ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/227670563

A new species of muntjac, Muntiacus putaoensis (Artiodactyla: Cervidae) from northern Myanmar

Article *in* Animal Conservation · February 1999

DOI: 10.1111/j.1469-1795.1999.tb00042.x

CITATIONS
39

READS
78

3 authors, including:

George Amato
American Museum of Natural History
115 PUBLICATIONS **2,994** CITATIONS

SEE PROFILE

Alan Rabinowitz
Panthera
50 PUBLICATIONS **2,040** CITATIONS

SEE PROFILE

All in-text references underlined in blue are linked to publications on ResearchGate, letting you access and read them immediately.

Available from: George Amato
Retrieved on: 19 September 2016

Animal Conservation (1999) **2**, 1–7 © 1999 The Zoological Society of London Printed in the United Kingdom

A new species of muntjac, *Muntiacus putaoensis* (Artiodactyla: Cervidae) from northern Myanmar

George Amato[1,2], Mary G. Egan[1,2,3] and Alan Rabinowitz[1]

[1]Wildlife Conservation Society, 2300 Southern Blvd., Bronx, New York 10460, USA
[2]Molecular Systematics Laboratory, American Museum of Natural History, 79th Street at CPW, New York, New York 10024, USA
[3]Department of Biological Sciences, Fordham University, 441 E. Fordham Road, Bronx, New York 10458, USA

(Received 18 May 1998; accepted 2 October 1998)

Abstract

A new species of barking deer (*Muntiacus* spp.) is described from northern Myanmar. Diagnostic DNA character data are presented along with preliminary information on morphology, distribution, and phylogenetic relationships. This discovery contributes significantly to our knowledge of this poorly studied group and highlights the importance of continued field surveys in remote regions. Similar studies have resulted in a significant increase in conservation efforts in other parts of South-east Asia, and argue for additional conservation efforts for this region.

INTRODUCTION

Five species of large mammals have recently been discovered or rediscovered in the Annamite Mountains region of Laos and Vietnam (Dung *et al*., 1993; Tuoc *et al*., 1994; Schaller, 1995; Groves *et al*., 1997; Amato *et al*., 1998; Giao *et al*., 1998), of which three are muntjac (*Muntiacus* spp.). These discoveries have led to assertions that this region is a unique Pleistocene refugium preserving exceptional levels of endemism (Dung *et al*., 1994; Groves *et al*., 1997). One of the results has been an enormous infusion of conservation funding for a very specific area. Reported here is the discovery and description of another muntjac using diagnostic DNA characters, not in the Annamite Mountains, but in a remote area of Myanmar. We suggest that this wave of discoveries reflects how little is known about both this particular taxonomic group, and the remote, relatively unexplored mountainous regions of Asia.

Northern Myanmar, between 24–28° N and 97–99° E, is situated along the western escarpment of Yunnan Province in China, originally part of the Tibetan Plateau to the north and the China Plateau to the east (Kingdon-Ward, 1944). This mountainous region contains floral communities of Miocene origin, isolated since the last glaciation (Kingdon-Ward, 1936, 1944). What little zoological data have been collected from this region (Dollman, 1932; Kinnear, 1934) indicate relatively high biodiversity within a transition zone of Indo-Malayan and Sino-Himalayan fauna.

All correspondence to G. Amato, Wildlife Conservation Society, 2300 Southern Blvd., Bronx, New York 10460, USA. Tel: 0718 220-6826; E-mail: gamato1@aol.com.

During a recent (February 23–April 29, 1997) biological survey expedition into northern Myanmar, an unfamiliar species of muntjac was observed in addition to the black muntjac (*M. crinifrons*) and the common muntjac *(M. muntjak*) (Rabinowitz & Khaing, 1998). The specimens were distinguished by their size, color, antler formation, and distribution. This unfamiliar taxon was called the 'leaf deer' by local hunters. We compared a 498 base-pair (bp) fragment of 16 S mitochondrial ribosomal (mt rDNA), a 380 bp fragment of 12 S mt rDNA, a 1183 bp fragment of cytochrome *b* (cyt b) mtDNA gene, and a 381 bp fragment of control region (Dloop) from known species of muntjac with samples from this unknown taxon. Also presented here are skull characteristics and measurements, pelage description based on the examination of a fresh specimen, and a cladogram, all consistent with the diagnostic character data from mitochondrial DNA indicating that the leaf deer differs from all currently described species of muntjac. Additionally, these data also indicate a close phylogenetic relationship between this newly discovered species and two other recently discovered species in Laos and Vietnam.

