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Identification of the South Korean Hermit Beetle (Coleoptera: Scarabaeidae: Cetoniinae)

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남한산 큰자색호랑꽃무지의 종 동정 (딱정벌레목, 풍뎅이과, 꽃무지아과)

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ABSTRACT: The South Korean hermit beetle has previously been identified as *Osmoderma opicum*, which is distributed in Japan. Because of its rarity, this species is classified as an endangered species. To date, however, the identity of this species in South Korea has not been conclusively confirmed. To assess the taxonomic status of the hermit beetle occurring in South Korea, we performed a comparative study with the beetle's Eurasian congeners, based on morphological examination and molecular analysis using *COI* gene sequences. The results clearly showed that the South Korean hermit beetle is identical to *Osmoderma caeleste*, which has been described from the Russian Far East. Therefore, we suggest that the taxonomic identity of the South Korean hermit beetle is *O. caeleste*.

Key words: Taxonomy, Cetoniinae, *Osmoderma caeleste*, Species identification

초 록: 남한산 큰자색호랑꽃무지는 그 동안 일본에 분포하는 *Osmoderma opicum*으로 동정되어 왔으며, 국내에서는 그 희귀성에 의해 멸종위기종으로 다루어지고 있지만, 남한 개체군에 대한 면밀한 종의 실체에 대한 구명은 없었다. 이에 대해, 남한산 큰자색호랑꽃무지와 유라시안 근연종에 대해 형태 및 COI유전자 염기서열을 비교를 실시하였다. 결과적으로, 남한산 개체군은 형태적으로 극동러시아에 분포하는 *O. caeleste*와 동일하였다. 따라서, 남한산 큰자색호랑꽃무지는 *O. caeleste*임을 제안한다.

검색어: 분류, 꽃무지아과, 큰자색호랑꽃무지, 종의 동정

The genus *Osmoderma* LePeletier de Saint-Fargeau and Serville consists of 12 species, which occur throughout the Palearctic (9 species) and Nearctic (3 species) regions (Audisio et al., 2007). Species of *Osmoderma* are commonly referred to as "hermit beetles" because they live in hollows in old broad-leaved trees and are rarely observed in nature (Audisio et al., 2009). European species of these beetles (mainly *O. eremita*) are regarded as one of the most important "flagship species" and "umbrella species" for the conservation of local saproxylic communities (Ranius, 2002a, 2002b; Audisio et al., 2009). Among these, five species are listed as near threatened to endangered in the IUCN Red List of Threatened Species by virtue of their rarity and association with a specific habitat type (Nardi and Micó, 2010; Nieto and Alexander, 2010).

In the Korean Peninsula, there are a total of four species and

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one subspecies in the genus *Osmoderma. O. opicum* Lewis, 1887 has been recorded in South Korea by Cho (1969), whereas three species, *O. barnabita* Motschulsky, 1845 described by Stebnicka in 1980, *O. davidis* forma *amurensis* Gusakov, 2002 described by Gusakov (2002), and *O. caeleste* Gusakov, 2002 described by Audisio in 2007, are listed in North Korea, although without any reliable specimen identification. Furthermore, recently, a new subspecies, *O. opicum coreanum*, was also described from North Korea by Tauzin (2013).

It is noteworthy that South Korean specimens of *Osmoderma* have been extremely rarely observed. Only four specimens, consisting of three females and a male, have been collected in South Korea since 1969 (Kim, 2011). The South Korean population, which is considered to be *O. opicum,* has accordingly been registered as a critically endangered species in the Korean Red Data Book (Lee, 2013). In order to determine accurate species traits of the South Korean hermit beetle, in 2012, we first obtained a Korean female specimen, which we assumed to be *O. opicum* (specimen no. 3257) and six authenticated Japanese specimens of *O. opicum*. However, these two geographically separated populations differed in several of their external features. It was accordingly suggested that the South Korean hermit beetle might have been incorrectly recorded as *O. opicum*. Therefore, over the past 4 years, we have attempted to obtain a larger number of specimens of the Korean hermit beetle as well as voucher specimens of the close relatives in neighboring regions. Recently, three appreciable studies on the European hermit beetles, carried out mainly using molecular and distributional data, have contributed to a reasonable and objectively supported taxonomic arrangement (Audisio et al., 2007, 2009; Landvik et al., 2013). It was therefore possible to compare our molecular data with the results obtained for European hermit beetles.

