

Determining the Specific Status of Korean Collared Scops Owls

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ABSTRACT

The collared scops owl that occurs in Korea is a protected species but its exact specific status has been questioned. To resolve the species status, a molecular phylogenetic analysis was conducted using two fragments of mitochondrial DNA, cytochrome *b* (cyt *b*, 891 bp) and NADH dehydrogenase subunit 2 (ND2, 627 bp) genes. Phylogenetic trees of cyt *b* revealed that all Korean specimens formed a monophyletic group with Japanese scops owl *Otus semitorques* with very low sequence divergence ($d=0.008$). We obtained a similar ND2 tree as well ($d=0.003$); however, the genetic distance between Korean individuals and *O. lempiji* from GenBank (AJ004026–7, EU348987, and EU601036) was very high and sufficient enough to separate them as species (cyt *b*, $d=0.118$; ND2, $d=0.113$). We also found that Korean species showed high differentiation from *O. bakkamoena* (AJ004018–20 and EU601034; cyt *b*, $d=0.106$; ND2, $d=0.113$) and *O. lettia* (EU601109 and EU601033, cyt *b*, $d=0.110$; ND2, $d=0.117$) as well. Therefore, we suggest that the Korean collared scops owl should be designated as *Otus semitorques*.

Keywords: collared scops owl, molecular phylogeny, taxonomy, mitochondrial DNA, *Otus semitorques*

INTRODUCTION

Accurate taxonomic information forms the basis of the protection and management policies for species conservation (Valdecasas and Camacho, 2003; Mace, 2004; Allendorf and Luikart, 2007). When a threatened species or subspecies is incorrectly classified and not legally protected, it can become extinct. Conversely, when common species are wrongly classified and overprotected, the budget and efforts that should be spent on endangered species may be wasted. The scops owl, designated as a natural monument (No. 324–7) and a protected species by the Korean Cultural Heritage Administration (CHA), is being threatened by many anthropogenic impacts collision, traffic accidents, habitat destruction, hunting and poisoning (Kim, 2006; CHA, 2010) finally, it is in the tendency of decreasing in population (The Korean Association for Bird Protection, 2012; CHA, 2010). Kim (2006) elucidated that collared scops owl is one of the most frequently rescued species accounted for 82.2% of avian records.

Collision and traffic accidents were the main causes and especially traffic accident is the representative cause to threaten nocturnal raptor species. 93.7% of rescued natural monuments were raptors and 63.7% of the raptors were owl species including scops owl. Then, the need of counterplan to reduce traffic accidents of nocturnal raptors was suggested implying this scops owl might be placed in the serious circumstance without any conservation or management strategies. In spite of its importance, basic data for Korean collared scops owl is lack. Even though, this species is reported as both winter visitor and resident race (Deignan, 1950; Lee et al., 2000), this statement was not verified (The Ornithological Society of Korea, 2009). Besides, the use of the scientific name of the Korean collared scops owls has been inconsistent.

The scops owl occur in Korea were traditionally referred as *Otus bakkamoena* (see Cumming, 1933; Nam, 1950; Fennell, 1952; Vaurie, 1965; Dickinson, 2003; The Ornithological Society of Korea, 2009), though Austin (1948) described that the scops owls in Korea are common spring and autumn

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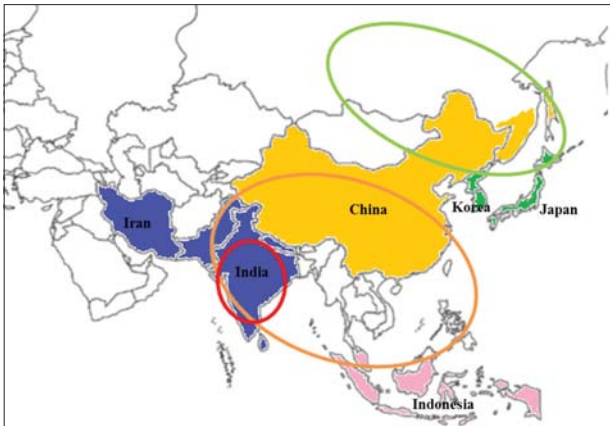


Fig. 1. Distribution map of *Otus* species; this map was drawn based on habitat information in Weick (2006). *Otus lempiji* (pink), *O. bakkamoena* (blue), *O. lettia* (yellow), and *O. semitorques* (green). *Otus lempiji* shares red circled areas with *O. bakkamoena*, *O. lempiji*, and *O. lettia* share orange circled zone and *O. lettia* shares yellow-green circled areas with *semitorques*.

