mootnick, 1983) and then *Hoolock* (Mootnick & Groves, 2005). Taxonomic variation between hoolock populations was first recognized by Groves (1967), who identified a major east-west morphological division and established the subspecies *Hylobates hoolock leuconedys* to distinguish eastern hoolock populations from their western counterparts. These two groups were interpreted as

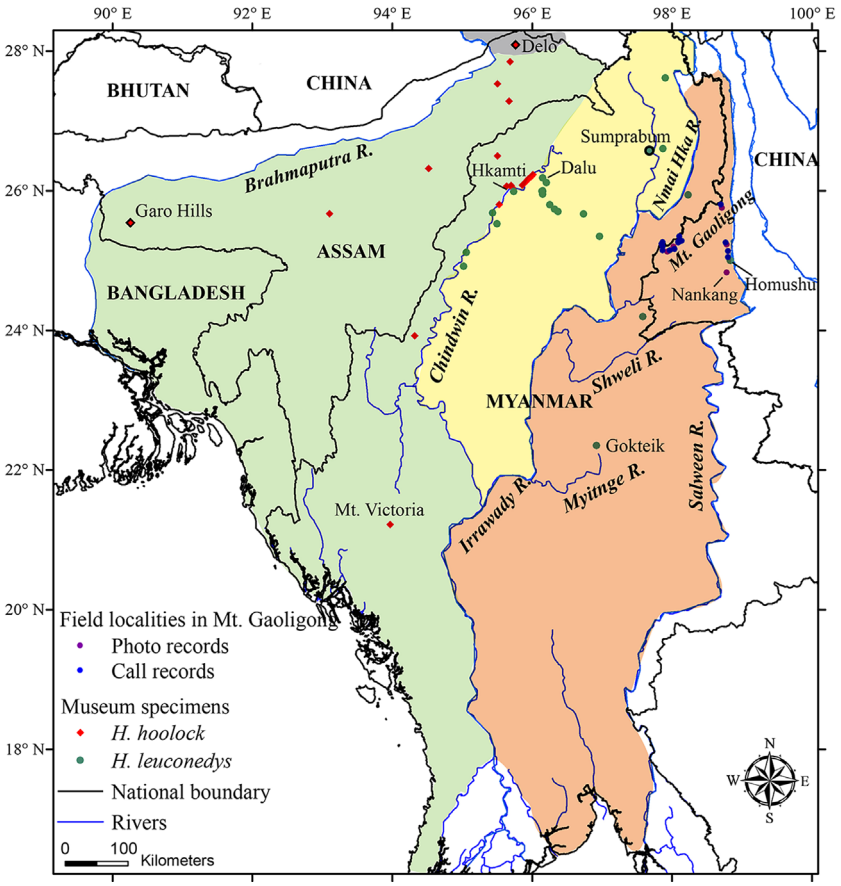

FIGURE 1 Field localities for eastern hoolocks, and collection localities for museum specimens of eastern and western hoolocks. The distribution and type localities of *H. hoolock hoolock* (red, Garo Hills), *H. h. mishmiensis* (gray, Delo), *H. leuconedys* (yellow, Sumprabum), and *H. tianxing* (blue, Homushu) are shown

subspecies for 40 years (Brandon-Jones et al., 2004; Groves, 1967), but are now recognized as distinct species, the western hoolock (*Hoolock hoolock*) and the eastern hoolock (*H. leuconedys*) (Geissmann, 2007; Groves, 2001; Mittermeier et al., 2013; Thinh, Mootnick, Geissmann et al., 2010). More recently, new hoolock populations found between the Lohit and Dibang Rivers in Assam and Arunachal Pradesh (Chetry & Chetry, 2010; Chetry, Chetry, Das, Loma, & Panor, 2008; Chetry, Chetry, Ghosh, & Singh, 2010; Das, Biswas, Bhattacharjee, & Mohnot, 2006) have been described as a new subspecies of western hoolock, the Mishmi Hills hoolock (*H. hoolock mishmiensis*) (Choudhury, 2013).

These different groups of *Hoolock* can be distinguished on the basis of external morphological characteristics (Figure 2); notably, the shape of the eyebrows and the color of the eye rings, beard, and preputial tuft (Choudhury, 2013; Groves, 1967, 1972). In *H. hoolock*, adult males are jet black with a black or faintly grizzled preputial tuft, closely spaced white brow streaks connected by white hair, and white hair on the chin or below the eyes, while females are buffy colored, the color of hands and feet being the same as the body (Groves, 1967, 1972, 2001). In *H. leuconedys*, adult males have a black coat with a white preputial tuft and well-separated brow streaks, and adult females have again a buffy pelage but with distinctly lighter brown hands and feet (Das et al., 2006; Groves, 1972). Adult males of *H. h. mishmiensis* differ from those of *H. h. hoolock* in having thick, closely connected eyebrows, a prominent black or grayish beard tuft, and a buffy or rufescent buff genital tuft. Choudhury (2013) also noted some slight differences in the white face ring of *H. h. mishmiensis* females, with the brows of this subspecies being transversely oriented above the orbital ridge, whereas they are slightly concave in *H. h. hoolock* and sharply downcurved in *H. leuconedys*. This series of external characters was used to identify *H. h. mishmiensis* as a new subspecies, and may therefore be helpful for assessing the taxonomic status of other little-studied hoolock populations.

Large rivers are considered to represent barriers for gibbon dispersal (Groves, 1967; Thinh, Rawson et al., 2010), not only because these primates do not swim and are largely restricted to the forest canopy, but also because forested environments in river valleys are generally unfavorable for gibbons (Groves, 1967; Thinh, Rawson et al., 2010). The Chindwin River in Myanmar was identified as the distributional boundary between *H. hoolock* and *H. leuconedys* (Groves, 1967), and the Lohit River may also act as a boundary between *H. h. hoolock* and *H. h. mishmiensis* (Choudhury, 2013). Large rivers may have played therefore an important role in the diversification and speciation of hoolocks and other hylobatids (Groves, 1967; Thinh, Rawson et al., 2010), and the taxonomic status of other hoolock populations that are isolated by large rivers needs to be critically assessed.

Mt. Gaoligong (or Gaoligongshan) is located between the Salween River (Nujiang in Chinese, Mae Nam Salawin in Thai, and Thanlwin in Burmese) and the Nmai Hka tributary of the Irrawaddy River in western Yunnan Province, China, and eastern Myanmar (Chaplin, 2005), and represents the easternmost end of the distribution of hoolock populations (Figure 1). Hoolocks were first recorded from Mt. Gaoligong by the American Museum of Natural History's Asiatic Zoological Expedition in 1917 (Allen, 1930; Anonymous, 1917) (Supplemental Figure S1), and gibbon specimens collected by the expedition from this region are now in the American Museum of Natural History (New York) and the Museum of Comparative Zoology (Harvard University, Cambridge, MA). More recently, hoolocks on Mt. Gaoligong have been the focus of extensive field research by PFF and his team (Fan, 2016; Fan et al., 2011, Fan, Ai, Fei, Zhang, & Yuan, 2013). These gibbons were originally identified as *H. leuconedys* (Groves, 1967, 2001). However, during long-term observations of wild individuals, PFF noticed that their external morphological characteristics differ from the typical morphology of *H. leuconedys*, which had been originally described by Groves (1967) on the basis of individuals located east of the Chindwin River.

