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# Asian Primates Journal

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Sumatran orangutan (*Pongo ebelii*)

**Photo by** Conservation International Indonesia

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# FOREWORD

After much delay and several hiccups, we welcome you to the first issue of *Asian Primates* – the Journal of the Asian Section of the IUCN/SSC Primate Specialist Group.

Unprecedented threat from destruction of tropical forests, and illegal hunting and trade of wildlife have are globally causing almost 30% of our closest living relatives to be in danger of extinction. The situation is even precarious in Asia where many non-human primate species and their habitats are severely threatened.

The situation in Asia is best summed by the fact that 11, or 44%, of the World's 25 Most Endangered Primates – a list compiled at the 21<sup>st</sup> Congress of the International Primatological Society in Entebbe – are Asian primates.

These 11 Asian primates comprise Western Hoolock gibbon (*Hoolock hoolock*), Horton Plains slender loris (*Loris tardigradus nycticeboides*), Western purple-faced langur (*Semnopithecus vetulus nestor*), Pig-tailed langur (*Simias concolor*), Siau Island tarsier (*Tarsius* sp.), Delacour's langur (*Trachypithecus delacouri*), Golden-headed langur (*Trachypithecus poliocephalus poliocephalus*), Grey-shanked douc (*Pygathrix cinerea*), Tonkin snub-nosed monkey (*Rhinopithecus avunculus*), Hainan black-crested gibbon (*Nomascus hainanus*), and the Sumatran orangutan (*Pongo abelii*).

The *Asian Primates* is a new journal committed to disseminating information relating to the research and conservation of non-human primates in Asia, will therefore also serve to highlight and draw the attention to issues relating to threatened primate species and their habitats.

The *Asian Primates* will be an important source of information not only amongst the IUCN/SSC Primate Specialist Group members in the region, but also to other professionals and those with a keen interest in primates and primate conservation. The journal further aims to provide a venue for developing the capacities of young Asian nationals by encouraging them to submit manuscripts in English.

As no one discipline can encapsulate the many aspects of primates, the *Asian Primates* thus encourage submissions that reflect inter- and multi-disciplinary perspectives to primates, thereby allowing the sharing of these perspectives, and the initiation of innovative and creative dialogues that help us learn more about our closest living relatives, and that help us to conserve them, their habitats, other denizens that share those habitats with them, and the ecosystem services these habitats provide us.

**Editors**

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## CURRENT STATUS OF THE HAINAN GIBBON (*Nomascus hainanus*): PROGRESS OF POPULATION MONITORING AND OTHER PRIORITY ACTIONS

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### ABSTRACT

We report on recent developments with respect to the status of the world's rarest ape, the Hainan Gibbon *Nomascus hainanus*. Since it was intensively surveyed in October 2003, the sole known population, at Bawangling National Nature Reserve, Hainan Island, has increased to 17-20 individuals, including two social groups and up to five solitary adults. The larger group, Group A, currently contains one adult male, two adult females, two subadults, two juveniles and two infants; the smaller Group B contains one adult male, two adult females, one subadult, one juvenile and one infant. The solitary gibbons, which are rarely seen, include up to three adult-sized black individuals (with two confirmed as males by their male solo calls) and up to two adult females; observations from recent years suggest individuals of both sexes can be black even beyond the point of migration from the natal group. An update is also given on other conservation actions, including patrolling, forest restoration, understanding threats, surveys of other forests, gathering of information relevant to landscape-scale restoration and management, nature-reserve capacity-building, and promoting public awareness. The progress in these directions provides a platform for future conservation efforts.

**Keywords:** *Nomascus hainanus*, Hainan gibbon, population, status, conservation actions.

### INTRODUCTION

Hainan Gibbon *Nomascus hainanus* (Thomas) is currently the world's rarest ape (Chan *et al.*, 2005; Zhou *et al.*, 2005)\*. Endemic to Hainan Island, its decline was rapid; the population was estimated to be over 2,000 in the 1950s but only 30 by the 1980s (Liu *et al.*, 1984). Scientific studies began in the 1960s (Liu *et al.*, 1984; Liu and Tan, 1990), and focused on the Bawangling forest area, which by 1990 would be the sole confirmed refuge (although it may have hung on into the 1990s in three to four other remote localities: Chan *et al.*, 2005). The known Bawangling gibbon population was considered as low as seven to eight individuals in the 1970s, but had reportedly recovered to 21 by the late 1980s, when it comprised four groups in an area of 1,200ha (Liu *et al.*, 1989; Wang, 1995). In the

early 1990s it had dropped again to 15 individuals (Zhang and Sheeran, 1994).

Local reserve staff, trained to accompany Prof. Liu Zhenhe in the field, continued intermittently to monitor the gibbons at Bawangling (since 1988 a National Nature Reserve) during the 1990s, but doubt grew internationally over the continued existence of the population, since local reports were not independently verified until the visit of the Hong Kong-based Kadoorie Farm & Botanic Garden (KFBG) team in 1998 (KFBG, 2001). At that time according to the most experienced of the Bawangling National Nature Reserve (BNNR) field staff, Chen Qing, who guided the KFBG team to see and photograph the gibbons, the known population was 17 (KFBG, 2001). An official status survey that year, however, reported 23 gibbons, in four groups

\* English nomenclature of this gibbon varies. It is sometimes called Hainan Crested Gibbon or Hainan Black Gibbon, though in these *Nomascus* gibbons both the full crest and black coloration are absent in the adult female. The widely used name Hainan Black Crested Gibbon is also easily confused with the name Hainan Black-crested Gibbon which includes redundancy as all extant crested gibbon taxa have black crests. Since the name Hainan Gibbon is self-explanatory and simple, we use it here.

(State Forestry Administration Survey & Planning Institute and the BNNR Management Office, 2001). A postgraduate study in 2001-2002, involving around three months of fieldwork and 17 sightings, estimated 12-19 individuals in four groups, of which the fourth was not seen (Wu *et al.*, 2004).

Postgraduate field research by one of us (ZJ) began in September 2002, when BNNR field staff recognized two social groups of gibbon. Through daily contact with the gibbons over two years (Zhou, 2007), the study confirmed the presence of only two groups, termed A and B, with an uncertain number of solitary males. In-depth work was conducted on Group B, which initially comprised one adult male, two adult females and one adolescent. At the time Group A comprised five individuals, which were also individually recognizable within the group. Immature animals could not be reliably sexed; external genitalia are similar and only full adult males and females can be sexed by their coloration (predominantly black and yellow respectively) (Pocock, 1905; 1927).

It is possible that two of the four groups reported in 1998 and 2001-2002 had disbanded by the end of 2002, when more intensive survey work began. But it seems more likely that the discrepancy in the number of groups stems from the lack of wide-scale simultaneous survey over the potential area of occupancy, and from unexpressed assumptions about group home ranges. Home ranges in the species as reported earlier (Liu *et al.*, 1989; Liu and Tan, 1990) were only 100-500ha. Those revealed by ZJ's study, based on months of direct tracking, were higher at 987ha and 548ha for Groups A and B respectively (Zhou, 2007); they also overlapped. This made it difficult to distinguish adjacent groups without either reliable individual recognition - very difficult for wild gibbons, especially after only brief visual contact - or simultaneous observations.

To resolve the uncertainty over the gibbon population, in October 2003 a comprehensive rapid survey was invited by Hainan Wildlife Conservation Centre (HWCC), the conservation division of Hainan Forestry Department. It was conducted with KFBG funding, and coordinated by one of us (BC). Trained by Thomas Geissmann, 16 teams of observers from HWCC (including

staff of BNNR and other Hainan nature reserves), KFBG, the China Programme of Fauna & Flora International (FFI-China), Northeast Normal University and Kunming Institute of Zoology were stationed at different fixed locations throughout the gibbons' known range, recording all vocalizations, for the whole morning call period, over 11 days (14-24 October). A supplementary survey followed on 25-29 October with 13 teams covering the well-forested Qichadaling and Yajiadaling parts of the reserve's new extension area; here no gibbons were found. The methodology assumed all mature gibbons would call at least once during the 11-day main survey period (and probably during the 5-day supplementary survey), as suggested by previous experience with other gibbons (Chan *et al.*, 2005); it should be noted that gibbon calling frequency can be depressed in areas of high human disturbance (Johns, 1985; Nijman, 2001) or low gibbon density (Chivers, 1974). The survey detected the two known social groups (A and B), by then with six and five individuals respectively, and two lone males, comprising a total of 13 individuals (Geissmann & Chan, 2004; Fellowes & Chan, 2004; Chan *et al.*, 2005; Zhou *et al.*, 2005).

Immediately after the comprehensive survey a first Workshop to Conserve the Hainan Gibbon was convened at Bawangling, again at HWCC's initiation, and with coordination and partial funding from KFBG (Fellowes & Chan, 2004; Chan *et al.* 2005). Among those invited were past and present researchers, field personnel, representatives of other interested parties including FFI-China, East China Normal University (ECNU) and the Zoological Society of Paris (ZSP), and officials from the province, counties and townships. Following a series of scientific presentations, participants discussed various constraints, actual or possible, on population recovery, including poaching, habitat quality, age structure, sex ratio, social and genetic barriers. While not all of these could be addressed, participants agreed a series of priority actions required to conserve the gibbons: to continue and intensify monitoring of the gibbon population in BNNR; to reinforce patrolling effectiveness; to afforest degraded habitats in strategic locations with tree species valuable to gibbons; to understand the direct threats to gibbon survival; to locate

any additional gibbons surviving in Hainan; to instigate a visionary strategy of ecological restoration; to build the capacity of BNNR to conserve the Hainan Gibbon and the forest ecosystem; and to implement a publicity campaign. We report here on recent developments with respect to these priority actions and the species' status.

## 1. Population Monitoring

Although BNNR has been unable to secure core funding for scientific monitoring, it has provided personnel. From 2003 two BNNR staff were funded by ECNU-ZSP to monitor Group A, while FFI-China funded one of us (ZJ) to monitor Group B from December 2003, on completion of his postgraduate fieldwork, until November 2004. Monitoring work met with difficulties in funding; KFBG has since early 2005 been funding ZJ to carry out ecological research on the two groups, and since April 2005 funding eight reserve staff (in two rotating teams of two) to conduct daily monitoring of the two groups, which has continued to the present day (May 2007).

These intensive efforts show that the population of Hainan Gibbon has increased in the past five years. The two groups have produced newborn infants every two years, as previously noted (Liu *et al.*, 1989), while a single individual departed from each group during the same

period (Table 1). These changes brought the composition of Groups A and B to nine and six individuals respectively.

A constant problem in monitoring the Hainan Gibbon has been the elusiveness of solitary individuals, and the difficulty of observing more than one group on the same date to exclude the possibility of double-counting. The 2003 survey detected two solitary males by call, but they were not seen (Geissmann & Chan, 2004). Sightings are rare even by the field staff who patrol the gibbon range daily. At present the field staff believe there could be up to five solitary individuals: up to three adult-sized black individuals (with two confirmed as males by their male solo calls) and up to two adult females. Not all, however, have been seen this year. There is also the possibility that individual group members occasionally stray further from their groups than usual, and so some doubt remains over these. If all five of these individuals are indeed solitary and are still alive, the population would appear to have reached 20 individuals.

In spite the normal birth rate, no new social group has been formed since 2000. Survival outside the natal group was suspected to be a limiting factor in 2003, but there remains a lack of evidence in this regard.

**Table 1.** Size of each group, and of extra-group population, 2002-2007.

	Initial number	Additions	Losses	Final number
<b>Group A</b>				
2002	5	0	0	5
2003	5	1 born ~Oct	0	6
2004	6	0	0	6
2005	6	2 born ~Apr	1	7
2006	7	0	0	7
2007	7	2 born ~Mar	0	9
<b>Group B</b>				
2002	4	1 born ~Nov	0	5
2003	5	0	0	5
2004	5	1 born ~Dec	1	5
2005	5	0	0	5
2006	5	0	0	5
2007	5	1 born ~Jan	0	6
<b>Solitary population</b>				
2002-2007	2-3	2	0-5	0-5
<b>Overall</b>				
2007				15-20

The recorded home ranges of Group A and Group B are 987ha and 548ha respectively; higher than the previously recorded ranges of 100 to 500ha (Liu *et al.*, 1989; Liu & Tan, 1990). These figures are also higher than those for most *Nomascus* (40-500ha) and far higher than those for other gibbons (20-40ha) (Chan *et al.*, 2005), although some past figures are minimum estimates. It is uncertain whether the large home ranges at Bawangling are a sign of suboptimal habitat or expansion due to lack of neighboring groups, or else a natural trait of the species. Larger groups have both higher nutritional needs and, presumably, greater competitive ability; during our observations since 2005, when the population of Group A increased to seven individuals they temporarily invaded one-third of the territory of Group B during the dry season of 2006 when food sources were scarce.

## 2. Patrolling Effectiveness

In 2003 opinions differed on the likelihood of poaching. It certainly occurred in the past at Bawangling; the Tropical Exposition Museum of Hainan of Hainan Forestry Department holds two gibbon specimens hunted by a local villager in 1984. There were rumors of gibbons being hunted in 2000 and 2003, but these were difficult to validate due to the high penalties attached to hunting such Class I Nationally Protected species. No systematic hunting of gibbons has occurred in recent years, as there has been little if any hunting mortality, but even occasional incidental losses would be significant. Reliable patrolling is therefore key, and is taken seriously by BNNR staff, who arrest hunters and destroy illegal camping shelters in the forest. To assist in these efforts KFBG bought a patrolling vehicle and a fine-scale satellite map of the gibbon range, and is producing a map with the detailed trail system and other landscape features. Since the introduction of the daily monitoring system, hunting in the core forest has decreased, with a much higher frequency of observing "game animals" such as the Black Giant Squirrel *Ratufa bicolor*, Hainan Giant Flying Squirrel *Petaurista hainanus*, and Wild Boar *Sus scrofa*, and much less frequent evidence of hunting including gunshots and gin traps.

Morale of patrol staff is a consideration, since monitoring and patrolling teams are obliged to stay in difficult conditions in the forest. Since 2003 KFBG has undertaken various interventions to boost morale, with the erection of permanent forest base camps for the monitoring teams, the introduction of a reserve logo for identity, field clothing, binoculars, long-zoom cameras, GPSs and other necessary field gear.

## 3. Forest Restoration in Areas Adjacent to the Core Gibbon range

The current area of continuous primary forest available to the gibbons is just 1,600ha, occurring between 800m and 1,280m asl, higher than the typical elevation of primary lowland rainforest in Hainan and with lower diversity and abundance of gibbon food plants. The monitoring revealed gibbon groups frequently forage in narrow strips of suitable lowland-forest habitat adjacent to degraded shrublands and monoculture pine plantations, indicating a shortage of food sources in the "safer" upland forest habitats. The 2003 workshop identified habitat extent and quality as a major potential constraint on the number of gibbons, and gibbon groups, that could be supported at Bawangling. Consequently BNNR agreed to stop the harvesting of resin in these pine plantations, and restore them to mixed native forest suitable for gibbons. A gibbon habitat restoration project with funding and technical support from KFBG was launched in early 2004; reserve staff, in particular members of the gibbon monitoring team, were asked to collect seeds of gibbon food plants from the forest, and a native tree nursery was established. In 2005-2006 a total of 32 species, the majority (>90%) of which were gibbon food trees, were planted: 14,410 saplings over 16ha in the rainy season of 2005 and 27,561 saplings over 57ha in 2006. Major gibbon food plants which grow relatively rapidly (e.g. *Acmena acuminatissima*, *Endospermum chinensis*, *Syzygium cumini*, *Bischofia javanica*, *Elaeocarpus dubius*, *Caryota ochlandra*, *Canarium album* and *Nephelium topengii*) were planted in especially large numbers, along with other fast-growing species to ensure a usable forest canopy within 10-15 years; the pine canopy has been progressively

thinned by ring-barking. Initial progress of the young trees has been encouraging, with only minor damage (by Sambar *Cervus unicolor* and Wild Boar) and over 80% survival after 2 years. BNNR has also planted some areas using similar techniques, with government funding. Further planting is planned for c. 100ha with c. 80,000 saplings, funded by KFBG and the Gibbon Conservation Alliance, and additional planting of other degraded areas is planned by BNNR pending funds from national and provincial sources. BNNR is monitoring the saplings planted, with KFBG's support.