The South Korean hermit beetle is an important insect species in the conservation management of threatened species comprising forest saproxylic communities. Nonetheless, as mentioned above, there is still a lack of accurate information on the exact species identity and ecological traits of the Korean population that we seek to conserve.

Materials and Methods

Specimen collection and morphospecies examination

To assess the exact species status of the South Korean hermit beetle, we obtained a total of 16 dried specimens belonging to three *Osmoderma* species: six South Korean specimens, which have been recognized as *O. opicum*, two of which were previously recorded as voucher specimens (nos. 7299 and 9572) by Kim (2011); five specimens of true *O. opicum* from Honshu, Japan; three specimens of true *O. caeleste* from Far East Russia; and two specimens of *O. barnabita* from West Russia. We were, however, unable to obtain any specimens of *O. opicum coreanum* and *O. davidis* (Table 1).

Table 1. The examined specimens and information for the successful sequences of *COI* gene

Sample no.	Species	Locality	Data collected	Sex	Voucher no.	GBAn of *COI*	Retrieving sequence of *COI* (657bp)	Reference
1	*Osmoderma caeleste* (?)	Yanggu, GW, South Korea	26. IX. 2011	M	3257	KX346561	1-657	This study
2	*Osmoderma caeleste* (?)	Garigol, Jogyeong-dong, Injae, GW, South Korea	26. VII. 2000	F	7299	KX346562	93-318	This study
3	*Osmoderma caeleste* (?)	Mt. Odae, Jinbu, Phyeong-chang, GW, South Korea	8. VII. 2010	F	7387	KX346563	1-657	This study
4	*Osmoderma caeleste* (?)	Mt. Ilweol, Ilweol-myeon, Yongyang, GB, South Korea	XI. 2008	F	9569	KX346564	1-657	This study
5	*Osmoderma caeleste* (?)	Yongdae-ri, Buk-myeon, Injae, GW, South Korea	31. VII. 2013	F	9571	KX346565	1-657	This study
6	*Osmoderma caeleste* (?)	Guun-ri, Sangseo-myeon, Hwacheon, GW, South Korea	9. VIII. 2000	M	9572	KX346566	1-657	This study
7	*Osmoderma opicum*	Hyogo, Honshu, Japan	VII. 2011	M	7293	KX346570	1-657	This study

Table 1. Continued

Sample no.	Species	Locality	Data collected	Sex	Voucher no.	GBAn of *COI*	Retrieving sequence of *COI* (657bp)	Reference
8	*Osmoderma opicum*	Mt. Hyonosen, Hyogo, Honshu, Japan	VI. 2007	M	7295	KX346571	1-657	This study
9	*Osmoderma opicum*	Mt. Hyonosen, Hyogo, Honshu, Japan	VI. 2007	M	7296	KX346572	1-657	This study
10	*Osmoderma opicum*	Mt. Hyonosen, Hyogo, Honshu, Japan	VI. 2007	F	7297	KX346573	1-657	This study
11	*Osmoderma opicum*	Mt. Hyonosen, Hyogo, Honshu, Japan	VI. 2007	F	7298	KX346574	1-657	This study
12	*Osmoderma caeleste*	Kamenushka vie, Ussurijsky dist., Primorskii krai, Far East of Russia	11. VII. 2010	F	9411	KX346567	1-657	This study
13	*Osmoderma caeleste*	S. Primorye, Far East of Russia	no data	M	9412	KX346568	1-657	This study
14	*Osmoderma caeleste*	Kamenushka vie, Ussurijsky dist., Primorskii krai, Far East of Russia	25. VIII. 2007	M	9413	KX346569	1-657	This study
15	*Osmoderma barnabita*	Nikiforovo village, Serpuhov dist., Moscow, Russia	20. VII. 2012	M	7362	KX346575	250-657	This study
16	*Osmoderma barnabita*	Nikiforovo village, Serpuhov dist., Moscow, Russia	20. VII. 2012	F	7372	KX346576	38-619	This study
17	*Osmoderma barnabita*	Croatia			OB1.1	AJ880684	1-657	Audisio et al. (2009)
18	*Osmoderma barnabita*	Greece			OB2.1	AM412378	1-657	Audisio et al. (2009)
19	*Osmoderma barnabita*	Germany			OB3.2	AM412379	1-657	Audisio et al. (2009)
20	*Osmoderma barnabita*	Slovakia			OB4.1	AM423160	1-657	Audisio et al. (2009)
21	*Osmoderma barnabita*	Finland			FIN1	KC476172	62-657	Landvik et al. (2013)
22	*Osmoderma barnabita*	Finland			FIN2	KC476173	62-657	Landvik et al. (2013)
23	*Osmoderma barnabita*	Finland			FIN3	KC476174	77-657	Landvik et al. (2013)
24	*Osmoderma barnabita*	Finland			FIN4	KC476175	114-657	Landvik et al. (2013)
25	*Osmoderma barnabita*	Finland			FIN6	KC476177	113-657	Landvik et al. (2013)
26	*Osmoderma barnabita*	Finland			FIN7	KC476178	77-657	Landvik et al. (2013)
27	*Osmoderma italicum*	Italy			OI1.1	AJ880686	1-657	Audisio et al. (2009)
28	*Osmoderma cristinae*	Italy, Sicily			OC1.1	AJ880679	1-657	Audisio et al. (2009)