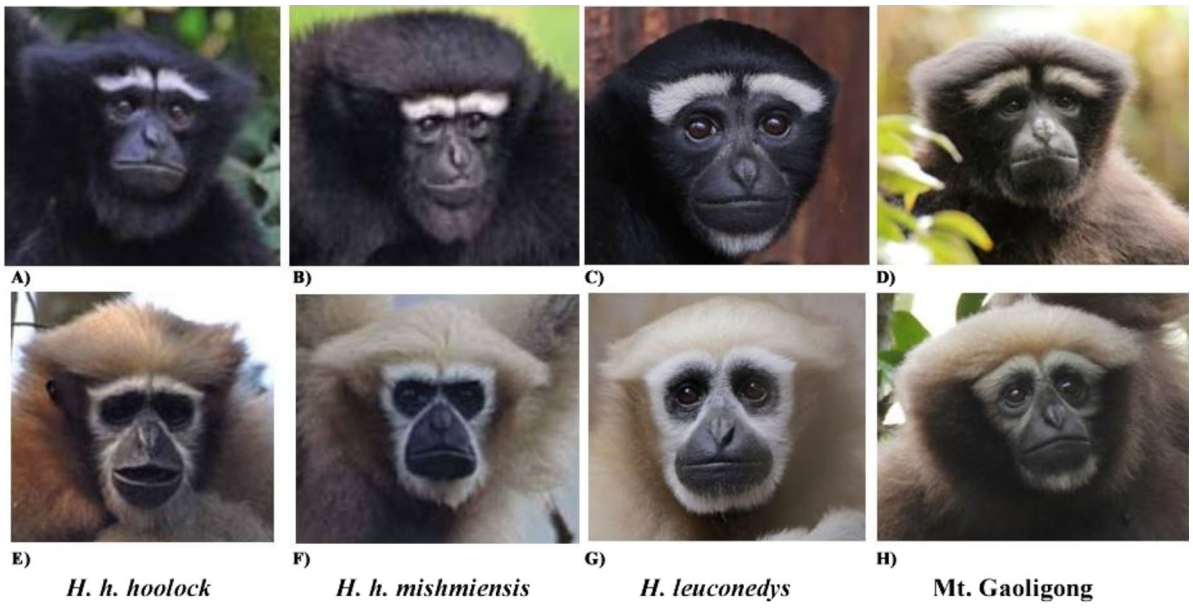

FIGURE 2 Photos of male (top row) and female (bottom row) hoolocks from different taxa and geographic populations. Photos of *H. h. hoolock* and *H. h. mishmiensis* are from Choudhury (2013)

Based on the observed morphology of hoolock individuals from Mt. Gaoligong, and the allopatric separation of this population from other hoolock populations by a large river system, we hypothesized that the population may represent a distinct, undescribed hoolock taxon. To test this hypothesis, we collected photographs and feces from both wild and captive individuals, examined museum specimens, and conducted multidisciplinary analyses using both DNA and morphological/morphometric data. Based on the results of this integrative study, we conclude that the Mt. Gaoligong population represents a new species of hoolock gibbon.

2 | METHODS

2.1 | Live animals

Intensive wild hoolock population surveys were conducted in Mt. Gaoligong, Yunnan, in 2008 and 2009 (Fan et al., 2011), with photographs taken of individuals in two family groups at Lamahe and Xiangbai (Supplemental Table S1). Further long-term ecological studies were then conducted on hoolocks at Datang (three groups, 10 months; Zhang, Yuan, Cui, & Fan, 2011), Nankang (one group and one solitary female, 15 months; Fan et al., 2013), Banchang (three groups, 46 months; Yin et al., 2016), and Xiangbai (two groups, 1 month; Chang-Yong Ma, unpublished data). One group (NA) and the solitary female (NB) at Nankang and one group (BB) at Banchang were habituated to researchers during these ecological studies, and photographs were taken of 14 habituated and unhabituated individuals. We also visited Dehong Wildlife Rescue Center (Mangshi County, Yunnan, May 2015), Yunnan Safari Park (Kunming, Yunnan, June 2013 and October 2014), Taibao Park (Baoshan, Yunnan, May 2015), three zoos (Kunming, October 2015; Zhengzhou, August 2015; Beijing, September 2015), and Pianma Ranger Station of Gaoligongshan National Nature Reserve (Nujiang, Yunnan, October 2015), which contained captive hoolock individuals, as well as a hoolock kept as a pet in Dulujiang, Nujiang County, Yunnan; we obtained photographs of 22 captive individuals (Supplemental Table S1).

2.2 | Morphological and morphometric analyses

We examined 122 hoolock specimens curated at: the American Museum of Natural History (AMNH), New York (n = 86); the Academy of Natural Sciences of Philadelphia (ANSP), Philadelphia (n = 1); the National Museum of Natural History (USNM), Smithsonian Institution, Washington, D.C. (n = 3); the Museum of Comparative Zoology (MCZ), Harvard University, Cambridge, Massachusetts (n = 2); the Natural History Museum (NHM), London (n = 21); the National Zoological Museum of China, Institute of Zoology (IOZ), Chinese Academy of Sciences, Beijing (n = 3); and the Kunming Natural History Museum of Zoology (KNHMZ), Kunming Institute of Zoology (KIZ), Kunming (n = 6), representing historical specimens collected from Mt. Gaoligong as well as specimens attributed to both *H. hoolock* and *H. leuconedys* (Supplemental Table S2). Specimens were identified following Groves (1967).

Twenty-three craniomandibular variables were measured from 77 adult hoolock individuals in AMNH, ANSP, MCZ, NHM, and USNM by CG (Supplemental Table S3). The provenance and measurements of all specimens are given in Supplemental Table S2. Morphometric variation was analyzed using principal component analyses (PCA) and discriminant function analyses (DFA) in SPSS v17.0 (SPSS, Inc., Chicago, IL), conducted on log10-transformed variables. For the DFAs, specimens of *H. hoolock* were assigned to their own group, and specimens of *H. leuconedys* from the west and the east of the Irrawaddy-Nmai Hka River were assigned to two groups based on their provenance and morphological and molecular differentiation (see below).

Taxonomic differences of the hoolock dentition were investigated using discrete morphological traits and geometric morphometrics (GM). Cusp nomenclature follows Swindler (2002). Special attention was paid to the lower P4 and the upper and lower molars, given their usefulness in hominoid alpha taxonomy (Bailey & Lynch, 2005; Frisch, 1965; Kitahara-Frisch, 1973; Martinon-Torres et al., 2006; Ortiz et al., 2015; Swindler, 2002; Uchida, 1996). Although we conducted GM analyses for all upper and lower molar types, we only present here the results of the M2s due to their reduced degree of dental wear (vs. M1s) and relatively large samples in museum collections. All photographs for GM analysis were taken by AO following Ortiz et al. (2015). We used tpsDig2 v2.22 (Rohlf, 2015) to place 10 and 11 semi-landmarks representing the outline of each upper and lower molar, respectively. Shape information was extracted using a Procrustes superimposition, as implemented in MorphoJ v1.06d (Klingenberg, 2011). Multivariate statistics (PCA and DFA) were implemented in MorphoJ v1.06d and SPSS v17.0.

2.3 | Molecular analyses

We obtained three recent hoolock soft tissue samples (one piece of muscle and two small pieces of pedal skin) from three different individuals housed at KIZ, as well as 25 fecal samples obtained from Chinese captive individuals, confiscated wild individuals, and wild individuals from two field localities in Mt. Gaoligong (Supplemental Table S1). These specimens represented individuals identified as *H. leuconedys* sensu stricto, and the Mt. Gaoligong hoolock population based on locality data and morphological characteristics. We also obtained one sample of *H. hoolock* from Dhaka Zoo, Bangladesh. Museum specimens were treated in a series of 24-hr washes in 75%, 50%, and 25% ethanol, followed by successive 24-hr immersions in ddH_2O. The fecal samples were collected using a "two-step" storage procedure following Nsubuga et al. (2004), and were preserved at −20°C after arrival in the laboratory. DNA was extracted from recent and historical tissue samples using a DNeasy blood and tissue kit (QIAGEN, Hilden, Germany). Fecal DNA was extracted using a QIAamp® stool mini kit (QIAGEN).

