Effects of habitat fragmentation and human disturbance on the population dynamics of the Yunnan snub-nosed monkey from 1994 to 2016

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In this study, we integrate data from field investigations, spatial analysis, genetic analysis, and GLMs to evaluate the effects of habitat fragmentation on the population dynamics, genetic diversity, and range shifts in the endangered Yunnan snub-nosed monkey (*Rhinopithecus bieti*). The results indicate that from 1994 to 2016, *R. bieti* population size increased from less than 2,000 to approximately 3,000 individuals. A primary factor promoting population recovery was the establishment of protected nature reserves. We also found that subpopulation growth rates were uneven, with the groups in some areas, and the formation of new groups. Both the fragmentation index, defined as the ratio of the number of forest patches to the total area of forest patches (e.g. increased fragmentation), and increasing human population size had a negative effect on population growth in *R. bieti*. We recommend that government conservation plans prioritize the protection of particular *R. bieti* populations, such as the Baimei and Jisichang populations, which have uncommon haplotypes. In addition, effective conservation strategies need to include an expansion of migration corridors to enable individuals from larger populations such as Guyoulong (Guilong) to serve as a source population to increase the genetic diversity of smaller *R. bieti* subpopulations. We argue that policies designed to protect endangered primates should not focus solely on total population size but also need to determine the amount of genetic diversity present across different subpopulations and use this information as a measure of the effectiveness of current conservation policies and the basis for new conservation policies.

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26 **ABSTRACT**

27 In this study, we integrate data from field investigations, spatial analysis, genetic analysis, and GLMs to

28 evaluate the effects of habitat fragmentation on the population dynamics, genetic diversity, and range shifts in

29 the endangered Yunnan snub-nosed monkey (*Rhinopithecus bieti*). The results indicate that from 1994 to 2016,

30 *R. bieti* population size increased from less than 2,000 to approximately 3,000 individuals. A primary factor

31 promoting population recovery was the establishment of protected nature reserves.   We also found that

32 subpopulation growth rates were uneven, with the groups in some areas, and the formation of new groups.

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34 patches (e.g. increased fragmentation), and increasing human population size had a negative effect on

35 population growth in *R. bieti*. We recommend that government conservation plans prioritize the protection of

36 particular *R. bieti* populations, such as the Baimei and Jisichang populations, which have uncommon

37 haplotypes.   In addition, effective conservation strategies need to include an expansion of migration corridors

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40 endangered primates should not focus solely on total population size but also need to determine the amount of

41 genetic diversity present across different subpopulations and use this information as a measure of the

42 effectiveness of current conservation policies and the basis for new conservation policies.

43 **Key words:** habitat fragmentation; human disturbance; *Rhinopithecus bieti*; population size; spatial analyst;

44 range shift; human population density; conservation policies; genetic diversity; land use

INTRODUCTION

Globally, anthropogenic activities resulting in deforestation, habitat loss, the extraction of natural resources, the introduction of invasive species, agricultural expansion, and climate change represent a major driver of the unprecedented decline in biodiversity (*Millennium Ecosystem Assessment, 2006; Magurran & Dornelas, 2010; Newbold et al., 2015*). Quantifying the effects of individual factors and developing solutions to protect environments and endangered is a key scientific challenge (*Mace et al., 2010; Magurran & Dornelas, 2010*). Anthropogenic activities have altered as much as 50% of terrestrial land cover, and land-use patterns, such as the expansion of croplands for industrial agriculture and pastures for grazing cattle and other domesticated animals, has led to a global reduction in the number of species (*Millennium Ecosystem Assessment 2006; McGill 2015*). This has led scientists to refer to the current period as the Anthropocene mass extinction event (*Ceballos, Ehrlich & Dirzo, 2017*), with species extinction rates estimated to be 100–1,000 times greater than during past evolutionary periods (*Raup, 1995; Pimm et al., 1995*). Protecting animal and plant survivorship ad biodiversity requires monitoring and managing changes in species population size and genetic diversity (*Dornelas et al., 2014*).