Table 1. Continued

Sample no.	Species	Locality	Data collected	Sex	Voucher no.	GBAn of *COI*	Retrieving sequence of *COI* (657bp)	Reference
29	*Osmoderma cristinae*	Italy, Sicily			OC2.1	AM412382	1-657	Audisio et al. (2009)
30	*Osmoderma cristinae*	Italy, Sicily			OC2.3	AM412383	1-657	Audisio et al. (2009)
31	*Osmoderma lassallei*	Greece			OL.1.1	AJ880685	1-657	Audisio et al. (2009)
32	*Osmoderma lassallei*	Greece			OL.2.1	AM412380	1-657	Audisio et al. (2009)
33	*Osmoderma lassallei*	Greece			OL.2.2	AM412381	1-657	Audisio et al. (2009)
34	*Osmoderma lassallei*	Greece			OL.3.1	AM423161	1-657	Audisio et al. (2009)
35	*Osmoderma eremita*	Italy			OE1.1	AJ880680	1-657	Audisio et al. (2009)
36	*Osmoderma eremita*	Sweden			OE2.1	AJ880681	1-657	Audisio et al. (2009)
37	*Osmoderma eremita*	Sweden			OE2.2	AJ880682	1-657	Audisio et al. (2009)
38	*Osmoderma eremita*	Italy			OE3.1	AJ880683	1-657	Audisio et al. (2009)
39	*Osmoderma eremita*	France			OE5.1	AM412372	1-657	Audisio et al. (2009)
40	*Osmoderma eremita*	France			OE6.1	AM412373	1-657	Audisio et al. (2009)
41	*Osmoderma eremita*	France			OE6.2	AM412374	1-657	Audisio et al. (2009)
42	*Osmoderma eremita*	France			OE7.1	AM412375	1-657	Audisio et al. (2009)
43	*Osmoderma eremita*	France			OE7.2	AM412376	1-657	Audisio et al. (2009)
44	*Osmoderma eremita*	France			OE7.4	AM412377	1-657	Audisio et al. (2009)
45	*Osmoderma eremita*	Italy			OE8.1	AM423158	1-657	Audisio et al. (2009)
46	*Osmoderma eremita*	Slovenia			OE9.1	AM423159	1-657	Audisio et al. (2009)
47	*Osmoderma eremita*	Germany			OE14.1	AM423156	1-657	Audisio et al. (2009)
48	*Osmoderma eremita*	Germany			OE15.1	AM423157	1-657	Audisio et al. (2009)
49	*Dicronocephalus adamsi**	Sangdaewon, Jungwon, Seongnam, GG, South Korea	6. VI. 2012	F	7258	KM390855	1-657	Lee et al. (2015)

*Denotes outgroup taxon extracted from GenBank. GBAn is denoted the gene bank accession number. Numbers of 18 to 50 are extracted from GenBank.

For simultaneous examination of both morphological and molecular characters, we first observed the general structure of the specimens under a stereoscopic microscope (MZ 16A and MZ 6; Leica, Solms, Germany) for morphospecies identification and diagnostic characteristic delimitation. All of the examined materials and genomic DNA stocks are preserved in the insect collection at the National Institute of Agricultural Science, Jeonju, Korea. The provincial abbreviations of the collecting sites within Korea are as follows: GW, Gangwon-do; GB, Gyeongsangbuk-do.