METHODS

The skulls and skull parts were obtained between 27°15'N, 97°30'E and 27°35'N, 97°50'E (Table 1). Morphological measurements follow those described by Groves & Grubb (1990) and Giao *et al*. (1998).

DNA was isolated from a large number of muntjac specimens providing a wide representation of genetic and geographic variation, including species sympatric

Table 1. Sample sources

Samples	Types of sample/location				Total number
M. putaoensis	Dried skin and bone – Myanmar (between 27°15N, 97°30 and 27°35N, 97°50)				8
	AMNH accession number	Location where sample purchased	Specimen details	DNA extracted from:	
	269939	Sinsagu	Skull, Female	Bone	
	269940	Atanga	Skull, Male	Bone	
	269941	Atanga	Skull, Male	Bone	
	269942 (Type)	Atanga	Skull, Male	Bone	
	269943	Atanga	Skull, Male	Bone	
	269944	Before Rabaw	Skull, Female	Skin from same individ. as skull	
	269945	Lanzatu	Male, frontlet[a]	Skin from frontlet	
	269946	Auranga	Male, frontlet	Skin from frontlet	
M. muntjak	Blood – India (1) Dried skin and bone – Laos (7) Phong Sali, Ban Mai (20°58'N,107°35'E), Attapeu province (14°58'N,107°23'E) Myanmar (11)				19
M. crinifrons/ gongshanensis	Dried bone – East China Normal University Museum (1) Dried tissue – Kunming Institute of Zoology (1) Dried skin and bone – Myanmar (12)				14
M. rooseveltorum	Skin and bone – Field Museum holotype, Laos; Ban Phuviang, Xiangkhoung province (19°49'N,103°45'E)				4
M. truongsonensis	Dried skin and bone fragment – Laos: Ban Tangyoum Sekong province (15°38'N,107°12'E)				2
M. vuquangensis[b]	Bone – Laos: Attapeu province (14°58'N,107°23'E)				2
M. feae	Blood – Thailand: Dusit Zoo				2
M. reevesi	Genbank; DNA, O. Ryder, San Diego Zoo				2
Elaphodus cephalophus	WCS				1
Cervus eldi	WCS				1

[a]Frontlet, frontal bone, pedicels, and antlers saved as trophy. [b]*M. vuquangensis*, *Megamuntiacus vuquangensis*.
WCS, Wildlife Conservation Society.

with the unknown taxon (Table 1). Most samples consisted of small pieces of dried tissue recovered opportunistically in the field. Samples of *M. crinifrons* and *M. gongshanensis* were obtained as small bone fragments from museum collections in China (Table 1). One of the *M. rooseveltorum* specimens was the holotype (Amato *et al*., 1998). DNA from hair and skin samples was extracted overnight at 56° C in Lifton's buffer (0.1 M Tris, 0.2 M sucrose, 0.05 M EDTA, 1% (w/v) SDS, pH 8.5) to which 1.4 mg/ml of Proteinase K was added. DNA was precipitated using standard phenol, chloroform and ethanol precipitation procedures (Sambrook, Fritsh & Maniatis, 1989). Bone samples were decalcified by soaking in 0.5 M EDTA pH 8.5 for several hours at 37° C prior to extraction and precipitation as described above. Details of the extraction procedures used for museum specimens appear in Rosenbaum *et al*. (1997) and Amato *et al*. (1998).

Amplifications were performed in a Perkin Elmer 9600 (cycling conditions: 38 cycles of 94° C denaturation for 45 s, 47° C annealing for 45 s, 72° C extension for 45 s) with the following reagent concentrations: 67 mM Tris, 3 mM $MgCl_2$, 16.6 mM $NH_4(SO_4)_2$, 0.8 mM premixed dNTPs, 0.2 μM of each primer and 1U of *Taq* DNA polymerase (Perkin Elmer). The following mtDNA primers were used for polymerase chain reaction (PCR) amplification and sequencing: 16 Sa, 16 Sb, 12 Sa, 12 Sb (Kocher *et al*., 1989), cyt b primers L14724, L14979, H15149, L15408, H15915R (Kocher *et al*., 1989; Irwin, Kocher & Wilson, 1991), and Dloop primers 1.5 (Arnason, Gullberg & Widegren, 1993) and Dlp5 (Baker *et al*., 1993). Amplification products were visualized using agarose gel electrophoresis and GeneCleaned (Bio101) according to manufacturer's instructions. Purified PCR products were cycle-sequenced using fluorescently labeled dideoxy terminators and run on Applied Biosystems 373A and 377 automated sequencers. Sequences were obtained from both strands and compared analytically.