transients which belong to *Otus asio ussuriensis*. On the other hand, *Otus lempiji* (Tomek, 1999; Lee et al., 2000) or *Otus lettia* (del Hoyo et al., 1999) were also used for the Korean scops owl. However, the distributions of these species are quite different (Fig. 1) (Weick, 2006). *Otus lempiji* is distributed in Southeast Asia, including the Malay Peninsula, the Southern Thai Peninsula, and Indonesia. *Otus bakkamoena*, known as the Indian or collared scops owl, is distributed in southeast Arabia and southeast Iran to southern Pakistan, part of Nepal, India, and Sri Lanka (Weick, 2006). The geographic range of *Otus lettia* (Hodgson, 1836) is from eastern Nepal to Sakhalin including Southeast Asian areas.

Based on its distribution within Korea, the existence of two populations of the scops owl has been also suggested in Korea: *O. bakkamoena ussuriensis* in the Korean Peninsula and *O. bakkamoena semitorques* on Jeju Island (Nam, 1950; Vaurie, 1965; The Ornithological Society of Korea, 2009). Therefore, some recent literatures also note these populations as two different species (*O. lempiji ussuriensis* and *O. semitorques semitorques*) (Clements, 2007) or even merge these groups into one single species *O. lempiji* (*O. lempiji ussuriensis* and *O. l. semitorques*) (Tomek, 1999) or *O. semitorques* (*O. semitorques ussuriensis* and *O. s. semitorques*) (König and Weick, 2008) which is distributed in southeast Siberia to Japan (del Hoyo et al., 1999; Weick, 2006; Fuchs et al., 2008). While there is record that Korean population has paler and grayer tones than Japanese population especially in its light markings (Deignan, 1950), when considering the geographic proximity and the possible existence of a local population belonging to Japanese population on Jeju island in Korea, the scops owl occurs in Korea may more

closely related with *O. semitorques* rather than *O. lempiji* and *O. bakkamoena*. However, all the previous literatures on the Korean scops owl were based on its morphological features only. Therefore, in spite of the uncertainty and complexity on its status, the *O. bakkamoena* is the recommended name until confirmative studies on the phylogenetic status of the scops owl in Korea would be conducted (The Ornithological Society of Korea, 2009).

The phylogeny of *Otus* remains unclear (Heidrich et al., 1995; del Hoyo et al., 1999; Ryu and Park, 2003; Proudfoot et al., 2007; Fuchs et al., 2008). To resolve the species status of the Korean collared scops owls, a molecular phylogenetic study was conducted. We compared sequences of Korean collared scops owls, *O. lempiji*, with *O. bakkamoena*, *O. lettia*, and *O. semitorques* using two regions of mitochondrial DNA and confirmed the species status of Korean owls as well as relationships among the other species.

MATERIALS AND METHODS

Sampling

We obtained 36 tissue samples of Korean collared scops owls from the Conservation Genome Resource Bank for Korean Wildlife (CGRB; CGRB constructed a network of wildlife rescue centers and animal hospitals where injured or dead wild animals are brought throughout Korea). We obtained three DNA samples of *O. semitorques* (from bird strikes or traffic accidents) from Japan. The sequences of the other *Otus* species, *O. lempiji* (EU348987, AJ004026–7 for cytochrome *b* [*cyt b*]; EU601036 for NADH dehydrogenase subunit 2 [ND2]), *O. bakkamoena* (AJ004018–20 for *cyt b*; EU601034 for ND2) and *O. lettia* (EU601109 for *cyt b*; EU601303 for ND2) for phylogenetic analysis, were obtained from GenBank (Table 1).

DNA extraction and polymerase chain reaction (PCR) molecular data

DNA was extracted using DNeasy Tissue and Blood Kits (Qiagen, Valencia, CA, USA). Two mitochondrial regions were amplified using the following primers: 1) *cyt b* gene using L14841 (Kocher et al., 1989) and H16065 (Brito, 2005) and 2) ND2 gene using L5219 and H6316 (Meng et al., 2008). Each 30 μ L PCR reaction contained \sim 50 ng of template DNA, 1.5 mM $MgCl_2$, 2.5 mM dNTPs, and 1 U (0.2 μ L) of i-star *Taq* polymerase (5 U/ μ L, iNtRON Biotechnology, Seongnam, Korea). Thermocycling conditions started with an initial denaturation of 94°C for 5 min, followed by the touch-down method of 20 cycles of 45 s at 94°C for denaturation, 45 s at 55°C for annealing (decreasing 0.5°C per cycle to 45°C), and 45 s at 72°C for extension, and 25 more cycles of 45 s at 94°C