DNA extraction, PCR strategy, and DNA analyses

To reconfirm the specific status of the specimens, we performed molecular analysis using sequences of the *cytochrome c oxidase subunit I* (*COI*) gene, which is useful in identifying species within a closely related species group (Han et al., 2012). For genomic DNA extraction, we removed the genitalia from the abdomens of both sexes and used them directly to extract genomic DNA using non-destructive methods (Han et al., 2012). The remaining genital structures were cleaned by heating with 10% KOH solution in a Wise Therm ®HB-48P heating block at 60℃ for 1-2 h and then preserved in microvials containing glycerine for morphological examination.

For PCR based on dried specimens, we first attempted to amplify the DNA barcoding region (658 bp) of the *COI* gene using the universal primer set LCO1490/HCO2198 (Folmer et al., 1994); however, we were unable to obtain amplicons for most samples; this should be expected considering the likelihood of DNA degradation in specimen maintained under unknown dried conditions (Goldstein and Desalle, 2003; Hajibabaei et al., 2006; Wandeler et al., 2007). To retrieve the barcoding regions of the *COI* gene, we adopted the PCR methodology focused on old museum specimens, as described by Han et al. (2014), and designed 13 new specific primers for the *COI* gene of *O. opicum* and *O. caeleste* using Primer3 (Untergrasser et al., 2012) (Table 2). PCR was conducted using AccuPower *ProFi Taq* PCR PreMix (Bioneer, Daejeon, Korea). The amplification profile consisted of pre-denaturation for 5 min at 95℃, 35-45 cycles of 30 s at 95℃ for denaturation, 30 s at 48-53℃ for primer annealing, and then 45 s at 72℃ for extension, with a final extension for 5 min at 72℃. To confirm product size and purity, the PCR amplicons were assessed by 0.7% agarose gel electrophoresis using Safe-Pinky (Gen

Table 2. Primers used in the study

Gene	Strategy	Primer name	Sequence (5'→3')	Reference
COI	LCO1490/HCOI2198 in first attempt	F: LCO1490	GGTCAACAAATCATAAAGATATTGG	Folmer at al. (1994)
		R: CO2198	TAAACTTCAGGGTGACCAAAAAATCA	Folmer at al. (1994)
	For difficult samples, OschF1/OschR197; OschF9/OschR233; OschF79/OschR329; OschF178/OschR389; schF307/OschR565, OschR622 and OschR683; OschF365/ OschR622 and OschR683	F: OschF1 (=LCO1490)	TTCRACAAACCACAAAGAYATTGG	This study
		R: OschR197	AATCARTTTCCAAATCCMCCA	This study
		F: OschF9	TTTTCTATTCGGCAGATGAGC	This study
		R: OschR233	AATGCTATRTCTGGGGCACCT	This study
		F: OschF79	ACWCCYGGDTCTYTAATTGG	This study
		R: OschR329	CATCCYGTTCCTGCTCCATT	This study
		F: OschF178	GGKGGATTTGGAAAYTGATTA	This study
		R: OschR389	GCYAAATCWACRGATGCYCCT	This study
		F: OschF307	GAAAATGGAGCAGGAACRGG	This study
		R: OschR565	TTGCYCCTGCAARAACWGGWA	This study
		R: OschR622	CKGCWGGATCAAARAAAGAA	This study
		R: OschR683 (=HCO2198)	TAAACTTCTGGRTGWCCAAARAATCA	This study
		F: OschF365	ATAGAGGRGCATCYGTWGATTT	This study

Numbers refer to the position of the primer 5'-end mapped on DNA barcoding region in *COI* gene (Hebert et al., 2003).

DEPOT, Barker, TX, USA). The amplicons successfully obtained were purified using the AccuPrep PCR Purification Kit (Bioneer, Daejeon, Korea) following the manufacturers protocol. Sequencing was performed commercially by Macrogen (Seoul, Korea). All sequences were generated In both directions. The quality and potential polymorphic sites of the analyzed sequences were checked using Chromas 2.33 (Technelysium Pty Ltd, Australia).

