

Applying DNA barcoding to conservation practice: a case study of endangered birds and large mammals in China

Biodiversity and Conservation

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Table 1 Collection information of the investigated samples, (a) birds, (b) mammals. The underlined samples failed to retrieve COI sequences and were discarded in the molecular identification. Class I and Class II referred to first-grade and second-grade state protection animals, respectively

(a)

Putative species	Taxonomy		Sample vouchers	Tissue	Location/Source	Collection date	No. of whole specimens	Remarks
	Order	Family						
<i>Lophophorus lhuysii</i>	Galliformes	Phasianidae	B04	muscle	Sichuan, Pixian	2007-9-28	1	Class I, endemic to China IUCN: Vulnerable
<i>Chrysolophus pictus</i>	Galliformes	Phasianidae	<u>B26</u>	feather	Shaanxi, Hanzhong, Yuhuang Mountain	2012-3-4	1	Class II IUCN: Least Concern
<i>Phasianus colchicus</i>	Galliformes	Phasianidae	B12, B35, <u>B36</u>	feather muscle	Tibet; Sichuan, Baoxing	2008-2-25 2007-12-12	3	IUCN: Least Concern
<i>Arborophila rufipectus</i>	Galliformes	Phasianidae	AR1, AR2, AR3, AR5, ARB3, ARB6	blood	Sichuan, Pingshan, Laojunshan National Nature Reserve	2008-5-2 2012-4-28 2012-5-24	2	Class I, endemic to China IUCN: Endangered
<i>Arborophila ardens</i>	Galliformes	Phasianidae	B10, <u>B11</u>	muscle†	Hainan	2009-3-25	1	Class I, endemic to China, IUCN: Vulnerable
<i>Arborophila brunneopectus</i>	Galliformes	Phasianidae	B33	muscle	Yunnan, Sipsongpanna	2012-1-14	1	IUCN: Least Concern
<i>Pandion haliaetus</i>	Falconiformes	Pandionidae	B02	muscle†	Sichuan, Pixian	2009-9-26	0	Class II

<i>Accipiter virgatus</i>	Falconiformes	Accipitridae	B03	feather	Chengdu*	2006-1-8	0	IUCN: Least Concern Class II
<i>Aquila clanga</i>	Falconiformes	Accipitridae	B16	muscle†	Sichuan, Aba	2005-10-12	0	IUCN: Least Concern Class II
<i>Falco cherrug</i>	Falconiformes	Falconidae	B05	muscle†	Sichuan, Ganzi	2010-10-26	0	IUCN: Vulnerable Class II
<i>Falco tinnunculus</i>	Falconiformes	Falconidae	B13	muscle	Sichuan, Chengdu	2008-10-21	1	IUCN: Endangered Class II
<i>Asio otus</i>	Strigiformes	Strigidae	B08	muscle†	Sichuan, Pixian	2011-9-26	0	IUCN: Least Concern Class II
<i>Athene noctus</i>	Strigiformes	Strigidae	B15	muscle	Gansu, Baiyin	2008-1-1	1	IUCN: Least Concern Class II
<i>Grus monacha</i>	Gruiformes	Gruidae	N1, N2, N25	blood	CRBGPB*	2011-12-12	3	IUCN: Vulnerable Class I
<i>Grus vipio</i>	Gruiformes	Gruidae	N15, N16	blood	CRBGPB*	2011-12-12	2	IUCN: Vulnerable Class II
<i>Grus nigricollis</i>	Gruiformes	Gruidae	N17, N23, N24	blood	CRBGPB*	2011-12-12	3	IUCN: Vulnerable Class I
<i>Grus leucogeranus</i>	Gruiformes	Gruidae	N18, N19, N29, N30	blood	CRBGPB*	2011-12-13	4	IUCN: Critically Endangered Class I
<i>Grus japonensis</i>	Gruiformes	Gruidae	N20, N21, N22, N33, N34	blood	CRBGPB*	2011-12-13	5	IUCN: Endangered Class I
<i>Anthropoides virgo</i>	Gruiformes	Gruidae	N27, N28	blood	CRBGPB*	2011-12-13	2	IUCN: Least Concern Class II
<i>Ciconia ciconia</i>	Ciconiiformes	Ciconiidae	N7, N8, N31, N32	blood	CRBGPB*	2011-12-14	3	IUCN: Least Concern Class I
<i>Leptoptilos crumenifer</i>	Ciconiiformes	Ciconiidae	N9, N10, N11	blood	CRBGPB*	2011-12-14	2	IUCN: Least Concern Class I
<i>Mycteria ibis</i>	Ciconiiformes	Ciconiidae	N12, N13, N14	blood	CRBGPB*	2011-12-15	3	IUCN: Least Concern Class I

(b)

Scientific name	Taxonomy		Sample vouchers	Tissue	Location/Source	Collection date	No. of whole specimens	Remarks
	Order	Family						
<i>Pseudois nayaur</i>	Artiodactyla	Bovidae	A11	heart	Ningxia, Yinchuan	2006-10-10	1	Class II IUCN: Least Concern
<i>Pseudois schaeferi</i>	Artiodactyla	Bovidae	A17	muscle	Sichuan, Batang	2006-4-5	1	Class II, endemic to China
<i>Pantholops hodgsonii</i>	Artiodactyla	Bovidae	A53	muscle	Tibet	2008-2-25	0	Class I, endemic to China IUCN: Endangered
<i>Cervus unicolor</i>	Artiodactyla	Cervidae	A23	fur	Sichuan, Baoxing	Unknown	0	Class II IUCN: Vulnerable
<i>Cervus albirostris</i>	Artiodactyla	Cervidae	A29	fur	Sichuan, Aba,	Unknown	0	Class I IUCN: Vulnerable
<i>Cervus nippon</i>	Artiodactyla	Cervidae	M1, Q2, H26	fur	Sichuan, Aba, Ruoergai	2007-8-1	0	Class I IUCN: Least Concern
<i>Mandrillus sphinx</i>	Primates	Cercopithecidae	S38, S62	kidney† blood	Qingdao Zoo Chengdu Zoo	1998-1-12 2010-6-3	2	IUCN: Vulnerable
<i>Cercopithecus mitis</i>	Primates	Cercopithecidae	S30,S36	liver†	CRBGPB*	1996-1-23	2	IUCN: Least Concern
<i>Chlorocebus aethiops</i>	Primates	Cercopithecidae	S64	blood	Chengdu Zoo	Unknown	1	IUCN: Least Concern
<i>Trachypithecus francoisi</i>	Primates	Cercopithecidae	S44, S63	muscle blood	CRBGPB* Chengdu Zoo	2008-12-1 2010-12-21	1	Class I IUCN: Endangered
<i>Papio sp.</i>	Primates	Cercopithecidae	S29 S61	liver† blood	CRBGPB* Chengdu Zoo	1995-12-29 2002-3-20	0	
<i>Saimiri sciureus</i>	Primates	Cebidae	S45,S50	muscle	CRBGPB*	2008-12-1	2	IUCN: Least Concern

<i>Cebus apella</i>	Primates	Cebidae	S46	muscle	CRBGPB*	2008-12-1	1	IUCN: Least Concern
<i>Nycticebus coucang</i>	Primates	Lorisidae	S49	fur	CRBGPB*	2008-12-1	0	Class I IUCN: Vulnerable
<i>Lemur catta</i>	Primates	Lemurida	S26	heart	CRBGPB*	1995-1-24	1	IUCN: Endangered
<i>Panthera leo</i>	Carnivora	Felidae	S14, S15, S18	blood	CRBGPB	Unknown	3	IUCN: Vulnerable
<i>Panthera pardus</i>	Carnivora	Felidae	S23,S28	liver blood	CRBGPB*	1994-9-22 1995-12-24	2	Class I IUCN: Near Threatened
<i>Panthera tigris amoyensis</i>	Carnivora	Felidae	S53,S54,S55,S56,S57, S58, S59	blood	CRBGPB*	Unknown	7	Class I IUCN: Endangered
<i>Felis bieti</i>	Carnivora	Felidae	<u>S7</u> , <u>S8</u> , <u>S9</u>	blood†	CRBGPB*	Unknown	1	Class II, endemic to China IUCN: Vulnerable
<i>Neofelis nebulosa</i>	Carnivora	Felidae	<u>S19</u> , <u>S20</u>	blood†	CRBGPB*	Unknown	0	Class I IUCN: Vulnerable
<i>Cuon alpinus</i>	Carnivora	Canidae	S51	fur	Sichuan, Kangding	1997-2	0	Class II IUCN: Endangered
<i>Ailuropoda melanoleuca</i>	Carnivora	Ailuridae	A3, A4, XJ, A144	blood feces	CRBGPB	2009-11-3	4	Class I IUCN: Endangered

* showed the source of samples rather than the locations. CRBGPB is an abbreviation of Chengdu Research Base of Giant Panda Breeding.