Table 1. Sample ID and collection locality of collared scops owl

No.	CGRB ID	Collection locality		Regions and haplotype ID	
				Cyt <i>b</i> Hap ID	ND2 Hap ID
1	cgrb1827	Seoul	South Korea	–	2
2	cgrb4486	Seoul	South Korea	–	4
3	cgrb4487	Seoul	South Korea	1	4
4	cgrb5419	Seoul	South Korea	–	2
5	cgrb1834	Gyeonggi	South Korea	1	4
6	cgrb1875	Gyeonggi	South Korea	1	4
7	cgrb5336	Gyeonggi	South Korea	1	4
8	cgrb5353	Gyeonggi	South Korea	1	4
9	cgrb6210	Gyeonggi	South Korea	–	2
10	cgrb2050	Gangwon	South Korea	1	2
11	cgrb4565	Gangwon	South Korea	–	5
12	cgrb4572	Gangwon	South Korea	1	2
13	cgrb1868	Chungbuk	South Korea	1	4
14	cgrb3105	Chungbuk	South Korea	1	4
15	cgrb1872	Chungnam	South Korea	7	2
16	cgrb2846	Chungnam	South Korea	3	4
17	cgrb1901	Jeonbuk	South Korea	8	4
18	cgrb1903	Jeonbuk	South Korea	1	4
19	cgrb1934	Jeonbuk	South Korea	4	2
20	cgrb148	Jeonnam	South Korea	1	4
21	cgrb173	Jeonnam	South Korea	–	4
22	cgrb1788	Jeonnam	South Korea	–	4
23	cgrb2731	Jeonnam	South Korea	–	4
24	cgrb3088	Jeonnam	South Korea	1	4
25	cgrb3376	Jeonnam	South Korea	–	4
26	cgrb3410	Jeonnam	South Korea	–	3
27	cgrb3417	Jeonnam	South Korea	–	1
28	cgrb5954	Jeonnam	South Korea	5	4
29	cgrb9454	Jeonnam	South Korea	–	4
30	cgrb9505	Jeonnam	South Korea	–	2
31	cgrb1792	Gyeongbuk	South Korea	–	4
32	cgrb1857	Gyeongbuk	South Korea	1	4
33	cgrb2863	Gyeongnam	South Korea	4	1
34	cgrb3581	Gyeongnam	South Korea	6	4
35	cgrb4855	Gyeongnam	South Korea	4	5
36	cgrb3819	Jeju	South Korea	–	2
37	cgrb10338	Kanakawa	Japan	9	6
38	cgrb10339	Kanakawa	Japan	9	6
39	cgrb10340	Kanakawa	Japan	9	6
40	EU348987	<i>Otus lempiji</i>	Java	11	–
41	AJ004027	<i>Otus lempiji</i>	Taiwan	12	–
42	AJ004026	<i>Otus lempiji</i>	Taiwan	12	–
43	AJ004018	<i>Otus bakkamoena</i>	Captive	10	–
44	AJ004019	<i>Otus bakkamoena</i>	Captive	10	–
45	AJ004020	<i>Otus bakkamoena</i>	Captive	10	–
46	EU601109	<i>Otus lettia</i>	Laos	13	–
47	EU601036	<i>Otus lempiji</i>	Captive	–	7
48	EU601034	<i>Otus bakkamoena</i>	Captive	–	7
49	EU601033	<i>Otus lettia</i>	Laos	–	8

Cyt *b*, cytochrome *b*; ND2, NADH dehydrogenase subunit 2; –, samples not analyzed.

for denaturation, 45 s at 45°C for annealing, and 45 s at 72°C for extension, ending with a final extension of 10 min at 72°C. PCR products were purified using a Zymoclean Gel DNA Recovery Kit (Zymo Research, Irvine, CA, USA) and

directly sequenced in both directions using ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems Inc., Carlsbad, CA, USA), followed by detection on an ABI 3730 XL DNA sequencer.

Phylogenetic analysis

The sequences were edited by Geneious Pro version 4.7 (Drummond et al., 2009) and aligned using Clustal X (Thompson et al., 1997). Phylogenetic trees were constructed using maximum-likelihood (ML) and maximum-parsimony (MP) methods by PAUP version 4.0 beta 10 (Swofford, 2001). The MP tree was constructed from 100 replicates using a heuristic search algorithm with tree-bisection-reconnection. In the ML analysis, HKY85+G model for *cyt b* and HKY85 model for ND2 were chosen as the best fit model by Modeltest 3.7 (Posada and Crandall, 1998) and heuristic search of 100 replicates was carried out. The neighbor-joining (NJ) method was also used to construct a phylogenetic tree with 1000 bootstrap replicates by MEGA version 4 (Tamura et al., 2007). The number of polymorphic sites (S), haplotype diversity (h), and nucleotide diversity (π) were calculated using DnaSp 4.10 software (Rozas et al., 2003). Pairwise sequence distances were calculated based on the Kimura two-parameter method (Kimura, 1980).