COI sequence analysis

In this study, sixteen *COI* sequences from 16 dried specimens were successfully generated. In addition, 32 *COI* sequences of five species published in two previous studies (Audisio et al., 2009; Landvik et al., 2013) were downloaded from the GenBank (http://www.ncbi.nlm.nih.gov/genbank) (Table 1). Data sets of the nucleotides of *COI* genes were aligned in MEGA 5.2 (Tamura et al., 2011) using ClustalW with the default settings (Gap Opening Penalty = 15, Gap Extension Penalty = 6.66 in both pairwise and multiple alignments). The anterior and posterior regions of uncertain alignment were eliminated from the data matrix. The *COI* alignment for reading frames was checked manually by translating sequences into amino acids to identify stop codons and potential shifts. Sequences were finally trimmed to 657-bp fragments. *COI* sequences generated in this study are available from GenBank under the accession numbers KX346561-76 (Table 1). For molecular species identification, a neighbor-joining (NJ) analysis (Saitou and Nei 1987) was performed using MEGA 5.2. Genetic distances within and between species of hermit beetle were calculated using Kimura's two-parameter test (Kimura, 1980) in accordance with Nei's empirical guidelines (Nei 1991, 1996).

Results and Discussion

Morphospecies identification of the Korean hermit beetle

We examined diagnostic morphological features for each specimen and species. The South Korean hermit beetle (Fig. 1 A and B) was revealed to be identical to the true *O. caeleste* from Far East Russia (Fig. 1C and D). These taxa share the same characteristics, such as an arched frontal margin of the

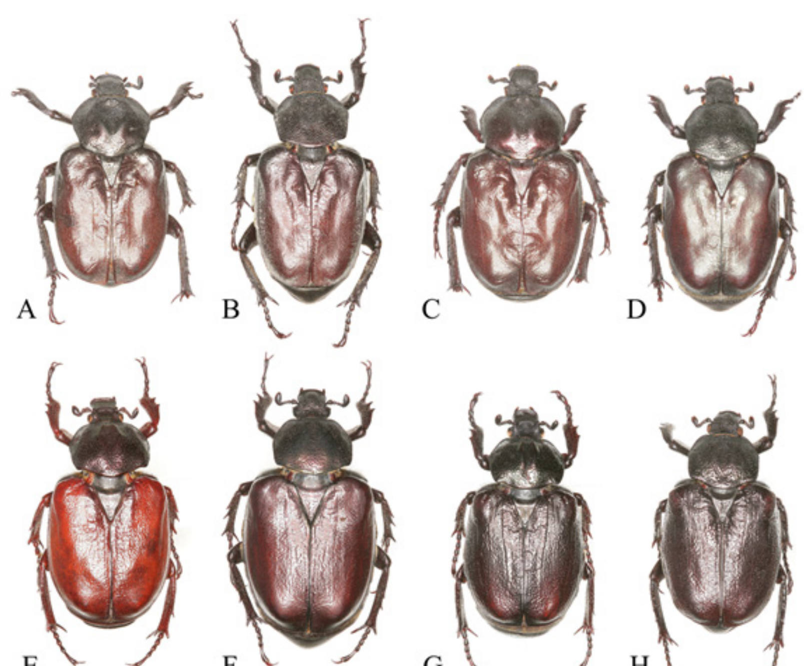

Fig. 1. *Osmoderma* spp. A and B: *O. caeleste* from Korea (A: no. 9572, male; B: no. 7387, female); C and D: *O. caeleste* from the Far East Russia (C: no. 9412, male; D: no. 9411, female); E and F: *O. opicum* from Japan (E: no. 7295, male; F: no. 7298, female); G and H: *O. barnabita* from West Russia (G: no. 7362, male; H: no. 7372, female).

head, a reverse triangular depression on the pronotum (Fig. 2A-D) and shortly developed parameres of the aedeagus (Fig. 3A and B). In contrast, *O. caeleste* can easily be distinguished from the true *O. opicum* on the basis of the truncated frontal margin of the head, the longitudinal and narrow groove on the pronotum (Fig. 2E and F), and the distinctly elongated parameres of the aedeagus (Fig. 3C). *O. barnabita* (Fig. 1G and H) is also distinctly separated from the South Korean hermit beetle by the darker chestnut color of the body, the longitudinal and narrow groove, the rather spare punctures on the pronotum (Fig. 2G and H), and the shortly developed, but with the lobe likely expanded base of parameres of the aedeagus (Fig. 3D). In the Far East Asia, additional related species, *O. davidis* is distributed in Russia, E. China and Mongolia. The South Korean hermit beetle was compared with this species according to the literatures by Boucher (2002) and Tauzin (2013). *O. davidis* has the ginkgo leaf-liked pronotal groove and the elongate parameres of the aedeagus. It is indicated that the South Korean hermit beetle and *O. davidis* are a distinctly separated species, respectively.