An important form of habitat degradation is fragmentation, which transforms large tracts of continuous habitat into smaller and spatially distinct patches immersed within a dissimilar matrix (*Didham, Kapos& Ewers, 2012; Wilson et al., 2016*). Fragmentation can create detrimental edge affects along the boundaries of habitat patches, alter ecological conditions resulting in the decline of native species, restrict animal movement and gene flow, and sever landscape connectivity (*Crooks & Sanjayan, 2006*) leading to local population extinction in response to insufficient viable "core" habitat (*Ewers & Didham, 2007*). Habitat fragmentation has continued at an alarming rate, and has impaired key ecosystem functions by decreasing biomass, limiting seed dispersal, shifting predator-prey relationships, and altering nutrient cycles (*Haddad et al., 2015*).

Several species of nonhuman primates are particularly affected by habitat fragmentation due to their dependence on intact and biodiverse forested landscapes to obtain a nutritionally balanced diet (*Arroyo-Rodríguez & Fahrig, 2014*). Currently, anthropogenic habitat modifications have resulted in more than half of

70   the world's primate species listed as Vulnerable, Endangered, or Critically Endangered (*Estrada et al., 2017;*

71   *Estrada et al., 2018*). Primate populations inhabiting small and isolated forest fragments are especially

72   vulnerable to extinction (*Benchimol & Peres, 2013*).   However, given evidence of behavioral plasticity, the

73   benefits of social learning, and the ability to exploit a broad range of different food types, many species of

74   primates are reported to survive, at least over periods of several years, in impacted landscapes by modifying

75   aspects of their activity budget, group size and composition, ranging patterns, and diet (*Onderdonk & Chapman,*

76   *2000*; *Wong & Sicotte, 2007; Boyle & Smith, 2010*).

77   The endangered Yunnan snub-nosed monkey (*Rhinopithecus bieti*), a large-bodied species of nonhuman

78   primate, that lives in social groups of several hundred individuals (*Kirkpatrick et al., 1998*). A survey in the

79   early 1990's indicated that this species was confined to a narrow region between the Yangtze and Mekong

80   rivers (98°37′ to 99°41′E, 26°14′ to 29°20′N).   Their population size was estimated to be less than 2,000

81   individuals, distributed across 19 distinct social groups (*Long et al., 1994*).   A study by Zhao et al. (2018)

82   integrating data on evolutionary genetics and biogeographical information concluded that both the historical

83   distribution (past 2000 years) and current population structure of the *R. bieti* appears to have been directly

84   impacted by human activities, principally   agricultural expansion resulting in severe habitat fragmentation of

85   the Tibetan Plateau and by hunting (*Liu et al., 2009*).

86   Recent studies modeling habitat change and agricultural expansion across *R. bieti's* range, predict an

87   increase in forest fragmentation and a decrease in habitat quality resulting in range contraction over the next

88   25-75 years (*Xiao et al., 2003*; *Wong et al., 2013; Li et al., 2018*). However, some of these studies were based

89   on data collected in the early 1990's and therefore may not accurately represent the current demographic and

90   ecological challenges and conservation pressures faced by *R. bieti*. The main purpose of our study is to: (1)

91   investigate the current population size and distribution of the *R. bieti*, (2) conduct a landscape and spatial

92   analysis of their distribution area, and (3) examine how present day habitat fragmentation influences *R. bieti*

93   population size, genetic diversity, and changes in geographical distribution.

METHODS

Study area and data collection

This field study was approved by State Forestry Administration of China. The study area is a narrow region of 17,000 km² in the northwest of Yunnan Province and the Tibet Autonomous Region. It is bounded by the Mekong River to the west and the Yangtze River to the east (*Long et al., 1994*). The elevation of the study area varies from 1,300 m to 5,400 m. Our study was conducted across all current distributional areas of *R. bieti* in Yunnan Province and Tibet. Each of the remaining wild groups was surveyed from January to November 2013 and April to September 2016. In order to obtain the most accurate information for this species, we conducted detailed surveys within its main distribution in three national nature reserves (Bamaxueshan, Honglaxueshan and Tianchi), one provincial nature reserve (Yunling), and two sites that are outside of protected reserves (Jinsichang and Bamei) (Fig. 1).