We first amplified and sequenced two mitochondrial gene regions, the cytochrome *b* (*CYT B*) and D-loop regions (Supplemental Table S4), for all 27 samples (Supplemental Table S1). Based on the

genetic diversity in our samples and preliminary phylogenetic analysis, we selected a subset of samples for whole mitochondrial genome (hereafter mitogenome) sequencing using either Sanger or next-generation sequencing (NGS). The mitogenomes of one *H. hoolock* (HHO) and one *H. leuconedys* (BSJO) were amplified using 19 pairs of primers (Supplemental Table S4) via individual PCRs. These PCR amplicons were 0.9–1.4 kb in length and overlapped in 100–500 bp. PCR amplicons were gel-purified and were sequenced on an ABI3130*xl* sequencer using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). For the remaining samples, amplicon sequencing (O'Neill et al., 2013) and hybridization-capturing (Horn, 2012) were employed, accompanied by the NGS technique. We first amplified the complete mitogenome of the modern muscle tissue sample using long-range PCR (Chan et al., 2010) using two pairs of primers (Supplemental Table S4). PCR products were used for NGS and for generating probes for capture-hybridization, following Horn (2012). For amplicon sequencing, PCR products were sheared using an IonShear kit (ThermoFisher Scientific, Waltham, MA), and ligated with adapters using Ion Plus Fragment Library and Ion Xpress Barcode Adapter kits (ThermoFisher Scientific). PCR products were also used to generate homemade probes using a BioNick Labeling Kit (Invitrogen, Carlsbad, CA). Stool DNA libraries were analyzed using the same Ion Xpress kits. Capture-hybridization was performed for each individual stool DNA library using the homemade probes and an Oligo aCGH Hybridization Kit (Agilent Technologies, Santa Clara, CA), following Horn (2012). Hybridization was performed in a Mastercycler nexus thermocycler (Eppendorf) at 65°C for 72 h. Because our samples were all modern or relatively recently collected, we did not conduct a second run of enrichment, as recommended for ancient DNA samples by Horn (2012). After quantification using a Qubit Fluorometric Quantitation (ThermoFisher Scientific), all libraries were pulled and sequenced using a 316 v2 chip with the Ion Torrent™ Personal Genome Machine® (PGM) system (ThermoFisher Scientific).

The Sanger sequencing results were assembled and edited using Lasergene v7.1 and aligned using MUSCLE. Available *CYT B* sequences of gibbons in GenBank were downloaded and included in our analyses (Supplemental Table S1). PGM results were initially analyzed and converted into FASTQ format using the Torrent Suite v4.0.2. Reads shorter than 60 bp were filtered and adapter sequences were trimmed. We de novo assembled the modern muscle tissue sample and mapped the reads to a hoolock mitogenome (NC_023377, withdrawn by the authors from GenBank; Atsushi Matsui personal communication) using Geneious v8.1. Reads generated for each fecal sample were mapped to NC_023377 and the assembly muscle tissue mitogenome. To control for contamination, we compared the *CYT B* and *D-loop* sequences generated by NGS and Sanger sequencing from the same samples; we considered mismatches of no more than 1 bp as indicating no contamination. Mitogenomes were aligned using MUSCLE and annotated using Geneious v8.1. We checked premature stop codons in each coding gene to avoid potential inclusion of pseudogenes. We also downloaded 20 mitogenomes representing 10 gibbon species, two gorilla species, two chimpanzee species, two orangutan species, three macaque species, and humans from GenBank for inclusion in analyses (Supplemental Table S1).

2.4 | Phylogenetic analyses

Phylogenetic analyses were performed using RAxML for maximum likelihood tree estimation, and BEAST v1.8 for Bayesian tree and divergence time estimation. We first analyzed a dataset comprising 31 complete or nearly complete mitogenomes, including 11 newly derived and 20 from GenBank. All tRNAs, the ND6 gene and the D-loop region were removed from the dataset alignment. The remaining 13,375 bp alignment, including all other coding genes and two rRNA genes, was subdivided into 38 data blocks based on gene and codon positions. Best-fit partitioning schemes and evolutionary models were determined simultaneously using PartitionFinder v1.0 (Lanfear, Calcott, Ho, & Guindon, 2012) under the Bayesian Information Criterion (BIC).

Although gibbons are present in the fossil record, it has been extremely difficult to determine the relationships of these extinct taxa with living species and whether they represent crown-group or stem-group gibbons (Benton et al., 2015). We therefore selected fossil primates with well-accepted phylogenetic relationships to calibrate the tree. To calibrate the root of the tree (crown Catarrhini), we used *Rukwapithecus fleaglei* from the Miocene Nsungwe Formation of Tanzania, which has been identified as the oldest stem hominoid and crown catarrhine yet known (Stevens et al., 2013). The minimum date for this fossil is 24.44 million years ago (Ma) based on the age of the Nsungwe Formation (Roberts et al., 2010). Using a uniform distribution for this calibration, we set the lower boundary to 24.44 Ma. We set the upper boundary to 34 Ma at the Eocene-Oligocene transition (Seiffert, 2006) following Benton et al. (2015). To calibrate the crown Hominoidea, we used *Sivapithecus indicus* from the Miocene Chinji Formation of Pakistan (Kappelman et al., 1991), which has been identified as a member of the Ponginae (Seiffert, 2006). The youngest estimated date for this fossil is 11.6 Ma based on the age of the Chinji Formation (Kappelman et al., 1991). Using an exponential distribution for this calibration, we set the lower boundary to 11.6 Ma with a mean value of 7.45, to allow the upper soft boundary to extend to the Eocene-Oligocene transition. The second calibration used the most recent common ancestor (MRCA) of humans and chimpanzees. *Sahelanthropus tchadensis* from Toros Menalla, northern Chad (Brunet et al., 2002) is currently interpreted as likely to represent the oldest crown-group hominin post-dating the human-chimpanzee split (Strait, 2013). The youngest date for this fossil is 6.5 Ma according to the relative chronology of the Nawata Formation (Deino, Tauxe, Monaghan, & Hill, 2002). Given that fossils dated between 7 and 10 Ma from the tribe Hominini are scarce and difficult to allocate to lower taxonomic categories, we used 10 Ma as the soft upper boundary (Benton et al., 2015). We used an exponential distribution for this calibration and set the lower boundary to 6.5 Ma with a mean value of 1.2 to allow the upper boundary to extend back to 10 Ma. The alignment was divided into seven partitions (Supplemental Table S5) according to the results of PartitionFinder. Each

BEAST analysis employed seven lognormal relaxed-clock models (i.e., one per partition), a birth–death tree prior, and was run for 40 million generations, sampling every 4,000 generations. Posterior distributions and effective sample sizes (ESSs) were calculated using Tracer v1.6.

We also analyzed a dataset including ~1,823 bp partial *CYT B* and D-loop regions for an extended sampling of 34 hoolocks including those retrieved from GenBank (Supplemental Table S1) in order to test whether the external morphological pattern we observed is also supported by molecular data. The partition scheme and evolutionary models were also estimated using PartitionFinder (Supplemental Table S5). We used an extensive Bayesian skyline tree prior.

Except for historical specimens, no gibbon was killed or captured during this research. All field research reported in this manuscript was permitted by the Management Bureau of Gaoligongshan National Nature Reserve, and adhered to the legal requirements of China and the American Society of Primatologists' principles for the ethical treatment of nonhuman primates.