#### 4. Understanding Direct Threats

Local human activity was an obvious potential threat to gibbon survival. In 2003-2004 a preliminary participatory rural appraisal (PRA) study in ten neighboring villages was conducted by FFI-China and Hainan Environmental Ecological Education Centre (HEEEEC). The appraisal revealed some conflict in land use between the reserve and neighboring communities. Hunting, collecting fuelwood, logging to provide coffins and building-materials, grazing and agricultural encroachment in lowland regenerating forest were all potentially inimical to gibbon conservation and forest biodiversity conservation. FFI subsequently organized two workshops attended by ten local community leaders, from villages closest to the gibbon territories, along with other local, provincial and national officials, and KFBG. Some residents reported perceptions different from those of reserve staff regarding reserve boundaries and laws, indicating a need for greater engagement (FFI China Programme, 2005; Gao, 2006).

Since 2003 scientific studies have been funded by KFBG to understand the food availability to the gibbons, by a better understanding of the ecology of food plants. To date 80 food plants have been detected (Lin *et al.*, 2006a & 2006b). Food availability is particularly likely to be a constraint between February and April when a limited number of the major foods are available, and less favored foods are eaten in larger quantities. Plant community quadrats and phenological studies showed the higher-altitude

home range of Group A has a less diverse and less abundant food plant community in comparison with that of Group B (Lin Jiayi, pers. comm., August 2006), which may explain its larger home range, and the temporary intrusion into Group B's territory during the winter lean season in 2006. This was taken into account during the habitat restoration actions above.

#### 5. Surveys for Additional Populations

In 2003 there were rumors of gibbons being heard or seen at several other sites in Hainan. Following concerted investigations by HWCC and KFBG, only two sites yielded consistent reports: the unprotected forest at Exianling to the southwest of Bawangling, and the extensive forest in the newly-established Yinggeling Nature Reserve to the southeast. A series of general surveys at Yinggeling were unable to confirm these reports, as was a specialized survey of Exianling between 13 and 19 March 2007 by HWCC and KFBG. While further work is under way at Yinggeling, it must be considered doubtful that any additional populations survive.

#### 6. Landscape-Level Restoration

It was clear in 2003 that if the Hainan Gibbon population was to recover from its current low level and restricted range to long-term viability, a larger-scale habitat restoration and *in-situ* population management would be required. Satellite maps of different scales were obtained by FFI and KFBG. In addition KFBG has supported two relevant doctoral research projects: an analysis of habitat selection and potential distribution of Hainan Gibbons, by a student of the Kunming Institute of Zoology, Yunnan Province; and a study on the phenology and ecology of the gibbons' main food plants by a student from South China Agricultural University, Guangdong Province. These studies are nearing completion at the time of writing, and will be useful references in future conservation and restoration work.

#### 7. Capacity Building of BNNR for Conservation

There has been gradual improvement in the capacity of BNNR in recent years. Through regular involvement KFBG has been able to



help identify gaps in the capacity of the nature reserve and support improvements (e.g. in species identification, survey and monitoring techniques, ecological and conservation concepts). In addition FFI conducted a training-needs assessment at Bawangling early in 2004, confirming the need for capacity building in many aspects of nature reserve management. New reserve management since 2005 has been directed by HWCC to give heightened attention to conserving the gibbons and their habitat; positive steps include the replacement of a road from Dongliu to Bawangling Town with a new one further from the core area, an enlarged team of reserve wardens, and new warden substations surrounding the forest. Perhaps most encouragingly, the field staff show increased signs of initiative and engagement in the work of gibbon conservation relative to 2003. There remains scope for improvements in various core competences.

## 8. Publicity and Community Engagement

Attempts to engage the local human population in gibbon conservation have included a scheme by Seacology, with the Zoological Society of Shanghai, ZSP and HEEEC, awarding scholarships to poor children in four villages to the south of the gibbon range in exchange for agreements to protect the forest and the gibbons (Anon., 2007); work by FFI and the Unicorn Foundation in Qingsong township, Baisha County to the east, including teacher training and the co-management workshops reported above; school-based activities with the distribution of Hainan Gibbon-themed calendars and fai chun (Chinese New Year posters) in various neighboring communities by KFBG; and donation of Hainan Gibbon T-shirts to local stakeholders by FFI. While no evaluation of the impact of this work has been done, it is likely that local communities have a more informed attitude toward the gibbons and the reserve than hitherto, although some of the community engagement work has suffered from unstable funding. In terms of raising visitor awareness, BNNR has begun discussions to establish an education centre, which has the potential to give visitors a better insight into the world of gibbons, their habitat and their plight.

Wider public awareness of the gibbons in Hainan has been fostered by HWCC and KFBG through close collaboration with the media including the Hainan Daily newspaper (whose readers also received the fai chun in 2005); HWCC also arranged the distribution of Hainan Gibbon calendars by volunteers in various Hainan cities. In 2005 KFBG, BNNR, the University of Hong Kong and Hainan Normal University organized university students from Hainan and Hong Kong to participate in a tree-planting activity to commemorate the commencement of the gibbon habitat restoration project (Lam *et al.*, 2005). Meanwhile international recognition is promoted through continued inclusion of the species in the "25 most endangered primates" list of Conservation International and the IUCN Primate Specialist Group (Mootnick *et al.*, 2005 and in press).

## OVERALL OUTLOOK

Although the Hainan Gibbon still breeds at natural birth intervals, the population has struggled to increase since the establishment of Bawangling Nature Reserve in 1980, after 27 years of forest protection by law. The various proactive conservation measures reported here are only a beginnings. Controlling illegal activity remains a challenge, and there is no room for complacency regarding basic protection. Habitat quality is still a concern, and it will be some 15 years before the initial success of the present restoration effort is known. There are also concerns regarding population viability, including social constraints on reproduction and group formation (with most surviving individuals suspected to be genetically related, and mate choice highly restricted), fertility (with at least one female apparently too old to give birth), genetic constraints, and the unconfirmed possibility of a male-skewed birth sex ratio (Liu *et al.*, 1989). These complex influences indicate the need for open scientific discussion of conservation options, and a second Hainan Gibbon conservation workshop is provisionally planned for late 2007. In the meantime the priorities remain strict protection of the gibbons and their recovering habitat, along with sustained positive engagement with local communities.

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## RELATIVE POPULATION DENSITY OF *Tarsius diana*e IN MAN-INFLUENCED HABITATS OF LORE LINDU NATIONAL PARK, CENTRAL SULAWESI, INDONESIA

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### ABSTRACT

The aim of this study was to know the impact of human activities on population density of *Tarsius diana*e by estimating the relative population density in four habitat types differently influenced by man. The study was conducted in the vicinity of Kamarora, at the northeastern boundary of Lore Lindu National Park, Central Sulawesi. Four different habitats were chosen: (H1) primary or old secondary forest; (H2) secondary forest  $\pm$  30 years after clearance with small-scale selective logging; (H3) forest with interspersed small coffee and cocoa plantations; and (H4) forest with selective logging and plantations. The tarsiers' sleeping sites were determined using triangulation. Relative population density was estimated by measuring the average distances between three nearest neighbors. The results suggest that different human-influenced habitat have different effects on tarsier's density. The smallest distances ( $116.2 \pm 18$  m) between sleeping sites, which represent the highest estimated population density (57.1 groups in one square km), were found in habitat type H1, the least disturbed habitat. Estimated population density in habitat type H3 or "forest plantations" was 38 groups/km<sup>2</sup>, followed by habitat type H2 or secondary forest with selective logging 36.4 groups/km<sup>2</sup>, and the smallest population density was estimated at 32.9 groups/km<sup>2</sup> in habitat type H4 or forest with selective logging and plantations.

**Keywords:** population density, *Tarsius diana*e, sleeping trees, *Ficus* spp., Sulawesi.

### INTRODUCTION

Eight species of tarsiers are currently recognized, six of which are endemic to Sulawesi and surrounding islands. These are: Dian's tarsier *Tarsius diana*e Niemitz *et al.*, 1991 (or perhaps synonymous *T. dentatus* Miller & Hollister, 1921 according to Brandon-Jones *et al.*, 2004), endemic to lower mountains of Central Sulawesi; the Pygmy tarsier *T. pumilus* Miller & Hollister, 1921, which is found in the high mountains in Central Sulawesi; Spectral Tarsier *T. spectrum* Pallas, 1778 (or perhaps the correct name for this species is *T. tarsier* Erxleben 1777 as mentioned by Brandon-Jones *et al.*, 2004 and Merker & Groves, 2006), endemic to lowlands, with the type locality in northern Sulawesi; Sangihe Island tarsier *T. sangirensis* Meyer, 1896, endemic to the Sangir-Talaud; Peleng Island tarsier *T. pelengensis* Sody, 1949; and the last described Lariang tarsier *T. lariang* Merker & Groves, 2006. The two species not found in Sulawesi are Philippine Tarsier *T. syrichta* Linnaeus, 1758 which live in the southern islands of the Philippines, and Western Tarsier *T.*

*bancanus* Horsfield, 1821 in Borneo, Bangka, Belitung, and southern parts of Sumatra. (Musser & Dagosto, 1987; Niemitz *et al.*, 1991; Tremble *et al.*, 1993; Groves, 1998; Supriatna & Wahyono, 2000).

*T. diana*e was described as distinct from other tarsier species morphologically, anatomically, and in vocalizations, by Niemitz *et al.* (1991). This species might be the predominant species in Central Sulawesi, based on vocalizations (Nietsch, 1999).

Like many other primates, tarsiers are arboreal and use trees and plants as feeding sites or for sleeping sites. The sleeping sites of tarsiers vary with habitat type. In primary forest, tarsiers are often found to sleep in tree cavities, especially of strangler figs (*Ficus* spp.). In secondary vegetation, tarsiers can sleep in tree cavities, bamboo stands, or shrubs (MacKinnon & MacKinnon, 1980; Tremble *et al.*, 1993; Leksono *et al.*, 1997). Gursky (1998) found *Ficus caulocarpa* was often used by Spectral tarsier as a sleeping tree in Tangkoko-Batuangus Nature Reserve.

*Tarsius diana* suffers the risk of population decline as primary rain forest in the island is converted into plantations and transmigration settlements. Sulawesi has lost over 20% of its forest cover during 1985–1997, which is 1,890 km<sup>2</sup> per year (Holmes, 2002). Forest clearance even occurs inside conservation areas, including Lore Lindu National Park in Central Sulawesi. Besides coffee and cocoa plantations, exploitation of woods, bamboo and rattan, are one of the most common human activities in Sulawesi.

Bynum (1999) identified some variables to detect and monitor the influence of human activities on lowland rain forest in Lore Lindu NP. Those variables, i.e. the number of stumps, exotic trees, large trees and rattan, are very useful to determine and monitor disturbances in lowland forest in Lore Lindu NP.

Although extinction risk of *Tarsius diana* is not high at the moment (IUCN category Lower Risk/conservation-dependent), increased human activities in Lore Lindu National Park may present a serious threat to this endemic species. While there have been many field studies about tarsiers (e.g. MacKinnon & MacKinnon, 1980; Crompton & Andau, 1986, 1987; Musser & Dagosto, 1987; Niemitz *et al.*, 1991; Tremble *et al.*, 1993; Gursky, 1998; Nietsch, 1999; Merker & Muehlenberg, 2000; Merker *et al.*, 2004 & 2005; Merker, 2006), information on the possible impacts of human activities on tarsier density is needed for a conservation program to be effective. Only MacKinnon & MacKinnon (1980), Merker & Muehlenberg (2000), and Merker *et al.* (2004 & 2005) showed that there can be a different population density in different habitats.

The main objectives in this study were to estimate the relative population density of *Tarsius diana* in four habitat types with different levels of human activities in Lore Lindu National Park. This information is needed to assess the conservation status and conservation action of the genus *Tarsius*.

## MATERIALS AND METHODS

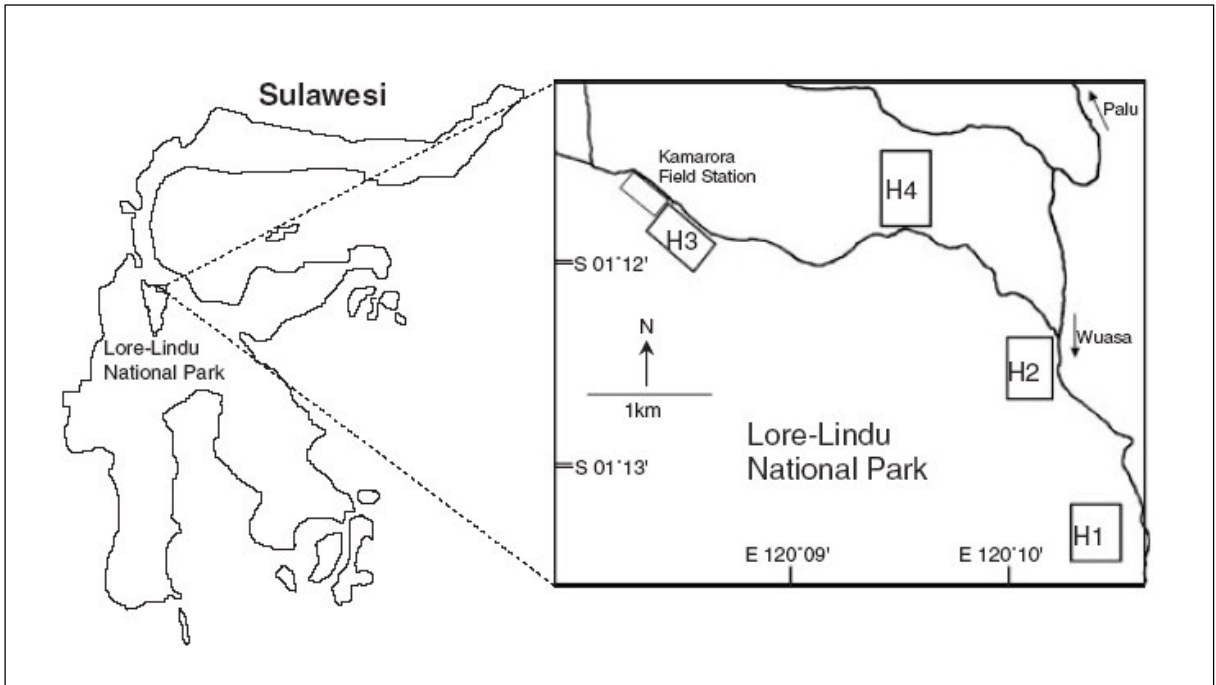
The study was conducted in the Kamarora field station, situated at approximately 700–900 m above sea level (asl) at the northeastern boundary of Lore Lindu National Park, Central

Sulawesi, about 70 km southeast of Palu (Figure 1). Kamarora is the type locality of *T. diana*. Data collection was carried out from February to June 2001. Four forest patches, each 10–20 ha, were selected as sampling sites. Those sites were: (H1) pristine forest or the “least disturbed forest”; (H2) secondary forest with small-scale selective logging, or “slightly disturbed forest”; (H3) secondary forest interspersed with several small (0.2–0.5 ha) old coffee and cocoa plantations, or “moderately disturbed forest”; and (H4) forest with either logging and intensive plantations still occurring or “heavily disturbed forest”.