Sequences of 12 S, 16 S, and cyt b for multiple individuals of each presumed species were aligned manually. There was only a single gap 1 base in length in *M. vuquangensis* allowing for constructing an unambiguous alignment of these regions by any method. Dloop sequences were aligned using the multiple sequence alignment program MALIGN 2.1 (Wheeler & Gladstein, 1994) using gap to change cost ratios of 2:1, 4:1, 6:1, 8:1 and 10:1. Ambiguous alignment regions were culled from the resulting alignments prior to analysis thus reducing the number of bases of Dloop analyzed to 381 aligned bases.

Individual bases were assessed using Population

Table 2. Measurements of the leaf deer, *Muntiacus putaoensis*

A. Craniometrics from skulls (in mm) of male *M. putaoensis*

Pedicel length		Antler length		Gap between antler tips	Gap between burrs	Beam circumference	
(L)	(R)	(L)	(R)			(L)	(R)
33	35	34	37	32	35	39	39
38	38	22	25	40	35	29	29
24	20	10	14	45	45	35	37
39	40	8	10	30	30	31	32
37	33	12	13	50	46	39	38
35	34	35	33	55	46	29	29
23	25	24	17	40	35	35	35

B. Craniometrics from skulls (in mm) of an adult male (top row) and adult female (bottom row) *M. putaoensis*

Skull length	Braincase width	Max. length of nasal	Max. width of nasal	Orbit horizontal		Orbit vertical		Interorbital breadth	P1–P3 upper		M1–M3 upper	
				(L)	(R)	(L)	(R)		(L)	(R)	(L)	(R)
16.5	5.3	4.5	1.8	3.2	3.2	3.2	3.3	3.8	5.5	5.5	3.3	3.3
16.0	4.5	4.5	1.5	3.0	3.0	2.9	2.9	3.2	5.0	5.0	3.0	3.0

L, left side; R, right side.

Aggregation Analysis (Davis & Nixon, 1992) to see if they diagnosed the unfamiliar taxon as a new species (Nixon & Wheeler, 1990; Davis & Nixon, 1992). The utility of examining a less variable region such as the 16 S mt rDNA for species diagnosis was explored. This fairly conservative region was chosen to minimize the problem of over diagnosis, which is a concern when dealing with small sample sizes. Multiple individuals of each presumed species were sequenced for 16 S. In some instances e.g. *M. putaoensis* and *M. rooseveltorum*, all known individuals of a species were sequenced. For the more widespread species we sequenced larger sample sizes from several localities across the distributional range of those species, as in the case of *M. muntjak* and *M. crinifrons* with samples from China, India, Myanmar, Laos and Bali. Well vouchered museum specimens or unambiguously identified field specimens were used to confirm the ability of this method to diagnose known species as well as identify new species. Numbers of individuals sequenced for each species are as follows: *M. putaoensis*, *n* = 8; *M. rooseveltorum*, *n* = 4; *M. truongsonensis*, *n* = 2; *M. vuquangensis*, *n* = 2; *M. crinifrons/ gongshanensis*, *n* = 14; *M. feae*, *n* = 2; *M. reevesi*, *n* = 2; *M. muntjak*, *n* = 19; *Elaphodus cephalophus*, n = 1; *Cervus eldi*, *n* = 1.

Additional mt gene regions (12 S, cyt b and Dloop) were also explored. Numbers of individuals for each taxon and for each additional gene region are as follows: *M. putaoensis* (12 S, *n* = 3; cyt b, *n* = 3 for the entire 1183 bp fragment, and an additional 5 individuals were sequenced for a 200 bp fragment; Dloop, *n* = 2); *M. rooseveltorum* (12 S, *n* = 3; cyt b, *n* = 2 for the entire 1183 bp fragment, and an additional 2 individuals including the Type specimen were sequenced for a 200 bp fragment; Dloop, *n* = 2); *M. truongsonensis* (12 S, *n* = 2; cyt b, *n* = 1 for the entire 1183 bp fragment and *n* = 2 for the 200 bp fragment; Dloop, *n* = 2); for *M. vuquangensis* and *M. feae* (*n* = 2 for each additional gene region); for *M. reevesi* , *Elaphodus cephalophus* and *Cervus eldi* (*n* = 1 for each additional gene region); *M. crinifrons/gongshanensis* (12 S, *n* = 5; cyt b, *n* = 13; Dloop, *n* = 4) and *M. muntjak* (12 S, *n* = 6; cyt b, *n* = 16; Dloop, *n* = 4).

A preliminary phylogenetic analysis was conducted by searching for minimum length cladograms using PAUP 3.1.1 (Swofford, 1993). Representatives of all muntjac taxa except *M. atherodes* were included in the phylogenetic analysis. A simultaneous analysis approach was used combining all four mtDNA gene regions. All characters were unordered and equally weighted. Branch support was determined by decay indices (Bremer, 1988, 1994) and bootstrap frequency was based on 1000 bootstrap replicates.