RESULTS

Mitochondrial sequence variability

The partial sequences of *cyt b* (891 bp, JN11958–81) and ND2 (627 bp, JN11982–12020) were successfully determined from 24 *cyt b* and 39 ND2 collared scops owl specimens. We compared the sequences with *O. lempiji* (EU348987, AJ004026–7 for *cyt b*; EU601036 for ND2), *O. bakkamoena* (AJ004018–20 for *cyt b*; EU601034 for ND2) and *O. lettia* (EU601109 for *cyt b*; EU601033 for ND2) from GenBank. There were no indels present in either gene. In the *cyt b* gene, there were eight polymorphic sites, resulting in seven haplotypes. ND2 sequences showed five polymorphic sites and five haplotypes. The overall haplotype diversity and nucleotide diversity of Korean specimens were 0.614 ± 0.116 and 0.111 ± 0.030 , respectively, in *cyt b* and 0.606 ± 0.073 and 0.132 ± 0.021 , respectively, in ND2 (Table 2).

Table 2. Summary of sample size (N), number of haplotype (H), number of variable sites (S), haplotype diversity (h), and nucleotide diversity (π) for the specimens used in this study (*cyt b*/ND2)

	Gene	N	H	S	h (SE)	π (\pm SE, %)
Korean	<i>Cyt b</i>	21	7	8	0.614 ± 0.116	0.111 ± 0.030
	ND2	36	5	5	0.606 ± 0.073	0.132 ± 0.021
Japanese	<i>Cyt b</i>	3	1	0	0	0
	ND2	3	1	0	0	0
<i>Otus lempiji</i>	<i>Cyt b</i>	3	2	32	0.667 ± 0.314	2.394 ± 1.129
	ND2	1	1	–	–	–
<i>Otus bakkamoena</i>	<i>Cyt b</i>	3	1	0	0	0
	ND2	1	1	–	–	–
<i>Otus lettia</i>	<i>Cyt b</i>	1	1	–	–	–
	ND2	1	1	–	–	–

Cyt b, cytochrome *b*; ND2, NADH dehydrogenase subunit 2; 0, When haplotype is one, no haplotype diversity and nucleotide diversity were determined; –, When sample size is one, no variable site was detected, therefore haplotype diversity and nucleotide diversity cannot be detected.

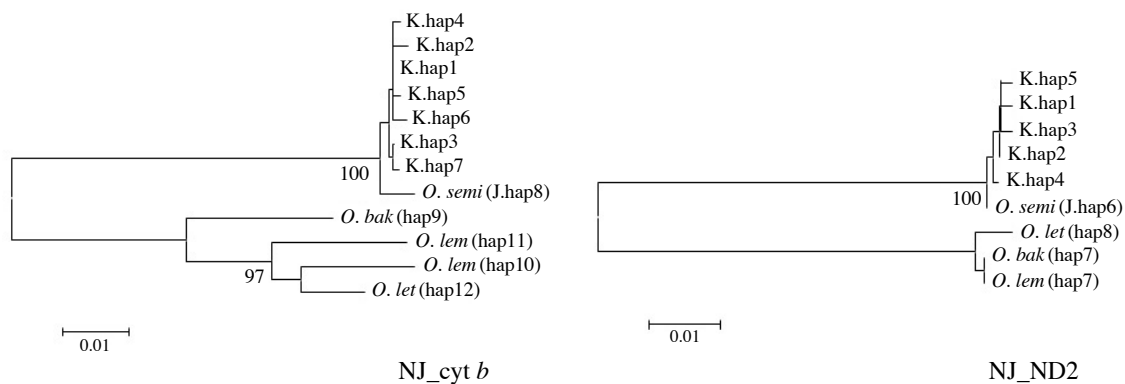


Fig. 2. Molecular phylogenetic trees of Korean collared scops owls, *Otus lempiji* (*O. lem*), *O. bakkamoena* (*O. bak*), *O. semitorques* (*O. semi*), and *O. lettia* (*O. let*). All maximum-likelihood (ML), maximum-parsimony (MP), and neighbor-joining (NJ) trees revealed same aspects. NJ tree of cytochrome *b* (*cyt b*, 891 bp) and NADH dehydrogenase subunit 2 (ND2, 627 bp) using haplotypes was showed (not shown ML and MP trees). K, Korean; J, Japanese.