To localize tarsier sleeping trees in a given area, we recorded all audible duet songs the animals performed every morning around dawn. By triangulating (Kenward, 1987; Muehlenberg, 1993) the positions of the call sources and multiple repetition of this procedure, tarsiers could be traced to their sleeping sites. Once all the sleeping sites in each habitat were known, mapping all sleeping sites in the investigated areas and measuring the distances between the groups resulted in relative population density estimates (Brockelman & Ali, 1987). To ensure their confinement to one specific habitat, only groups living in the center of each forest patch were considered. We assumed that one sleeping site represent one groups. The average distance of three nearest neighbors was used to estimate the range size of one group and then to estimate the population density in a given area (detail method in Merker, 2003 and Merker *et al.*, 2004).

Sixteen randomly-selected point samples were evaluated to assess the level of human disturbance in each habitat (Bynum, 1999; Merker *et al.*, 2004 & 2005). At each point, a 10 m x 10 m quadrat was made, and we measured the number of stumps, exotic trees, and trees of diameter at breast height > 50 cm. The disturbance parameters were determined in each and then integrated into a disturbance index (described in Merker, 2003 and Merker *et al.*, 2004) to compare the relative intensities of anthropogenic influence.

To determine the extent to which food abundance and locomotor support affect the distribution of tarsier, the availability of insects



**Figure 1.** Map of Sulawesi and location of the study plots along the northeastern boundary of Lore Lindu National Park (after Merker, 2006).

and substrate density were also assessed. At eight sample points in each habitat type, the abundance of insects eaten by Sulawesi tarsiers (Nietsch, 1993; Tremble *et al.*, 1993) was estimated using two methods (Merker, 2003; Merker *et al.*, 2004): (1) to count moths, winged termites and mantids, a Petromax kerosene pressure lantern was fastened at 1.5 m in a small tree, and during the subsequent 5 min all visible insects with body length >1 cm attracted by the light were counted; by differentiating the animals by species, size and location it was possible to avoid repeat counts of single specimens. (2) to count grasshoppers, crickets and cicadas, insects vocalizing within a 10 m radius around the sample point within 5 min were counted; repeated counts were avoided by noting specific vocalization patterns and the locations of individuals. At each of the eight sample points three replicate counts, each on a different evening, were made over 19.00-21.00.

Based on the results of the habitat-use analysis by Crompton & Andau (1986 & 1987) and Merker (1999 & 2003), the branch/trunk/sapling trees with a diameter between 1-4 cm were measured, in vertical and horizontal orientation, to analyse the substrate for locomotion

abundance. The sampling method used PCQ, with 16 randomly placed points in each habitat.

All data sets were tested for normality using the Kolmogorov-Smirnov test. For normally distributed data, ANOVA and the Least Significant Difference Post Hoc Test were used, and the non-parametric Median Test was used to detect significant differences between habitats. Spearman's  $r_s$  was used to test correlations between parameters. All tests are two-tailed. (Sokal and Rohlf, 1995)

## RESULTS

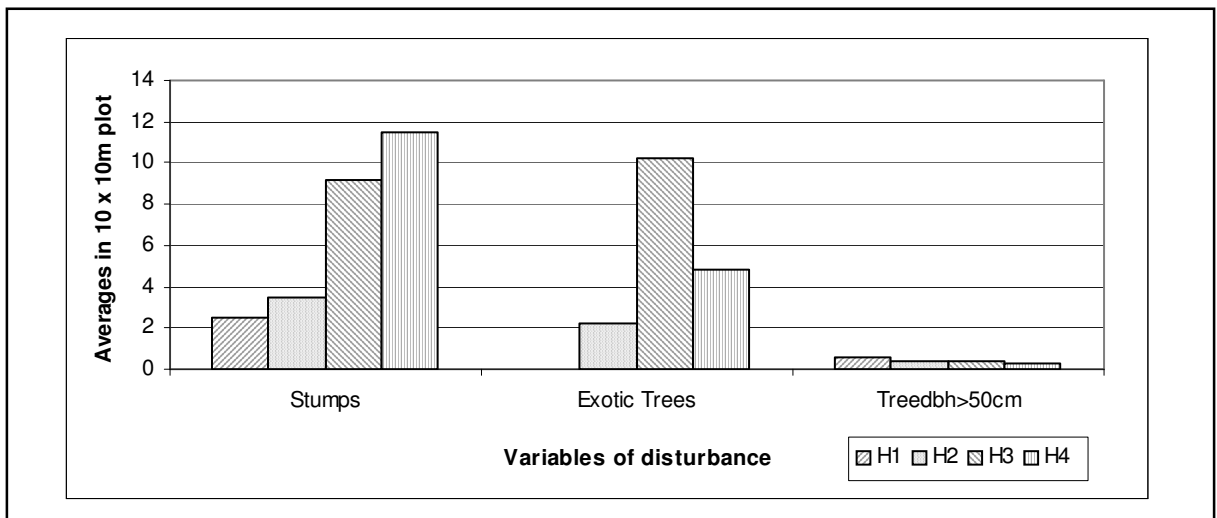
Observed groups of *Tarsius diana* always came back to their initial sleeping site each morning. However, there is a tendency that tarsier moved to alternate sleeping sites when disturbed. Average distance to three nearest neighbors and estimated population densities are shown in Table 1. The smallest distances to nearest neighbors ( $116.2 \pm 18.0$  m,  $n = 9$  groups), which is calculated and estimated to the highest density  $57.1$  groups/km<sup>2</sup>, were found in H1, the least disturbed habitat. Meanwhile, in habitat where both logging and plantations still occurred (H4), distances between sleeping sites was the greatest ( $152.9 \pm 7.1$  m,  $n = 9$  groups), giving the estimated tarsier population

**Table 1.** The average distances between three nearest neighbors, relative population density of *Tarsius diana*, number of stumps, insects density and locomotor support abundance in four different habitats at Lore Lindu National Park, Central Sulawesi.

	Habitat type			
	H1 (primary forest)	H2 (second forest with small scale logging)	H3 (second forest with small-scale interspersed plantations)	H4 (second forest with logging and plantations)
Average distances to three nearest neighbors $\pm$ SD (m)	116.2 $\pm$ 18.0	145.5 $\pm$ 13.7	142.4 $\pm$ 11.2	152.9 $\pm$ 7.1
No. of groups observed (n)	9	10	10	9
Population density $\pm$ SD (groups/km <sup>2</sup> )	57.1 $\pm$ 21.5	36.4 $\pm$ 6.8	38.0 $\pm$ 5.8	32.9 $\pm$ 3.1
Average stumps density per 100 m <sup>2</sup>	2.5 $\pm$ 4.4	3.5 $\pm$ 4.3	9.2 $\pm$ 10.3	11.5 $\pm$ 3.1
Average insects density $\pm$ SD (ind./5 min./point)	11.6 $\pm$ 1.7	12.1 $\pm$ 1.9	13.5 $\pm$ 2.6	10.3 $\pm$ 2.4
Locomotor support abundance $\pm$ SD (substrates per 100 m <sup>2</sup> )	32.7 $\pm$ 4.2	115.0 $\pm$ 14.6	67.2 $\pm$ 8.5	77.8 $\pm$ 9.9

density as small as 32.9 groups/km<sup>2</sup>. An intermediate number of groups were found where both secondary forest with small-scale logging (H2) and secondary forest with old plantations (H3) were present. Table 1 also shows the disturbances (represented by average number of stumps per 100 m<sup>2</sup> - while the other disturbance parameters is shown in Figure 2),

insect abundance (individuals/5 minute/sampling-point), and locomotors supports density (substrates per 100 m<sup>2</sup>) in each habitat. The average distance between the sleeping site of a group and the sleeping sites of its three nearest neighbouring groups differed significantly between studied habitat type (ANOVA,  $F_{3,19}=3.76$ ,  $P<0.05$ ).



**Legend explanation:** H1 = least disturbed forest; H2 = slightly disturbed forest; H3 = moderately disturbed forest; H4 = heavily disturbed forest.

**Figure 2.** The average of some human activities variables in four habitat types at Lore Lindu National Park, Central Sulawesi.

There was no significant difference between habitat types in the number of insects (ANOVA,  $P > 0.05$ ). The results indicate relative population density was lower where stump density was higher (Spearman rank correlation  $r_s = 0.8$ ). Population density was positively related to the abundance of insects but the relations were weak (Spearman rank correlation  $r_s = 0.4$ ).

## DISCUSSION

Different habitat types, with different human activities, had different relative population densities of *T. diana*, although no replicates were performed in each type so the differences recorded cannot be firmly attributed to habitat type. Primary forest, the least disturbed habitat, had the highest relative population density (Table 1). The relative population density of tarsier in primary forest (57.1 groups/km<sup>2</sup>) was significantly greater compare to each other habitat types that are influenced by human land-use (ANOVA,  $P < 0.05$ ). There are no significant differences between tarsier population densities in the secondary forest with small-scale logging (H2) or secondary forest with small-scale interspersed plantations (H3) and secondary forest with both logging and plantations (H4).

Some studies mentioned that *Tarsius bancanus* was commonly found in secondary forest (i.e. Fogden, 1974; Niemitz, 1984). MacKinnon & MacKinnon (1980) also found that in Tangkoko-Batuangus, the population density at sea level of *Tarsius spectrum* in shrubby forest (10 indiv./ha) was greater than in primary forest at 1,000 m asl (only 5 indiv./ha). However, Gursky (1998) reported that *T. spectrum* were more abundant in the conservation area in Tangkoko-Batuangus (lowland rainforest, sea level).

Merker & Muehlenberg (2000) also found that forest with small-scale interspersed plantations had higher estimated population density of tarsier than other habitat types. Ganzhorn (1987) reported that population density of lemur species in Madagascar tended to be higher in old plantations than other habitat types, but not as high as in primary forest. Our research found that relative population density of *Tarsius diana* in primary forest was

higher than other habitat types. Unfortunately, considering the differences in methodology and specific site, it was not possible to make a direct comparison between this study and the other studies.

If we compare to the results in the year 1998 (Merker and Muehlenberg, 2000), there are a decreasing tendency on population density of *T. diana* in Lore Lindu National Park. Merker and Muehlenberg (2000) reported that the lowest population density was 5.6 groups/10 ha, which is similar to the highest number in our results 57.1 groups/km<sup>2</sup>. Indeed, there is no information about forest loss or change to other human land-use especially between years 1998 to 2001. Merker *et al.* (2004) showed a subsequent decline in *T. diana* population densities and suggested that human activities affect the population and survival of *T. diana* in Lore Lindu NP. Merker *et al.* (2005) mentioned that population density decreased with increasing anthropogenic influences. They also stated that focusing solely on population density, primary forest is the most important habitat for tarsier conservation. As mentioned by Merker *et al.* (2005), it is not clear what causes the lower abundance of tarsiers in the slightly disturbed habitats (H2) even though resources are plentiful. One possible reason may be the high susceptibility of these animals to visual and acoustic disturbance in their environment (Merker & Muehlenberg, 2000).

Conservation strategy for this unique and endemic species should consider the differences of human activities. Tarsiers can adapt to traditional land uses such as small-scale plantations or selective logging (Merker & Muehlenberg, 2000; Yustian, 2007). Slight disturbance may open up the forest canopy and result in a greater heterogeneity of the forest and subsequently a higher arthropod diversity and density. Insect abundance was found to be highest in the slightly disturbed habitats, H2 and H3, and lowest in the mixed-species plantation H4. The increased prey density in H2 and H3 may balance the adverse effects of selective logging and acoustic disturbance at these sites (Merker & Muehlenberg, 2000). More research is needed to study the role of small-scale plantations as the support habitat for tarsiers.



## CONCLUSIONS

Different habitats with different level of human activities were found to have different relative population densities of *T. diana*. The highest relative density was in primary forest, the least disturbed habitat ( $57.1 \pm 21.5$  groups/km<sup>2</sup>), followed by relatively more disturbed habitats: forest with small-scale old plantations ( $38.0 \pm 5.8$  groups/km<sup>2</sup>), secondary forest ( $36.4 \pm 6.8$  groups/km<sup>2</sup>), and forest with both logging and plantations ( $32.9 \pm 3.1$  groups/km<sup>2</sup>).

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## REDISCOVERY OF THE CRITICALLY ENDANGERED EASTERN BLACK CRESTED GIBBON *Nomascus nasutus* (HYLOBATIDAE) IN CHINA, WITH PRELIMINARY NOTES ON POPULATION SIZE, ECOLOGY AND CONSERVATION STATUS

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### ABSTRACT

The Critically Endangered Eastern Black Crested Gibbon *Nomascus nasutus* is rediscovered in China. Acoustic records and direct observation confirmed gibbon occurrence in the Bangliang Limestone Forest of Jingxi County in Guangxi Zhuang Autonomous Region of southwest China, along the Sino-Vietnam border. We recorded a total of 19 gibbon groups including three family groups plus a solitary male over a five-day survey; all groups had two females in adult pelage and carried infants, and at least one group was bigynous with both adult females carrying infants. Vocalizations were concentrated in the early hours from dawn till 07:00, especially immediately after sunrise (71% of total song bouts). The average length of a song bout was 22.4 minutes (n = 14 song bouts), and a single song bout comprised up to 11 female great calls. The Bangliang Limestone Forest constitutes about one-third of a contiguous transboundary limestone forest block, and two of the three groups were observed traveling between China and Vietnam, making collaborative transboundary conservation imperative. Priority conservation actions to preserve this population should focus on (1) establishing a protected area in Bangliang Limestone Forest; (2) forming a close working relationship with Vietnamese counterparts; (3) strengthening the enforcement of forest and wildlife protection regulations inside the gibbon forest; and (4) restoring degraded areas inside and around the gibbon forest to accommodate expected population expansion. Very little is known about the ecology of this species; a better understanding of its life with studies on its social, breeding and feeding ecology should be a priority.

**Keywords:** *Nomascus nasutus*, Eastern Black Crested Gibbon, China, Guangxi, survey, population, ecology, vocalization, conservation.

### INTRODUCTION

The crested gibbons of the genus *Nomascus* are restricted to moist forest of Indochina, and are amongst the least studied of the Hylobatidae (Preuschoft *et al.*, 1984). Their taxonomy is still in a state of flux. From north to south they include the Western Black Crested Gibbon *N. concolor*; the *nasutus* group, east of the Red River; the Northern White-cheeked Gibbon *N. leucogenys*; Yellow-cheeked Gibbon *N. gabriellae*. A fifth taxon, known as the Southern White-cheeked Gibbon *N. leucogenys siki* (or *N. siki*), intermediate between *N. leucogenys* and *N. gabriellae* in both distribution and several morphological/vocal/molecular characters, has been suggested to be of hybrid origin (see discussions in Brandon-Jones *et al.*, 2004; Groves, 2004; Roos, 2004). The occurrence of possible additional taxa within the *leucogenys-*

*gabriellae* complex has recently been suggested (e.g. Konrad & Geissmann, 2006; Ruppell, 2007), but more work has to be done to resolve the confusion.

Indochina has a long history of human settlement and forest-based warfare; thus many range countries of *Nomascus* gibbons are subjected to intense pressures from habitat loss and poaching. As such, all recognized crested gibbon taxa are globally threatened. The status of the world's gibbon species was reassessed during a recent Asian Primate Red List workshop held in Cambodia (Geissmann, 2007); while *N. hainanus* and *N. nasutus* are unequivocally recognized as the two most threatened living apes (and both Critically Endangered), *N. concolor* and *N. leucogenys* are also categorized as Critically Endangered, and *N. gabriellae* as Endangered.