† represented tissues with different degree of decay.

Table 2 PCR primers used in this study

Primers	Primer sequence (5'-3')	Direction	Primer source
BirdF1	TTCTCCAACCACAAAGACATTGGCAC	F	Kerr et al. 2007
BirdR1	ACGTGGGAGATAATTCCAAATCCTG	R	Kerr et al. 2007
FalcoFa	TCAACAAACCACAAAGACATCGGCAC	F	Kerr et al. 2007
BirdR2	ACTACATGTGAGATGATTCCGAATCCAG	R	Kerr et al. 2007
GF_1	TCAACYAACCACAAAGATATCGGAAC	F	Originally designed
GR_1	ACGTGTGAGATGATTCCAAAACCTG	R	Originally designed
VF1	TTCTCAACCAACCACAAAGACATTGG	F	Ward et al. 2005
VR1	TAGACTTCTGGGTGGCCAAAGAATCA	R	Ward et al. 2005
VF1d	TTCTCAACCAACCACAARGAYATYGG	F	Ivanova et al. 2006
VR1d	TAGACTTCTGGGTGGCCRAARAAYCA	R	Ivanova et al. 2006

Table 3 Downloaded barcodes of (a) birds, (b) mammals used in the study

(a)

Species	Order	Family	Sample No.	GenBank accessions
<i>Grus monacha</i>	Gruiformes	Gruidae	2	NC020578, EF515778
<i>Grus nigricollis</i>	Gruiformes	Gruidae	1	NC020579
<i>Grus grus</i>	Gruiformes	Gruidae	4	GU571424, GU571425, GU571916, NC020577
<i>Grus japonensis</i>	Gruiformes	Gruidae	1	NC020575
<i>Grus canadensis</i>	Gruiformes	Gruidae	2	NC020582, JQ174965
<i>Grus antigone</i>	Gruiformes	Gruidae	1	NC020581
<i>Grus vipio</i>	Gruiformes	Gruidae	2	NC021368, KF939579
<i>Grus leucogeranus</i>	Gruiformes	Gruidae	3	NC020574, KF958462, KF939578
<i>Anthropoides virgo</i>	Gruiformes	Gruidae	2	NC020573, JQ174043
<i>Mycteria leucocephala</i>	Ciconiiformes	Ciconiidae	2	HM804932, HM804933
<i>Mycteria americana</i>	Ciconiiformes	Ciconiidae	3	FJ027865, DQ433030, JQ175426
<i>Ciconia nigra</i>	Ciconiiformes	Ciconiidae	1	AY567882
<i>Ciconia ciconia</i>	Ciconiiformes	Ciconiidae	4	GU571816, GU571817, NC002197, AY567881
<i>Ciconia boyciana</i>	Ciconiiformes	Ciconiidae	1	NC002196
<i>Microhierax caerulescens</i>	Falconiformes	Falconidae	1	JQ175370
<i>Falco tinnunculus</i>	Falconiformes	Falconidae	5	GQ481882, GQ481883, GU571393, GU571886, NC011307
<i>Falco rusticolus</i>	Falconiformes	Falconidae	4	GU571882, KF525372, GU571390, DQ432927
<i>Falco peregrinus</i>	Falconiformes	Falconidae	4	JX029991, JQ282801, GU571880,

				GU571388
<i>Falco columbarius</i>	Falconiformes	Falconidae	3	GU571878, GU571384, KF537263
<i>Falco amurensis</i>	Falconiformes	Falconidae	2	GQ481872, GQ481873
<i>Falco vespertinus</i>	Falconiformes	Falconidae	1	JQ174839
<i>Falco naumanni</i>	Falconiformes	Falconidae	3	GU571386, GQ481875, GQ481876
<i>Falco subbuteo</i>	Falconiformes	Falconidae	5	GU571392, GU571884, GQ481877, GQ481878, GQ481880
<i>Pandion haliaetus</i>	Falconiformes	Pandionidae	5	DQ434696, DQ433863, GU571998, GU571516, NC008550
<i>Accipiter gentilis</i>	Falconiformes	Accipitridae	6	GQ922623, GQ922625, NC011818, GU571207, AY666498, DQ433279
<i>Accipiter nisus</i>	Falconiformes	Accipitridae	4	GU571210, JF312191, JF312194, GQ922642
<i>Accipiter soloensis</i>	Falconiformes	Accipitridae	3	JF312282, JF312184, JF312185
<i>Accipiter gularis</i>	Falconiformes	Accipitridae	3	JF792341, JF792343, JQ173889
<i>Accipiter virgatus</i>	Falconiformes	Accipitridae	3	GQ481352, GQ481253, JF312186
<i>Buteo buteo</i>	Falconiformes	Accipitridae	5	GQ922641, GQ481407, NC003128, GU571289, GU571769
<i>Buteo hemilasius</i>	Falconiformes	Accipitridae	2	GQ922627, GQ922628
<i>Buteo lagopus</i>	Falconiformes	Accipitridae	3	AY666502, GU571771, GU571292
<i>Aquila clanga</i>	Falconiformes	Accipitridae	1	GU571739
<i>Aquila chrysaetos</i>	Falconiformes	Accipitridae	3	GU571264, GU571265, GU571737
<i>Haliaeetus indus</i>	Falconiformes	Accipitridae	1	HM639875
<i>Otus sunia</i>	Strigiformes	Strigidae	3	GQ482286, GQ482287, GQ482289
<i>Otus scops</i>	Strigiformes	Strigidae	1	JQ175647
<i>Ninox scutulata</i>	Strigiformes	Strigidae	1	EU251422
<i>Aegolius funereus</i>	Strigiformes	Strigidae	3	GU571226, GQ482195, GU571703
<i>Glaucidium cuculoides</i>	Strigiformes	Strigidae	2	JQ174938, JQ174939
<i>Glaucidium passerinum</i>	Strigiformes	Strigidae	2	GU571423, GU571915
<i>Bubo bubo</i>	Strigiformes	Strigidae	3	GU571764, GU571285, GQ922629
<i>Bubo scandiacus</i>	Strigiformes	Strigidae	4	GU571287, EU525330, EU525331, EU525332
<i>Strix nebulosa</i>	Strigiformes	Strigidae	4	KF525370, DQ434173, GU572107, GU572108
<i>Strix leptogrammica</i>	Strigiformes	Strigidae	1	NC021970
<i>Strix aluco</i>	Strigiformes	Strigidae	3	GU571634, GU572104, GU572106
<i>Strix uralensis</i>	Strigiformes	Strigidae	5	GQ482681, GQ482682, GU571637, GU572109, GU572110
<i>Strix davidi</i>	Strigiformes	Strigidae	1	GQ922637
<i>Ithaginis cruentus</i>	Galliformes	Phasianidae	2	GQ922649, NC018033
<i>Arborophila</i>	Galliformes	Phasianidae	3	NC012453, GQ922643, GQ922644

<i>rufipectus</i>					
<i>Arborophila ardens</i>	Galliformes	Phasianidae	1	NC022683	
<i>Arborophila brunneopectus</i>	Galliformes	Phasianidae	1	NC022684	
<i>Arborophila rufogularis</i>	Galliformes	Phasianidae	1	NC020584	
<i>Arborophila gingica</i>	Galliformes	Phasianidae	1	FJ752425	
<i>Tetraogallus altaicus</i>	Galliformes	Phasianidae	1	GQ482760	
<i>Francolinus pintadeanus</i>	Galliformes	Phasianidae	1	NC011817	
<i>Francolinus francolinus</i>	Galliformes	Phasianidae	2	JF498852, JF498851	
<i>Francolinus pondicerianus</i>	Galliformes	Phasianidae	3	JF498853, JF498854, JF498855	
<i>Francolinus erckelii</i>	Galliformes	Phasianidae	2	JF498849, JF498850	
<i>Gallus gallus</i>	Galliformes	Phasianidae	3	AP003580, KC189864, KC189866	
<i>Tetraophasis obscurus</i>	Galliformes	Phasianidae	1	NC018034	
<i>Tetraophasis szechenyii</i>	Galliformes	Phasianidae	4	NC020613, GQ922645, GQ922646, GQ922647	
<i>Lophophorus sclateri</i>	Galliformes	Phasianidae	1	NC020589	
<i>Lophophorus lhuysii</i>	Galliformes	Phasianidae	1	NC013979	
<i>Tragopan temminckii</i>	Galliformes	Phasianidae	4	NC020586, GQ922633, GQ922634, GQ922635	
<i>Tragopan caboti</i>	Galliformes	Phasianidae	1	NC013619	
<i>Pucrasia macrolopha</i>	Galliformes	Phasianidae	2	NC020587, GQ922648	
<i>Syrmaticus ellioti</i>	Galliformes	Phasianidae	1	NC010771	
<i>Syrmaticus humiae</i>	Galliformes	Phasianidae	1	NC010774	
<i>Syrmaticus reevesii</i>	Galliformes	Phasianidae	1	NC010770	
<i>Phasianus colchicus</i>	Galliformes	Phasianidae	4	NC015526, JN850750, GQ482363, GQ922650	
<i>Phasianus versicolor</i>	Galliformes	Phasianidae	1	NC010778	
<i>Chrysolophus pictus</i>	Galliformes	Phasianidae	3	NC014576, GQ922630, FJ752433	
<i>Chrysolophus amherstiae</i>	Galliformes	Phasianidae	4	GQ922603, GQ922604, GQ922608, FJ752434	
<i>Lophura nycthemera</i>	Galliformes	Phasianidae	3	NC012895, GQ922618, GQ922620	
<i>Lophura swinhoii</i>	Galliformes	Phasianidae	1	KF218954	
<i>Crossoptilon auritum</i>	Galliformes	Phasianidae	3	GQ922639, GQ922640, NC015897	
<i>Crossoptilon crossoptilon</i>	Galliformes	Phasianidae	3	NC016679, GQ922613, GQ922614	