RESULTS

Skull character measurements from a nearly complete male and female (Table 2) are similar to those of *M. rooseveltorum* (Osgood, 1932) and a recently described taxon from Vietnam, *M. truongsonensis* (Giao *et al*., 1998), but clearly differentiate this species qualitatively from the sympatric *M. muntjak* and *M. crinifrons* (Groves & Grubb, 1990). Informal observations of the recently hunted specimen reveal chestnut colored pelage in contrast to the nearly black color of the osteologically similar *M. truongsonensis* (Fig. 1).

Fourteen DNA characters (12 S(1), 16 S(2), cyt b(7), and Dloop(4)) unambiguously diagnose this taxon from all other species of muntjac (Table 3). Additional substitutions differentiate this taxon individually from previously described species (i.e. 40 sites from *M. truongsonensis*, 95 sites from *M. vuquangensis*, 106 sites from *M. crinifrons*, Table 4)

Two equally most parsimonious trees were recovered from the simultaneous analysis of the four mtDNA gene regions (GenBank Accession numbers: AF108031–

Table 3. Sites in mtDNA that diagnose *Muntiacus putaoensis* as distinct from all other muntjac species

Species	Nucleotide position													
	12 S	16 S		cyt b								Dloop		
	288	199	259	22	687	744	762	850	1178	1180	14	124	349	351
M. putaoensis	**A**	**A**	**C**	**C**	**T**	**G**	**T**	**C**	**G**	**G**	**A**	**T**	**C**	**C**
M. rooseveltorum	C	T	T	T	C	A	C	T	A	A	G	C	T	T
M. truongsonensis	T	T	T	T	C	A	C	T	A	A	G	C	T	T
M. vuquangensis	C	T	T	T	C	A	C	T	A	A	G	C	T	T
M. crinifrons/gongshanensis	T	T	T	T	C	A	C	T	A	A	G	C	T	T
M. feae	T	T	T	T	C	A	C	T	A	A	G	C	T	T
M. reevesi	C	T	T	T	C	A	C	T	A	A	G	C	T	T
M. muntjak	T	T	T	T	C	A	C	T	A	A	G	C	T	T

Fig. 1. *Muntiacus putaoensis* from northern Myanmar showing chestnut colored pelage. (Photograph by A. Rabinowitz)

AF108041). A strict consensus tree is presented in Fig. 2. The monophyly of the genus *Muntiacus* is well supported with the giant muntjac (*Megamuntiacus vuquangensis*) nested well within this group. Four lineages (1. *M. reevesi*; 2. *M. muntjak;* 3. *M.crinifrons/ gongshanensis* + *feae*; and 4. (((*M. putaoensis* + *M. truongsonensis*) + *M. rooseveltorum*) + *M. vuquangensis*)) are supported, however relationships within these lineages are less well supported. *Muntiacus gongshanensis* is not resolved as a separate taxon from *M. crinifrons*. A sister taxon relationship between *M. crinifrons* and *M. feae* is well supported. Addition-ally, this tree also reveals a close phylogenetic relationship between this new species (*M. putaoensis*), *M. truongsonensis*, *M. rooseveltorum*, and *M. vuquangensis* (Fig. 2).

Table 4. Number of additional mtDNA substitutions that differentiate *Muntiacus putaoensis* from *M. truongsonensis*, *M. vuquangensis*, and *M. crinifrons*

	12 S	16 S	cyt b	Dloop
M. truongsonensis	3	1	21	15
M. vuquangensis	6	9	47	33
M. crinifrons	9	8	69	20

SPECIES DESCRIPTION

Muntiacus putaoensis new species

Type

AMNH 269942, skull only, male (incomplete), purchased by A. R. at Atanga village, 30 km east of Putao (27°21'N, 97°24'E), northern Myanmar.

Paratypes

AMNH 269939, –40, –41, –43, –44, –45, –46 (incomplete) skulls (Table 1).

Diagnosis

A small muntjac species with chestnut colored coat pelage, including upper surface of tail, resembling common muntjac, *M. muntjak*, but with short, thin pedicels (<40 mm length, <39 mm circumference) and short, unbranched antlers (see Table 2). There appear to be no obvious differences in the craniometrics and canine lengths between available male and female specimens. Also, diagnostic mt rDNA fragments are reported here (see Table 3).