## 1. Background of the Eastern Black Crested Gibbon *Nomascus nasutus*

Classification of the nasutus group of crested gibbons has been problematic and dynamic (Fooden *et al.*, 1987; Geissmann, 1989; Geissmann *et al.*, 2000; Geissmann *et al.* 2003; Nadler, 2003; Brandon-Jones *et al.*, 2004; Groves, 2004; Roos, 2004). Contemporary primatologists agree there are at least two extant nasutus gibbon taxa. The Hainan Gibbon (*N. nasutus hainanus*) is endemic to the island of Hainan, with a single population of less than 20 individuals (Geissmann & Chan, 2004; Chan *et al.*, 2005; Wu *et al.*, 2005; Zhou *et al.*, 2005); and work in progress suggest it merits species status. This would mean the Eastern Black Crested Gibbon (*N. n. nasutus*), which ranged at least from southwest Guangxi Zhuang Autonomous Region (hereafter Guangxi Province) in China to northeast Vietnam (Dao, 1983; Fooden *et al.*, 1987; Geissmann *et al.*, 2000), would also be a distinct species. Experts participated in the 2006 Asian Primate Red List workshop already recognized the two as distinct species, namely *N. hainanus* and *N. nasutus*.

Gibbons were believed exterminated in Guangxi since the 1950s (Tan, 1985). In Vietnam it was also feared extinct until scientists from Fauna & Flora International (FFI) rediscovered a population in the limestone forest of Phong Nam-Ngoc Khe Communes in the northernmost Trung Khanh District, Cao Bang Province, northeast Vietnam along the border with Guangxi Province, China. At least five gibbon groups with 26 individuals were counted in a survey conducted in August 2002 (Geissmann *et al.*, 2002 & 2003), and 37 individuals in eight groups were recorded in September 2004 (Trinh Dinh Hoang, 2004).

The first author and colleagues visited Trung Khanh to study the gibbons in October 2005 (Chan & Ng, 2006), and observed that the gibbons occur very close to the China border. An investigation of the area's satellite maps indicated that the forest extends across the border into Jingxi County, Guangxi Province. Shortly afterwards we started interview surveys to search for possible gibbon occurrence around the area in China. In May 2006, we received reports from locals of the Bangliang-Daxing

Villages in Jingxi County that gibbons still exist in a limestone forest area. At around the same time, research groups from FFI-China and Guangxi University visited the site; while they were unable to observe the gibbons, the latter group heard the gibbon calls, but it was not clear whether the gibbons they heard were within Chinese territory. With enough reliable information at hand, we conducted a survey in September 2006 to collect first-hand field data.

## 2. Survey Area

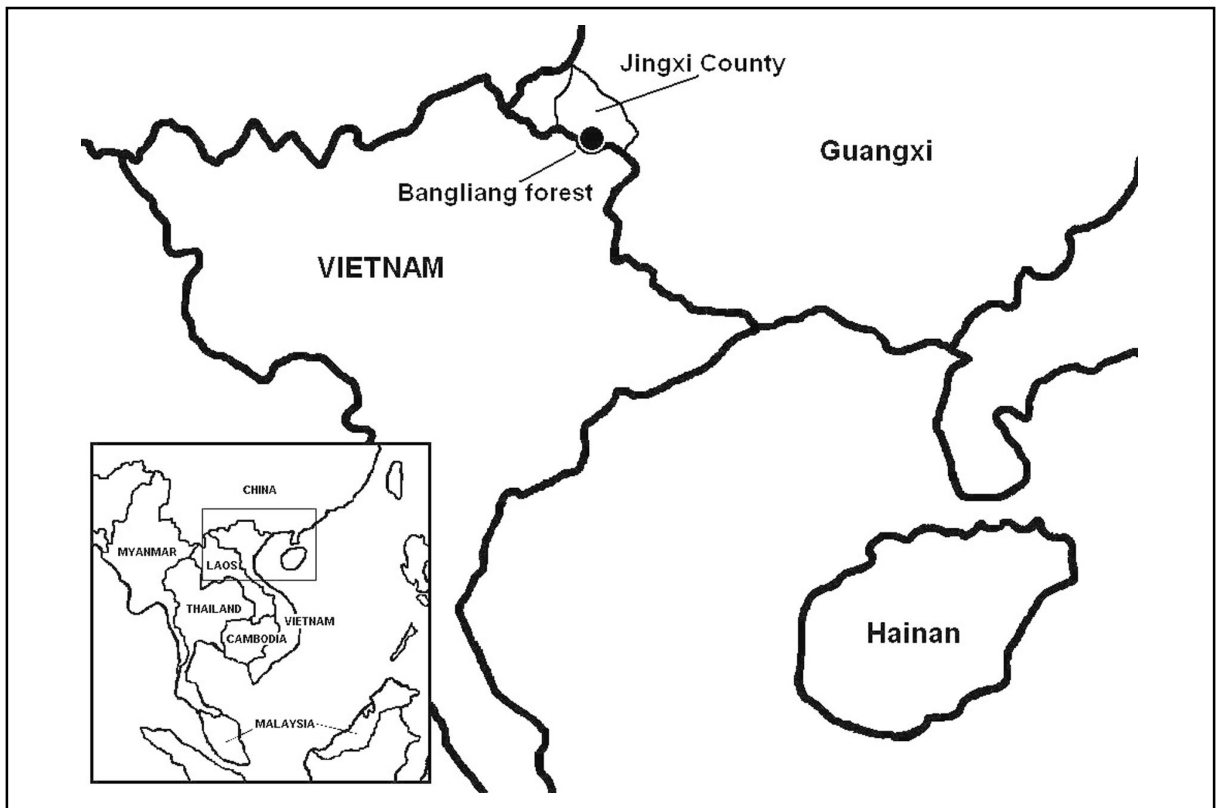
The Sino-Vietnam border area shared by Guangxi and the eastern part of Yunnan Province of China, and Cao Bang, Lang Son and Ha Giang Provinces of Vietnam, is dominated by karst limestone formations, which support one of the largest and most intact tropical karst mountain forest ecosystems in the world, but much of the forest in this area has been severely degraded by uncontrolled logging and agricultural encroachment, particularly in the 1980s-1990s. The zonal vegetation of the region is tropical limestone monsoon rainforest. About twenty years ago, the forest in southwest Guangxi was dominated by trees 25-35 m in height, dominant species including *Excentrodendron hsienmu*, *Cephalomappa sinensis*, *Garcinia paucinervis*, *Parashorea sinensis* and *Deutzianthus tonkinensis* (Su *et al.*, 1988). Vertical zonation of the plant community is not obvious due to the relatively low altitudinal range in the area. The region has a seasonal northern tropical monsoon climate with distinct wet (May to September) and dry (October to April) seasons. Annual mean temperature is c. 23°C, and the annual average rainfall is c. 1,500mm.

The survey area is the northern portion of a larger limestone forest complex shared by Jingxi County of Guangxi Province, China and Trung Khanh District of Cao Bang Province, Vietnam. The area has a picturesque karst landscape consisting of densely packed steep limestone outcrops interspersed with lowland depressions (called "Nong" or "Lung", by the same Long ethnic subgroup of the Zhuang minority in China and Vietnam, respectively), with an altitude range of c. 500-930 m asl. The forest block runs in a northwest-southeast direction, with two rivers acting as a natural boundary, merging where the forest ends in

Vietnam. The forest measures c. 12km at its longest and c. 4km at its widest, with a total area of c. 48km<sup>2</sup>; c. 18km<sup>2</sup> of it is within Chinese territory and the rest is on Vietnamese soil. Our survey area is surrounded by the Bangliang and Daxing Villages, to the west and east respectively (hereinafter Bangliang Limestone Forest; 22°55'N 106°30'E). Almost all valuable timber trees (e.g. *Excentrodendron hsienmu*) in the Bangliang Limestone Forest have been extracted and most other large trees were felled for charcoal production, therefore very little pristine forest remains. The current vegetation consists mainly of evergreen broadleaf forest with an average canopy height of less than 18m and a dbh of no more than 40cm, and climbing vines (e.g. *Tetrastigma planicaule* and *Gnetum hainanense*), sometimes covering extensive areas, are prominent elements in the area's flora. Fortunately, some of the gibbons' food plants (e.g. *Ficus* spp. and *Choerospondias axillaris*) are of little economic value, thus larger trees (up to 25m tall) can be found scattered around the area. Figure 1 illustrates the geographic location of the Bangliang Limestone Forest.

## METHODS

We conducted reconnaissance visits of the gibbon forest from June to August 2006, to understand gibbon ranging patterns and to determine the best observation posts. A full gibbon survey, with participants from Guangxi, Hainan and Guangdong provinces, was conducted between 11 and 15 September 2006, using primarily the fixed-point call-based method (e.g. Brockelman and Ali, 1987; Brockelman and Srikosamatara, 1993). Data collected during the morning auditory survey basically followed Geissmann *et al.* (2007). A total of 33 personnel were involved in the field survey; eight teams of two were posted at pre-selected vantage points on peaks or ridges roughly 500 m apart, and the type and timing of all gibbon vocalizations were recorded from predawn (i.e. c. 06:00) to 11:00 on five consecutive mornings. All eight teams had at least one member with prior experience (mainly with the closely related Hainan Gibbon) in surveying wild gibbons. An additional six teams, with basic training on identifying gibbons and their calls prior to the survey, conducted various biodiversity studies



**Figure 1.** Geographic location of the Bangliang Limestone Forest, Guangxi Province, China.

**Table 1.** Gibbon observation events in the present survey. Group composition: AM = Adult male; AF = Adult female; SJ = Subadult/juvenile; IN = Infant. Coordinates were recorded using hand-held GPS.

Date	Duration	Distance between observers and gibbons (m)	Group composition (number in each age-sex class)	Coordinates
11-Sept	17:45-17:49	300	AM(1), AF(1), SJ(2), IN(2)	--
12-Sept	10:06-10:26	200	AM(1), AF(1), IN(1)	22°55'23.0" N, 106°29'52.9" E
12-Sept	10:11-11:13	400	AM(1), AF(1), SJ(2)	22°55'43.8" N, 106°30'16.3" E
13-Sept	08:06-08:28	150	AM?(1)	22°55'15.4" N, 106°30'47.9" E
14-Sept	06:45-07:01	150	AM(1), AF(2), SJ(3), IN(2)	22°55'48.3" N, 106°30'11.7" E
14-Sept	07:02-07:24	800	AM(1), AF(2), SJ(3), IN(2)	22°55'46.3" N, 106°30'13.8" E
14-Sept	07:15-07:45	200	AM(1), AF(2), SJ(3), IN(1)	22°55'15.4" N, 106°30'47.9" E
14-Sept	15:30-16:20	70	AM(1), AF(2), SJ(3), IN(2)	22°56'02.6" N, 106°30'15.4" E
15-Sept	08:30-08:43	300	AM(1), AF(2), SJ(3), IN(2)	22°56'02.6" N, 106°30'15.4" E
15-Sept	10:05-10:21	50	AM(1), AF(2), SJ(3), IN(1)	22°55'14.6" N, 106°30'49.3" E

in the same vicinity, covering a larger distance within the forest each morning, and they collected gibbon sighting/vocal data whenever gibbons were detected. All team members also conducted fieldwork each afternoon within the gibbon forest during the survey period, and therefore our survey covered the full active hours of gibbons. A one-day training, based on materials collected during the Trung Khanh visit by the first author, on gibbon recognition and vocalization was delivered to all participants, and standardized record sheets provided prior to the survey to ensure accuracy of data collected. Minimum population size was estimated by triangulation of vocalization data and confirmed with direct observations. Behavioral data was collected by the more experienced team members during morning fixed-point survey or opportunistic encounters.

## RESULTS

During the survey period, the weather was cool (nighttime temperature down to 13°C) but sunny. The time of dawn was around 06:15, and time of sunrise was around 06:30. A total of 14 song bouts were heard (excluding the uncertain and extremely brief solo male calls), and gibbons were observed directly on ten occasions (Table 1). Based on locations of observation/vocalization, overlapping song bouts, and group composition, three groups, including one group with eight individuals, one group with seven, and a group consisting of a pair and their single infant, plus a solitary male, could be confirmed, making a total of 19 individual gibbons (Table 2).

### 1. Vocalizations

A male solo song from far away (>1km) was heard for eight minutes between 17:20 and

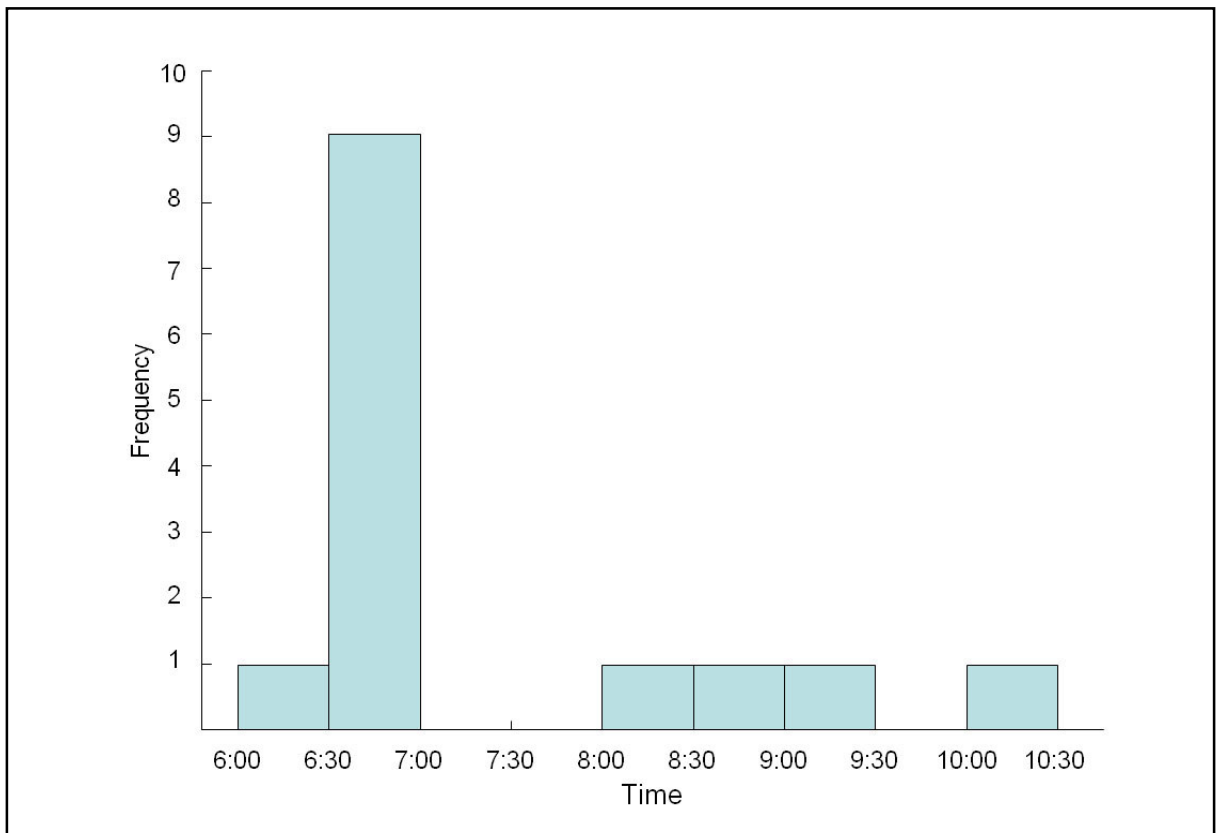
**Table 2.** Gibbon group composition recorded in the present survey.

	Group composition				Total no.
	Adult males	Adult females	Subadults/Juveniles	Infants	
Group A	1	2	3	1	7
Group B	1	1	--	1	3
Group C	1	2	3	2	8
<b>Total no.</b>	<b>3</b>	<b>5</b>	<b>6</b>	<b>4</b>	<b>18</b>

17:28 on 10 September 2006, but the timing was outside *Nomascus gibbons'* normal calling hours and the listeners were uncertain due to the long distance. There was a solitary male emitting an extremely brief solo call on two days (11 and 14 September), lasting for only one minute each time. These records are not included for the following analysis.