3 | RESULTS

3.1 | External morphology

All black hoolock individuals from Mt. Gaoligong have white eyebrows, which is a distinctive feature of the genus. However, they differ from the holotype of *H. leuconedys* (NHM ZD.1950.391) in four characteristics: (i) the eyebrow streaks are thinner and separated by a large gap; (ii) the beards are completely black or brown instead of white; (iii) white hair is absent in the suborbital area; (iv) the genital tufts are black, brown, or dark gray instead of whitish (Table 1; Figure 2C and D and Figure 3; Supplemental Figure S2). Adult females from Mt. Gaoligong are characterized by incomplete white face rings, with only sparse white hairs present on the lateral orbital and suborbital regions ($n = 7$; Figure 2H), which are much less conspicuous than those of typical *H. leuconedys* females ($n = 7$; Figure 2G). We also found geographic variation among *H. leuconedys* specimens from east and west of the Irrawaddy River. Consistent with the holotype of *H. leuconedys*, all individuals from the west of the river display thick eyebrows, a white or silvery genital tuft in males, and whitish hair around the orbital and suborbital regions in females. In contrast, males from the east of the Irrawaddy River (MCZ 26474, 30383; AMNH M-43068; NHM ZD.1933.7.29.15) resemble Mt. Gaoligong hoolocks in displaying thin, well-separated eyebrows and a dark genital tuft, as well as in lacking white hair in the suborbital area and lacking a white beard. The genital tuft of two old adult males (identified from their heavily worn lower molars) from this region (MCZ 30383 and IOZ 25965, Supplemental Figure S3) is gray in color, paler than individuals from Mt. Gaoligong but still much darker than that of NHM ZD.1933.7.29.15. Although female hoolocks from this region (e.g., AMNH M-43065; USNM 257988) exhibit whitish hair on the suborbital regions, it is less conspicuous or absent lateral to the orbits.

3.2 | Craniodental variation

Results of the PCA using 23 craniodental variables indicate that the first principal component (PC1) accounts for 40.30% of the variance and is positively correlated with all variables (loading >0.24) except for the palate breadth at upper M2 (loading = 0.10), reflecting a size effect (Supplemental Table S3). The second principal component (PC2) represents 10.23% of the variance and is dominated by the breadth at upper M2, the breadth at upper M3, and face height (loading >0.52). The plot of the first two components revealed extensive overlap between specimens representing different species/allopatric populations (Figure 4A). Similar patterns were observed when using the shape outline of upper and lower M2s (Figure 4C and E). These results suggest that skull and tooth shape are conserved among hoolock taxa, a general pattern observed in hylobatids (Jablonski & Chaplin, 2009). Figure 4B, D, and F illustrate the scatter plots of the first two discriminants using the same craniodental dataset, showing that *H. hoolock* and *H. leuconedys* from east and west of the Irrawady River are more clearly separated from each other, although there is still some overlap between the three groups. The likelihood of individuals being correctly assigned to their own taxon/group ranges between 87% and 93% (Supplemental Table S6).

The lower p4 shows patterns of morphological variation corresponding to the different hoolock species and geographic

TABLE 1 Comparison of external characteristics between *Hoolock tianxing* and other hoolock taxa

	H. leuconedys	*H. tianxing*	*H. h. hoolock*	*H. h. mishmiensis*
Ventral pelage in males	Brownish	Brownish	Black	Black
Gap between brow-streaks in males	Wide	Widest	Narrow	Narrow
Brow-streaks in males	Thick	Thin	Thick	Thickest
Genital tuft in males	White or silvery	Black or brown	Black or faintly grizzled	Black with buffy or rufescent buff
Beard on chin in males	Less prominent White or buffy	Less prominent Black or brown	Prominent Black	Prominent Black
Gap between brow-streaks in females	Conspicuous	Conspicuous	Inconspicuous	Inconspicuous
Brow-streaks in females	Downturned More white between eyes	Downturned Less white between eyes	Slightly concave	Horizontal

FIGURE 3 A hoolock specimen from Homushu Pass, Mt. Gaoligong (AMNH M-43068, top row) and the holotype of *H. leuconedys* (NHM ZD.1950.391, bottom row), showing (left to right) eye brows and suborbital area, beard, and genital tuft

populations (Figure 5). The lower p4 of *H. hoolock* individuals from Myanmar is trapezoidal-shaped, and the talonid is generally wider than the trigonid because (i) the metaconid and protoconid are relatively small; (ii) the hypoconid is generally present and equal in size to the entoconid; and/or (iii) both the entoconid and hypoconid are moderate or large in size (Figure 5a). *H. hoolock* individuals from Assam, India, differ slightly from their counterparts from Myanmar in that the lower p4 is more square-shaped in overall outline because (i) the protoconid is well-developed; (ii) the entoconid and hypoconid are present but not strongly developed; and/or (iii) the talonid and trigonid are similar in width (Figure 5b). In *H. leuconedys* individuals from the west of the Irrawaddy River, the lower p4 has a rhomboidal shape (Figure 5c and d), and the trigonid is usually wider than the talonid because (i) the two mesial cusps are very large, with the metaconid being as large as the protoconid and (ii) the hypoconid and entoconid are greatly reduced and in some cases absent (especially the hypoconid). In hoolock individuals from the east of the Irrawaddy River, the lower p4 is generally oval-shaped because (i) the teeth are mesiodistally shorter; (ii) the talonid and trigonid are of equal width; and/or (iii) distal cusps are present, but not well-developed (Figure 5e and f).

3.3 | Genetic variation

We obtained partial *CYTB* and D-loop sequence data for all 27 soft tissue and fecal samples (~1,823 bp; 3.7% missing data). The two samples selected for mitogenome sequencing using Sanger and 12 samples selected for NGS were all successfully sequenced. New sequences obtained in this study were submitted to GenBank (accession numbers: KY250036–KY250074). We obtained 14,776 reads for the tissue DNA library, and 21,336–109,463 reads for the fecal DNA libraries. At least 95% of the amplicon sequencing reads could be mapped to the hoolock mitogenome. Mean coverage (depth/site) of the modern tissue samples was 248.1 (SD = 126.7). Hybrid capture yielded mean coverages of 35.2–222.5. Between 5.00% and 39.92% of the reads could be mapped to the reference mitogenome. We obtained complete (or nearly complete; ≤0.5% missing data) mitogenomes for nine individuals, and partial mitogenomes for the other three individuals (5.5–16.6% missing data; Supplemental Table S7).

The mitogenome gene tree is well-supported for all interspecific relationships (posterior probabilities [PP] ≥0.98) except for the root of Hylobatidae (Figure 6). *Hylobates* and *Symphalangus* are fully supported as sister groups (PP = 1.0). The MRCA of Hylobatidae is estimated as occurring 6.79 Ma (95%CI = 7.64–6.01 Ma). The single sample representing *H. hoolock* diverged from the other samples at ca. 1.14 Ma (PP = 1.0; 95%CI = 1.38–0.93). Two distinct clades are detected within samples previously classified as *H. leuconedys* from both east and west of the Irrawaddy River (PP = 1.0), which diverged ca. 0.49 Ma (0.60–0.39 Ma).