Description

This small muntjac, called the leaf deer by local villagers, is described as the smallest of three sympatric species that inhabit the mountainous area between the Mali Kha and Mai Kha rivers of northern Myanmar. Hunters claim this animal resides 'on the mountain tops' while the other two larger sympatric species, the common muntjac, *M. muntjak*, and the black muntjac, *M. crinifrons*, are found lower down. DNA sequence data indicate that it is the sister taxon to *M. truongsonensis*,

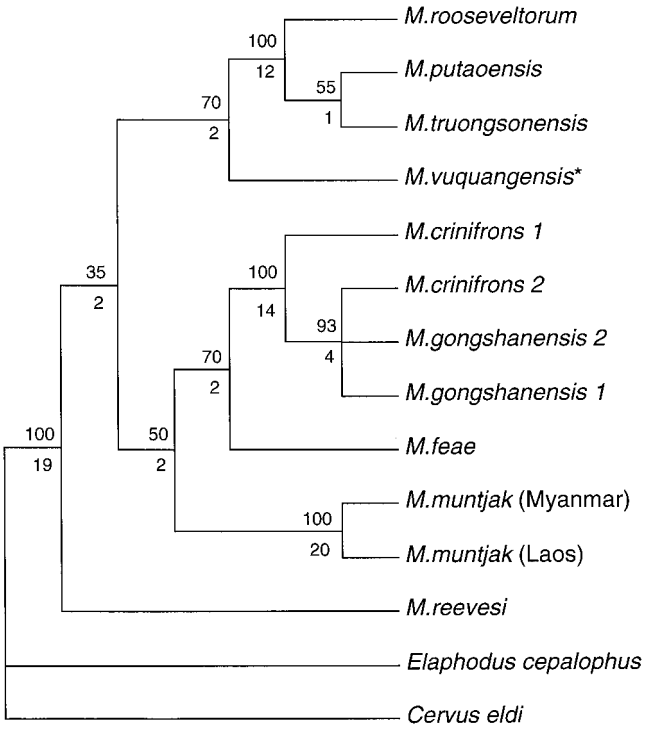

Fig. 2. Parsimony simultaneous analysis of mitochondrial gene regions (16 S, cyt b, 12 S, and Dloop). Strict consensus of two equally most parsimonious trees (length = 536, CI = 0.521, RI = 0.595 ignoring uninformative characters). Bootstrap frequencies as percentages of 1000 bootstrap replicates are shown above branches and decay indices (Bremer, 1988, 1994) are shown below branches. The monophyly of the genus *Muntiacus* is supported inclusive of the giant muntjac, *Megamuntiacus vuquangensis*(*). A sister group relationship of *M. crinifrons* and *M. feae* and a clade consisting of ((*M. putaoensis* + *M. truongsonensis*) *M. rooseveltorum*) are supported.

despite differences in coat and tail coloration. Preliminary morphological measurements are consistent with this hypothesis.

Etymology

The specific name, *M. putaoensis*, refers to the most northern town in Myanmar, Putao, the closest reference point for this species.

DISCUSSION

A great deal of confusion currently surrounds muntjac taxonomy (Groves & Grubb, 1982, 1987, 1990; Ma, Wang & Xu, 1986; Corbet & Hill, 1992) reflecting in part a lack of morphological differentiation in this group. DNA sequence data suggest that all extant species of muntjac may represent a recent radiation from this ancient lineage of cervids (Groves & Grubb, 1987; Miyamoto, Tanhauser & Laipis, 1989; Cronin *et al.*, 1996). Eight species are generally recognized (Ma *et al.*, 1986; Corbet & Hill, 1992). Data in this study diagnose a new species of muntjac from northern Myanmar in relation to these recognized species, and we propose the name *M. putaoensis* for this taxon. The description includes diagnostic molecular characters, morphological characters, and distribution data.

mtDNA proved to be useful for identifying diagnostic characters for all species of muntjac examined. Using multiple samples from most taxa allowed us to confidently treat these as characters as defined by Davis & Nixon (1992) providing information for species delimitation under the clearly articulated phylogenetic species concept (Cracraft, 1989; Nixon & Wheeler, 1990). Identification of diagnostic sites in the conserved 16 S and 12 S regions, in addition to those in the more variable cyt b and Dloop fragments, reduced our concerns about over diagnosing taxa for which there were only a small number of samples available. Similarly, the consistency of diagnostic sites in these regions for the 19 widely separated samples of *M. muntjak* further supported the utility of this approach.