A total of 14 song bouts were heard during the survey period. The gibbon groups produced morning songs on all days during the study period. We heard all three groups plus the solo male call on 14 September 2006, and only Group A was heard on 15 September. Daily song bout frequency ranged from one to four song bouts (12 September) per day. Vocalization started just after dawn; the earliest song bout started at 06:28 on 15 September, and the last one at 10:06 on 12 September, with a peak during the first 30 minutes after sunrise (71.4% of total song bouts,  $n = 14$ ). Figure 2 illustrates the frequency distribution of the start time of vocalization for *Nomascus nasutus* in Guangxi.

On some occasions, the male started a solo but the female was reluctant to contribute, and it took the adult male of Group A 14 minutes to induce the female to begin a great call on 14 September 2007. In comparison with the Hainan Gibbon, with which the survey team has much experience, the Eastern Black Crested Gibbons' elaborate song appears to be longer. Duet song bouts lasted for 22.4 minutes on average ( $n = 14$  song bouts), and varied from 9 minutes (Group C at 06:58-07:07 on 11 September) to 38 minutes (Group B at 09:12-09:50 on 12 September). The song duration is much longer than those documented for the *Nomascus "siki"* of Bach Ma National Park, which averaged seven minutes (Geissmann *et al.*, 2007), and those of Phong Nha-Ke Bang National Park, which averaged c. 13 minutes (Ruppell, 2007). Females participated in the duet more frequently in comparison to the Hainan Gibbon (Bosco Chan, unpubl. data), with a maximum of 11 great-call phrases recorded during a single song bout of Group C, on 12 September 2006 between 06:50-07:11.



**Figure 2.** Frequency distribution of the start time of vocalization for *Nomascus n. nasutus* in Guangxi Province, China ( $n = 14$  song bouts). First arrow at bottom is the average time of dawn, second arrow the average time of sunrise.

As with other gibbons, e.g. the Hainan Gibbons (Bosco Chan, unpubl. data) and the Javan Gibbons (Haag, 2007), the whole family participated in a song with much shaking of branches and brachiating during the female great calls. This activity was video-taped during our survey.

## 2. Movement Pattern

Gibbon groups traveled between China and Vietnam, at least for Group A and Group C. On 14 September, Group C was observed by two different observation teams moving from China towards Vietnam in the early morning after their first vocalization of the day (06:45 by the first team, then 07:02 by the second team). The gibbon group moved out of sight from 07:24, but returned from Vietnam via the same route at 15:30 on the same day, feeding on a fruiting *Choerospondias axillaris*, and possibly spent the night on nearby sleeping trees. The same group was observed again at the same spot in the following morning at 08:30. Although Group A was only sighted from one observation post close to the international border inside Chinese territory, the same observation team heard it from as close as 200m (on 11 and 13 September) to as far as over 1km on the intervening date, which means the group is most likely to have a home range which transverses international boundary, but it cannot be confirmed due to the complex topography of its home range, and the uncertainty of where the actual international border lies. Our data suggest there are two peaks for feeding activity; one in the early morning after the first morning song, and another from 15:30 probably until the time they retreat into their sleeping trees.

## 3. Inter-group Interaction

It was interesting to observe neighboring gibbon groups interact when singing. Groups A and C have their territory boundaries in a lowland depression and when one group started calling, the other, if present in the same general area, would start singing soon and both groups approached each other while continuing to sing. On one occasion, Group C started singing close to their common boundary, and when Group A heard its neighbor singing close to its territory it started to sing from the other side of

the mountain and the whole group quickly climbed over the mountain, approaching Group C to a minimum distance of c.200 m. When Group C finished the song bout, Group A did not remain at the conflict zone for long and swiftly moved uphill, disappearing back to the other side of the mountain, where the group might have resumed feeding.

## 4. Sympatric Animals

At our study site a total of four primate species were present; we also observed the Rhesus Monkey *Macaca mulatta* and the Assam Macaque *Macaca assamensis* in the wild during our survey, and local people reported that the Francois's Leaf Monkey *Trachypitecus francoisi* also occurs in the region, but only on outcrops with cliffs and caves. Although some of the observation posts were situated in areas with such landscape features, we were unable to locate the leaf monkeys during our short survey. The survey team also recorded the Maritime Striped Squirrel *Tamiops maritimus*, Red-bellied Squirrel *Callosciurus erythraeus*, Black Giant Squirrel *Ratufa bicolor* and the Masked Palm Civet *Paguma larvata*, as well as droppings of a flying squirrel on tree branches. In addition, villagers reported the presence of Asian Palm Civet *Paradoxurus hermaphroditus* and a second species of flying squirrel. All the above are frugivorous/herbivorous and may compete with the gibbons for food. Two large mammalian predators, the Asiatic Black Bear *Ursus thibetanus* and the Leopard *Panthera pardus*, are reported to be present. Presence of the Asiatic Black Bear was confirmed by the survey team, with evidence of fresh claw marks and feeding signs. At the predawn period on 13 September 2006, one of our teams heard a big animal traveling on trees and breaking branches. A group of Rhesus Monkeys was seen with the first light from the source of the sound and the local guide suggested that it was a leopard hunting the troop of macaques.

## DISCUSSION

Based exclusively on interview results and remains of skins from animals reported to be hunted locally, gibbons were said to occur along the Sino-Vietnam border of Guangxi's Longzhou, Daxin, Jingxi and Napo counties in



the fairly recent past (e.g. up to the 1950s) (Tan, 1985; Zhang, 1997). As far as we are aware, however, there has been no definitive field-based scientific record of wild-living gibbons from Guangxi in modern times, and the source of the original claim (see Tan, 1985, followed by Fooden *et al.*, 1987 and subsequent workers) appears not substantiated. Our result is thus of considerable value to ascertain the distribution of gibbons in this province, making Guangxi the fourth province in China (after Hainan, Yunnan and Tibet) supporting extant gibbon populations. However, there is ambiguity in exactly where the international border lies at the local scale, and it is possible that our survey data includes some gibbon groups/individuals from Vietnam. This is, however, not the highest concern, as the gibbons are living on the international border and failure to protect the gibbons and their habitat from either side of the tiny forest block will effectively jeopardize the population as a whole.

Our survey covered an area of c. 6km<sup>2</sup>, and we found three groups of 18 individuals. Using a very crude calculation we estimated a density of 0.5 gibbon groups/km<sup>2</sup> within the existing habitat, which has been under considerable human disturbance. Our survey timing seems to have been very appropriate to survey for gibbons (at least for this species), as the gibbon groups were observed and produced morning songs on all days during the study period, and they were encountered opportunistically several times during afternoon transect walks, suggesting September may be a high-activity month for the gibbons. Unfortunately, because of the brevity of survey time, local topography and dense vegetation, we were unable to identify many of the gibbons' food plants, but they were seen feeding on fruits of *Ficus* spp., *Choerospondias axillaris* and various climbing vines. During our visit to Trung Khanh, we observed the gibbons feeding on the fruits of *Caryota urens* and *Bridelia fordii*.

## Conservation

Prior to our discovery, the Bangliang Limestone Forest was not considered a priority site for conservation, although the national logging ban on natural forest and rifle confiscation campaign appear to have curbed rampant

forest clearance and poaching. In fact it is still not a legally protected area and a formal management structure is still lacking, in spite of regular patrolling to combat illegal activities by the county authorities. During our survey, hunting did not seem to be a major issue as game animals such as civets, macaques and squirrels were still regularly seen, as were large predators such as the Asiatic Black Bear and (possibly) Leopard. In addition, we did not find any signs of hunting activity.

On the other hand, removal of large trees, mainly for illegal charcoal production, must be immediately stopped because the abundant food sources these few remaining large trees provide is vital for the continued survival of the gibbons. The gibbons at Bangliang appear to be breeding well and the population should increase should the gibbons and their habitat be saved from further human disturbances. This leads to another problem which must be tackled with some immediate actions, because of the time it takes to be effective; the size of suitable forest in the Bangliang Limestone Forest is limited and less than 10km<sup>2</sup> is currently of good enough quality to support gibbons - by our crude estimation, enough to support no more than five groups. The rest, at the periphery of the forest core surrounded by villages, is poor-quality shrubland subjected to regular human disturbances. Even within the small patch of good forest many of the lowland depressions have been severely logged, either as raw material of charcoal or as fuel for charcoal burning, and a rather large depression deep inside the gibbon range is currently under cultivation as maize fields. Straight after the five-day survey, part of our survey team did a reconnaissance visit in adjacent areas of limestone forest, which turned out to be interspersed by extensive fields, hundreds of households, village roads and a few highways, and the hillsides are at their most degraded stage (what limestone scientists term "rock desertification"). Because of the geology and landscape, karst habitats are fragile ecosystems and particularly susceptible to degradation. Forest restoration in these degraded areas, as well as better protection of the peripheral buffer zone, are thus important if the gibbons' future population is not to be limited by habitat

availability. Much can be learned in this regard from the reforestation experience around the Hainan Gibbon habitat at Bawangling, Hainan Island, which is a collaborative project of KFBG and Bawangling National Nature Reserve (Fellowes *et al.*, this volume).

In addition to the immediate surroundings of Bangliang, there are ten protected areas along the Sino-Vietnam border in Guangxi Province (Chunxiu, Defu, Diding, Dizhou, Encheng, Gulongshan, Laohutiao, Nonggang, Qinglongshan and Xialei), and eight of them (all except Defu and Diding) cover similar limestone ecosystems (although it is doubtful the Eastern Black Crested Gibbon is a limestone specialist). Most of these reserves are small and isolated from each other, and there is an urgent need to step up protection of these reserves and ensure habitat continuity between them. Bangliang Limestone Forest constitutes about one-third of a contiguous transboundary limestone forest block, and two of the three groups were observed traveling between China and Vietnam, making collaborative transboundary conservation imperative. It should be borne in mind that the thriving gibbon population on the Vietnamese side is "locked in" by the two branches of a large river flowing from China southwards into Vietnam, which effectively prevent the gibbons from expanding further into Vietnam. Thus the population, should it increase, could expand only into China, making the restoration of habitat there more crucial. Very little is known about the ecology of this species; for instance no formal study has been conducted on its feeding ecology, and we do not even have a food plant checklist for such a critically endangered species. A better understanding of its life, with studies on its ecology and social, breeding and foraging behavior should be a priority. Chan & Ng (2006) provided a preliminary checklist of its (potential) food plant species based on a quick survey at Trung Khanh, and work is in progress to more thoroughly describe the species' habitat characteristics and potential food plants based on botanical studies in both China and Vietnam (Ng & Chan, in prep.).

Priority conservation actions to preserve the apparently healthy population of the Eastern Black Crested Gibbon should focus on (1)

establishing a protected area in Bangliang Limestone Forest in the immediate future; (2) forming a close working relationship with Vietnamese counterparts, both at government and NGO levels; (3) strengthening the enforcement of forest and wildlife protection regulations in the gibbon forest and its buffer zone; and (4) restoring degraded areas inside and around the gibbon forest to accommodate expected population expansion.

## ACKNOWLEDGMENTS

We want to express our sincere thanks to those who participated in the September 2006 survey; the forest has no permanent water and everyone worked under extremely tough conditions. We are especially grateful to the participation of fieldworkers with prior gibbon survey experience from the Hainan Forestry Department and Hainan Bawangling National Nature Reserve. Logistical support was kindly provided by the Guangxi Forestry Department and the Jingxi Forestry Bureau. Ng Sai-chit kindly provided botanical information, Lee Kwok Shing and Anthony Wong assisted in data collation, and John Fellowes gave useful comments on the draft. FFI-Vietnam kindly guided us to observe the Cao Vit Gibbons in 2005. This work was co-funded by Guangxi Forestry Department and Kadoorie Farm & Botanic Garden.

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## IS *Presbytis* A DISTINCT MONOPHYLETIC GENUS: INFERENCE FROM MITOCHONDRIAL DNA SEQUENCES

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### ABSTRACT

We present a molecular study to examine whether the genus *Presbytis* is monophyletic and distinct from *Trachypithecus*. We sequenced 2300 base pairs of the mitochondrial ND3, ND4L, ND4 and associated tRNAs genes. Five species of *Presbytis* were used including *Presbytis melalophos*, *P. thomasi*, *P. comata*, *P. hosei*, and *P. rubicunda*. *Trachypithecus*, represented by *T. cristatus* and *T. obscurus* and *Nasalis larvatus*, *Pygathrix nemaues*, *Colobus guereza*, *Macaca nemestrina* and *M. fascicularis* were used as outgroups. Our interpretation based on character and distance analyses suggests that *Presbytis* forms its own monophyletic clade distinct from the genus *Trachypithecus*. Relative genetic distance and bootstrap support values from the mtDNA region further confirm the monophyly of *Presbytis*.

**Keywords:** *Presbytis*, *Trachypithecus*, mitochondrial DNA, monophyletic, molecular systematics.

### INTRODUCTION

At present, very little work has been done on the molecular systematics of Asian colobines. Because of this, Asian colobine systematics has been based on ecological, behavioral and morphological data (Oates *et al.*, 1994; Jablonski, 1998; Yan-Zhang *et al.*, 1993; Groves, 2001). Of the little molecular work that has been done, most of it has focused on *Trachypithecus* and some of the odd-nosed leaf monkeys (Rosenblum *et al.*, 1997; Wang *et al.*, 1997; Yaping & Ryder, 1998; Stewart & Disotell, 1998), rather than *Presbytis* itself. Therefore, phylogenetic relationships among the Asian leaf monkeys, particularly *Presbytis* and its relationship to *Trachypithecus*, are not well defined.

Many morphologists and ecologists do not agree on a common delimitation of species within the *Presbytis* group (Groves, 1989; Brandon-Jones, 1995). Formerly, *Semnopithecus* and *Trachypithecus* were grouped into *Presbytis* (Pocock, 1928; Napier, 1985; Wolfheim, 1983). Some Chinese primatologists agree with this arrangement (Peng *et al.*, 1988; Li, 1993). Hill

(1934) separated these groups from *Presbytis*, at the genus level, and Hooijer (1962) and Eudey (1987) subsequently agree with this assignment. However, Brandon-Jones (1984), Strasser and Delson (1987) and Delson (1994) recognize *Trachypithecus* as the subgenus of the *Semnopithecus*.

The separation of *Trachypithecus* from *Presbytis* has also been adopted by several other researchers (Nowak, 1991; Oates *et al.*, 1994; Brandon-Jones *et al.*, 2004). However, the variability in the use of the *Presbytis*-*Trachypithecus* clades and their presumed relationship to one another has produced taxonomic and phylogenetic confusion. For this reason, these taxa should be reanalyzed using other systematic approaches such as those provided by molecular analysis. In this study, we examined whether *Presbytis* is a monophyletic group distinct from *Trachypithecus*. This was done by using molecular techniques to determine whether gene sequences found in species of *Presbytis* are phylogenetically distinct from gene sequences found in representative species of the genus *Trachypithecus*, whose members

used to be categorized as members of the genus *Presbytis*.

A robust molecular systematic study should include a phylogenetic analysis of DNA sequences from mitochondrial DNA (mtDNA). Melnick *et al.* (1992) have summarized the uses of mtDNA in primate evolutionary studies. We selected the mitochondrial ND3, ND4L, ND4 genes and three tRNA genes flanking or separating them, because they have been shown in previous studies to resolve Asian primate phylogenetic relationships (Wang *et al.*, 1997; Evans *et al.*, 1999). Using mtDNA gene sequences with its own unique inheritance pattern offers the greatest opportunity to capture the phylogenetic information present in a group of species genetic material.