In the gene tree based on partial mitochondrial hoolock sequence data, the primary division is between one sample of *H. h. hoolock* from Dhaka Zoo (Bangladesh) and all other samples (PP = 1.0; Figure 7). It should be noted that all these other samples were identified as *H. leuconedys*, except for two individuals from GenBank, which were identified as *H. h. hoolock* (Y13304, Y13305). Two monophyletic clades are again strongly supported (namely clades I and II, PP ≥ 0.97). All individuals in clade I, from various sources with known taxonomic

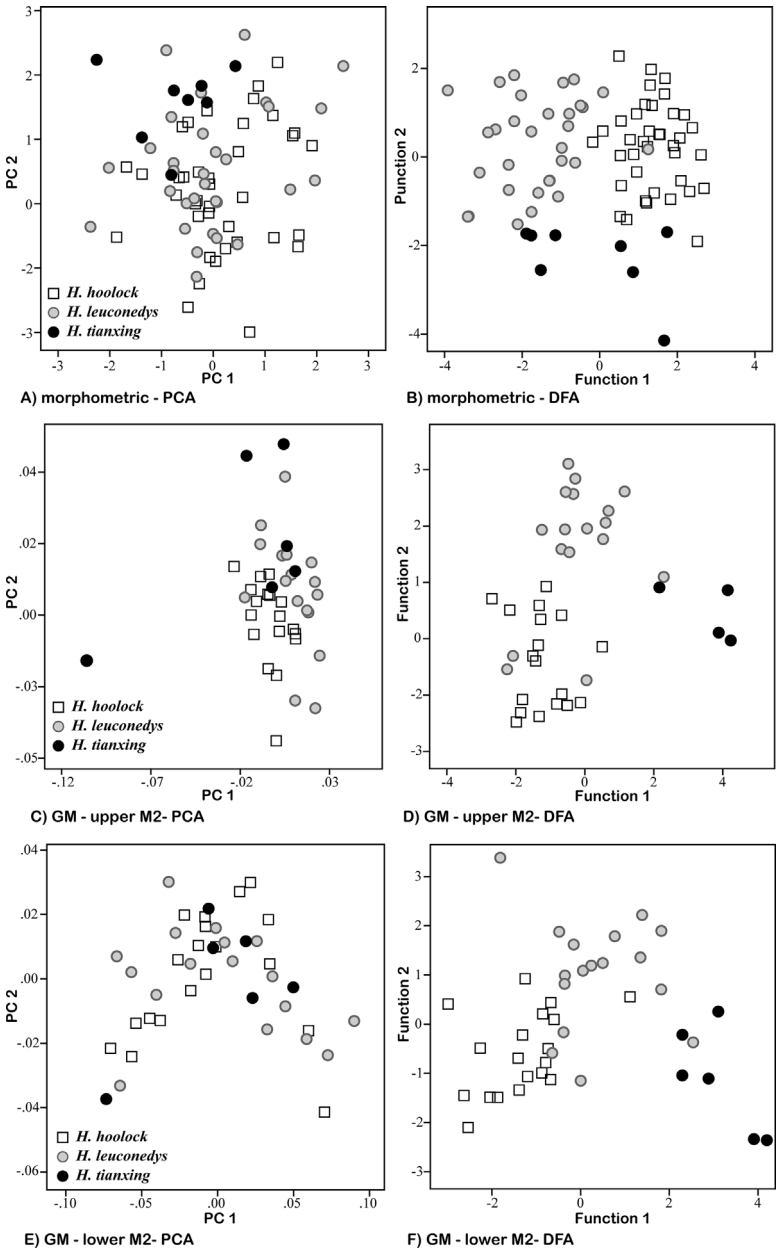

FIGURE 4 PCA and DFA for hoolock taxa, using craniodental measurements (A and B), shape of the outline of the upper M2 (C and D), and shape of the outline of the lower M2 (E and F)

identities, show typical *H. leuconedys* morphology. Clade II consists of all five wild individuals from Mt. Gaoligong, together with fecal or soft tissue samples from five other animals from Chinese museums and captive centers (Supplemental Table S1). Except for the GenBank sequences of unknown identity, all wild-born captive individuals are morphologically similar to the Mt. Gaoligong population. The Kimura 2-parameter (K2P) genetic distance (Kimura, 1980) of the complete *CYT B* dataset between the Bangladesh *H. hoolock* samples and *H. leuconedys* sensu lato was 2.9%, and the K2P distance between clades I and II was 1.2%.

Based on our examination of museum specimens and observation of wild animals, morphological and morphometric comparisons, and genetic analyses, we demonstrate that eastern hoolocks from the east of the Irrawaddy River are distinguishable according to a range of external and molecular characteristics from individuals from the west of the Irrawaddy River. We therefore recognize the hoolock population distributed to the east of the Irrawaddy River and to the west of the Salween River as a new species.

3.4 | Systematic biology

Order Primates Linnaeus (1758)
Family Hylobatidae Gray (1870)
Genus *Hoolock* Mootnick and Groves (2005)
Hoolock tianxing sp. nov.
Hylobates hoolock leuconedys: Groves (1967): 276 (part).
Skywalker hoolock gibbon (天行长臂猿) or Gaoligong hoolock gibbon (高黎贡白眉长臂猿).

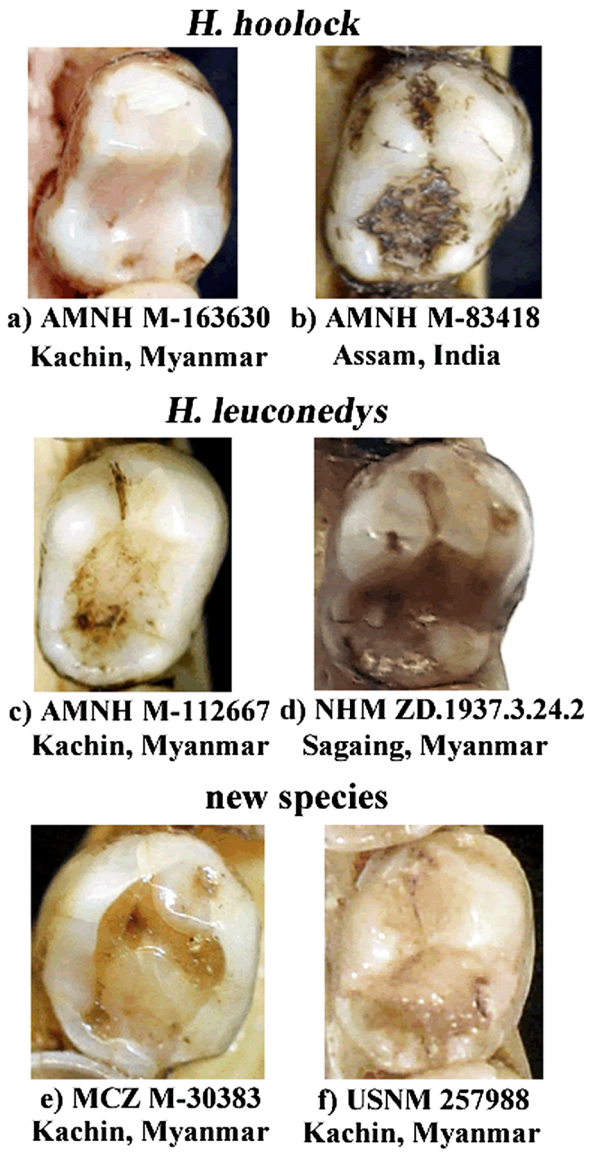

FIGURE 5 Lower p4 of different hoolock species and geographic populations

3.4.1 | Holotype

AMNH M-43068 (adult male, skin only; Figure 3), collected by Roy Chapman Andrews and Yvette Borup Andrews on April 5, 1917 during the American Museum of Natural History's Asiatic Zoological Expedition (Allen, 1938).

3.4.2 | Type locality

Ho-mu-shu (=Hongmushu) Pass, Baoshan, Yunnan, China (25.00 N, 98.83 E).