Tauzin (2013) described a subspecies, *Osmoderma opicum coreanum*, from Hamgyong province, North Korea based on a male holotype, which was provided Dr. Jingke Li (see Tauzin 2013, p. 2). However, we must point out to have doubt on the

Fig. 2. The dorsal aspects of the head and the pronotum of *Osmoderma* spp. A and B: *O. caeleste* from Korea (A: no. 9572, male; B: no. 7387, female); C and D: *O. caeleste* from the Far East Russia (C: no. 9412, male; D: no. 9411, female); E and F: *O. opicum* from Japan (E: no. 7295, male; F: no. 7298, female); G and H: *O. barnabita* from West Russia (G: no. 7362, male; H: no. 7372, female).

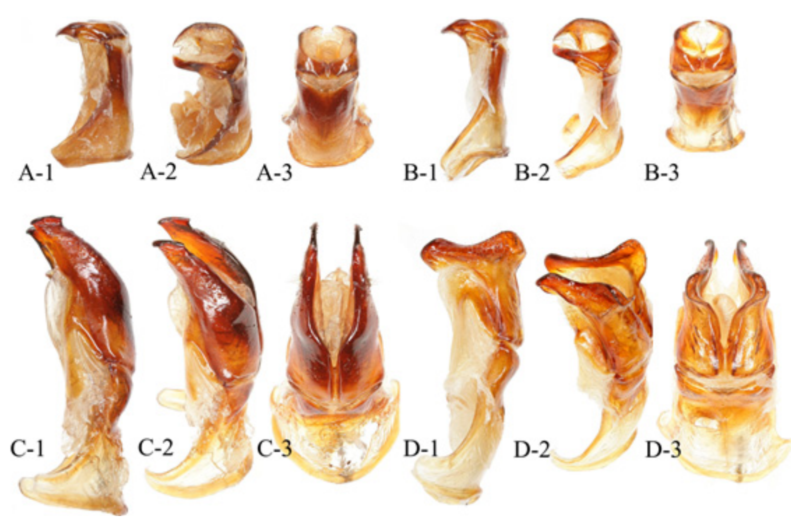

Fig. 3. Aedeagus of *Osmoderma* spp. A: *O. caeleste* from Korea (A: no. 9572, male); B: *O. caeleste* from the Far East Russia (B: no. 9412); C: *O. opicum* from Japan (C: no. 7295, male); D: *O. barnabita* from West Russia (D: no. 7362, male).

original locality of the specimen of the new subspecies, because Dr. Li has often provided to purchasers many insect specimens collected from adjacent countries of Asia such as China, Russia, and Japan as though they were from North or South Korea. The existence of this subspecies in Korea is needed to reconfirm in the further study.

COI sequence analysis

A total of 16 *COI* sequences representing three morpho-speices, *O. opicum*, *O. barnabita*, and *O. caeleste* including the Korean population, were successfully generated. Before analyzing the *COI* sequences, we confirmed the absence of any putative Numts and heteroplasmies from the analyzed sequences. One sequence (specimen no. 7299), only partly sequenced in 226 bp fragment, was confirmed to be identical with the other sequences of the Korean hermit beetle, but not including in the calculation of genetic distance and in NJ analysis.

The NJ tree topology (Fig. 4) and the pairwise distances of *COI* sequences (Table 3) showed that the Korean specimens were strongly clustered to those of the Far East Russian *O. caeleste* with low intraspecific (range: 0-0.3%). But, this clade was distinctly separated from other species clades by large interspecific distances (range: 13.7-19.4%). This result indicates that the South Korean hermit beetle and the true *O. caeleste* from the Far East Russia are the same species. The Japanese species, *O. opicum*, was also solely clustered with low intraspecific (range: 0-0.8%) and large interspecific distances (range: 12.1-16.0%). The interspecific distances between *O. caeleste* and *O. opium* were significantly large, ranging from 16.6% to 17.2%. This result insinuates that these two species have undergone an independent evolution, respectively, and more early separated from the extant European species. Two West Russian specimens of *O. barnabita* analyzed in this study were clustered with the previous sequences of *O. barnabita* (Audisio et al., 2009; Landvik et al., 2013). Therefore, the South Korean hermit beetle is identified to *O. caeleste* based on both of morphological and DNA barcoding analyses.