## METHODS

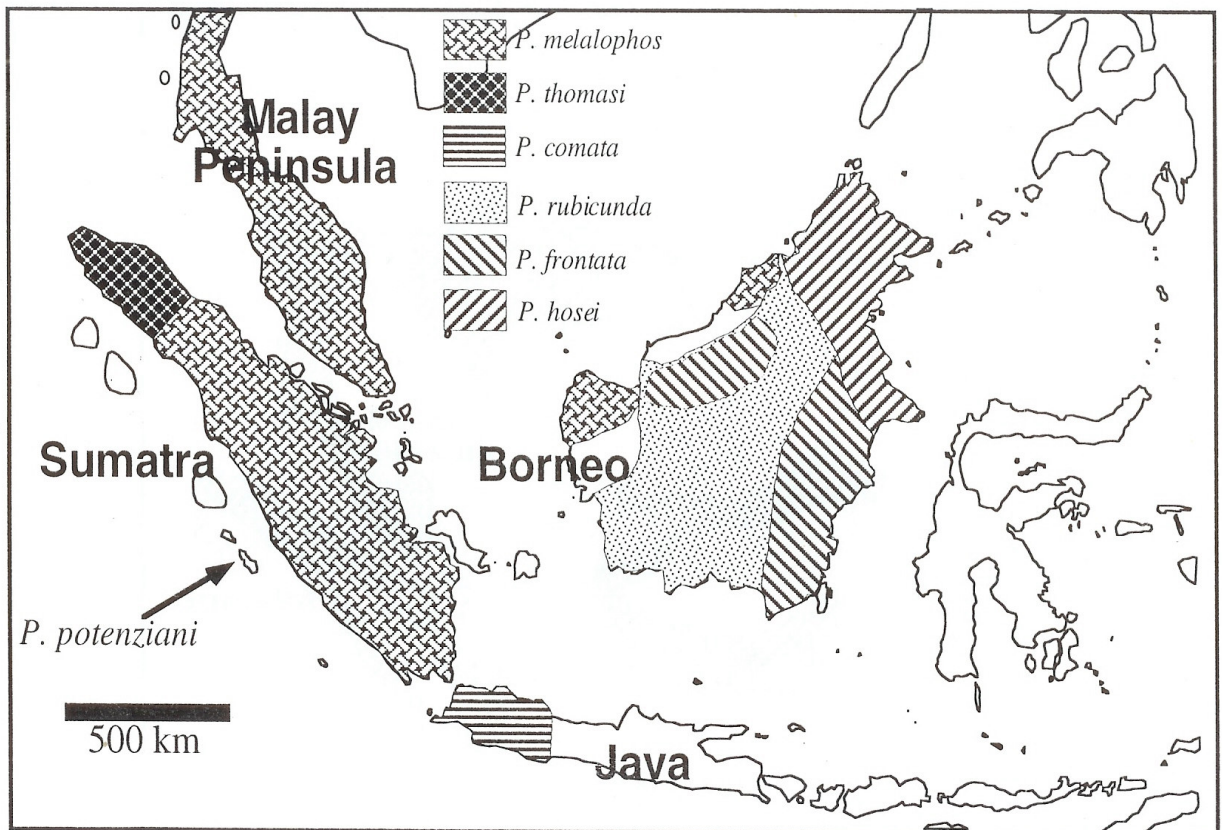
### 1. Samples

We used five species to represent the genus *Presbytis*: including *P. hosei*, *P. rubicunda*, *P. melalophos*, *P. thomasi* and *P. comata* (Figure 1). Five subspecies of *P. melalophos* were selected, including *P. m. femoralis*, *P. m. robinsoni*, *P. m.*

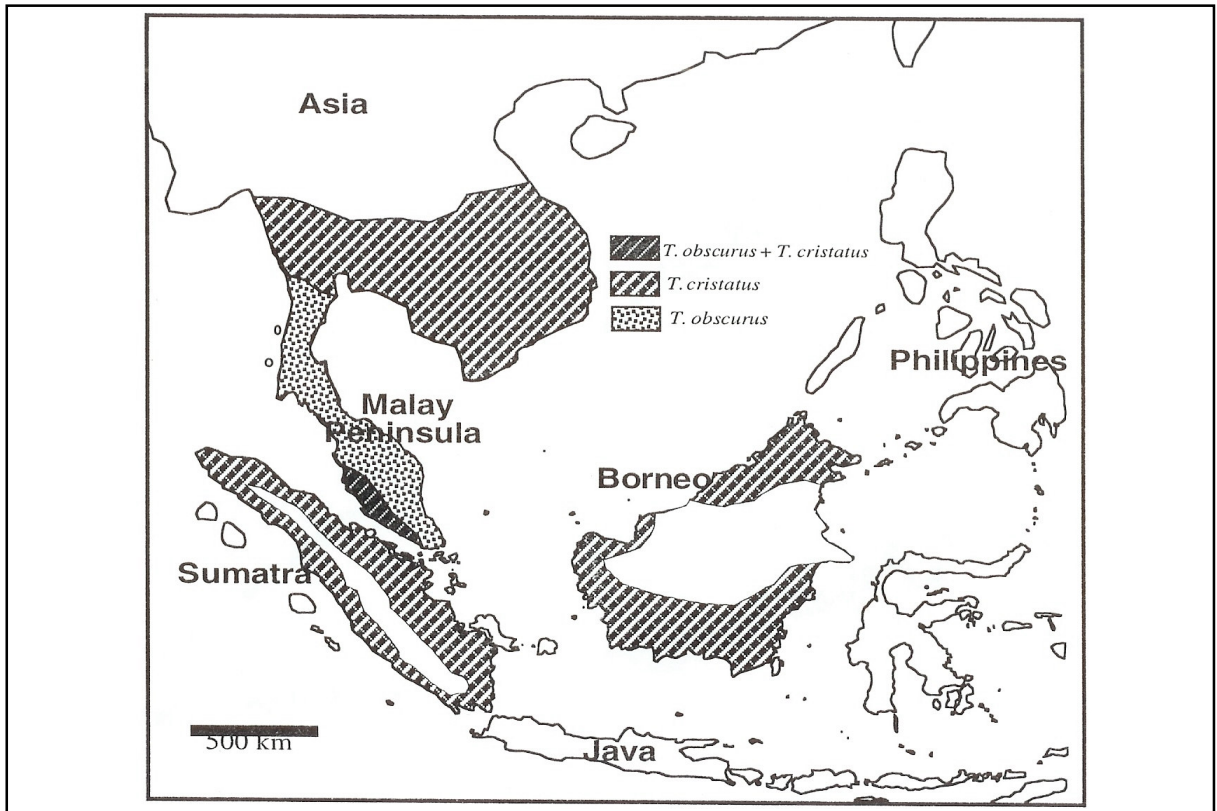
*siamensis*, *P. m. natunae* and *P. m. mitrata*. We used *T. cristatus* and *T. obscurus* as representatives of the genus *Trachypithecus*. *T. cristatus* has a narrow distribution on the Malay Peninsular and Central Thailand, but is more geographically widespread on Borneo, Sumatra and Indochina (Figure 2). The range of *T. obscurus* is more restricted, extending from the Isthmus of Kra to the Malay Peninsular. We also used *Nasalis larvatus*, *Pygathrix nemaeus*, *Colobus guereza*, *Macaca nemestrina* and *M. fascicularis* as outgroups in order to properly "root" the relationships between the two formerly congeneric groups. Details of genetic samples are in table 1.

### 2. DNA Sequencing

Total genomic DNA was extracted from tissue or blood using the Qiagen tissue kit with small modifications of standard blood and tissue procedures. We used highly specific primers (T-46PF and T-2409PR), developed by D. T. The, to amplify a segment of mitochondrial DNA spanning the tRNA<sup>glyf</sup>, ND3, tRNA<sup>arg</sup>, ND4L, ND4 and tRNA<sup>his</sup> genes.



**Figure 1.** Distribution of species of the genus *Presbytis* (based on Oates *et al.*, 1994).



**Figure 2.** Distribution of the *T. cristatus* and *T. obscurus* (based on Oates *et al.*, 1994).

**Table 1.** Details of genetic samples.

Taxon	Code	Origin
<i>P. melalophos siamensis</i>	BM24	Besut, Terengganu, Malaysia
<i>P. melalophos robinsoni</i>	BM33	Selama, Perak, Malaysia
<i>P. melalophos femoralis</i>	BM36	Kluang, Johor, Malaysia
<i>P. melalophos mitrata</i>	DM4630	Simpai, Sumatra, Indonesia
<i>P. melalophos natunae</i>	DM4609	Natuna Islands, Indonesia
<i>P. rubicunda</i>	Tawau	Tawau, Sabah, Malaysia
<i>P. thomasi</i>	DJ4626	North Sumatra, Indonesia
<i>P. comata</i>	DJ4572	West Java, Indonesia
<i>P. hosei</i>	BM67	Tawau, Sabah, Malaysia
<i>T. cristatus</i>	BM1B	Kuala Selangor, Malaysia
<i>T. cristatus</i>	BM1A	Kota Kuala Muda, Malaysia
<i>T. obscurus</i>	BM8	Sik, Kedah, Malaysia
<i>T. obscurus</i>	BM4B	Taiping, Perak, Malaysia
<i>T. obscurus</i>	BM5B	Kota Kuala Muda, Malaysia
<i>N. larvatus</i>	BM91	Bintagor, Sarawak, Malaysia
<i>N. larvatus</i>	BM93	Kuching, Sarawak, Malaysia
<i>N. larvatus</i>	BM94	Simunjan, Sarawak, Malaysia
<i>Py. nemaesus</i>	DJ9018	Cuc Puong Center, Vietnam
<i>Py. nemaesus</i>	No.2.the	Quang Nam, Vietnam
<i>C. guereza</i>	Cg	Kenya, Africa
<i>M. fascicularis</i>	DM9042	Hanoi, Vietnam
<i>M. nemestrina</i>	BM96	Kuching, Sarawak, Malaysia

**Table 2.** Oligonucleotide primer pair used in this study and their PCR conditions.

ND3, ND4L, ND4,tRNAs	Forward/Reverse Primer Sequences T-46PF (5'- CTT CCA ATT AGCTAGTTT CGATA-3') T-2409PR (5'-GCA TGG ATT AGC AGT CCTTGC AAG CT-3')
PCR conditions	Thermocycling parameters were 35 cycles of denaturing at 94°C (1 min), annealing at 56°C (1 min) and extension for 3 min at 72°C.

We carried out 50µl amplifications in a Perkin Elmer Model 480 thermal cycler. A sample of DNA was subjected to 35 cycles of amplification. Each PCR reaction contained 1.0 units of Taq DNA polymerase (Perkin Elmer), 20 pm/µl of each primer, 1µl of dNTPs, 8µl of Buffer A, and 0.5µl of DMSO. Table 2 lists conditions that were used to successfully amplify genes mtDNA region. We loaded our PCR products onto 1.5% agarose gels for electrophoresis. When amplifying the mitochondrial genome, we took precautions to reduce the possibility that our analysis would be affected by nuclear insertions of mtDNA pseudogenes. To do this, we followed the methods of Morales & Melnick (1998). First, our initial amplifications were of very long segments (>2kb). Second, we ran our PCR products in agarose gels and made sure that there was only a single bands we cut out the correctly sized band, which was consistently the strongest

amplification product before conducting subsequent amplifications or sequencing. Finally, our results from these steps resulted in mtDNA sequences congruent with the other studies of some of the same taxa for the same region (Wang *et al.*, 1997; Evans *et al.*, 1999).

Final PCR products were cleaned using the Qiagen PCR Purification Kit and made ready to proceed with cycle sequencing. We performed cycle sequencing with sets of internal primers (Table 3) and the Big-Dye sequencing kit (Perkin Elmer) using the protocols supplied by the manufacturers with the modification that all reaction volumes equal 9µl. We cleaned sequencing products of excess dyes with CentriSep Spin Columns (Princeton separations). We electrophoresed the sequencing products on a 4.25% polyacrylamide gel (19:1 Acryl/Bis gel stock, AMRESCO) and scored the results on an ABI 377 PRISM automated DNA sequencer (Perkin Elmer).

**Table 3.** List of internal primer sequences.

ND3-ND4	Sequence (5' to 3')
GLYF	ACT TCC AAT TAG CTA GTT T
ND4#1	CTT CTA ACA CTR ACC GCC TGA CT
NAP2M	GGA GCT TCA ACG TGG GCT TT
ARGREV2	TAG ATT ART ATG CCT AGG AGT G
ND4LM	CTA ATA TGC YTA GAA GGA ATA AT
ND4SREV2	AAG AAT TAT TTT TAG CATTG
NEWND4M	AAT ACC CCT ATA TGG YCT ACA CCT ATG
LEAF1	CCC TGA AGC TTY ACT GGC GCT AT
FORMREVLPRIM2	CTT CAR AAG GCT ATT AGT GG
	TAC ATG TAC ATT ACA ACC CAA CGA GG
T-577F	CTC ACT CCT GGG CAT ATT
T-729F	CTC ACT CCT GGG CAT ATT
T-1089F	CAG CAG TAG GCC TTG C
T-1465F	AAA GCC CAT GTT GAA GC
T-1731F	TGA AGCTTT ACT GGC GC
T-1798R	CGG CTG TGG GTT CGT TC
T-1210R	GCG TTG AGG CGT TCT GCT TG
T-827R	TGG AAA ATC ATG TTG TTG GT
T-304R	GTT GTT TGG AGG GCT CAT GG



### 3. Analyses

We identified the ambiguous flanking regions of each sequence and removed them from the data using the FACTURA program (ABI, Perkin Elmer). We overlaid all sequences of a particular gene using the AutoAssembler software and then aligned them by eye against the homologous regions in the human mtDNA and nuclear genomes (e.g. Anderson *et al.*, 1981).

We analyzed our data using two primary methods to discern phylogenetic relationships: maximum parsimony (MP) and neighbor joining (NJ). These analyses were conducted using PAUP version 4.0 (Swofford, 1999). For unweighted MP, we obtained trees by heuristic searches treating all nucleotide substitutions as unordered. Heuristic searches used random addition of sequences with ten replications. Our data were also subjected to bootstrap analysis with 2000 replications to assess the strength of support for any particular clade (Felsenstein, 1985). We further analyzed the mtDNA data using a weighted MP in accordance with the proportions (3:8:1) we calculated from transition and transversion (TI/TV) ratios in the first, second and third codon position using MacClade 3.0 (Maddison & Maddison, 1992). We also constructed trees using the NJ method by employing the Tajima and Nei distance option of PAUP. We quantified homoplasy using the consistency index (CI) and the homoplasy index (HI). We calculated TI/TV ratios using PAUP based on pairwise base differences.

## RESULTS

### 1. Sequence Variation

We excluded the sequences of tRNA<sup>gly</sup> and tRNA<sup>his</sup>, since these regions contained only a small highly conserved part of the fragment analyzed and only partial tRNA<sup>gly</sup> and tRNA<sup>his</sup> gene sequences were obtained. The complete DNA sequence for the ND3, tRNA<sup>arg</sup>, ND4L and ND4 contains 2080 base pairs. We combined sequences from these regions because these four loci are tightly linked to each other and the combination gave better phylogenetic resolution (Wang *et al.*, 1997). The following analyses are based on approximately 2.1Kb of DNA sequence.

We plotted numbers of transitional and transversional changes against uncorrected p-distances using Microsoft Excel. Our graphs show a linear relationship (Figure 3) implying that no saturation has occurred in these sequences. Table 4 compiles data on TI/TV ratios. Our results indicate that region of ND3, tRNA<sup>arg</sup>, ND4L and ND4 possess high ratio of TI/TV (5.67:1) as compared to nuclear regions (Md-Zain, 2001). Since our data sets include *Presbytis*, *Trachypithecus*, *Nasalis*, *Pygathrix*, *Colobus* and *Macaca*, these results indicate that the mtDNA region evolves more rapidly in these six genera compared to the autosomal and Y-chromosome regions (Md. Zain, 2001).