3.4.3 | Paratypes

AMNH M-43065 (adult female, skin only; Supplemental Figure S1) and MCZ 26474 (=AMNH M-43067, skin and skull, relocated to MCZ in September 1930), collected at the same locality as the holotype (Allen, 1938). IOZ 25965 (adult male, skin and skull; Supplemental Figure S3), collected on 4 June, 1965 at Tengchong, Yunnan, China. MCZ 30383 (adult male, skin and skull; Supplemental Figure S3) collected on 15 January, 1932, ca. 40 miles east of Bhamo, northern Myanmar, during the Brooke Dolan expedition.

3.5 | Etymology

Tianxing constitutes the pinyin (standard mainland Chinese phonetic alphabet) transliteration of 天行, meaning heaven's movement or skywalker (*xing*, movement, can act as either a noun or a verb), a name referring to the unique locomotory mode of gibbons (brachiation; Figure 8) and derived from the text of the I Ching, an ancient Chinese work of divination: 天行健君子以自强不息 ("As heaven's movement is ever vigorous, so must the scholarly gentleman (君子, "*junzi*") ceaselessly strive for self-improvement"). Gibbons were widely regarded as a symbol of scholar-officials or *junzi* in ancient China, as the perceived "noble" characteristics of gibbons were considered to accord with the aesthetic taste of both Daoism and traditional Chinese scholars (van Gulik, 1967; Ye & Heule, 2013).

3.6 | Diagnosis

Hoolock tianxing is a hoolock gibbon distinguished from other described hoolock species by a combination of external and dental characters. In males, the ventral pelage is brownish, resembling that of *H. leuconedys* but differing from *H. hoolock*. The eyebrows are relatively thinner than in *H. hoolock* and *H. leuconedys*, and well-separated, differing from the condition in *H. hoolock*, where there is only a narrow gap between the eyebrows. White hairs are absent in the suborbital area, differing from *H. leuconedys*, which has white hairs in the suborbital area. The beards of males are black or brown, differing in color from *H. leuconedys*, which has a whitish or buffy beard, and not as prominent as in *H. hoolock*. The black, brown or grayish genital tuft in males differs in color from *H. leuconedys*, which has a white or silvery tuft. The face rings in females are incomplete, differing from the condition in both *H. hoolock* and *H. leuconedys*. The crown outline of the lower p4 is oval, making it distinct from *H. leuconedys* and *H. hoolock* individuals from Myanmar and more similar to *H. hoolock* from Assam.

3.7 | Description

In adult males, the ventral pelage is generally dark brown, and the dorsal pelage has a brownish overlay, especially apparent under bright light (Supplemental Figure S2); eyebrows thin and well-separated; white hairs absent in the suborbital area; beard not conspicuous, black or brown in color, not contrasting with the color of the chest or body; genital tuft prominent, usually black or dark brown in color with a few white hairs present, not contrasting with the color of the groin. In older animals, the genital tuft is fainter and light brownish in color (Supplemental Figure S4). In adult females, pelage color is generally yellowish, but varies with age (yellowish white to reddish blonde); eye rings incomplete; white hair typically not present on the lateral orbital region, or if present, not as conspicuous as on the brows on the lateral orbital region; white hair sometimes also not present on the suborbital region (Supplemental Figure S5). Juveniles do not have white hair on the chin or under the eyes; eyebrows are not always well-separated. Lower p4 is generally mesiodistally short and oval-shaped, with the

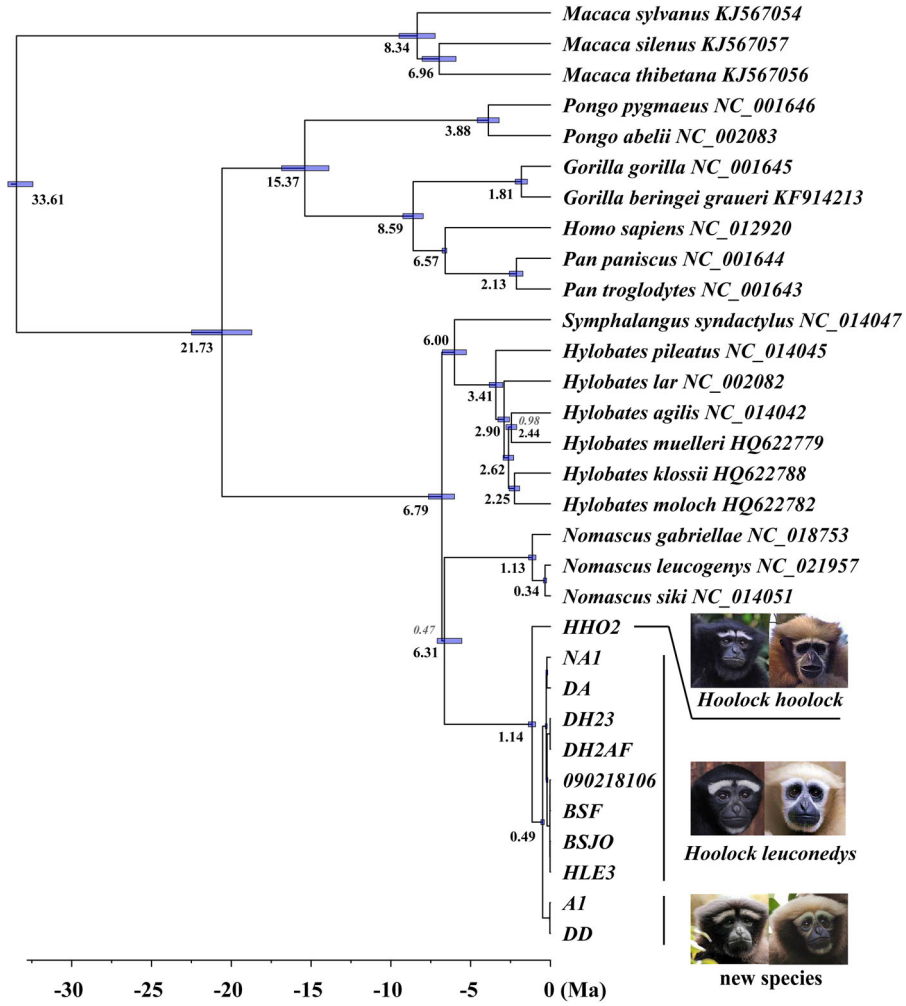

FIGURE 6 Bayesian tree of various catarrhines estimated using complete mitochondrial genome sequence data. Branch lengths represent time. Node bars indicate the 95%CI for the clade age. Unless specified, all interspecific relationships are strongly supported (PP = 1.0). PPs lower than 1.0 are shown in gray. Numbers above the nodes indicate Bayesian posterior probabilities, numbers below the nodes refer to median ages

talonid and trigonid of equal buccolingual width. Distal cusps are present, but not well-developed (Figure 5e and f).

3.8 | Distribution

Between the Irrawaddy-Nmai Hka River and the Salween River in China and Myanmar. The Dulongjiang valley, the upper tributary of the Nmai Hka River, may serve as a dispersal barrier for hoolocks. Wild individuals are confirmed to occur on Mt. Gaoligong, and historical museum specimens are also known from further south at Gokteik, Shan State, northern Myanmar. Geissmann et al. (2013) estimated that a healthy population with ca. 50,000 individuals of eastern hoolock live in Shan State subtropical forests, and ca. 16,000 individuals live in montane rainforest in Kayah-Kayin (see below).

3.9 | Comments

Although Groves (1967) suggested that the color of the hands and feet is lighter than the body color in *H. leuconedys*, we found no difference in coloration between the hands, feet, or bodies in examined individuals of either *H. leuconedys* or *H. tianxing*. The two specimens in our study sample from Gokteik, Shan State, Myanmar (USNM 257988 and ZD.1933.7.29.15), which represents the southernmost record of *H. tianxing*, show minor morphological differences from individuals from Mt. Gaoligong; the male specimen is very similar to the holotype of *H. tianxing*, but the female possesses more white hair on the suborbital region than individuals from Mt. Gaoligong. Gokteik is 300 km southwest of Mt. Gaoligong, indicating that these observed differences may represent allopatric differentiation between hoolock populations in this region. However, more specimens from Shan, Kayah, and Kayin States need to be examined to assess whether this apparent variation is a genuine population-level characteristic.