Based on information of group location, a survey was conducted and the number of monkeys per group was censused. Specific locations of *R. bieti* groups were obtained from local forest rangers and officials who regularly patrol the reserves. *R. bieti* were counted directly using auxiliary telescopes to assist in observations (*Wu et al., 1988*). Each monkey group was observed for $14-21$ days, counting all individuals in the group (*Wu et al., 1988*). The counting of monkeys in each group was based on the following method. Travel in *R. bieti* occurs both on the ground and in trees. However, when crossing open areas, the monkeys often walk slowly in a single or nearly single. A total of 17 groups were censused. *R. bieti* live in groups called multilevel societies (MLS), which are composed of several harem social and reproductive units (*Qi et al., 2014*). Each harem contains a single adult male, several adult females, and offspring. These harems travel, feed, and range together throughout the day and form a large band. Bands are followed by one or more all male units (AMU) that contain juvenile, sub-adult, and adult bachelor males. Because each band or MLS has been tracked by patrol officers for several years, we were able to obtain an accurate count of its size. This was accomplished by first counting the number of harems in each band and then estimating the number of monkeys per harem and per AMU to obtain a final total. In order to increase census accuracy, at least 3 researchers participated in each

119 survey and counted/estimated the number of harems and harem sizes.

120 To monitor changes over time in *R. bieti* demography and distribution, we obtained information on *R. bieti*

121 population size and location in 1990 from Long *et al.* (1994) and compared it with our current data. To assess

122 relationships among suitable habit, population size, and gene flow, we conducted an analysis of genetic

123 diversity among groups. The genetic diversity for each group was calculated using FSTAT 3.2 (*Goudet,1995*)

124 based on microsatellite dates from the analyses of 157 individuals' samples (blood, muscle and faecal) in 2007

125 (*Liu et al. 2007, 2009*). Genetic information was obtained from 11 of the 17 known groups of *R. bieti*.

126 **Landscape data and spatial analysis**

127 We used a land cover map (30″×30″ resolution) derived from an assemblage of 13 SPOT5 images across the

128 known distribution of *R. bieti* for 1990 and 2016. This covered an area of 17, 000 km^2 including the entire *R.*

129 *bieti* range. Vegetation types suitable for *R. bieti* were defined as dark-coniferous forest dominated by species

130 such as *Abies georgei* and mixed coniferous and broadleaf forest (*Kirkpatrick et al., 1998; Xiao et al., 2003*; *Li*

131 *et al., 2015*).   We then verified our habitat classification accuracy using data from the Conservation Information

132 Centre of The Nature Conservancy of China. Fragmentation metrics for *R. bieti* were calculated using

133 FRAGSTATS 4 (*McGarigal & Marks, 1995*). Patch density (PD, the number of patches divided by total

134 landscape area) was used as indices of fragmentation, with low PD indicating a more connected landscape and

135 high PD indicating a more fragmented landscape (*Olsoy et al., 2016*). A fragment was considered vegetatively

136 suitable to sustain a population of *R. bieti* if it included dark-coniferous forest and mixed coniferous and

137 broadleaf forest. Given the potential effect that habitat fragmentation can have on whether an area is suitable

138 for *R. bieti*. Supplementary information of forested land, cropland, pasture land, and land reclamation (defined

139 as primary forest converted into a secondary forest, pasture and cropland), as well as information on human

140 population density, was extracted from the History Database of the Global Environment (the pixel of size of

141 these data were 0.25 x 0.25 resolution ; HYDE3.1: *Goldewijk et al., 2011*; *He, Li & Zhang, 2015*; *Li, He &*

142 *Zhang, 2016*). We extracted values of human population density, cropland, and pasture (HYDE3.2; *Goldewijk,*

143 *2016*), all indicators of habitat fragmentation, between the years 1990 and 2016 with the function "extract" in R.