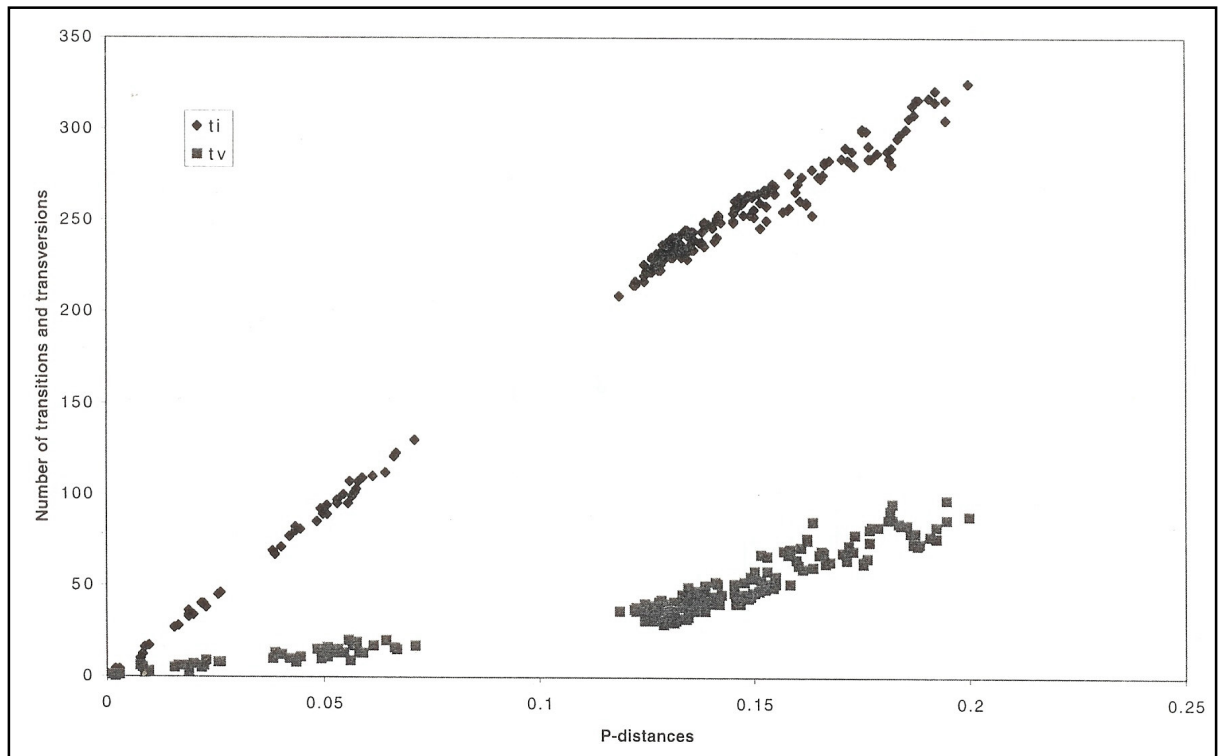
### 2. Phylogenetic Resolution

Figure 4 and Figure 5 show the tree topologies obtained from unweighted MP and NJ analyses of the complete mtDNA dataset. These two topologies are remarkably congruent with respect to the phylogenetic position of the *Presbytis* genus, its member species, and the outgroups *Trachypithecus*, *Nasalis* and *Pygathrix*. Unweighted MP analysis produced a single bootstrap tree (length=1773, CI=0.5324, HI=0.4676) with 100% bootstrap support for a single clade containing *P. hosei*, *P. rubicunda*, *P. comata*, *P. thomasi* and *P. melalophos*. *T. cristatus* and *T. obscurus* formed a separate monophyletic *Trachypithecus* clade, also with 100% bootstrap support. Weighted MP analysis (tree not shown) also supports the tree topology from unweighted MP in terms of the monophyletic position of the *Presbytis* species. Finally, *Nasalis* and *Pygathrix* also sorted to distinct monophyletic clades each with 100% bootstrap support. The NJ tree topology is somewhat more resolved than the MP tree, but it still supports a distinct monophyletic clade for the *Presbytis* species. We did not employ maximum likelihood analysis as NJ and MP analyses have already portrayed the distinct monophyletic clades with high bootstrap support.

## DISCUSSION

We have generated tree topologies from mtDNA region using character state and distance methods of analysis. All tree topologies agree that *Presbytis* species form a single





**Figure 3.** Plot of number of transitions and transversions vs. pairwise uncorrected p-distance from mtDNA data set.

monophyletic clade, distinct from the genus *Trachypithecus*. This distinction is strongly supported by 100% bootstrap values. Therefore, we argue these results strongly support the taxonomic arrangement of Oates *et al.* (1994), Groves (2001) and Brandon-Jones *et al.* (2004) in which *Presbytis* species are separated from *Trachypithecus* species with each being placed in their own distinct genus.

Besides looking at tree topologies and bootstrap values, support for a distinct monophyletic relationship among *Presbytis* species can also be derived from the Tajima and Nei distance matrix. Table 5 summarizes the

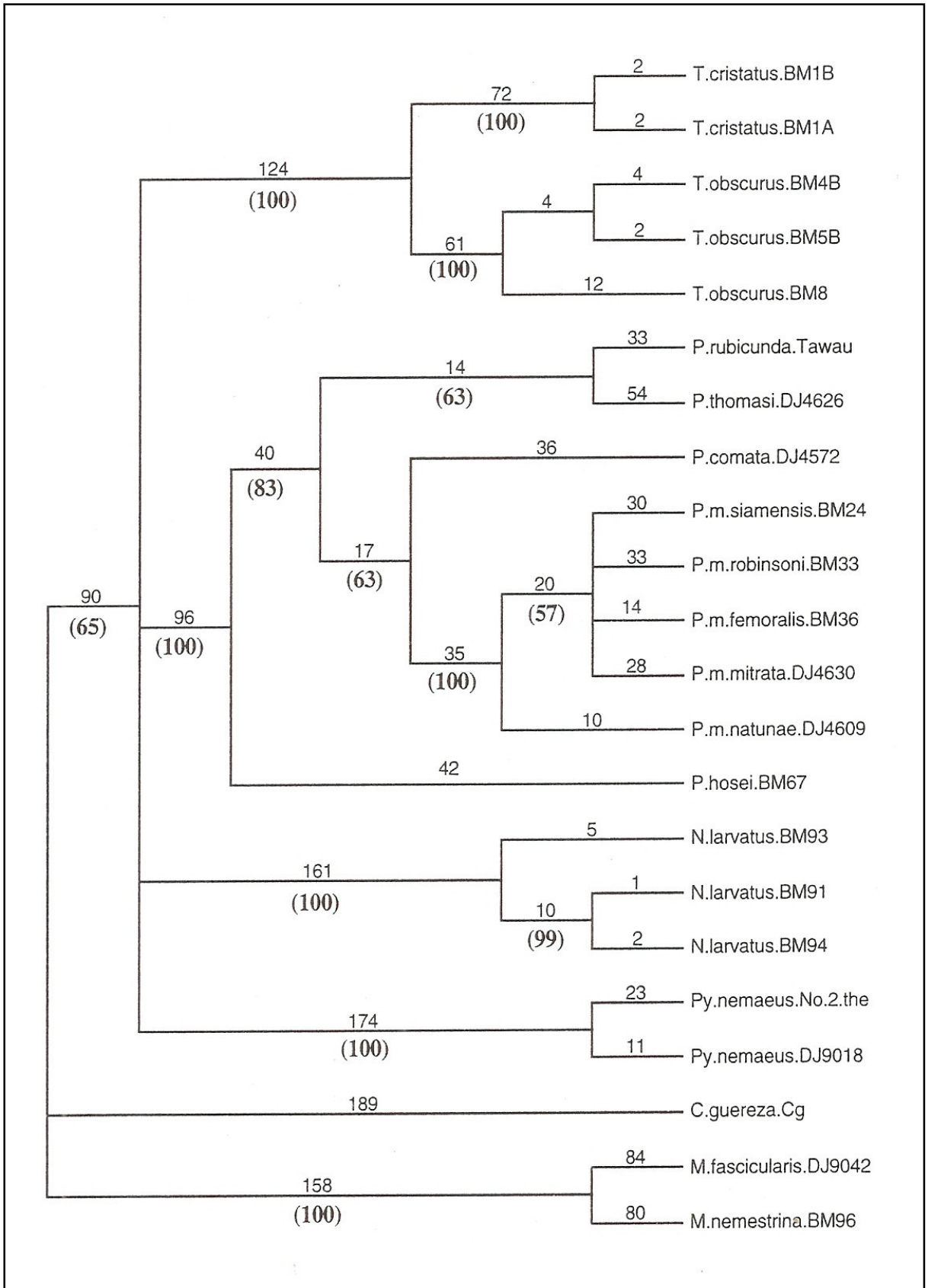
average percentage sequence divergence values calculated using Tajima and Nei's algorithm (Tajima & Nei, 1984) among *Presbytis* species, *Trachypithecus* species, *Nasalis* and *Pygathrix*, as well as between these groups. The average intra-generic genetic distance ranges from 4.50% in the *Presbytis* genus to 4.63% in the *Trachypithecus* genus. The average genetic distance between *Presbytis* and *Trachypithecus* species is 15.17%.

These results, while not conclusive, show that intergeneric differences between *Presbytis* and *Trachypithecus* are much greater than the interspecific differences in either genus: three

**Table 4.** Summary of variations along the sequences across taxa<sup>a</sup>.

	mtDNA
Total characters	2080
Constant characters	1276
Parsimony-uninformative characters	154
Parsimony-informative characters	650
% informative No. characters	31.25
Ratio TI/TV from pair wise base differences (PAUP)	5.67
Tree length	1773

<sup>a</sup> All gaps were excluded from analyses. The numbers of unambiguous transitions and transversions were generated from pairwise base differences using PAUP.



**Figure 4.** The maximum parsimony heuristic bootstrap tree. The bootstrap support values are shown below the branches of the parsimony tree.

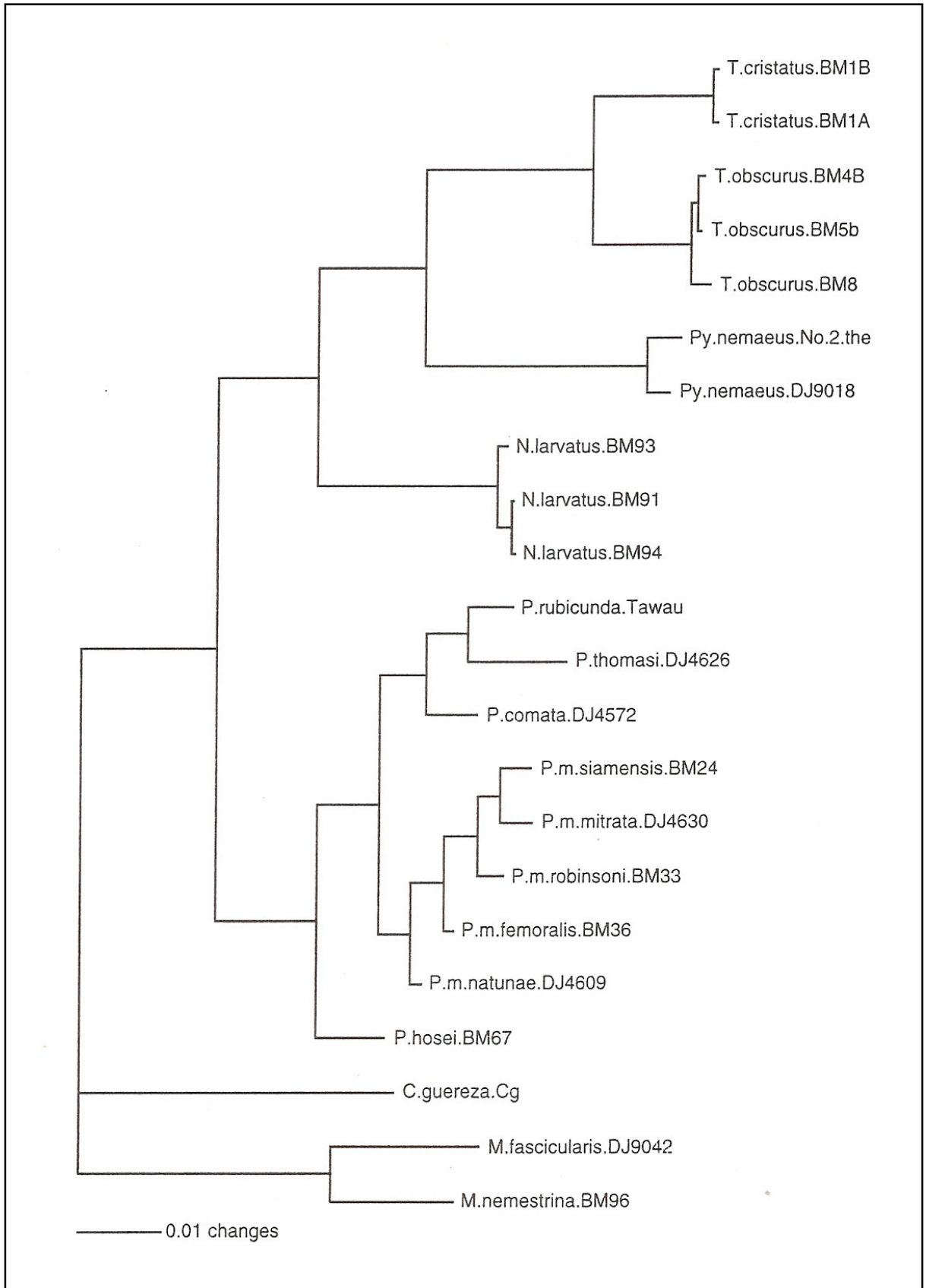


Figure 5. The neighbor-joining tree.

**Table 5.** Average percentage of genetic distance among and between *Presbytis*, *Trachypithecus*, *Nasalis*, and *Pygathrix* using the Tajima and Nei distance.

mtDNA	<i>Presbytis</i>	<i>Trachypithecus</i>	<i>Nasalis</i>	<i>Pygathrix</i>
<i>Presbytis</i>	4.496			
<i>Trachypithecus</i>	15.170	4.626		
<i>Nasalis</i>	14.790	16.770	-	
<i>Pygathrix</i>	16.571	18.087	18.378	-

times for mtDNA region. Data clearly show considerably greater genetic divergence between *Trachypithecus* and *Presbytis* than within either clade. Therefore, we strongly argue that these phenetic differences further validate the phylogenetic separation of *Trachypithecus* and *Presbytis* into two separate genera. In addition, the genetic distance values we obtain, for example between genus *Trachypithecus* and *Pygathrix* (18.1%), are in the range of those found by Wang *et al.* (1997) (e.g. 17.6%) using the same genes (ND3-ND4). Similarly, Rosenblum *et al.* (1997) found a genetic distance between *P. comata* and *T. cristatus* of 20.4%, while we found a distance of 15.5% between these same species at the same loci. These latter estimates are probably not significantly different given Rosenblum *et al.* (1997) used restriction site data as opposed to DNA sequence data to estimate genetic sequence divergence. Thus, we gain further confidence that our estimates of between-genus sequence divergence are accurate and the separation of *Presbytis* species and *Trachypithecus* species into two separate genera is well supported.

## CONCLUSION

Frequently, *Semnopithecus* and *Trachypithecus* have been grouped with *Presbytis* (Groves, 1970; Wolfheim, 1983) as one large heterogeneous genus. Some primatologists agree with this arrangement (Peng *et al.*, 1988; Li, 1993). However, Hill (1934) and Hooijer (1962) subdivided the genus *Presbytis* by elevating the subgenera *Semnopithecus* and *Trachypithecus*, to the generic level and retaining *Presbytis* for a distinct subset of species. Ecological, behavioral, and morphological data clearly support the separation of *Trachypithecus* from *Presbytis* (Hooijer, 1962; Weitzel & Groves, 1985; Oates *et al.*, 1994; Nowak, 1991; Groves, 2001; Brandon-Jones *et al.*, 2004). Our molecular data have

further corroborated this taxonomic distinction. DNA sequence data from mitochondrial region have distinguished *Trachypithecus* from *Presbytis*. Tree topologies from different kinds of phylogenetic analyses clearly indicate that *Presbytis* and *Trachypithecus* form their own distinct monophyletic clades. Bootstrap values strongly support the topologies obtained, which in turn support the phylogenetic hypothesis of two separate genera. Genetic distance patterns are also congruent with these results.