Hoolocks no longer survive at the type locality of *H. tianxing*. The nearest well-documented population occurs at Nankang (N24° 49′, E98°46′, H: 1800–2300 m a.s.l.), 20 km away from Hongmushu in the southern part of Gaoligong National Nature Reserve. The vegetation in this region consists of humid montane evergreen broad-leaved forest dominated by species of Lauraceae, Fagaceae, Theaceae, and Magnoliaceae. Mean annual temperature in this

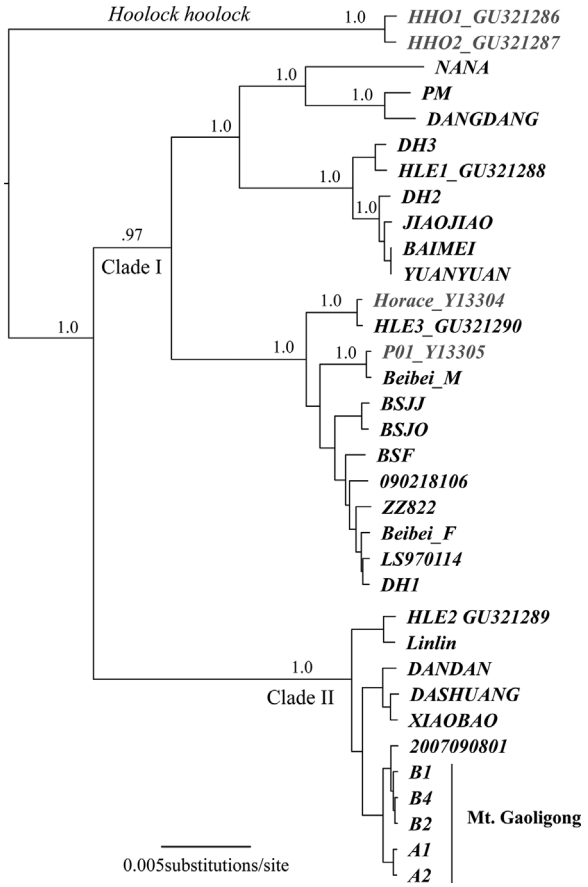

FIGURE 7 Mitochondrial gene tree for hoolocks, showing two major clades within *Hoolock leuconedys* sensu lato. Specimens shaded in gray were originally identified as *H. hoolock*. Node numbers indicate Bayesian posterior probabilities. Branch lengths represent substitutions/site

region between October 2010 and September 2011 was 13.3°C; the lowest recorded mean monthly temperature was 6.4°C in January 2011, and the highest was 20.3°C in August 2010 (Fan et al., 2013). Annual rainfall was 1801.4 mm during this period; rainfall was greater than 200 mm in each rainy season month from May to October, except in September 2011 (198.1 mm), and was less than 100 mm in each dry season month from November to April (Fan et al., 2013).

FIGURE 8 A juvenile male of *H. tianxing* from Mt. Gaoligong jumping across trees. Photo taken by Lei Dong

4 | DISCUSSION

4.1 | Confidence of the molecular results

Genomic-scale hybridization capture has been demonstrated to constitute a valid and powerful approach to recover endogenous DNA for ancient and non-invasive sampling, and is extremely useful for conservation of threatened species (Perry, Marioni, Melsted, & Gilad, 2010). In this study, our enrichments were able to recover whole mitogenomes efficiently for 8 of the 11 fecal samples. One potential issue might be so-called nuclear mitochondrial DNA sequences (NUMTs), which commonly exist in primates (Karanth, 2008); the NUMTs, however, should be at low enough levels to not influence base calling or subsequent assemblage accuracy (Li, Schroeder, Ko, & Stoneking, 2012).

The relationships between hylobatid genera are highly supported in our analyses (PP = 1.0); but they are characterized by short internal branches (Figure 6), a finding similar to previous studies (Kim et al., 2011; Springer et al., 2012; Thinh, Mootnick, Geissmann et al., 2010). This finding also matches the conclusions of the recent study using whole gibbon genome sequences by Carbone et al. (2014), who suggested a near-instantaneous diversification among the living hylobatid genera.

According to our mitogenomic analyses, the MRCA of living gibbons lived around 6.79 Ma, which is slightly older than the estimate of 5 Ma based on nuclear genome data Carbone et al. (2014). Our result is very similar to the gibbon MRCA age estimate given by Springer et al. (2012). The timing of divergence between *H. hoolock* and taxa previously classified as *H. leuconedys* was around 1.14 Ma (1.38–0.93 Ma), overlapping with the estimates given in two previous studies (1.42 [1.90–0.97] in Thinh, Mootnick, Geissmann et al. [2010]; 1.96 [4.4–0.22] in Springer et al. [2012]).

4.2 | Support for the new taxon

Groves (1967) recognized *H. hoolock* and *H. leuconedys* based on characters shown by a series of skulls, as well as on four soft tissue characters, namely the color of the preputial tuft, the shape of the eyebrow streaks, the color of the suborbital hair, and the color of the chin hair. His taxonomic assessments were further confirmed by molecular phylogenetic analyses (Thinh, Mootnick, Geissmann et al., 2010). Here, we found that these characters are equally prominent and distinguishable between *H. leuconedys* sensu stricto and *H. tianxing*. Skull shape in hominoids is generally conserved while the shape of postcanine teeth is usually variable (Uchida, 1996). Nevertheless, the 87–93% correct assignments of individual specimens using each of the morphometric and GM data in the DFAs support the occurrence of morphological differentiation. Similarly, the differentiation of the lower p4 is not clear-cut by itself. However, clear morphological differentiation between populations is apparent when considering these characteristics together.

Morphological discrimination is also congruent with divergence of the mitochondrial genomes. We acknowledge that mitogenomic gene trees can differ from nuclear genomic trees, as seen in primates for example in recent analysis of the "odd-nosed" Asian colobines (Liedigk

TABLE 2 Estimated divergence times between gibbon and other Asian primate sister species based on mitochondrial data

Sepcies group	Estimated divergence time (Ma)	References
Hoolock tianxing and *H. leuconedys*	0.49	This study
Nomascus leucogenys and *N. siki*	0.55	Thinh, Mootnick, Geissmann et al. (2010)
	0.34	This study
Trachypithecus francoisi and *T. leucocephalus*	0.27–0.46	Liu et al. (2013)
Trachypithecus francoisi and *T. poliocephalus*	0.25–0.50	Liu et al. (2013)
Trachypithecus obscurus and *T. phayrei*	0.36	He et al. (2012)
Trachypithecus cristatus and *T. germaini*	0.55	He et al. (2012)
Rhinopithecus bieti and *R. strykeri*	0.24	Liedigk et al. (2012)
	0.30	Zhou et al. (2014)
Pygathrix cinerea and *P. nemaeus*	0.23	Liedigk et al. (2012)

et al., 2012). However, the two clades representing *H. leuconedys* and *H. tianxing* are each strongly supported as monophyletic in our analysis, and diverged in the middle Pleistocene (ca. 0.49 Ma), suggesting long-term matrilineal isolation. In addition, the K2P distance between *H. leuconedys* and *H. tianxing* (1.2%) is similar to the differentiation observed between other gibbon sister species, for example, between *Nomascus annamensis* and *N. gabriellae* (1.26%) and between *N. leucogenys* and *N. siki* (1.0%) (Thinh, Mootnick, Thanh, Nadler, & Roos, 2010). Similarly, the estimated divergence time between *H. leuconedys* and *H. tianxing* is similar to or greater than estimated divergence times between other primate species in Asia (Table 2). All of these taxa are recognized as full species in the most recent taxonomic review of the world's primates (Mittermeier et al., 2013).