144   We used Spearman correlations to check for simple linear relationships between the growth rate of the *R. biet*

145   population and all environmental and landscape variables (fragmentation index, population density, area of

146   cropland, area of pasture, density of households, and density of roads). Then, we applied multiple linear

147   regression analyses to determine the partial and interactive effects of factors affecting *R. bieti* population size.

148   Finally, we applied GLMs to determine the factors affecting *R. bieti* population size.

149   **RESULTS**

150   Based on a census of 17 *R. bieti* subpopulation size isolated from each other and troop size ranged from 40-450

151   individuals. The mean number of harems per band was 12 ± 3.5. Across all subpopulations, the total number of

152   *R. bieti* in 2016 was estimated at approximately 3000 individuals. We found that population growth had varied

153   significantly by region. Current population estimates in the central and southwestern zones of the species'

154   range (Fig.1) (Baimaxueshan Nature Reserve and Yunling Nature Reserve) were 147% higher compared to

155   values reported in 1994 (including the formation of three new groups: Gehuaqing, Baijixun(Yongan),

156   Shikuadi). In contrast, size estimates for the southeastern and northern (Honglaxueshan Nature Reserve) areas

157   increased by only 24% and 21% respectively, from earlier estimates. This increase in population size was the

158   result of an increase in the size of bands rather than the number of *R. bieti* bands. We found that 6 groups with

159   populations sizes of less than 100 individuals in 1994 disappeared by 2016. These included those in Bajia,

160   Adong, Houziqin, Heishan, Dapingzi, and Moziping. The population sizes of three groups (Cikatong, Milaka,

161   and Akou (Anyi)) were found to decrease by 20-50% between 1994 and 2016 (Table 1). Four new groups were

162   identified in our census, Zhina, Gehuaqing, Baijixun (Yongan), and Shikuadi. These bands inhabit the northern

163   and southern regions of the species distribution and ranged in size of from 40 to 450 individuals (Table 1).

164   Our analysis of habitat fragmentation indicates that the southern populations of *R. bieti* (Tianchi nature

165   reserve, Yunling nature reserve and Jinsichang, fragmentation index: 0.99), live in more fragmented habitats

166   and are characterized by smaller band size compared to the Central (N=9) and northern (N=3) populations

167   (Baimaxueshan nature reserve and Honglaxueshan nature reserve, fragmentation index: 0.59). In general, the

168   GLMs indicated that the fragmentation index, human population size, and the area within a group's range

169   devoted to pasture was negatively correlated with *R. bieti* population size. In this case, after model selection

170   based on AIC, only a few variables remained (population size=0.85−3.09fragmentation index −2.39 population

171 density−0.49 pasture, R^2=0.52, $P<0.05$).

DISCUSSION

173 The results of our census indicate that the construction of protected nature reserves (87% of the *R. bieti* live in

174 protected areas) has effectively limited human disturbance and reduced hunting, leading to a population

175 increase from less than 2000 to almost 3000 individuals over the past 25 years. During the 1980's, populations

176 of *R. bieti* faced a set of severe anthropogenic challenges. For example, approximately 200,000 m^3 of

177 commercial logs were removed annually from their range, an entire band was killed by poachers (*Long et al.,*

178 *1994*; *Ding, Yang & Liu, 2003*), and the area of suitable habitat decreased by 31% from 1958 to 1997 as large

179 tracts of forests were converted into pastures for cattle grazing (*Xiao et al., 2003*).   In addition, in Deqin

180 country alone, approximately 430 *R. bieti* were killed for meat, fur, and medicine from 1970 to 1979, and 68

181 pieces of *R. bieti* fur or bone were sold in local stores (*Bai, 1987*). With the establishment of the Baimaxueshan

182 Nature Reserve, the Honglashan Nature Reserve, the Yunling Nature Reserve and the Tianchi Nature Reserve

183 between 1983 and 2003, *R. bieti* is now protected and this has effectively prevented poaching, the collection of

184 forest products, and livestock grazing within their range. Simultaneously the Chinese government has banned

185 all guns beginning in 1990s, and this has dramatically reduced poaching. Each of these measures has served to

186 protect *R. bieti* and to promote their population recovery over the past 25 years.