Phylogenetic analysis

All ML, MP, and NJ trees of *cyt b* and ND2 sets were very similar to each other, with only minor differences. Through phylogenetic analysis, we confirmed that trees in both regions consisted of two clades with 100% bootstrap values: Clade 1, Korean species and *O. semitorques*; Clade 2, *O. lempiji*, *O. bakkamoena* and *O. lettia* (Fig. 2 representative NJ tree; ML/MP trees not shown). All the Korean collared scops owl haplotypes were grouped together with very low genetic differences (*cyt b*, $d=0.001-0.005$; ND2, $d=0.002-0.003$) and were allied with *O. semitorques* (*cyt b*, mean $d=0.004$; ND2, mean $d=0.003$) (Tables 3, 4). When compared with *O. lempiji*, the genetic distance between the Korean samples and *O. lempiji* was extremely high (*cyt b*, $d=0.118$; ND2, $d=0.113$) (Tables 3, 4) and could be considered a species level difference. We also found a high genetic difference between the Korean species and *O. bakkamoena* (*cyt b*, $d=0.106$; ND2, $d=0.113$)/*O. lettia* (*cyt b*, $d=0.110$; ND2, $d=0.117$), respectively (Tables 3, 4). However, moderate (*cyt b*, 0.043) or very low (ND2, 0.003) genetic differences were observed among *O. lempiji*, *O. bakkamoena* and *O. lettia*, even, no genetic difference of ND2 gene between *O. lempiji* and *O. bakkamoena* was shown. No regional separation was observed among the Korean samples.

DISCUSSION

The collared scops owl, a natural monument and protected species of Korea, is the subject of questions concerning its formal scientific name and phylogenetic status. The taxonomic status of this species with other related *Otus* species is not clear. Several studies have been performed to resolve this issue (Heidrich et al., 1995; Wink and Heidrich, 2000;

Ryu and Park, 2003; Wink et al., 2004; Fuchs et al., 2008), but these efforts highlighted other *Otus* species or related species without including the Korean collared scops owl. As a consequence, *O. lempiji*, *O. bakkamoena*, *O. lettia*, and *O. semitorques* are still of ambiguous phylogenetic status (König et al., 1999; Weick, 2006; Fuchs et al., 2008; Lok et al., 2009). Their population has been decreasing gradually owing to many anthropogenic factors especially collision and traffic accidents (Kim, 2006; CHA, 2010). Moreover, if these threatening factors are not removed, the Korean species may become endangered. At this point, unclear classification could be an obstacle to conservation strategies. The aim of the present study was to clarify the scientific name, to clear the confusion surrounding the taxonomic status of the Korean collared scops owl, and to provide basic information for its conservation. We also sought to verify relationships among the three *Otus* species using mitochondrial *cyt b*, which has been used for owl systematic classification (Heidrich et al., 1995; Griffiths, 1997; Mindell et al., 1997; Ryu and Park, 2003; Wink et al., 2004), and additional ND2 sequences.

All the ML, MP, and NJ trees of both regions (*cyt b* and ND2) confirmed that the Korean species is clearly different from *O. lempiji* and *O. bakkamoena*, which are the scientific names used officially for the collared scops owl in Korea. Instead of clustering with *O. lempiji*, *O. bakkamoena*, or *O. lettia*, they aligned with the morphologically similar species, *O. semitorques* (Fig. 2). This implies that the Korean species is the same as *O. semitorques*. Further genetic and ecological data are necessary to verify this association. A slight genetic difference between the Korean collared scops owl and *O. semitorques* was detected in *cyt b* gene (Table 3), which may be considered as a regional variation (Deignan, 1950; Weick, 2006).

In the *cyt b* gene, *O. lempiji* (hap11) was allied with *O. lettia*

Table 3. Pairwise genetic distance of *cyt b* region by Kimura two-parameters

	K.hap1	K.hap2	K.hap3	K.hap4	K.hap5	K.hap6	K.hap7	<i>O. semi</i> J.hap8	<i>O. bak</i> hap9	<i>O. lem</i> hap10	<i>O. lem</i> hap11	<i>O. let</i> hap12
K.hap1												
K.hap2	0.002											
K.hap3	0.001	0.003										
K.hap4	0.001	0.003	0.002									
K.hap5	0.001	0.003	0.002	0.002								
K.hap6	0.002	0.005	0.003	0.003	0.003							
K.hap7	0.002	0.005	0.001	0.003	0.003	0.005						
<i>O. semi</i> J.hap8	0.007	0.009	0.008	0.008	0.008	0.009	0.009					
<i>O. bak</i> hap9	0.105	0.108	0.105	0.107	0.107	0.105	0.104	0.108				
<i>O. lem</i> hap10	0.119	0.119	0.119	0.12	0.118	0.122	0.118	0.122	0.051			
<i>O. lem</i> hap11	0.116	0.118	0.116	0.117	0.117	0.119	0.114	0.119	0.058	0.037		
<i>O. let</i> hap12	0.109	0.112	0.109	0.111	0.111	0.112	0.108	0.112	0.048	0.026	0.038	

Matrix to sequence divergence of mtDNA of Korean and Japanese collared scops owls with the other two species of *Otus* from GenBank was used. Haplotypes were used to compare genetic difference.