(b)

Species	Order	Family	Sample No.	GenBank accessions
<i>Cervus nippon</i>	Artiodactyla	Cervidae (Cervinae)	7	EU835712, EU835714, GQ329010, JF700150, JF700148, NC013834, AB116261
<i>Cervus albirostris</i>	Artiodactyla	Cervidae (Cervinae)	4	NC016707, GQ329003, GQ329005, JN632690
<i>Cervus unicolor</i>	Artiodactyla	Cervidae (Cervinae)	5	GQ411197, KF317912, GQ329017, GQ329016, JN714175
<i>Cervus elaphus</i>	Artiodactyla	Cervidae (Cervinae)	5	NC014703, NC013836, KF317906, KF317914, NC007704
<i>Elaphurus davidianus</i>	Artiodactyla	Cervidae (Cervinae)	5	GQ329018, GQ329019, GQ329025, GQ329026, NC018358
<i>Muntiacus muntjak</i>	Artiodactyla	Cervidae (Muntiacinae)	2	NC004563, JN714193
<i>Muntiacus crinifrons</i>	Artiodactyla	Cervidae (Muntiacinae)	4	GQ329035, GQ329036, GQ329037, NC004577
<i>Muntiacus reevesi</i>	Artiodactyla	Cervidae (Muntiacinae)	2	NC004069, NC008491
<i>Elaphodus cephalophus</i>	Artiodactyla	Cervidae (Muntiacinae)	1	NC008749
<i>Pseudois nayaur</i>	Artiodactyla	Bovidae (Caprinae)	4	HQ269457, HQ269458, HQ269459, NC020632
<i>Pseudois schaeferi</i>	Artiodactyla	Bovidae (Caprinae)	4	HQ269424, HQ269423, JX120620, NC016689
<i>Pantholops hodgsonii</i>	Artiodactyla	Bovidae (Caprinae)	3	HQ269460, HQ269461, NC007441
<i>Naemorhedus caudatus</i>	Artiodactyla	Bovidae (Caprinae)	1	NC013751
<i>Naemorhedus goral</i>	Artiodactyla	Bovidae (Caprinae)	4	HQ269425, HQ269426, HQ269427, NC021381
<i>Naemorhedus baileyi</i>	Artiodactyla	Bovidae (Caprinae)	1	NC020722
<i>Capricornis sumatraensis</i>	Artiodactyla	Bovidae (Caprinae)	3	NC020629, HQ269431, HQ269456
<i>Capricornis swinhoei</i>	Artiodactyla	Bovidae (Caprinae)	1	NC010640
<i>Ovis ammon</i>	Artiodactyla	Bovidae (Caprinae)	1	NC020656

<i>Lemur catta</i>	Primates	Lemuridae	1	NC004025
<i>Eulemur macaco</i>	Primates	Lemuridae	1	JF444301
<i>Nycticebus coucang</i>	Primates	Lorisidae	2	GQ259900, NC002765
<i>Nycticebus pygmaeus</i>	Primates	Lorisidae	1	GQ259902
<i>Saimiri sciureus</i>	Primates	Cebidae (Saimiriinae)	4	JF459275, AF312705, HQ005491, NC012775
<i>Saimiri ustus</i>	Primates	Cebidae (Saimiriinae)	1	AF312704
<i>Saimiri boliviensis</i>	Primates	Cebidae (Saimiriinae)	2	NC018096, NC021966
<i>Cebus xanthosternos</i>	Primates	Cebidae (Cebinae)	1	NC021961
<i>Cebus apella</i>	Primates	Cebidae (Cebinae)	5	NC016666, JF459118, FJ402879, FJ402870, FJ392287
<i>Cebus albifrons</i>	Primates	Cebidae (Cebinae)	1	NC002763
<i>Papio papio</i>	Primates	Cercopithecidae (Cercopithecinae)	2	NC020009, AY972684
<i>Papio hamadryas</i>	Primates	Cercopithecidae (Cercopithecinae)	2	NC001992, Y18001
<i>Papio anubis</i>	Primates	Cercopithecidae (Cercopithecinae)	4	FJ713433, KC757406, JX946197, NC020006
<i>Papio ursinus</i>	Primates	Cercopithecidae (Cercopithecinae)	1	NC020010
<i>Mandrillus sphinx</i>	Primates	Cercopithecidae (Cercopithecinae)	1	NC021956
<i>Cercopithecus kandii</i>	Primates	Cercopithecidae (Cercopithecinae)	1	JQ256968
<i>Cercopithecus albogularis</i>	Primates	Cercopithecidae (Cercopithecinae)	1	NC021944
<i>Cercopithecus mitis</i>	Primates	Cercopithecidae (Cercopithecinae)	2	NC023961, AY972790
<i>Chlorocebus aethiops</i>	Primates	Cercopithecidae (Cercopithecinae)	4	NC007009, AY972651, AY972687, DQ069713
<i>Chlorocebus tantalus</i>	Primates	Cercopithecidae (Cercopithecinae)	1	NC009748
<i>Chlorocebus pygerythrus</i>	Primates	Cercopithecidae (Cercopithecinae)	1	NC009747
<i>Chlorocebus sabaeus</i>	Primates	Cercopithecidae (Cercopithecinae)	1	NC008066
<i>Macaca sylvanus</i>	Primates	Cercopithecidae (Cercopithecinae)	3	JF444309, NC002764, JF444310
<i>Macaca nemestrina</i>	Primates	Cercopithecidae (Cercopithecinae)	1	EF568609
<i>Macaca mulatta</i>	Primates	Cercopithecidae (Cercopithecinae)	3	EF568612, EF568611, NC005943
<i>Macaca fuscata</i>	Primates	Cercopithecidae (Cercopithecinae)	1	JF444308

<i>Macaca fascicularis</i>	Primates	Cercopithecidae (Cercopithecinae)	2	NC012670, KF305937
<i>Macaca thibetana</i>	Primates	Cercopithecidae (Cercopithecinae)	1	NC011519
<i>Macaca assamensis</i>	Primates	Cercopithecidae (Cercopithecinae)	1	NC023795
<i>Trachypithecus francoisi</i>	Primates	Cercopithecidae (Colobinae)	1	NC023970
<i>Trachypithecus hatinhensis</i>	Primates	Cercopithecidae (Colobinae)	1	NC019579
<i>Rhinopithecus roxellana</i>	Primates	Cercopithecidae (Colobinae)	1	NC008218
<i>Rhinopithecus bieti</i>	Primates	Cercopithecidae (Colobinae)	2	NC015486, HQ287798
<i>Rhinopithecus brelichi</i>	Primates	Cercopithecidae (Colobinae)	2	NC018057, JN540032
<i>Rhinopithecus avunculus</i>	Primates	Cercopithecidae (Colobinae)	2	NC015485, JF293093
<i>Ursus arctos</i>	Carnivora	Ursidae (Ursinae)	5	JF443542, JF443544, GQ901981, GQ901983, JF499381
<i>Ursus thibetanus</i>	Carnivora	Ursidae (Ursinae)	3	NC009971, JF681229, JF681227
<i>Ursus maritimus</i>	Carnivora	Ursidae (Ursinae)	5	JX196375, JX196389, JF499382, JF443549, NC003428
<i>Tremarctos ornatus</i>	Carnivora	Ursidae (Ursinae)	2	FM177764, NC009969
<i>Ailuropoda melanoleuca</i>	Carnivora	Ursidae (Ailuropodinae)	1	NC009492
<i>Panthera leo</i>	Carnivora	Felidae (Pantherinae)	2	JF444382, KF776494
<i>Panthera pardus</i>	Carnivora	Felidae (Pantherinae)	2	JF444283, NC010641
<i>Panthera onca</i>	Carnivora	Felidae (Pantherinae)	1	NC022842
<i>Panthera tigris</i>	Carnivora	Felidae (Pantherinae)	6	FJ455123, FJ461530, NC010642, KF297576, FJ461531, NC014770
<i>Uncia uncia</i>	Carnivora	Felidae (Pantherinae)	2	NC010638, JF444483