Precisely what defines a species has long been a contentious issue among biologists (Cracraft, 1989; Avise & Ball, 1990; Amato & Gatesy, 1994). It has been suggested that different definitions reflect differences in specific utility and applicability for sub-disciplines within biology (Endler, 1989; Templeton, 1989). However, in spite of the fact that it is unclear how to precisely test species status under other species concepts (e.g. biological species concept: Mayr, 1963; Avise & Ball, 1990), the number of additional sites that distinguish this species from previously described species is consistent with the genetic differentiation that is observed in other closely related biological species for the regions examined (Irwin *et al.*, 1991; Gatesy *et al.*, 1997). The geographic distribution of this taxon in relation to its sister taxon further supports species delineation by most definitions. Both *M. putaoensis* and *M. truongsonensis* are confined to mountainous areas of remnant, specific forest types. However, the distance between northern Myanmar and the Annamite Mountains in Laos/Vietnam suggest that there could not have been any contact between these groups since the last major climatic change. Finally, phylogenetic analysis of multiple individuals (including eight *M. putaoensis*) always provides a topology that is concordant with species status.

This study is representative of a relatively new approach in conservation biology. Biodiversity surveys have been initiated in remote and understudied regions in order to rapidly assess attributes such as levels of endemism and species richness in order to establish conservation area priorities. Molecular genetics can be an important tool in such surveys. A phylogenetic species concept framework (Davis & Nixon, 1992; Cracraft *et al.*, 1998) using DNA characters to diagnose species is particularly useful in these surveys, especially when many of the samples acquired are not suitable for traditional morphological assessments. This should not be controversial since species descriptions have always been based on diagnostic characters, and it is not obvious why

morphological diagnostic characters would be qualitatively superior to DNA characters. This approach, though not clearly articulated, has been used successfully in a number of recent studies (Dung *et al*., 1993; Tuoc *et al*., 1994; Amato *et al*., 1998; Giao *et al*., 1998). However, the studies by Dung *et al*. (1993), Tuoc *et al*. (1994), and in particular Giao *et al*. (1998) suffer from poor taxonomic sampling for both describing new species and phylogenetic analysis. Specifically the lack of data, or even discussion of the sympatric *M. rooseveltorum*, weakened the conclusions of the Giao *et al*. (1998) paper until the completion of the Amato *et al*. (1998) study. Additional problems with this study include not using *Elaphodus* as an outgroup for phylogenetic analysis, confusion as to how they define what constitutes a species or subspecies, and morphological assessments on incomplete samples presented as analysis. In this study we have sought to avoid similar problems. While the DNA character data presented here allow us to confidently diagnose a new species, it is clear that morphological analysis must await the collection of additional complete specimens from a number of muntjac species.

Phylogenetic relationships of major muntjac lineages are well resolved. The monophyly of *Muntiacus* with respect to *Elaphodus* is well supported. This study further supports Schaller & Vrba (1996) and Giao *et al*. (1998) in calling for the abandonment of *Megamuntiacus* in favor of *Muntiacus* for *M. vuquangensis*. The phylogenetic analysis also questions the validity of one taxon (*M. gongshanensis*). *Muntiacus gongshanensis* has always been problematic since it was described on the basis of a single odd karyotype (C. P. Groves, pers. comm.). Here *M. crinifrons* and *M. gongshanensis* are treated as a single taxon because our data from multiple specimens (and other molecular data not presented here) do not diagnose *M. gongshanensis* as distinct. Further study is warranted. Other relationships within lineages remain less resolved. Additional studies of nuclear regions are currently underway in our laboratory to further explore these relationships.

One area that is well resolved is the close relationship between *M. putaoensis*, *M. truongsonensis*, and *M. rooseveltorum*. It is interesting to note that all three species appear to be confined to old growth forests in mountainous areas (Amato *et al*., 1998; Giao *et al*., 1998; Groves & Schaller, 1998) supporting the argument that such areas should be conservation priorities (Giao *et al*., 1998; Rabinowitz, Amato & Khaing, 1998). In addition to this noted endemism, these results also identify areas of species richness. Five years ago it was believed that there was only a single species of muntjac in the Annamite Mountains. We now know that there are four. Similarly, only one species of muntjac had been reported from northern Myanmar until the survey by Rabinowitz and colleagues found evidence of two more (Rabinowitz *et al*., 1998).

Perhaps the most important aspect of this discovery is that this new species of large mammal was found in another remote region of Asia outside the Annamites. This highlights the importance of continuing rigorous biological surveys in relatively unstudied areas. The fact that wildlife, as well as the habitats themselves, are currently disappearing at an alarming rate adds a sense of urgency to such research. Identifying yet another new species of muntjac also demonstrates how little we know about this group. The diagnostic molecular character approach presented here provides a useful, objective framework for further study.