187   Despite population increases, the conservation status of *R. bieti* continues to be negatively affected by forest

188 fragmentation and the presence of human settlements in and around their range. Our results indicate that *R.*

189 *bieti* population growth rates varied across its distribution with both increased habitat fragmentation and

190 increased human population density negatively affecting *R. bieti* population growth rates. This was most

191 evident in the southern region (Yunling and Tianchi Nature Reserves), where suitable habitats are highly

192 fragmented and *R. bieti* population growth rates are low. The situation faced by *R. bieti* of Fragmentation-

193 induced reduction in habitat quality and resource availability also has adversely affected the long-term viability

194 of other primate species (*Wahungu et al., 2005*; *Arroyo-Rodríguez & Mandujano, 2014*) and this has

195 contributed to a primate extinction crisis worldwide (*Estrada et al., 2017, Estrada et al., 2018; Li et al., 2018*). .

196

197   In the case of *R. bieti*, forest fragmentation spatially isolates subpopulations and impeding gene flow.

198   Moreover, the conversion of natural habitat to agricultural fields and pasture land leads to the local extinction

199   of small populations, as in the case of the Adong, Houziqin, Heishan, Dapingzi, and Moziping *R. bieti* bands.

200   Fragmentation also leads to reduction in the availability of large food patches, changes in tree species

201   composition and diversity, and introduces edge effects leading to changes in soil moisture and local climate,

202   resulting in a reduction in overall habitat quality (*Laurance et al., 2000*). Lichen and fruit are important

203   components of the diet of *R. bieti*, accounting for 50% of total feeding time (*Grueter et al., 2009*). Across most

204   forested environments, ripe fruit is characterized by a patchy distribution in space and time. Spatial and

205   temporal fruit patchiness is exacerbated in fragmented landscapes, limiting the ability of species (*Estrada &*

206   *Coates-Estrada, 1996*). For example, mantled howler monkeys (*Alouatta palliata*) living in more fragmented

207   landscapes spent more time traveling and searching for food than did groups living in less fragmented

208   landscapes (*Estrada & Coates-Estrada, 1996*).   We assume that given the large size of *R. bieti* bands,

209   increased fragmentation results in increased challenges in locating sufficient food.   Similarly, lichen is known

210   to be negatively impacted by human-induced environmental change such as air pollution, and a decline in the

211   production of lichen is likely to have a severely negative effect on *R. bieti* nutrient intake, reproduction,

212   population growth rates, and survivorship (*Grueter et al., 2008*).

213   Our results also indicated that the population growth rate of *R. bieti* was negativity correlated with human

214   population density and human accessibility to wildlife habitats. Based on our field observations, we noted that

215   during the *R. bieti* breeding season (month 2 to month 6), local people go into the forest to pick cordyceps,

216   which frightened and affects *R. bieti* breeding behavior by making *R. bieti* spending more time traveling. In

217   addition, human accessibility to wildlife habitats has the potential to become a vector for the transmission of

218   zoonotic diseases between humans, domesticated animals, and wildlife, and rapidly extirpate entire primate

219   subpopulations from local areas (*Patz et al., 2008; Lambin et al., 2010; Murray & Daszak, 2013; Gottdenker et*

220   *al., 2014; Estrada et al., 2018*). We found that in areas with human population densities of greater than 46

221 human/ km², *R. bieti* growth rates either increased very slowly (rate of 11.4%) or some small bands exhibited

222 negative growth and disappeared from areas such as Bajia, Adong, Houziqin, Heishan, Dapingzi, and Moziping.