The weight of all evidence strongly supports the separation of *Presbytis* and *Trachypithecus* into two separate clades, and possibly genera. What now needs to be done is further work to define the molecular phylogenetic position of *Semnopithecus* with respect to the distinct *Presbytis* and *Trachypithecus* clades. Since *Semnopithecus* has been previously grouped with *Presbytis* (Pocock, 1928; Groves, 1970; Wolfheim, 1983) and some primatologists have placed *Trachypithecus* as a subgenus of *Semnopithecus* (Strasser & Delson, 1987; Delson, 1994; Brandon-Jones, 1996), it is of considerable interest as to where *Semnopithecus* fits in relation to these other Asian colobine genera. We, therefore, suggest that further molecular analysis be done to resolve this important, related phylogenetic issue.

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## DISTRIBUTION SURVEY OF BENGAL SLOW LORIS *Nycticebus bengalensis* IN TRIPURA, NORTHEASTERN INDIA

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### ABSTRACT

A survey of Bengal slow loris, *Nycticebus bengalensis*, was carried out in June to July 2007 at Thrisna and Sepahijala Wildlife Sanctuaries in Tripura, Northeastern India. This survey is intended to estimate distribution and population status of the Data Deficient Bengal slow loris based on IUCN Red List 2007. Using recce sampling method, the encounter rate is 0.22 individuals/km<sup>2</sup>, which falls within the range seen in Assam (0.03-0.33 individuals/km<sup>2</sup>). Most animals were detected at around 8-15 m in height at the interior of moist deciduous forest.

**Keywords:** Bengal slow loris, *Nycticebus bengalensis*, distribution and population surveys, Tripura-Northeastern India.

### INTRODUCTION

Bengal slow loris *Nycticebus bengalensis* has the largest geographic range among the slow lorises and has been recorded from the forests of northeastern India, Myanmar, Cambodia, southern China, Laos, northern Thailand, and Vietnam (Nekaris *et al.*, 2006). Groves (2001) recognized the Bengal slow loris as a distinct species, separate from *Nycticebus coucang*, which is distributed in Indonesia, Malaysia, and Thailand (Nekaris *et al.*, 2006). Bengal slow loris is listed under Schedule I of the Wildlife (Protection) Act of India, 1972 and classified as Data Deficient species in the IUCN Red List (2007) due to limited information on its distribution and population status.

Preliminary observations show that slow lorises in India are found in subtropical and tropical evergreen and semi-evergreen habitats at altitudes up to 2400 m asl (Choudhury, 2001; Gupta, 2001). Habitat destruction due to *jhum* cultivation, tree felling, road kills and hunting have been reported as major threats to the species (Choudhury, 1992; Srivastava, 1999; Gupta, 2001; Radhakrishna *et al.*, 2006). Several studies have highlighted the need for better information on distribution, abundance, ecology, and conservation status of the species (e.g. Gupta, 2001; Radhakrishna *et al.*, 2006).

This paper presents results of a preliminary survey for Bengal slow loris *Nycticebus bengalensis* in the state of Tripura, northeastern India. The specific objectives of the study were to establish the distribution status of the species in the protected areas of Tripura and to assess conservation threats affecting its long-term survival.

### METHODS

The study was carried out in Trishna Wildlife Sanctuary and Sepahijala Wildlife Sanctuary, Tripura from June 10, 2007 to July 8, 2007. As time available for the survey was limited, the method employed was that of an encounter rate survey using recce sampling (Walsh and White, 1999; Radhakrishna *et al.*, 2006).

Local people and Forest Department employees were interviewed to gather information on recent and past sightings of slow lorises, rescued individuals, their preferred habitat and diet. Based on the information gathered from these interviews and the available records of vegetation cover; particular trails, beat paths and roads were selected for the survey.

The survey was conducted between 1900h and 0100h. The surveying team comprised four individuals among who three were involved in searching the area thoroughly. The speed of walking was maintained around 1 km/h.

From previously conducted surveys it is known that slow lorises emit a bright orange eye shine in response to flashed light (Radhakrishna *et al.*, 2006). Headlamps were hence used regularly during the survey to detect loris eye shine. All heights from ground level to the upper canopy were searched thoroughly during each walk.

Upon spotting potential eye shine powerful hand-held lights were used to identify the animal. If the individual was identified to be a slow loris, information was collected on parameters such as time of sighting, activity of the animal, height at which sighted, tree species on which sighted, and vegetation structure of the habitat.

Whenever possible, photographs were also taken of the sighted individual. A Garmin GPS unit was used to record geographic locations and the survey distances. During the survey, all signs of disturbance such as tree felling, bamboo collection, poaching and those indicating human presence and activity were recorded.

## RESULTS

A total of 41 km was covered in Trishna Wildlife Sanctuary and 16.5 km in Sepahijala Wildlife Sanctuary. Nine slow lorises were sighted in Trishna Wildlife Sanctuary (Table 1, Figure 1). Although slow loris was not sighted in Sepahijala Wildlife Sanctuary, their presence was confirmed from interviews with local people and employees of the Sepahijala Zoological Park.

Most sightings occurred in the interior areas of the forest where the vegetation was dense; tree species were largely moist deciduous (*Grewia microcos*, *Schima wallichii*, *Careya arborea*, *Artocarpus chaplasha*, *Syzygium* sp., *Dillenia pentagyna*, *Vitex penduncularis*, *Ficus* sp., *Terminalia bellirica*, *Gmelina arborea*, *Delonix regia*, *Terminalia chebula*, etc.), around 8–15 m in height, and overgrown with climbers and vines.

The average height at which individuals were sighted was 8.33 m ( $n = 9$ ; range = 5–15; SD = 2.94). The mean number of slow lorises

**Table 1.** Slow loris sightings in Trishna Wildlife Sanctuary, Tripura, Northeastern India.

Individuals*	Time of sighting (hours)	Tree species / height on tree	Geographical location	~ distance from nearest village (km)
1	2215	<i>Terminalia bellirica</i> / 15m	N 23°15'42.8" E 091°22'06.4"	5.5
2	2302	Unidentified / 5m	N 23°15'27.8" E 091°21'58.3"	5
3	2350	Unidentified / 5m	N 23°15'10.1" E 091°21'54.6"	4.5
4	2235	<i>Ficus</i> sp. / 8m	N 23°18'31.0" E 091°22'16.8"	1.5
5	2150	<i>Bursera serrata</i> / 8m	N 23°15'04.8" E 091°26'10.1"	2.5
6	2307	<i>Ficus</i> sp. / 10m	N 23°15'04.5" E 091°21'54.6"	4
7	0045	<i>Artocarpus chaplasha</i> / 8m	N 23°14'25.0" E 091°22'04.3"	3
8	2230	<i>Ficus</i> sp. / 10m	N 23°16'32.1" E 091°22'39.3"	3
9	2250	<i>Lagerstromea flosregine</i> / 6m	N 23°16'40.7" E 091°22'35.8"	3

\* as identified in Figure 1.





**Figure 1.** Slow loris sightings in Trishna Wildlife Sanctuary, Tripura, Northeastern India.

(0.22/km) can be considered as an index of relative abundance of the species in Trishna Wildlife Sanctuary. Seven out of the nine loris sightings in this sanctuary occurred clustered in a small area of 1.71 km<sup>2</sup> (the area shaded white in Figure 2).

## DISCUSSION

The encounter rates of Bengal slow loris from different areas of Assam ranged from 0.03 – 0.33 individuals/km (Radhakrishna *et al.*, 2006). Density counts for the closely related *Nycticebus coucang* from two studies in Malaysia vary more widely. Wiens and Zitzmann (2003) recorded a density of c. 80 individuals/km<sup>2</sup> in Segari Melintang Forest Reserve in West Malaysia, while Barrett (1981) estimated a density of c. 20 individuals/km<sup>2</sup> for the Malay Peninsula (Barrett, 1981 *in* Wiens and Zitzmann, 2003).

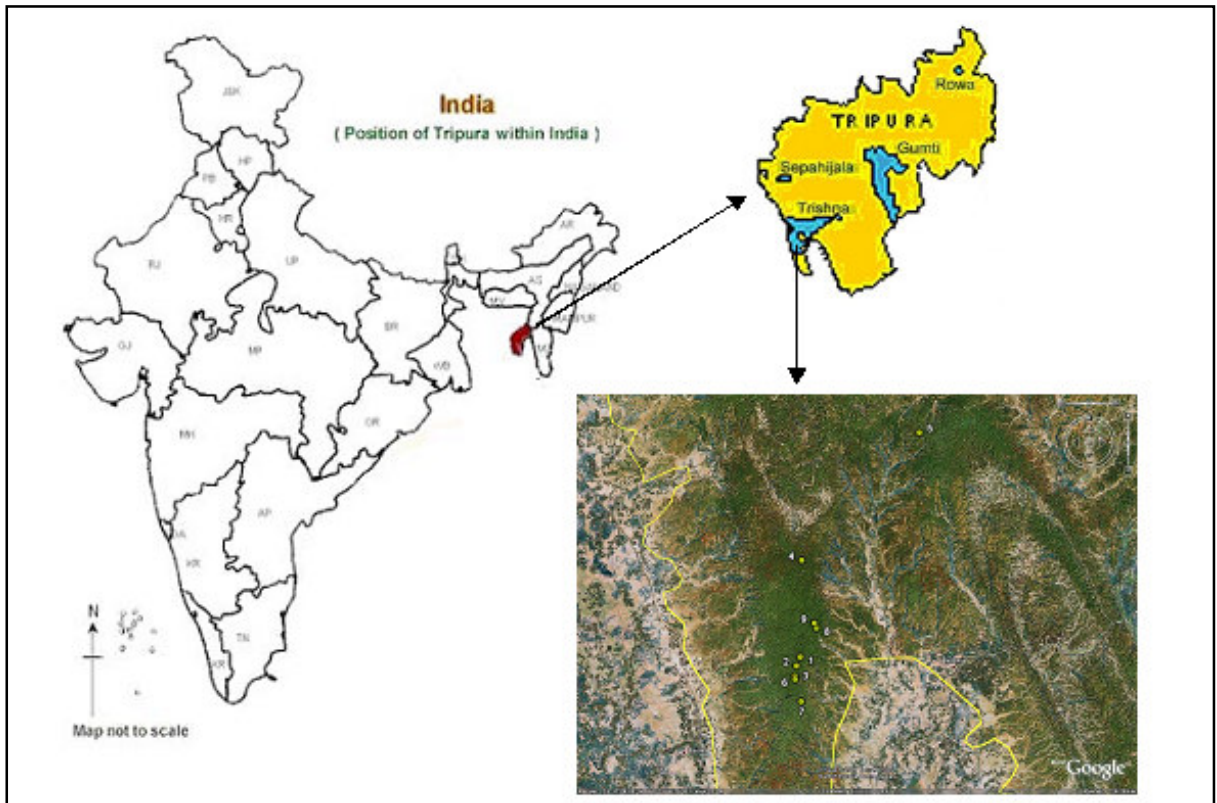
The present study showed an encounter rate of 0.22 individuals/km. Although this number falls within the range seen in an earlier survey in Assam, it is significant that a majority of the sightings (seven out of nine) occurred in a small

area of 1.71 km<sup>2</sup>. We strongly recommend a long-term study of the behavioural ecology of this slow loris population and surveys in other parts of Tripura to investigate whether the high density in the small area within Trishna WLS is a localized phenomenon, and if so, the factors contributing to this density.

A more rigorous examination of disturbance factors and slow loris densities in other parts of Tripura may also reveal the role of environmental disturbances in affecting slow loris densities. It has been reported that encounter rates for *Nycticebus bengalensis* are 5-15 times lower than for *Nycticebus coucang* (Nekaris and Nijman, 2007). A more detailed population and behavioural study of the slow loris in Tripura will reveal whether low densities for the Bengal slow loris is typical of the species or an effect of environmental stresses.

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**Figure 2.** Bengal slow loris sighting locations in Trishna Wildlife Sanctuary, Tripura, Northeastern India.

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The journal/newsletter aims to provide a basis for conservation information relating to the primates of the Asian. We welcome texts on any aspect of primate conservation, including articles, thesis abstracts, news items, recent events, recent publications, primatological society information and suchlike.

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Each issue of **ASIAN PRIMATES JOURNAL** will include up to six full articles, limited to the following topics: Taxonomy, Behavior, Genetics (when relevant for systematics), Biogeography, Ecology and Conservation. Texts for full articles should not exceed about 20 pages length (1.5 spaced, and including the references). Please include an abstract, Tables and Illustrations should be limited to six, excepting only the cases where they are fundamental for the text (as in species descriptions, for example). Full articles will be sent out for peer-review.

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There are usually reviewed only by the editors. A broader range of topics is encouraged, including such as behavioral research, in the interests of informing on general research activities which contribute to our understanding of platyrrhines. We encourage reports on projects and conservation and research programs (who, what, where, when, why, etc.) and most particularly information on geographical distributions, locality records, and protected areas and the primates which occur in them. Texts should not exceed 10 pages in length (1.5 spaced, including the references).

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### Chapter in book

Hohmann, G.M. and Fruth, B. 1995. Loud calls in great apes: sex differences and social correlates. **In:** *Current Topics in Primate Vocal Communication*, E. Zimmerman, J.D. Newman, and U. Juergens (eds.), pp. 161-184. New York: Plenum Press.

### Book

Niemitz, C. 1984. *The Biology of Tarsiers*. Stuttgart: Gustav Fischer.

### Thesis/Dissertation

Wallace, R.B. 1998. The behavioural ecology of black spider monkeys in north-eastern Bolivia. Doctoral thesis, University of Liverpool, Liverpool, UK.

### Report

Muckenhirn, N.A., Mortensen, B.K., Vessey, S., Fraser, C.E.O., and Singh, B. 1975. Report on a primate survey in Guyana. Unpublished report, Pan American Health Organization, Washington, DC.

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**FOREWORD** ..... 1

## **ARTICLES**

CURRENT STATUS OF THE HAINAN GIBBON (*Nomascus hainanus*): PROGRESS OF POPULATION MONITORING AND OTHER PRIORITY ACTIONS

John R. Fellowes, Chan Bosco Pui Lok, Zhou Jiang, Chen Shenghua, Yang Shibin and Ng Sai Chit ..... 2

RELATIVE POPULATION DENSITY OF *Tarsius diana* IN MAN-INFLUENCED HABITATS OF LORE LINDU NATIONAL PARK, CENTRAL SULAWESI, INDONESIA

Indra Yustian, Stefan Merker, Jatna Supriatna, and Noviar Andayani ..... 10

REDISCOVERY OF THE CRITICALLY ENDANGERED EASTERN BLACK CRESTED GIBBON *Nomascus nasutus* (HYLOBATIDAE) IN CHINA, WITH PRELIMINARY NOTES ON POPULATION SIZE, ECOLOGY AND CONSERVATION STATUS

Chan Bosco Pui Lok, Tan Xue-feng and Tan Wu-jing ..... 17

IS *Presbytis* A DISTINCT MONOPHYLETIC GENUS: INFERENCES FROM MITOCHONDRIAL DNA SEQUENCES

Badrul Munir Md. Zain, Juan Carlos Morales, Mohd. Nordin Hasan, Jasmi Abdul, Maklarin Lakim, Jatna Supriatna, and Don J. Melnick ..... 26

DISTRIBUTION SURVEY OF BENGAL SLOW LORIS *Nycticebus bengalensis* IN TRIPURA, NORTHEASTERN INDIA

Swapna N., Atul Gupta, and Sindhu Radhakrishna ..... 37