Table 4 Ambiguous identifications in the online nucleotide BLAST for (a) birds, (b) mammals

(a)

Sample vouchers	Outputs of online nucleotide BLAST			
	Classified species	Max score	Query cover (%)	Identity (%)
B03*	<i>Accipiter gularis</i>	1197	100	100
	<i>Accipiter virgatus</i>	1197	100	100
N9*,N10*,N11*	<i>Ciconia maguari</i>	854-856	99-100	90-91

	<i>Mycteria ibis</i>	854	100	90
N1, N2, N25	<i>Grus monacha</i>	1315-1321	100	99
	<i>Grus nigricollis</i>	1277-1282	100	99
N17, N23, N24	<i>Grus nigricollis</i>	1321-1327	100	99
	<i>Grus monacha</i>	1271-1277	100	99

(b)

Sample vouchers	Outputs of online nucleotide BLAST			
	Classified species	Max score	Query cover (%)	Identity (%)
S29	<i>Papio anubis</i>	1243	100	99
	<i>Papio hamadryas</i>	1238	100	99
S30, S36	<i>Cercopithecus mitis</i>	1205	100	98
	<i>Cercopithecus</i>	1199	100	98
	<i>albogularis</i>			
S61	<i>Papio hamadryas</i>	1192	100	99
	<i>Papio anubis</i>	1186	100	99

* The samples failing to be identified by online nucleotide BLAST.

Table 5 The individuals deviating from the average genetic divergence level, (a) with very large intraspecific distance, (b) with very low interspecific distance

(a)

Species (sample No.)	samples	Intraspecific K2P distances (%)		
		Min	Mean	Max
<i>Falco columbarius</i> (3)	KF537263	2.05	2.05	2.05
<i>Accipiter gentilis</i> (6)	AY666498	2.52	2.57	2.69
	DQ433279			
<i>Accipiter gularis</i> (4)	JQ173889	2.04	2.04	2.04
<i>Strix nebulosa</i> (4)	KF525370	3.02	3.02	3.02
	DQ434173			
<i>Ithaginis cruentus</i> (2)	GQ922649	2.37	2.37	2.37
<i>Cebus apella</i> (6)	NC016666	31.08	31.45	31.69
<i>Saimiri sciureus</i> (7)	NC012775	2.86	2.88	3.02
<i>Cercopithecus mitis</i> (4)	NC023961	4.50	4.72	4.82
<i>Papio anubis</i> (5)	JX946197	2.88	3.07	3.35
<i>Chlorocebus aethiops</i> (4)	NC007009	6.83	6.87	6.93
<i>Cervus elaphus</i> (5)	NC014703	2.37	2.48	2.53
	NC013836			
<i>Elaphurus davidianus</i> (5)	NC018358	13.31	13.36	13.49
<i>Muntiacus muntjak</i> (2)	JN714193	3.35	3.35	3.35
<i>Panthera leo</i> # (5)	S14, S15, S18	6.38	6.53	6.73

(b)

Species A (Sample No.)	Species B (Sample No.)	Interspecific K2P pairwise distance (%)		
		Min	Mean	Max
<i>Falco vespertinus</i> (1)	<i>Falco amurensis</i> (2)	1.57	1.57	1.57
<i>Accipiter virgatus</i> (3)	<i>Accipiter gularis</i> #(4)	0	0.94	2.04
<i>Buteo buteo</i> (5)	<i>Buteo hemilasius</i> #(4)	0.31	0.62	1.09
<i>Strix davidi</i> (1)	<i>Strix uralensis</i> (5)	1.09	1.34	1.41
<i>Syrnaticus ellioti</i> (1)	<i>Syrnaticus humiae</i> (1)	0	0	0
<i>Saimiri sciureus</i> #(7)	<i>Saimiri boliviensis</i> (2)	0	2.47	3.02
<i>Papio anubis</i> #(5)	<i>Papio hamadryas</i> #(3)	0.15	0.90	2.86
<i>Chlorocebus sabaues</i> (1)	<i>Chlorocebus aethiops</i> (4)	0	1.73	6.93
<i>Pseudois nayaur</i> #(5)	<i>Pseudois schaeferi</i> #(5)	1.09	1.56	1.73
<i>Capricornis swinhoei</i> (1)	<i>Capricornis sumatraensis</i> (3)	1.57	1.62	1.73
<i>Cervus unicolor</i> #(5)	<i>Cervus elaphus</i> (5)	1.25	2.65	3.68
<i>Cervus elaphus</i> (5)	<i>Cervus nippon</i> #(10)	1.73	2.71	3.35
<i>Panthera leo</i> #(5)	<i>Panthera onca</i>	2.20	3.81	6.24

Putative species inferred from DNA barcoding identification for queried samples.

Table 6 Diagnostic formulas of the endangered birds and mammals generated by BLOG for species identification

Scientific name	Diagnostic nucleotide formulas	Score
<i>Lophophorus lhuysii</i>	pos363=T & pos399=G	0.035
<i>Phasianus colchicus</i>	pos30=T & pos363=T & pos399=C	0.068
<i>Arborophila rufipectus</i>	pos207=T & pos528=C	0.079
<i>Arborophila ardens</i>	pos528=C & pos627=C	0.035
<i>Arborophila brunnepectus</i>	pos519=T & pos528=G	0.025
<i>Falco rusticolus</i>	pos399=T & pos528=G	0.057
<i>Falco tinnunculus</i>	pos399=T & pos528=A & pos627=C	0.068
<i>Buteo hemilasius</i>	pos66=C & pos84=T & pos627=T OR pos207=C & pos282=G	0.035
<i>Pandion haliaetus</i>	pos84=A & pos627=T	0.057
<i>Asio otus</i>	pos363=A & pos399=G & pos444=C	0.024
<i>Athene noctus</i>	pos207=A & pos444=C & pos627=A	0.024
<i>Grus monacha</i>	pos49=T & pos453=C & pos519=C & pos627=T	0.047
<i>Grus vipio</i>	pos54=T & pos282=T & pos444=A	0.047
<i>Grus nigricollis</i>	pos282=T & pos453=T	0.047
<i>Grus leucogeranus</i>	pos207=T & pos399=G	0.068
<i>Grus japonensis</i>	pos49=T & pos627=C	0.057
<i>Anthropoides virgo</i>	pos444=G	0.035
<i>Ciconia ciconia</i>	pos54=T & pos66=T & pos207=A & pos519=C & pos627=T	0.047

<i>Leptoptilos crumenifer</i>	pos66=C & pos207=T & pos627=T	0.047
<i>Pseudois nayaur</i>	pos333=C & pos360=T	0.071
<i>Pseudois schaeferi</i>	pos333=T & pos360=T & pos644=T	0.071
<i>Pantholops hodgsonii</i>	pos27=G & pos423=A	0.058
<i>Cervus unicolor</i>	pos207=T & pos264=T & pos418=T	0.071
<i>Cervus albirostris</i>	pos78=T & pos192=T & pos210=T	0.058
<i>Cervus nippon</i>	pos78=T & pos423=G & pos495=C OR pos192=C & pos225=C & pos423=G	0.097
<i>Mandrillus sphinx</i>	pos27=T & pos495=C	0.044
<i>Cercopithecus mitis</i>	pos78=C & pos117=G OR pos117=G & pos333=T	0.058
<i>Cercopithecus aethiops</i>	pos264=C & pos644!=T OR pos207=G & pos423=A	0.044
<i>Trachypithecus francoisi</i>	pos312=A & pos333=A & pos423=G	0.056
<i>Trachypithecus hatinhensis</i>	Pos312=G & pos333=A & pos418=C	0.029
<i>Papio anubis</i>	pos27=T & pos360=A & pos423=G OR pos27=T & pos264=T & pos423=A	0.058
<i>Papio hamadryas</i>	pos27=T & pos192=G & pos423=A	0.044
<i>Saimiri sciureus</i>	pos78=G & pos117=C	0.069
<i>Cebus apella</i>	pos78!=C & pos78!=G & pos153!=A & pos264=A & pos495=T & pos644!=A	0.071
<i>Nycticebus pygmaeus</i>	pos192=G & pos333=A	0.044
<i>Lemur catta</i>	pos333=T & pos423=C	0.044
<i>Panthera pardus</i>	pos333=A & pos495=C	0.058
<i>Panthera leo</i>	pos117=C & pos210=C & pos423=T	0.071
<i>Panthera tigris amoyensis</i>	pos207=G & pos423=T	0.071
<i>Ailuropoda melanoleuca</i>	pos210=G	0.071

Table 7 The conclusion of DNA barcoding identifications of (a) birds, (b) mammals, combined with morphological classification. Grey shadowed lines indicated samples with inconsistent morphological and molecular identification. Underlined samples indicated those just classified to generic level based on DNA barcoding