Cyt b, cytochrome b; K, Korean; *O. semi* J., *O. semitorques*, Japanese; *O. bak*, *O. bakkamoena*; *O. lem*, *O. lempiji*; *O. let*, *O. lettia*.

Table 4. Pairwise genetic distance of ND2 region by Kimura two-parameters

	K.hap1	K.hap2	K.hap3	K.hap4	K.hap5	<i>O. semi</i> J.hap6	<i>O. bak/lem</i> hap7	<i>O. let</i> hap8
K.hap1								
K.hap2	0.002							
K.hap3	0.003	0.002						
K.hap4	0.003	0.002	0.003					
K.hap5	0.003	0.002	0.003	0.003				
<i>O. semi</i> J.hap6	0.003	0.002	0.003	0.003	0.003			
<i>O. bak/lem</i> hap7	0.114	0.112	0.114	0.110	0.114	0.118		
<i>O. let</i> hap8	0.118	0.116	0.118	0.114	0.118	0.114	0.006	

Matrix to sequence divergence of mtDNA of Korean and Japanese collared scops owls with the other two species of *Otus* from GenBank was used. Haplotypes were used to compare genetic difference.

ND2, NADH dehydrogenase subunit 2; K, Korean; *O. semi* J., *O. semitorques*, Japanese; *O. bak*, *O. bakkamoena*; *O. lem*, *O. lempiji*; *O. let*, *O. lettia*.

more closely than another *O. lempiji* (hap12), moreover, in the ND2 region, *O. lempiji* and *bakkamoena* had the same sequence conformation (Tables 3, 4, Fig. 2), which may be due to the fact that the length of the sequences was not long enough to discriminate between the two species. Alternatively, sampling error can be another explanation. *O. lempiji* is often considered a race of *O. bakkamoena* or *O. lettia* (Weick, 2006) and members of the genus *Otus* are similar in plumage. Species discrimination of some taxa relies only on vocalization (Weick, 2006; Fuchs et al., 2008); therefore, individuals described as *O. lempiji* and *O. lettia* (cyt b)/*O. bakkamoena* (ND2) may be of the same species.

We had similar results with the MHC II gene (534 bp, nuclear DNA, unpublished to date), which grouped Japanese specimens together with Korean collared scops owls with very low genetic differentiation ($d=0.004$). No regional difference between Korean and Japanese specimens was detected. According to previous *Otus* species studies, mitochondrial genetic difference value among different species was 0.031–0.186 (Heidrich et al., 1995; Ryu and Park, 2003). Thus, we confirmed that the genetic distance between the Korean samples and *O. lempiji/bakkamoena/lettia* falls within the general interspecific differentiation range (Tables 3, 4).

Justification for changing the scientific name of the Korean collared scops owl to *O. semitorques* is supported by the following evidence: First, Deignan (1950) mentioned that the Korean species defined as *O. b. ussuriensis* seems to be inseparable from the Japanese specimens based on plumage color and wing length, and suspected that *semitorques* would prove to be the resident race of Southern Korea; second, there was a phylogenetic study of the family Strigidae which referred to the Korean species as *semitorques* (Ryu and Park, 2003) and also indicated unclear divergence among *Otus* species; third, in the Owls (Weick, 2006), we can definitely confirm the Korean scops owl is systematically classified as a subspecies of *semitorques*. According to Weick (2006), *O. lempiji*, *O. bakkamoena*, *O. lettia*, and *O. semitorques* are

distributed in different areas. Moreover, *lempiji* is frequently considered as *O. bakkamoena* or *O. lettia*, although they have different vocalizations. In the case of *O. semitorques*, it is often regarded as conspecific with the morphologically variable *O. bakkamoena*; however, they differ in vocalizations and the color of irises (*O. semitorques* has red irises). Finally, König and Weick (2008) described Korean species as *O. semitorques* in recent years.