Mt. Gaoligong, situated along the border of China and Myanmar, is a hotspot of new species discovery, with recent discoveries including other species of primates (Geissmann et al., 2011), as well as other vertebrates, for example, amphibians (Yang, Wang, & Chan, 2016; Yang, Wang, Chen, & Rao, 2016). This high discovery rate at least partly reflects the fact that these mountains have been difficult to access in the past, so that few expeditions have been carried out, and subsequently most animal groups have never been studied in detail. Most of these new species are locally endemic; Mt. Gaoligong is the westernmost part of the Hengduan Mountain Chain, which was formed during the uplift of the Himalayas (Zhong & Ding, 1996), and is geographically isolated from the other mountains in southwestern China by the Salween River valley. The "sky-island" topography and associated unfavorable valley habitats are likely to have driven extensive physical isolation, allopatric speciation, and high endemism in vertebrate populations (He & Jiang, 2014). Our description of *H. tianxing* provides further evidence for the unique local fauna of Mt. Gaoligong, and it is very likely that new species are still to be described in other taxonomic groups, many of which remain understudied and need to be re-examined.

Groves (1967) and Choudhury (2013) noticed morphological differences in hoolocks from the east and west of the Irrawaddy River. Groves (1967) also reported that three of the 22 *H. leuconedys* specimens he examined did not show white chins, and eight specimens did not have white hair under the eyes. However, he hesitated to erect any further hoolock taxa because at the time "too few specimens of either sex are available from the east of the Irrawaddy River to determine whether further splitting may be required." Following our analysis of a further eight historical specimens and 14 wild animals from the east of the river, we support the suggestion that the Irrawaddy-Nmai Hka River is likely to act as a barrier for different hoolock taxa, on the basis of external and craniodental morphological differences and the divergence of mitochondrial genomes.

4.3 | Individuals or specimens of particular significance

Based on a studbook of captive hoolock gibbons compiled in 2011, we identified a hybrid hoolock family in Kunming Zoo (Hehe♂ × Maomao♀). This pair reproduced five times, and was the most successful captive breeding hoolock pair in any Chinese zoo. Unfortunately, the adult pair and three of their five offspring (KNHMZ 2007090801, KNHMZ 2007082102, and another juvenile) died in 2007, possibly due to a flu-like infection, although two male offspring (Dandan and Xiaobao) still survive. The male had the typical white beard of *H. leuconedys* (YQL, personal observation), but a photograph of the adult female shows the typical morphology of *H. tianxing*. Their three male offspring (Xiaobao, Dandan, and KNHMZ 2007090801; Supplemental Figure S6a and b) all have white hair on their chins and genital tufts, but do not have white hair under their eyes, and their white beards are not as conspicuous as in typical *H. leuconedys* males (Supplemental Figure S2h–j). Genetic analysis of maternally inherited mitochondrial sequence data places them in the *H. tianxing* clade (Figure 7). We conclude that these offspring are *H. leuconedys* × *H. tianxing* hybrids. Xiaobao is now paired with a morphologically typical *H. leuconedys* female (Baimei) in Kunming Zoo; mitochondrial genetic analysis of the offspring of this pair (Jiaojiao and Yuanyuan) places them as expected in the *H. leuconedys* clade (Figure 7).

One skin specimen of an adult female (KIZ LS970114) was placed in the *H. leuconedys* clade in our phylogenetic analysis (Figure 7). Morphologically, this specimen resembles *H. leuconedys* in having thick white hair between its eyes (Figure 2c); it was, however, reportedly collected from Tengchong County, Yunnan, which is within the geographic distribution of *H. tianxing*. Its original collection record contains no further information on either the collector, collection date, and skull or body measurements. We consider it is highly possible that

this specimen in fact originated in Myanmar, and was bought in Tengchong.

4.4 | Conservation implications

The eastern hoolock, based on an assessment comprising populations of both *H. leuconedys* and *H. tianxing*, is currently listed as Vulnerable on the IUCN Red List (Brockelman & Geissmann, 2008), because a large population of 310,000–370,000 individuals (estimated based on very limited field surveys) has been reported from Myanmar (Geissmann et al., 2013). As the hoolock population on the east bank of the Irrawaddy River represents a new species, its formal conservation status must also be re-evaluated. According to the most recent available survey data from 2008 and 2009, the total population size of *H. tianxing* in China is less than 200 individuals, and the population is highly fragmented across different forest areas (Fan et al., 2011). Illegal hunting, habitat destruction, degradation and fragmentation, and the stochastic effects of small population size and isolation all threaten the future of *H. tianxing* in China (Fan et al., 2011; Fan, 2016). Based on average group density and area of suitable habitat, Geissmann et al. (2013) estimated the total population of hoolocks in Kachin State to be 240,000–290,000 individuals. This estimate is likely to include both *H. tianxing* and *H. leuconedys*, although hoolocks have a limited distribution on the east bank of the Nmai Hka River, suggesting that most of the Myanmar hoolocks in this estimate are likely to be *H. leuconedys*. Three infants or small juvenile hoolocks have been confiscated by the Chinese border police in the last 2 years, and one small juvenile hoolock from Myanmar was raised as a pet by a woman in Dulongjiang, Yunnan; all these individuals were *H. leuconedys*. The population of *H. tianxing* in Kachin State is therefore likely to be very small if it even still survives. A larger population of *H. tianxing* might still survive in the southern part of its proposed range; Geissmann et al. (2013) estimated that approximately 50,000 hoolocks occur in the subtropical forest of Shan State and 16,000 individuals occur in the montane rainforest of Kayah and Kayin States. These populations are distributed on the east bank of the Irrawaddy River, and therefore are likely to represent *H. tianxing*. These populations, however, face a series of threats including hunting, illegal trade, and rapid habitat loss (Geissmann et al., 2013). It is difficult to evaluate the conservation status of *H. tianxing* without more robust information on the status of these poorly known populations, but we propose that *H. tianxing* should probably be assessed as Endangered on the IUCN Red List, under criterion A4acd (IUCN, 2001).

Only 21 captive hoolock individuals are recorded in Chinese zoos in the hoolock studbook (Yang, 2011). We surveyed 22 captive hoolocks in China during this study, most of which were, however, not listed in the studbook. We found that only two of these individuals can be assigned to *H. tianxing*. Although it is likely that other captive hoolocks that we did not survey may also be *H. tianxing* individuals, the total number of captive individuals of this species must be very small, and we know of no captive *H. tianxing* females in China.

Only two pairs of either eastern hoolock species are known to have bred in China before 2011: in Kunming Zoo and Beijing Zoo (Yang, unpublished). The pair in Kunming Zoo died in 2007, and the other pair and another adult female in Beijing Zoo died in 2005. Kunming Zoo currently has a new hoolock pair (Xiaobao♂ × Baimei♀), which has bred successfully three times, and another hoolock pair in Dehong Wildlife Rescue Center (DH3♂ × DH2♀) gave birth in 2015; as discussed above, however, Xiaobao is a *H. leuconedys* × *H. tianxing* hybrid, whereas Baimei, DH3, and DH2 are all *H. leuconedys* individuals. Further investigation of hoolocks currently held in Chinese captive facilities, together with accurate species identification of captive hoolock individuals, is necessary in order to establish a national conservation breeding program for *H. leuconedys*, and to evaluate whether a similar conservation breeding program is feasible for *H. tianxing*.

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