(a)

Sample vouchers	Online BLAST		Online BOLD			NJ tree	DNA barcoding identification	Morphological identification
	Species	Identity (%)	Species	Similarity (%)	Distance			
N1, N2, N25	<i>Grus monacha</i>	99	<i>Grus (Grus monacha)</i>	100 (99.86)	<i>Grus monacha</i>	<i>Grus monacha</i>	<i>Grus monacha</i>	<i>Grus monacha</i>
N15, N16	<i>Grus vipio</i>	100	<i>Grus vipio</i>	100	<i>Grus vipio</i>	<i>Grus vipio</i>	<i>Grus vipio</i>	<i>Grus vipio</i>
N17, N23, N24	<i>Grus nigricollis</i>	99	<i>Grus (Grus nigricollis)</i>	100 (99.86)	<i>Grus nigricollis</i>	<i>Grus nigricollis</i>	<i>Grus nigricollis</i>	<i>Grus nigricollis</i>
N18, N19, N29, N30	<i>Grus leucogeranus</i>	99	<i>Grus leucogeranus</i>	99.40~99.58	<i>Grus leucogeranus</i>	<i>Grus leucogeranus</i>	<i>Grus leucogeranus</i>	<i>Grus leucogeranus</i>
N20-N22, N33, N34	<i>Grus japonensis</i>	99	<i>Grus japonensis</i>	99.70~100	<i>Grus japonensis</i>	<i>Grus japonensis</i>	<i>Grus japonensis</i>	<i>Grus japonensis</i>
N27, N28	<i>Anthropoides virgo</i>	100	<i>Anthropoides virgo</i>	100	<i>Anthropoides virgo</i>	<i>Anthropoides virgo</i>	<i>Anthropoides virgo</i>	<i>Anthropoides virgo</i>
N7†, N8†, N31†, N32†	<i>Ciconia boyciana</i>	100	<i>Ciconia boyciana</i>	100	<i>Ciconia boyciana</i>	<i>Ciconia boyciana</i>	<i>Ciconia boyciana</i>	<i>Ciconia ciconia</i>
<u>N9*-N11*</u>	<u><i>Ciconia maguari</i></u>	91	<u><i>Leptoptilos crumenifer</i></u>	99.80	<u><i>Leptoptilos crumenifer</i></u>	Ciconiidae	<u><i>Leptoptilos crumenifer</i></u>	<u><i>Leptoptilos crumenifer</i></u>
<u>N12*-N14*</u>	<u><i>Mycteria leucocephala</i></u>	97	<u><i>Mycteria (Mycteria leucocephala)</i></u>	97.80~98.00 (98.00)	<u><i>Mycteria leucocephala</i></u>	<i>Mycteria sp.</i>	<i>Mycteria sp.</i>	<i>Mycteria ibis</i>
B02	<i>Pandion haliaetus</i>	97	<i>Pandion haliaetus</i>	100	<i>Pandion haliaetus</i>	<i>Pandion haliaetus</i>	<i>Pandion haliaetus</i>	<i>Pandion haliaetus</i>
<u>B03*</u>	<u><i>Accipiter gularis/ Accipiter virgatus</i></u>	100	<u><i>Accipiter (Accipiter gularis)</i></u>	100 (100)	<u><i>Accipiter sp.</i></u>	<i>Accipiter sp.</i>	<i>Accipiter sp.</i>	<i>Accipiter virgatus</i>
B05*†	<i>Falco rusticolus</i>	100	<u><i>Falco (Falco biarmicus)</i></u>	100 (100)	<i>Falco rusticolus</i>	<i>Falco rusticolus</i>	<i>Falco rusticolus</i>	<i>Falco cherrug</i>
B08*	<i>Asio otus</i>	99	<i>Asio (Asio otus)</i>	100 (100)	<i>Asio otus</i>	Strigidae	<i>Asio otus</i>	<i>Asio otus</i>
B13	<i>Falco tinnunculus</i>	99	<i>Falco</i>	100 (100)	<i>Falco tinnunculus</i>	<i>Falco tinnunculus</i>	<i>Falco tinnunculus</i>	<i>Falco tinnunculus</i>

B15*	<i>Athene noctua</i>	100	(<i>Falco tinnunculus</i>) <i>Athene noctua</i>	100	<i>Athene noctua</i>	Strigidae	<i>Athene noctua</i>	<i>Athene noctua</i>
B16††	<i>Buteo hemilasius</i>	99	<i>Buteo</i> (<i>Buteo hemilasius</i>)	100 (100)	<i>Buteo hemilasius</i>	<i>Buteo hemilasius</i>	<i>Buteo hemilasius</i>	<i>Aquila clanga</i>
B04	<i>Lophophorus lhuysii</i>	99	<i>Lophophorus lhuysii</i>	99.16	<i>Lophophorus lhuysii</i>	<i>Lophophorus lhuysii</i>	<i>Lophophorus lhuysii</i>	<i>Lophophorus lhuysii</i>
B12, B35	<i>Phasianus colchicus</i>	99	<i>Phasianus colchicus</i>	99.90	<i>Phasianus colchicus</i>	<i>Phasianus colchicus</i>	<i>Phasianus colchicus</i>	<i>Phasianus colchicus</i>
AR1-AR3, AR5, ARB3, ARB6	<i>Arborophila rufipectus</i>	100	<i>Arborophila rufipectus</i>	99.00~100	<i>Arborophila rufipectus</i>	<i>Arborophila rufipectus</i>	<i>Arborophila rufipectus</i>	<i>Arborophila rufipectus</i>
B10*	<i>Arborophila ardens</i>	99	No match	-	<i>Arborophila ardens</i>	<i>Arborophila ardens</i>	<i>Arborophila ardens</i>	<i>Arborophila ardens</i>
B33*	<i>Arborophila brunneopectus</i>	99	No match	-	<i>Arborophila brunneopectus</i>	<i>Arborophila brunneopectus</i>	<i>Arborophila brunneopectus</i>	<i>Arborophila brunneopectus</i>

(b)

Sample vouchers	Online BLAST		Online BOLD		Distance	NJ tree	DNA barcoding identification	Morphological identification
	Species	Identity (%)	Species (best match)	Similarity (%)				
A11	<i>Pseudois nayaur</i>	99	<i>Pseudois</i> (<i>Pseudois nayaur</i>)	100 (100)	<i>Pseudois nayaur</i>	<i>Pseudois nayaur</i>	<i>Pseudois nayaur</i>	<i>Pseudois nayaur</i>
A17	<i>Pseudois schaeferi</i>	100	<i>Pseudois</i> (<i>Pseudois schaeferi</i>)	100 (100)	<i>Pseudois schaeferi</i>	<i>Pseudois schaeferi</i>	<i>Pseudois schaeferi</i>	<i>Pseudois schaeferi</i>
A53	<i>Pantholops hodgsonii</i>	100	<i>Pantholops hodgsonii</i>	100	<i>Pantholops hodgsonii</i>	<i>Pantholops hodgsonii</i>	<i>Pantholops hodgsonii</i>	<i>Pantholops hodgsonii</i>
A23	<i>Cervus unicolor</i>	99	<i>Cervus</i> (<i>Cervus unicolor</i>)	100 (99.69)	<i>Cervus unicolor</i>	<i>Cervus unicolor</i>	<i>Cervus unicolor</i>	<i>Cervus unicolor</i>
A29	<i>Cervus albirostris</i>	99	<i>Cervus</i> (<i>Cervus albirostris</i>)	100 (100)	<i>Cervus albirostris</i>	<i>Cervus albirostris</i>	<i>Cervus albirostris</i>	<i>Cervus albirostris</i>
M1, Q2, H26	<i>Cervus nippon</i>	99	<i>Cervus</i> (<i>Cervus nippon</i>)	100 (100)	<i>Cervus nippon</i>	<i>Cervus nippon</i>	<i>Cervus nippon</i>	<i>Cervus nippon</i>
S38, S62	<i>Mandrillus sphinx</i>	100	<i>Mandrillus sphinx</i>	100	<i>Mandrillus sphinx</i>	<i>Mandrillus sphinx</i>	<i>Mandrillus sphinx</i>	<i>Mandrillus sphinx</i>
S30, S36	<i>Cercopithecus mitis</i>	98	<i>Cercopithecus</i>	97.70 97.66	<i>Cercopithecus mitis</i>	<i>Cercopithecus sp.</i>	<i>Cercopithecus mitis</i>	<i>Cercopithecus mitis</i>