Since owls are nocturnal, their morphological characteristics are hard to observe so classification can be overlooked (Wink et al., 2004). Even though half of the *Otus* taxa are recognized as different species according to morphological traits, other species are still a matter of debate (Heidrich et al., 1995). Thus far, distinctive vocalization is used as a major key for discriminating between *Otus* species (Hekstra, 1982; König, 1991, 1994). If genetic information is added to morphological and vocal characteristics, phylogenetic relationships of owls will be better defined. Research of *Otus atricapillus* complex using mitochondrial DNA is a remarkable example to define four independent species which are morphologically very similar, have been considered as a single species (Heidrich et al., 1995). Even though there is abundant data to support the reclassification of the Korean collared scops owl as *O. semitorques*, there have not been any studies verifying this information to confirm that the incorrect scientific name has been used. We performed molecular analysis with mitochondrial DNA and acquired persuasive results. Based on our study, we suggest that the formally used scientific name of the Korean collared scops owl should be changed to *Otus semitorques* and this finding will be used as the basis for developing effective conservation or management strategies.

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REFERENCES

- Allendorf FW, Luikart G, 2007. Conservation and the genetics of populations. Blackwell Publishing, Oxford, pp. 6-14.
- Austin OL Jr, 1948. The birds of Korea. Bulletin of Museum of Comparative Zoology at Harvard College, 101:1-301.
- Brito PH, 2005. The influence of Pleistocene glacial refugis on tawny owl genetic diversity and phylogeography in Western Europe. *Molecular Ecology*, 14:3077-3094.
- Clements JF, 2007. The Clements checklist of the birds of the world. 6th ed. Christopher Helm, London, p. 163.
- Cultural Heritage Administration (CHA), 2010. A review of designating cultural monument animals (species) and preparation for effective management strategy. Cultural Heritage Administration, Daejeon, p. 326.
- Cumming DJ, 1933. Notes on Korean birds. *Transactions of the Royal Asiatic Society, Korea Branch*, 22:1-94.
- Deignan HG, 1950. The races of the collared Scops owl, *Otus bakkamoena* pennant. *Auk*, 67:189-201.
- del Hoyo J, Elliott A, Sargatal J, 1999. Barn owls to hummingbirds. In: *Handbook of the birds of the world*. Vol. 5 (Eds., Marks JS, Cannings RJ, Mikkola H). Lynx Edicion, Barcelona, pp. 76-243.
- Dickinson EC, 2003. The Howard and Moore complete checklist of the birds of the world. 3rd ed. Christopher Helm, London, pp. 1-1040.
- Drummond AJ, Ashton B, Buxton S, Cheung M, Cooper A, Heled J, Kearse M, Moir R, Stones-Havas S, Sturrock S, Thierer T, Wilson A, 2009. Geneious v4.7 [Internet]. Bio-matters Ltd., Auckland, Accessed 21 Sep 2012, <<http://www.geneious.com>>.
- Fennell CM, 1952. Some observations on the birds of southern Korea. *Condor*, 54:101-110.
- Fuchs J, Pons JM, Goodman SM, Bretagnolle V, Melo M, Bowie RCK, Currie D, Safford R, Virani MZ, Thomsett S, Hija A, Cruaud C, Pasquet E, 2008. Tracing the colonization history of the Indian Ocean scops-owls (Strigiformes: *Otus*) with further insight into the spatio-temporal origin of the Malagasy avifauna. *BMC Evolutionary Biology*, 8:197.
- Griffiths CS, 1997. Correlation of functional domains and rates of nucleotide substitution in cytochrome *b*. *Molecular Phylogenetics and Evolution*, 7:352-365.
- Heidrich P, König C, Wink M, 1995. Molecular phylogeny of South American Screech owls of the *Otus atricapillus* complex (Aves: Strigidae) inferred from nucleotide sequences of the mitochondrial cytochrome *b* gene. *Zeitschrift Fur Naturforschung, Section C, Biosciences*, 50:294-302.
- Hekstra GP, 1982. Description of twenty-four new subspecies of American Otus (Aves, Strigidae). *Bulletin of Zoological Museum of the University of Amsterdam*, 9:49-63.
- Hodgson BH, 1836. Scops lettia. *Asiatic Research*, 19:176-177.
- Kim YJ, 2006. Pattern analysis of the wildlife casualties in Korea. MS thesis, Seoul National University, Seoul, Korea, pp. 13-76.
- Kimura M, 1980. A simple method for estimating evolutionary rates of base substitutions through the comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16:111-120.
- Kocher TD, Thomas WK, Meyer A, Edwards SV, Pääbo S, Villablanca FX, Wilson AC, 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proceedings of National Academy of Sciences of the United States of America*, 86:6196-6200.
- König C, 1991. Zur Taxonomie und Ökologie der Sperlingskäuze (*Glaucidium* spp.) des Andenraumes. *Ökologie der Vögel*, 13:15-76.
- König C, 1994. Lautäußerungen als interspezifische Isolationsmechanismen bei Eulen der Gattung Otus (Aves: Strigidae) aus dem südlichen Südamerika. *Stuttgarter Beiträge zur Naturkunde Serie A (Biologie)*, 511:1-35.
- König C, Weick F, 2008. Owls of the world. 2nd ed. Helm Identification Guides, London, pp. 1-528.
- König C, Weick F, Becking JH, 1999. Owls: a guide to the owls of the worlds. Pica Press, Sussex, pp. 39-42.
- Lee WS, Koo TH, Park JY, 2000. A field guide to the birds of Korea. LG Evergreen Foundations, Seoul, p. 184.
- Lok AFSL, Lee TK, Lim KC, 2009. The biology of *Otus lempiji cnephaues* Deignan, the Sunda scops-owl in Singapore. *Nature in Singapore*, 2:31-38.
- Mace GM, 2004. The role of taxonomy in species conservation. *Philosophical Transactions of Royal Society of London B, Biological Sciences*, 359:711-719.
- Meng Y, Dai B, Ran JH, Li J, Yue B, 2008. Phylogenetic position of the genus *Tetraophasis* (Aves, Galliformes, Phasianidae) as inferred from mitochondrial and nuclear sequences. *Biochemical Systematics and Ecology*, 36:626-637.
- Mindell DP, Sorenson MD, Huddleston CJ, Miranda HCJ, Knight A, Sawchuk SJ, Yuri T, 1997. Phylogenetic relationships among and within select avian orders based on mitochondrial DNA. In: *Avian molecular evolution and systematics* (Ed., Mindell DP). Academic Press, San Diego, CA, pp. 213-247.
- Nam TK, 1950. A hand-list of the Korean birds. MS thesis, Seoul National University, Seoul, Korea, p. 103.
- Posada D, Crandall KA, 1998. MODELTEST: testing the model of DNA substitution. *Bioinformatics*, 14:817-818.
- Proudfoot GA, Gehlbach FR, Honeycutt RL, 2007. Mitochondrial DNA variation and phylogeography of the Eastern and Western Screech-Owls. *Condor*, 109:617-627.
- Rozas J, Sánchez-DelBarrio JC, Messeguer X, Rozas R, 2003. DnaSP, DNA polymorphism analyses by the coalescent and other methods. *Bioinformatics*, 19:2496-2497.