Systematic Accounts

Family Scarabaeidae Latreille

Subfamily Cetoniinae Leach

Tribe Trichiini Fleming

Genus *Osmoderma* LePeletier and Audinet-Serville

***Osmoderma caeleste* (Gusakov, 2002)**

Osmoderma barnabitum var. *castaneum* Tauzin, 1994: 204; Tauzin, 2006: 43 and 44.

Gymnodus caelestis Gusakov, 2002: 13 and 39 (Primorski Krai, Far East Russia).

Osmoderma sikhotense Boucher, 2002: 426 (Sikhote-Aline, Far East Russia).

[Korean records]

Osmoderma opicum Lewis: Cho, 1969: 688 (Atlas with a female photo, misidentification); Kim, 1978: 379 (Distributional map, listed); Shin and Yoon, 1994: 153 (Check list); Kim, 1998: 358 (List); Kim, 2001: 132 (Examined five specimens, misidentification); Kim, 2011: 33 (Examined five specimens, misidentification).

Material examined. South Korea: 1 male, Gangwon-do, Yanggu, 26. IX. 2011, collector unknown (voucher no. 3257); 1 female, Gangwon-do, Injae, Jogyeong-dong, Garigol, 26. VIII. 2000, H. N. Seok (voucher no. 7299); 1 female, Gangwon-do, Injae, Buk-myeon, Yongdaeri, 31. VII. 2013, Lim et al. (voucher no. 9571); 1 male, Gangwon-do, Hwacheon, Sangseo-myeon, Guunri, 9. VIII. 2000, J. R. Cha (voucher no. 9572); 1 female, Gangwon-do, Pyeongchang, Jinbu-myeon, Mt. Odae, 8. VII. 2010, collector unknown (voucher no. 7387); 1 female, Gyeongsangbuk-do, Yongyang, Ilweol-myeon, Mt. Ilweol, XI. 2008, Y. Y. Baik (voucher no. 9596).

Distribution. South Korea, North Korea (?), E. China (?), Mongolia (?), and the Far East Russia.

Remarks. We examined the female photo represented in Cho (1969) and two materials (nos. 7299 and 9572) used in Kim (2001, 2011). Based on our morphological and molecular examinations, three previous South Korean records were misidentifications of *O. caeleste*. Tauzin (2013) mentioned that *O. caeleste* is distributed in the Far East Russia, Beijing in China, Inner Mongolia and Korea. Among them, the distributions of China, Mongolia and Korea were listed having no the basis of examined specimen records. Therefore, it is needed that the exact distributional range of this species has to reexamine in the further study.