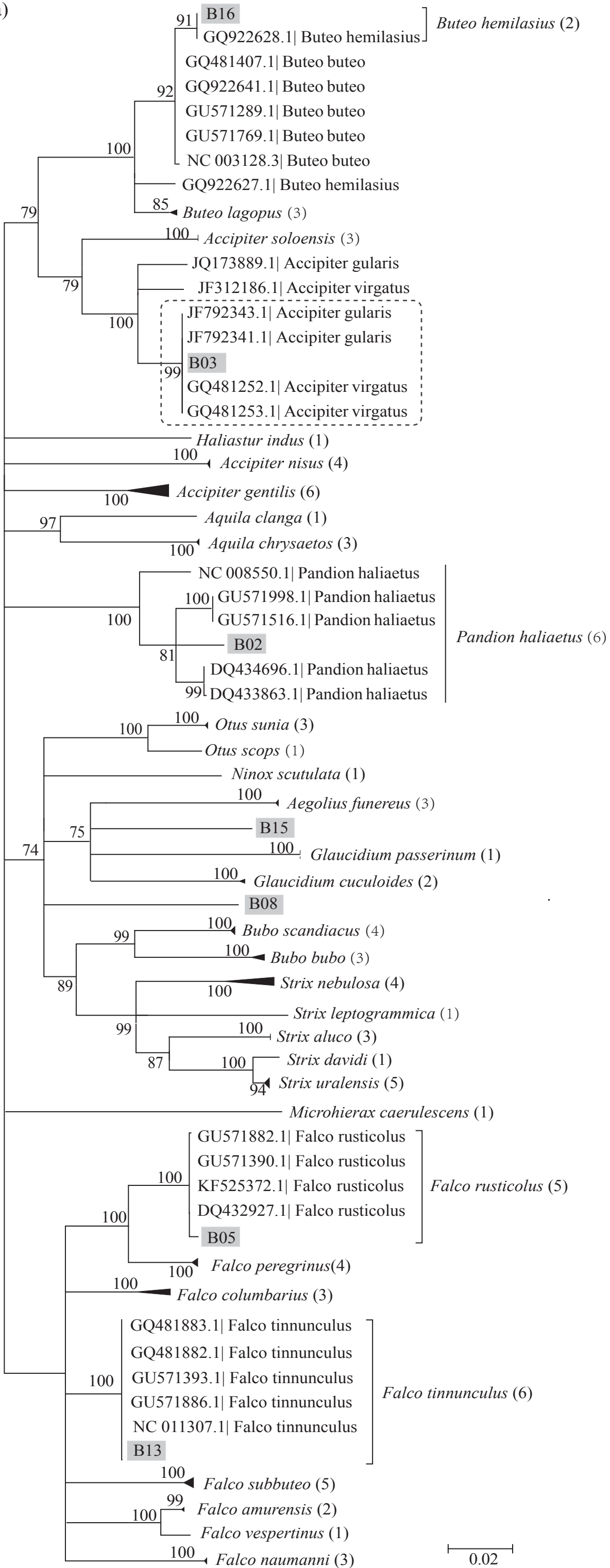
(<i>Cercopithecus mitis</i>)								
S64*	<i>Chlorocebus aethiops</i>	98	<i>Cercopithecus aethiops</i>	98.42	<i>Chlorocebus aethiops</i>	<i>Chlorocebus sp.</i>	<i>Chlorocebus aethiops</i>	<i>Chlorocebus aethiops</i>
S44	<i>Trachypithecus francoisi</i>	97	<i>Trachypithecus francoisi</i>	99.32	<i>Trachypithecus francoisi</i>	<i>Trachypithecus francoisi</i>	<i>Trachypithecus francoisi</i>	<i>Trachypithecus francoisi</i>
S63†	<i>Trachypithecus hatinhensis</i>	99	<i>Trachypithecus hatinhensis</i>	100	<i>Trachypithecus hatinhensis</i>	<i>Trachypithecus hatinhensis</i>	<i>Trachypithecus hatinhensis</i>	<i>Trachypithecus francoisi</i>
S29*	<i>Papio anubis</i>	99	<i>Papio</i> (<i>Papio hamadryas</i>)	100 (99.83)	<i>Papio anubis</i>	<i>Papio sp.</i>	<i>Papio anubis</i>	<i>Papio sp.</i>
S61*	<i>Papio hamadryas</i>	99	<i>Papio</i> (<i>Papio hamadryas</i>)	100 (99.84)	<i>Papio hamadryas</i>	<i>Papio sp.</i>	<i>Papio hamadryas</i>	<i>Papio sp.</i>
S45, S50	<i>Saimiri sciureus</i>	100	<i>Saimiri sciureus</i>	99	<i>Saimiri sciureus</i>	<i>Saimiri sciureus</i>	<i>Saimiri sciureus</i>	<i>Saimiri sciureus</i>
S51††	<i>Saimiri sciureus</i>	100	<i>Saimiri sciureus</i>	99	<i>Saimiri sciureus</i>	<i>Saimiri sciureus</i>	<i>Saimiri sciureus</i>	<i>Cuon alpinus</i>
S46	<i>Cebus apella</i>	100	<i>Cebus apella</i>	100	<i>Cebus apella</i>	<i>Cebus apella</i>	<i>Cebus apella</i>	<i>Cebus apella</i>
S49*†	<i>Nycticebus pygmaeus</i>	99	No match	-	<i>Nycticebus pygmaeus</i>	<i>Nycticebus pygmaeus</i>	<i>Nycticebus pygmaeus</i>	<i>Nycticebus coucang</i>
S26	<i>Lemur catta</i>	99	<i>Lemur catta</i>	100	<i>Lemur catta</i>	<i>Lemur catta</i>	<i>Lemur catta</i>	<i>Lemur catta</i>
S23, S28	<i>Panthera pardus</i>	100	<i>Panthera pardus</i>	100	<i>Panthera pardus</i>	<i>Panthera pardus</i>	<i>Panthera pardus</i>	<i>Panthera pardus</i>
S14*, S15*, S18*	<i>Panthera leo</i>	99	<i>Panthera</i> (<i>Panthera leo</i>)	100 (99.26~99.46)	<i>Panthera sp.</i>	Felidae	<i>Panthera leo</i>	<i>Panthera leo</i>
S53-S59	<i>Panthera tigris amoyensis</i>	99	<i>Panthera tigris</i> (<i>Panthera tigris amoyensis</i>)	100 (99.41~99.71)	<i>Panthera tigris amoyensis</i>	<i>Panthera tigris amoyensis</i>	<i>Panthera tigris amoyensis</i>	<i>Panthera tigris amoyensis</i>
A3, A4, A144, XJ	<i>Ailuropoda melanoleuca</i>	100	<i>Ailuropoda melanoleuca</i>	100	<i>Ailuropoda melanoleuca</i>	<i>Ailuropoda melanoleuca</i>	<i>Ailuropoda melanoleuca</i>	<i>Ailuropoda melanoleuca</i>

* showed the samples that obtained species assignments by concluding the results from multiple methods.

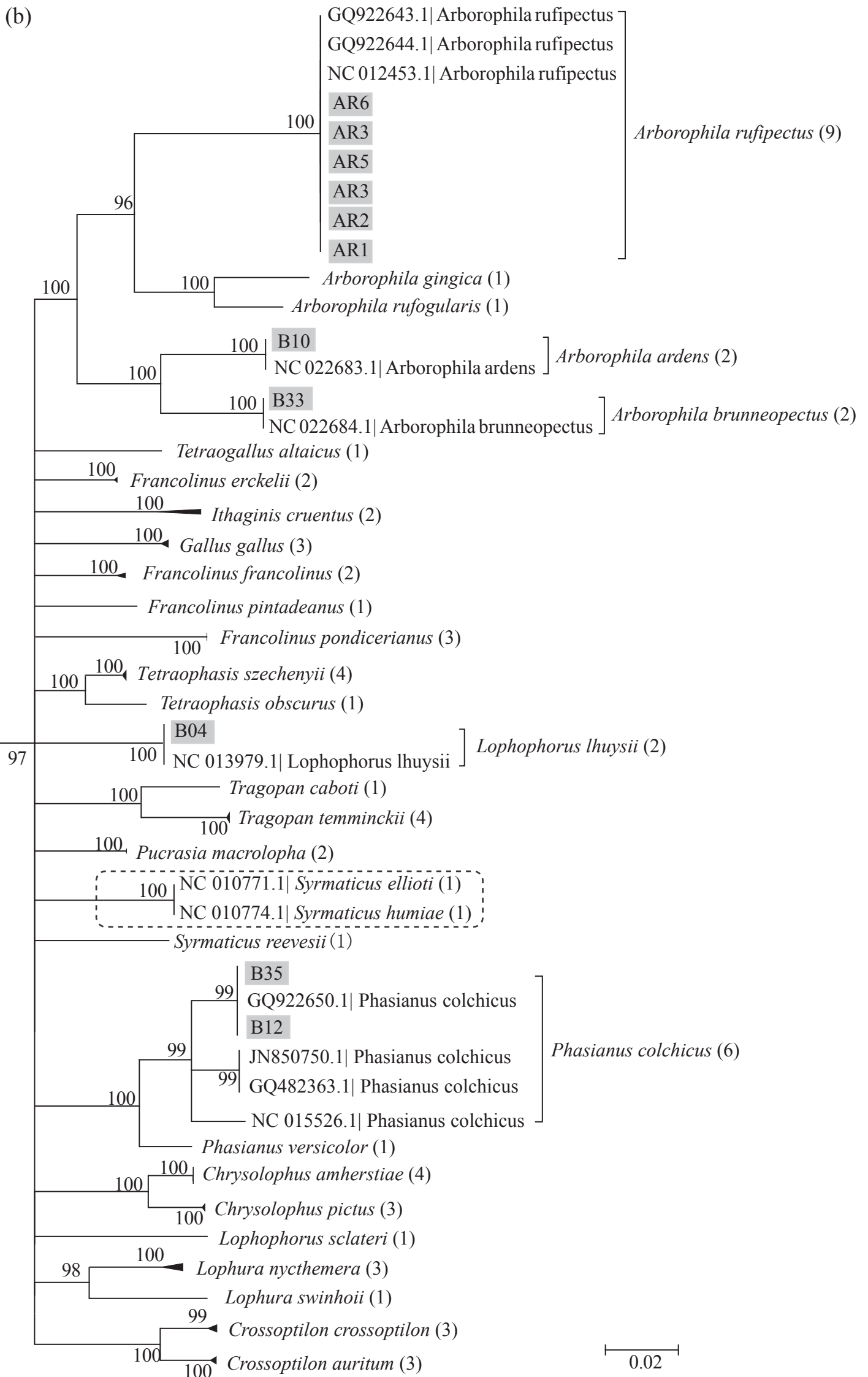
† showed potential morphologically-misidentified samples.