- Ryu SH, Park HC, 2003. Molecular phylogeny of the Family Strigidae (Aves) based on mitochondrial cytochrome *b* gene. Korean Journal of Systematic Zoology, 19:297-304.
- Swofford R, 2001. PAUP*: Phylogenetic analysis using parsimony (and other methods) 4.0 Beta [Internet]. Sinauer Associates, MA, Accessed 21 Sep 2012, <<http://www.sinauer.com/detail.php?id=8060>>.
- Tamura K, Dudley J, Nei M, Kumar S, 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Molecular Biology and Evolution, 24:1596-1599.
- The Korean Association for Bird Protection. Accessed 12 Aug 2012, <<http://www.bird.or.kr>>.
- The Ornithological Society of Korea, 2009. Checklist of the birds of Korea. Ornithological Society of Korea, Seoul, pp. 39-40.
- Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG, 1997. The Clustal_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Research, 24:4876-4882.
- Tomek T, 1999. The birds of North Korea. Non-Passeriformes. Acta Zoologica Cracoviensia, 42:1-217.
- Valdecasas AG, Camacho AI, 2003. Conservation to the rescue of taxonomy. Biodiversity and Conservation, 12:1113-1117.
- Vaurie C, 1965. The birds of the Palearctic fauna: Non-Passeriformes. H. F. & G. Witherby Ltd., London, pp. 603-604.
- Weick F, 2006. Owls (Strigiforms). Springer-Verlag, Berlin, pp. 67-68.
- Wink M, Heidrich P, 2000. Molecular systematics of owls (Strigiforms) based on DNA sequences of the mitochondrial cytochrome *b* gene. In: Raptors at risk (Eds., Chancellor RD, Meyburg BU). WWGBP/Hancock House, London, pp. 819-828.
- Wink M, Sauer-Gürth H, Fuchs M, 2004. Phylogenetic relationships in owls based on nucleotide sequences of mitochondrial and nuclear marker genes. In: Raptors worldwide (Eds., Chancellor RD, Meyburg BU). WWGBP/MME, Budapest, pp. 517-526.

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