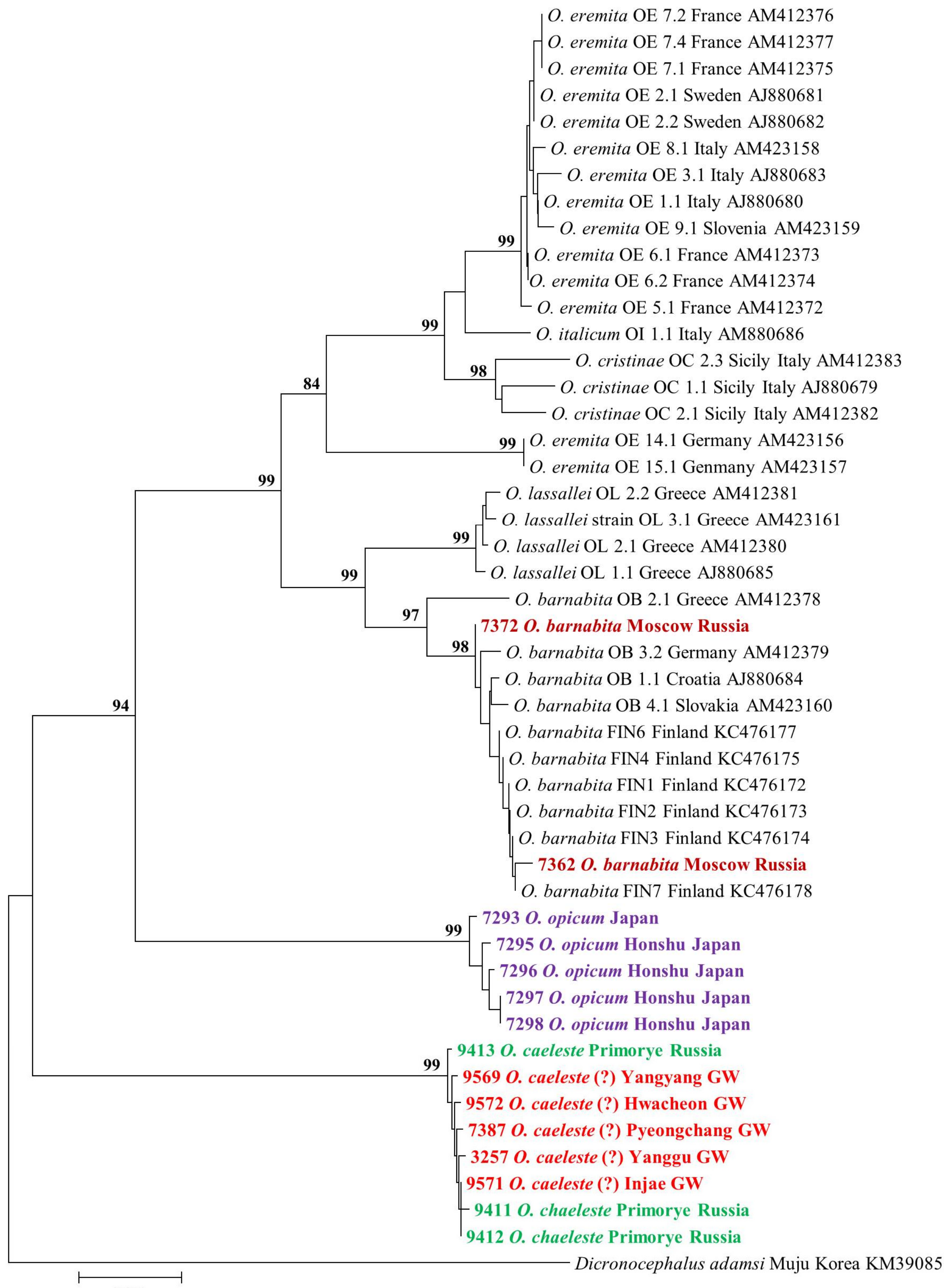

Fig. 4. Neighbor-Joining tree of *Osmoderma* spp. inferred from *cytochrome c oxidase subunit I* (*COI*). Terminal taxa in bold denote the examined specimens in this study.

Table 3. Pairwise distances of *COI* within and between *Osmoderma* spp.

	No. of samples	Within subspecies & species	Between subspecies & species						
			O. eremita clae A	*O. italicum**	*O. cristinae*	*O. eremita* clade B	*O. lassallei*	*O. barnabita*	*O. opicum*
O. eremita clade A	12	0.004 (0-0.009)							
*O. italicum**	1	-	0.027 (0.025-0.030)						
O. cristinae	3	0.023 (0.019-0.028)	0.041 (0.036-0.048)	0.033 (0.030-0.036)					
O. eremita clade B	2	0	0.079 (0.076-0.083)	0.085 (0.085)	0.085 (0.082-0.087)				
O. lassallei	4	0.005 (0.003-0.006)	0.090 (0.085-0.099)	0.089 (0.086-0.092)	0.095 (0.090-0.099)	0.099 (0.096-0.101)			
O. barnabita	12	0.008 (0-0.035)	0.094 (0.084-0.107)	0.098 (0.089-0.110)	0.098 (0.087-0.114)	0.091 (0.083-0.096)	0.051 (0.041-0.056)		
O. opicum	5	0.005 (0-0.008)	0.151 (0.143-0.160)	0.154 (0.151-0.156)	0.155 (0.149-0.158)	0.134 (0.127-0.137)	0.142 (0.136-0.147)	0.137 (0.121-0.146)	
O. caeleste + *O. caeleste* (?)	9	0.002 (0-0.003)	0.180 (0.143-0.194)	0.179 (0.143-0.186)	0.179 (0.148-0.188)	0.174 (0.147-0.180)	0.169 (0.137-0.175)	0.174 (0.159-0.188)	0.169 (0.166-0.172)

Numbers are indicated as mean (minimum-maximum) of the pairwise distance.

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