†† showed mislabelled samples.

(a)



0.02



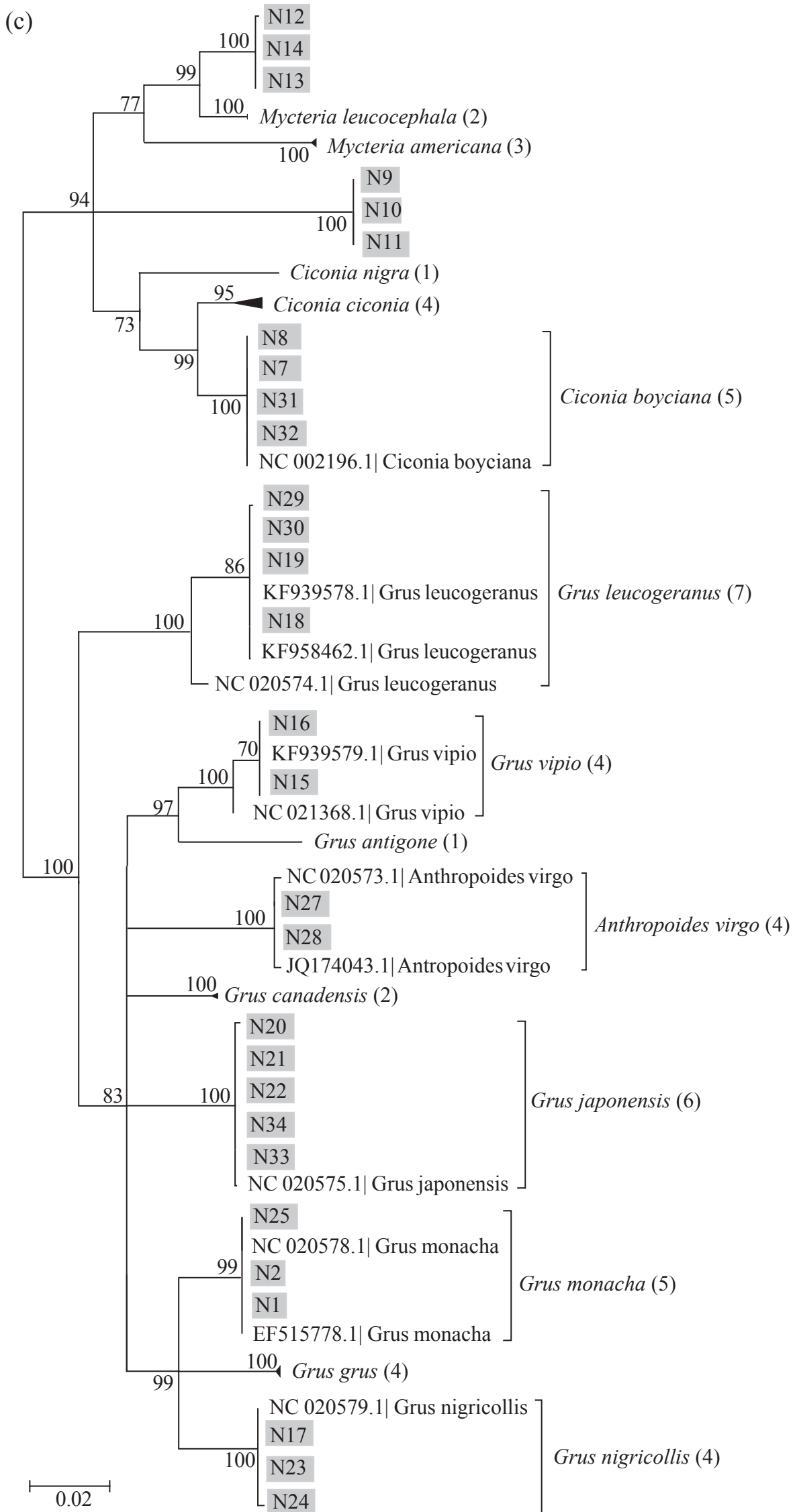
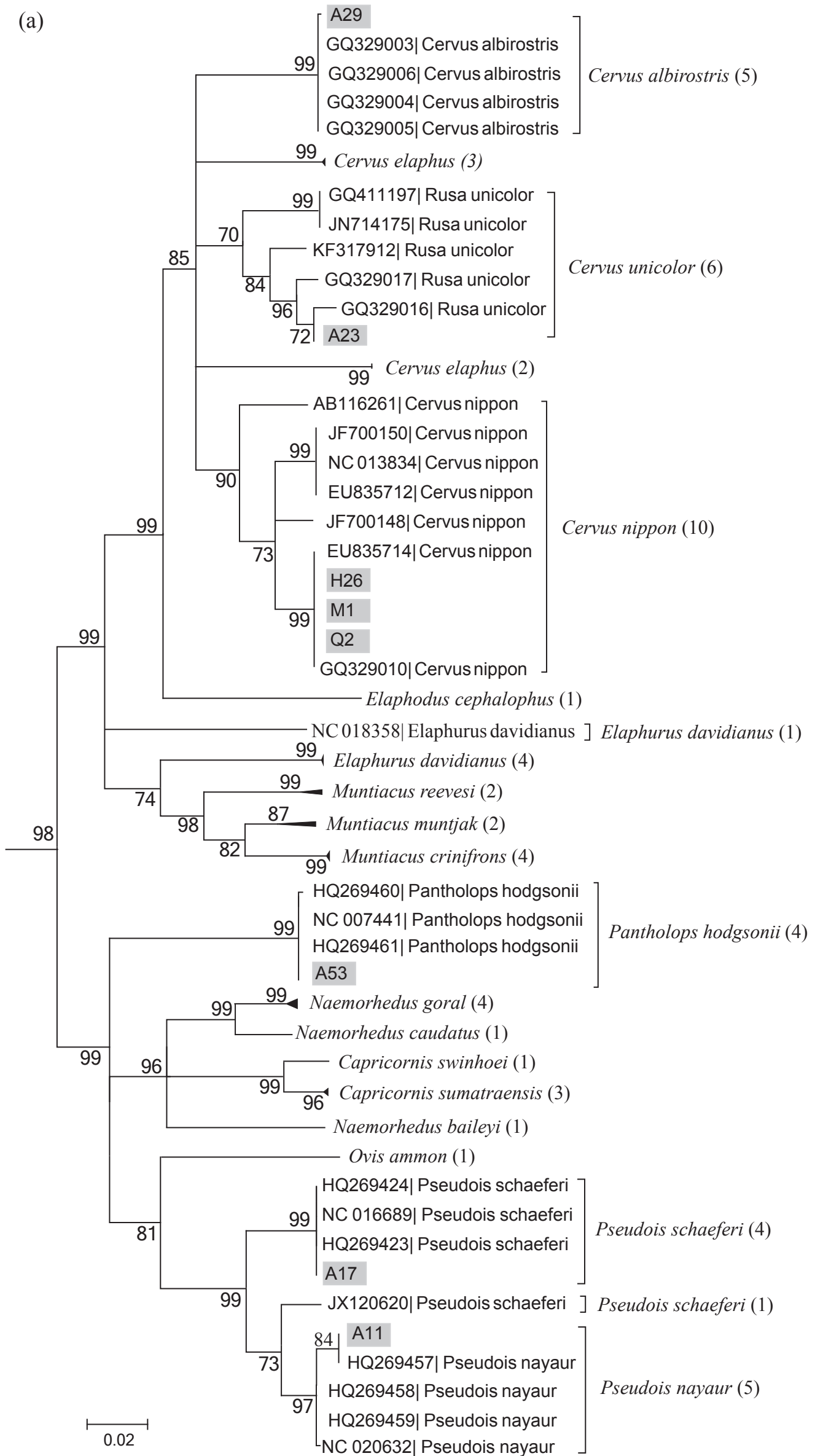
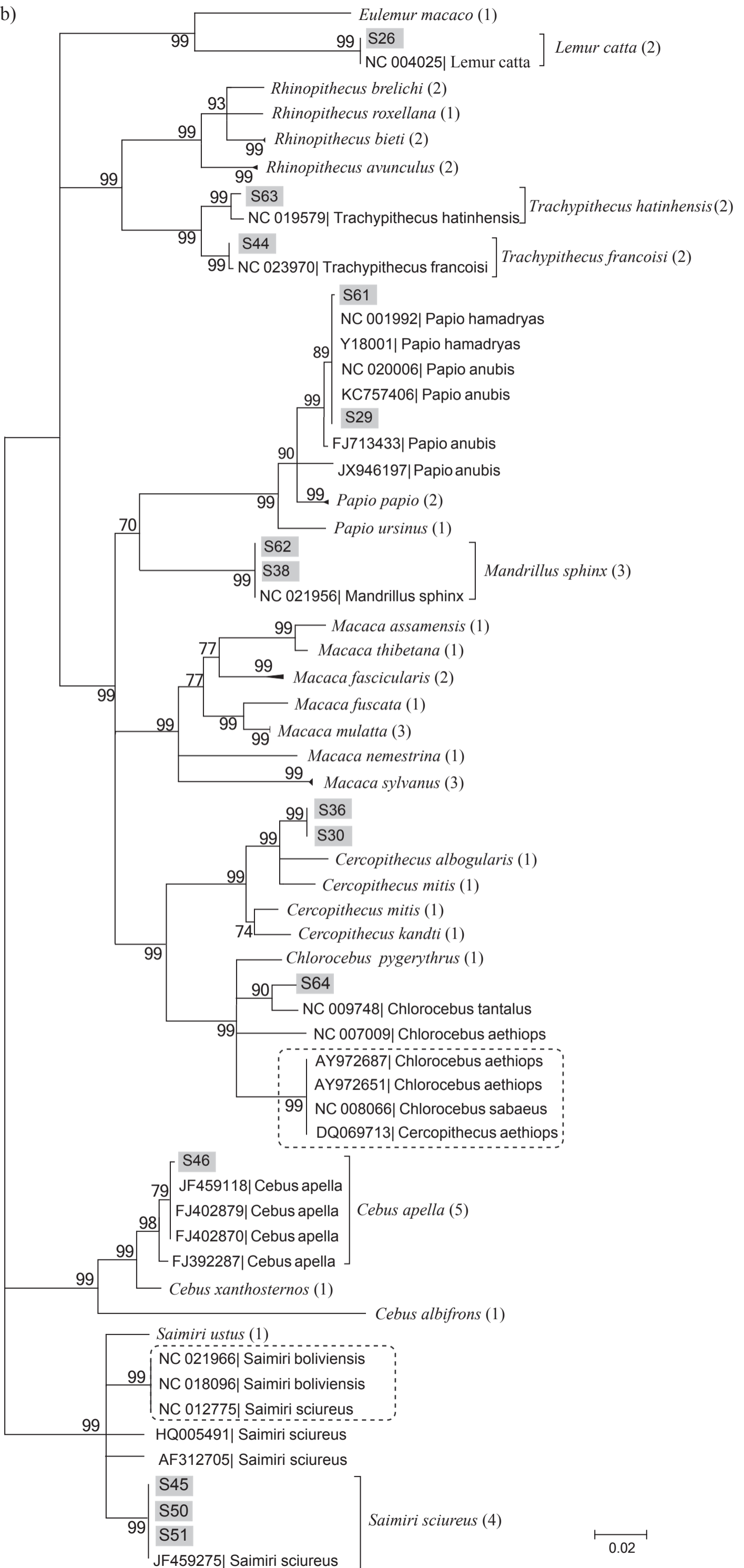