223 This reduction has been offset, in part, by the addition of four newly formed bands (Zhina, Gehuaqing, Baijixun

224 (Yongan), Shikuadi) and other bands increasing in size. Moreover, pastures serve as a barrier to migration for *R.*

225 *bieti* (*Kirkpatrick et al., 1998; Grueter et al., 2010*) and therefore severely limit the movement of individuals

226 across these highly transformed landscapes and the opportunity to expand into unoccupied habitats and form of

227 new bands.

228   Our results revealed that *R. bieti* populations in the Yunling Nature Reserve and in Jinshichang are the most

229 isolated, and characterized by certain unique haplotypes (haplotype M1-M4, M6-M11 and M29-30) (*Liu et al.,*

230 *2007*).   Moreover, despite the fact that total population size has increased dramatically over the past 25 years,

231 the population size of three bands (Milaka, Cikatong, and Akou (Anyi)) has markedly decreased, exacerbating

232 the loss of genetic diversity (Table.1).We suggest that management decisions for endangered species should

233 not focus solely on population size but also consider subpopulation genetic diversity. We recommend that

234 effective conservation policies for this species should prioritize protecting certain targeted populations such as

235 Milaka, Cikatong, and Akou (Anyi) that have high intraspecific genetic diversity but low growth rates (Table

236 1). In addition, government policies that promote the alleviation of human poverty, especially in rural

237 communities, represent an important conservation tool that indirectly protects *R. bieti*. In this regard, the

238 Chinese government has encouraged local farmers to return land to natural forest, prohibited logging, and

239 relocate from Nature Reserves.   This serves to reduce human interference and protect habitats that are critical

240 to the survival of *R. bieti* as well as others threatened taxa.

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374   **Figure legends**

375   **Figure 1**. The study area and locations of the bands of *R. bieti* from 1994 to 2016.

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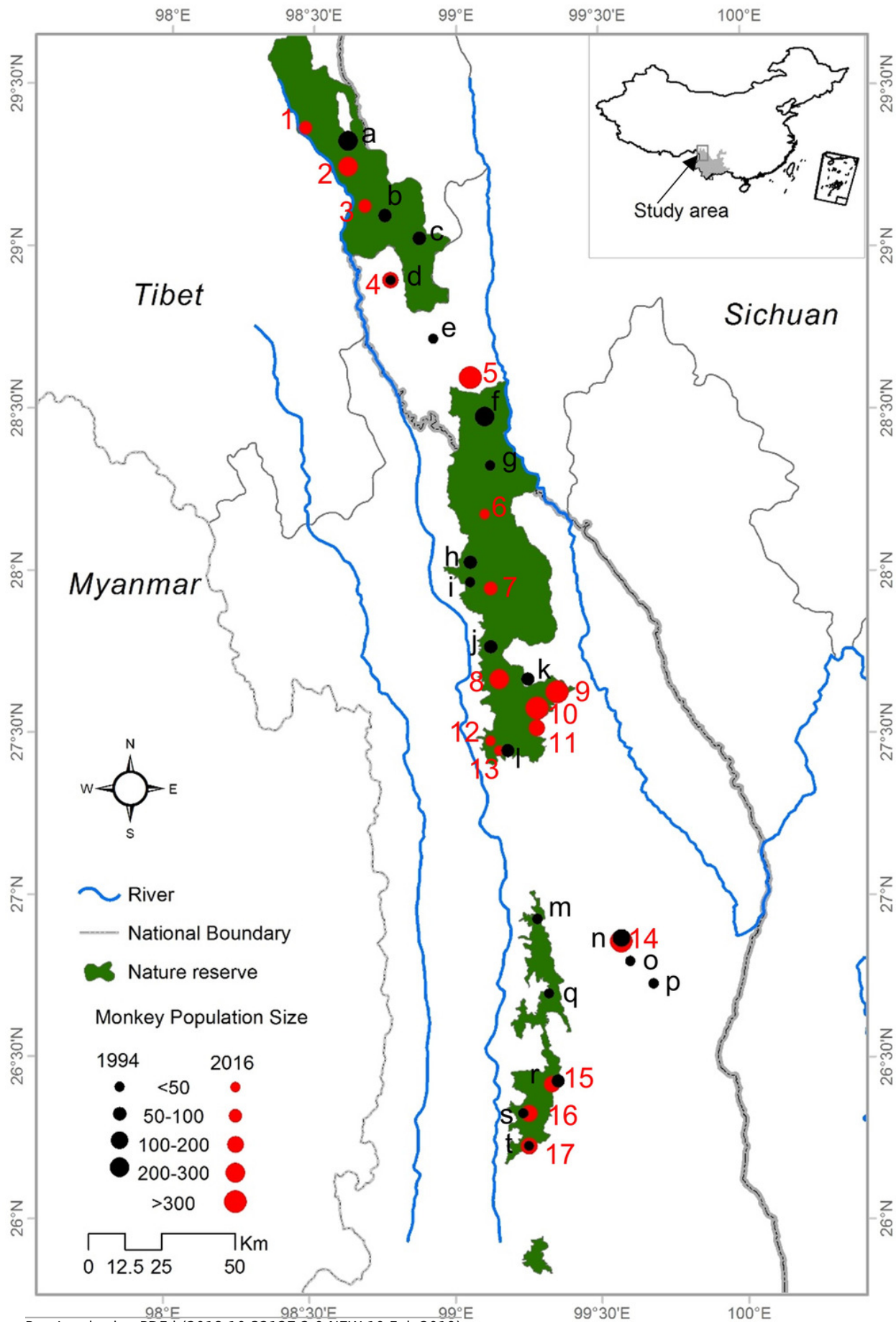
Figure 1