Acknowledgments

We thank U Saw Tun Khaing, Ministry of Forestry in Myanmar, Myanmar Forest Department for contributions to the fieldwork. Samples were provided by Zoological Park Organization Thailand, Kunming Institute of Zoology, East China Normal University, Field Museum of Natural History Chicago, and G. B. Schaller. R. Baker and H. Rosenbaum made important technical contributions. R. DeSalle and P. Goldstein provided useful comments on the manuscript. S. Rabinowitz aided in manuscript preparation.

REFERENCES

Amato, G., Egan, M. G., Schaller, G. B., Baker, R., Rosenbaum, H. C., Robichaud, W. G. & DeSalle, R. (1998) The rediscovery of Roosevelt's barking deer (*Muntiacus rooseveltorum*). *J. Mammal.* (in press)

Amato, G. & Gatesy, J. (1994). PCR assays of variable nucleotide sites for identification of conservation units. In *Molecular ecology and evolution: approaches and applications:* 215–226. Schierwater, B., Streit, B., Wagner, G. P. & DeSalle, R. (Eds). Basel: Birkhauser.

Arnason, U., Gullberg, A. & Widegren, B. (1993). Cetacean mitochondrial DNA control region: sequences of all extant baleen whales and two sperm whale species. *Mol. Biol. Evol.* **10**: 960–970.

Avise, J. C. & Ball, R. M. (1990). Principles of geneaological concordance in species concepts and biological taxonomy. *Oxford Surv. Evol. Biol.* **7**: 45–67.

Baker, C. S., Perry, A., Bannister, J. L., Weinrich, M. T., Abernethy, R. B., Calambokidis, J., Lien, J., Lambertsen, R. H., Urban Ramirez, J., Vasquez, O., Clapham, P. J., Alling, A., O'Brien, S. J. & Palumbi, S. R. (1993). Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. *Proc. Nat. Acad. Sci. USA* **90**: 8239–8243.

Bremer, K. (1988). The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42**: 795–803.

Bremer, K. (1994). Branch support and tree stability. *Cladistics* **10**: 295–304.

Corbet, G. B. & Hill, J. E. (1992). *Mammals of the Indomalayan region.* Oxford: Oxford University Press.

Cracraft, J. (1989). Speciation and its ontology: the empirical consequences of alternative species concepts for understanding patterns and processes of differentiation. In *Speciation and its consequences:* 28–59. Otte, D. & Endler, J. A. (Eds). Sunderland, MA: Sinauer Associates, Inc.

Cracraft, J., Feinstein, J., Vaughn, J. & Helm-Bychowski, K. (1998). Sorting out tigers (*Panthera tigris*): mitochondrial sequences, nuclear inserts, systematics, and conservation genetics. *Anim. Conserv.* **1**: 139–150.

Cronin, M., Stuart, R., Pierson, B. & Patton, J. (1996). k-casein gene phylogeny of higher ruminants (Pecora, Artiodactyla). *Mol. Phylogenet. Evol.* **6**: 295–311.

Davis, J. I. & Nixon K. C. (1992). Populations, genetic variation, and the delimitation of phylogenetic species. *Syst. Biol.* **41**: 421–435.

Dollman, J. G. (1932). Mammals collected by Lord Cranbrook and F. Kingdon-Ward in upper Burma. *Proc. Linn. Soc. Lond.* **145**: 9–11.

Dung, V., Giao, P., Chin, N. N., Tuoc, D., Arctander, P. & MacKinnon, J. (1993) A new species of living bovid from Vietnam. *Nature, Lond.* **363**: 443–445.

Dung, V., Giao, P. M., Chinn, N. N., Tuoc, D. & MacKinnon, J. (1994). Discovery and conservation of the Vu Quang ox in Vietnam. *Oryx* **28**: 16–21.

Endler, J. A. (1989). Conceptual and other problems in speciation. In *Speciation and its consequences:* 625–648. Otte, D. & Endler, J.A. (Eds). Sunderland, MA: Sinauer Associates, Inc.

Gatesy, J., Amato, G., Vrba, E., Schaller, G. B. & DeSalle, R. (1997). A cladistic analysis of mitochondrial ribosomal DNA from the Bovidae. *Mol. Phylogenet. Evol.* **7**: 303–319.

Giao, P., Tuoc, D., Dung, V., Wikramanayake, E., Amato, G., Arctander, P. & MacKinnon, J. (1998). Description of *Muntiacus truongsonensis*, a new species of muntjac (Artiodactyla: Muntiacidae) from Central Vietnam and implications for conservation. *Anim. Conserv.* **1**: 61–68.

Groves, C. P. & Grubb, P. (1982). The species of muntjac (genus *Muntiacus*) in Borneo. *Zool. Meded. Leiden* **56**: 203–214.