Fig. 1 The Neighbour Joining tree based on the DNA barcodes of endangered birds, (a) raptor, (b) pheasant, (c) crane and stork. Our samples are showed in grey shadow. Numbers refer to nodal support values inferred from NJ bootstrap $\geq 70\%$. The samples which shared DNA barcode with hetero-specific individuals are shown in the dashed boxes

(a)



(b)



(c)

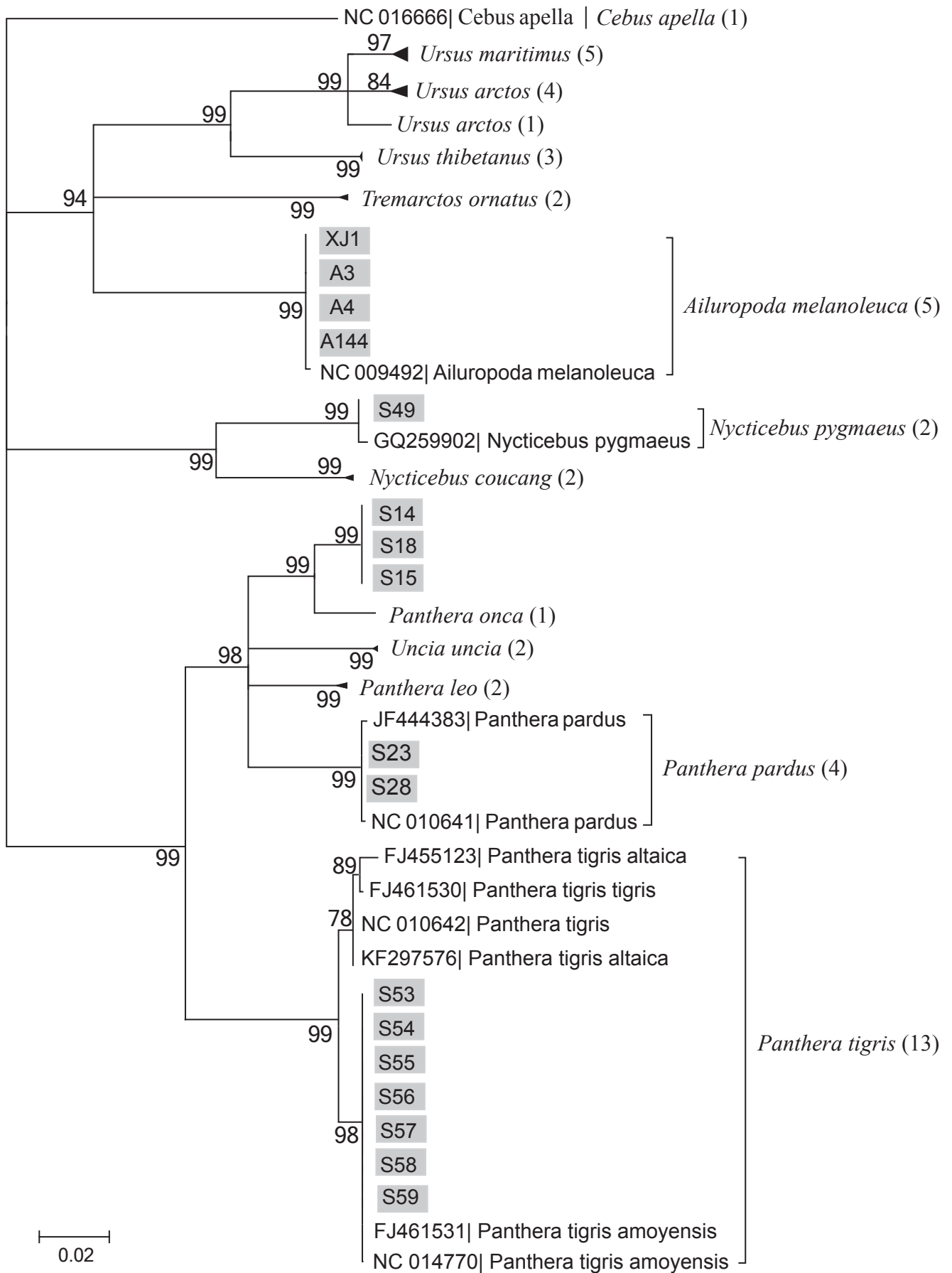


Fig. 2 The Neighbour Joining tree based on the DNA barcodes of endangered mammals, (a) deer and sheep, (b) Old World monkey, (c) carnivore. Our samples are showed in grey shadow. Numbers refer to nodal support values inferred from NJ bootstrap $\geq 70\%$. The samples which shared DNA barcode with hetero-specific individuals are inferred in the dashed boxes