The study area and locations of the bands of *R. bieti* from 1994 to 2016

Changes in the location of natural bands of *R. bieti* between 1994 to 2016

1 **Table.1** Changes in the location of natural bands of *R. bieti* between 1994 to 2016

Site	Latitude	Longitude	1994	2016	Change group	Intraspecific genetic diversity	
						Unbiased Hz	Obs Hz
Zhina	29.37	98.47	-	80	new	0.54	0.50
Xiaochangdu	29.33	98.62	> 200	280	unchanged	-	-
Milaka	29.13	98.75	<100	60	unchanged	0.58	0.64
Bajia	29.03	98.87	<100	-	disappeared	-	-
Bamei	28.9	98.77	<50	100	unchanged	0.59	0.54
Adong	28.72	98.92	<50	-	disappeared	-	-
Wuyapuya	28.48	99.1	> 200	400	unchanged	0.63	0.64
Cikatong	28.03	99.05	50-100	50	unchanged	0.63	0.65
Guyoulong(guilong)	27.97	99.05	<50	80	unchange	0.70	0.69
Shiba	27.77	99.12	50-100	200	Unchanged	-	-
Guomorong(Xiangguqibg)	27.67	99.25	50-100	480	unchanged	0.65	0.63
Akou(Anyi)	27.45	99.18	50-100	40	unchanged	0.66	0.59
Jinsichang	26.87	99.57	100-150	310	unchanged	0.57	0.54
Neidaqin(Fuhe)	26.43	99.35	50-100	120	unchanged	0.66	0.71
Lashashan	26.33	99.23	<50	130	unchanged	-	-
Houziqin	26.93	99.28	<50	-	disappeared	-	-
Longma	26.23	99.25	<50	140	unchanged	0.64	0.72
Heishan	26.7	99.32	<50	-	disappeared	-	-
Dapingzi	26.73	99.68	<50	-	disappeared	-	-
Moziping	26.8	99.6	<50	-	disappeared	-	-
Gehuaqing	27.58	99.28	-	450	new	-	-
Shikuadi	27.52	99.28	-	120	new		
Pantiange	27.47	99.15	-	-	unchanged	-	-

Baijixun(Yongan)	27.48	99.12	-	40	new	-	-