Groves, C. P. & Grubb, P. (1987). Relationships of living *Cervidae*. In *Biology and management of the Cervidae:* 21–59. Wemmer C. M. (Ed.). Washington: Smithsonian Institute.

Groves, C. P. & Grubb, P. (1990) Muntiacidae. In *Horns, pronghorns, and antlers:* 134–168. Bubenik, G. & Bubenik, A. (Eds). New York: Springer-Verlag.

Groves, C. & Schaller, G. B. (1998). The phylogenetic and biogeographic position of the newly-discovered Annamite Artiodactyls. In *Antelopes, deer, and relatives: fossil record, behavioral ecology, systematics, and conservation:* E. Vrba (Ed.). New Haven, CT: Yale University Press (in press).

Groves, C., Schaller, G. B., Amato, G. & Khounboline K. (1997). Rediscovery of the wild pig *Sus bucculentus*. *Nature, Lond.* **386**: 335.

Irwin, D. M., Kocher, T. D. & Wilson, A. C. (1991). Evolution of the cytochrome b gene of mammals. *J. Mol. Evol.* **32**: 128–144.

Kingdon-Ward, F. (1936). The Irrawaddy plateau. *Geogr. J. (Lond.)*. **94**: 293–308.

Kingdon-Ward, F. (1944). A sketch of the botany and geography of North Burma. *J. Bombay Nat. Hist. Soc.* **44**: 550–574.

Kinnear, N. B. (1934). On the birds of the Adung Valley, Northeast Burma. *J. Bombay Nat. Hist. Soc.* **37**: 347–368.

Kocher, T. D., Thomas, W. K., Meyer, A., Edwards, S. V., Paabo, S., Villablanca, F. X. & Wilson, A. C. (1989). Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA* **86**: 377–382.

Ma, S., Wang, Y. & Xu, L. (1986). Taxonomic and phylogenetic studies on the genus *Muntiacus*. *Acta Theriol. Sinica*. **6**: 191–209 [in Chinese].

Mayr, E. (1963). *Animal species and evolution*. Cambridge, MA: The Belknap Press.

Miyamoto, M., Tanhauser, S. & Laipis, P. (1989). Systematic relationships in the artiodactyl tribe Bovini (family Bovidae), as determined from mitochondrial DNA sequences. *Syst. Zool.* **38**: 342–349.

Nixon, K. C. & Wheeler, Q. D. (1990). An amplification of the phylogenetic species concept. *Cladisitics* **6**: 211–223.

Osgood, W. (1932). Mammals of the Kelley-Roosevelts and Delacour Asiatic expiditions. *Field Mus. Nat. Hist. Publ. 312, Zool. Ser*. **18(10)**: 192–339.

Rabinowitz, A., Amato, G. & Khaing, U. S. T. (1998). Discovery of the black muntjac, *Muntiacus crinifrons* (Artiodactyla, Cervidae), in north Myanmar. *Mammalia* **62**: 105–108.

Rabinowitz, A. & Khaing, U. S. T. (1998). Status of selected mammal species in north Myanmar. *Oryx* **32**: 201–208.

Rosenbaum, H. C., Egan, M. G., Clapman, P. J., Brownell, R. L. & DeSalle, R.(1997). An effective method for isolating DNA from historical specimens of baleen. *Mol. Ecol*. **6**: 677–681.

Sambrook, J., Fritsh, E. F. & Maniatis, T. (1989). *Molecular cloning: a laboratory manual*. (2nd edn). New York: Cold Spring Harbor Laboratory Press.

Schaller, G. B. (1995). An unfamiliar "bark". *Wildl. Conserv*. **98**: 8.

Schaller, G. B. & Vrba, E. S. (1996). Description of the giant muntjac (*Megamunticus vuquangensis*) in Laos. *J. Mammal*. **77**: 675–683.

Swofford, D. (1993). *PAUP: phylogenetic analysis using parsimony, version 3.1.1*. Champaign, IL: Illinois Natural History Survey.

Templeton, A. R. (1989). The meaning of species and speciation: a genetic perspective. In *Speciation and its consequences:* 3–27. Otte, D. & Endler, J. A. (Eds). Sunderland, MA: Sinauer Associates, Inc.

Tuoc, D., Dung, V., Dawson, S., Arctander, P. & MacKinnon, J. (1994). *Introduction of a new large mammal species in Vietnam.* Science and Technology news. Forest Inventory and Planning Institute (Hanoi), March 4–13 [in Vietnamese].

Wheeler, W. & Gladstein, D. (1994). *MALIGN, version 2.1*. New York: American Museum of Natural History.