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Original article

A DNA barcode library of the beetle reference collection (Insecta: Coleoptera) in the National Science Museum, Korea

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ABSTRACT

Coleoptera is a group of insects that are most diverse among insect resources. Although used as indicator species and applied in developing new drugs, it is difficult to identify them quickly. Since the development of a method using mitochondrial DNA information for identification, studies have been conducted in Korea to swiftly and accurately identify species. The National Science Museum of Korea (NSMK) has been collecting and morphologically identifying domestic reference insects since 2013, and building a database of DNA barcodes with digital images. The NSMK completed construction of a database of digital images and DNA barcodes of 60 beetle species in the Korean National Research Information System. A total of 179 specimens and 60 species were used for the analysis, and the averages of intra-specific and interspecific variations were $0.70 \pm 0.45\%$ and $26.34 \pm 6.01\%$, respectively, with variation rates ranging from 0% to 1.45% and 9.83% to 56.23%, respectively.

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Introduction

Insects are the most species-rich animal group, accounting for 66% of the entire population of the animal kingdom, and are composed of approximately 1 million described species worldwide (Zhang 2011). There are approximately 14,000 species that have been described in the Korean peninsula (Paek et al 2010), and considering species that are newly reported every year, it is estimated that there are approximately 40,000 insect species in Korea. Among these, Coleoptera exhibit the greatest diversity, with approximately 390,000 species recorded to date, and experts of biodiversity estimate that there are approximately 1,100,000 species in the world (Chapman 2009; Yeates et al 2003; Zhang 2011). Beetles are distributed on all continents and major islands, except Antarctica, and used in various areas, including climate-sensitive

biological indicator species, natural enemies, cultural insects, food resources, and new-drug development (Cloyd et al 2009; Jäch 2003; Otavo et al 2013). However, there are not enough taxonomists to morphologically identify species due to their diversity in sibling species, sexual dimorphism, different life stages (larvae, pupae and adults), and interspecific variations.

To overcome such limitations, DNA-barcode researchers proposed a standardized method to identify species through distinct genetic characteristics (DeWalt 2011). The benefits of genetic markers are that they can be applied to all taxa, enable one to distinguish genetic differences between species, and can be used as important evidence for not only morphologically unidentifiable species, but also unknown and new species. In the animal kingdom, the cytochrome C oxidase subunit I (COI) gene (658 bp) of mitochondria DNA is used to differentiate between species (Hebert et al 2003, 2004a, 2004b), and it has been regarded as one of the most effective analytical approaches for species identification to date. However, compared to foreign countries, not many studies on DNA barcodes have been published in Korea, and no initiative has been made to systematically integrate DNA information on domestic species and establish a database.

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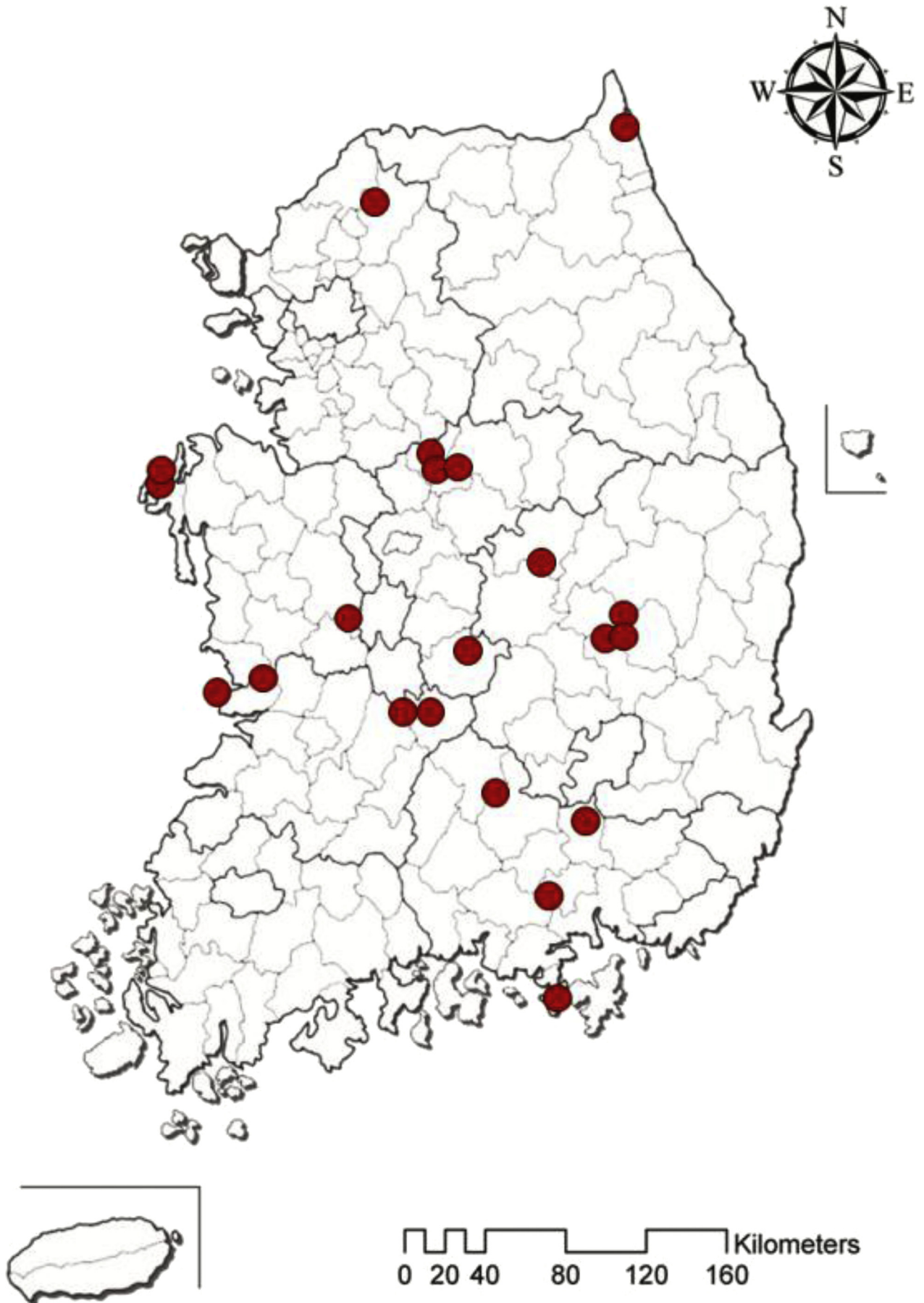


Figure 1. A map of collecting sites in South Korea.

Table 1. Primers used in this study.

Primer	Sequence	References
LCO1490	5'- GTC AAC AAA TCA TAA AGA TAT TGG -3'	Folmer et al 1994
HCO2198	5'- TAA ACT TCA GGG TGA CCA AAA AAT CA -3'	Folmer et al 1994
jjgLCO1490	5'- TTT CIA CIA AYC AYA ARG AYA TTG G -3'	Folmer et al 1994
jjgHCO2198	5'- TAI ACY TCI GGR TGI CCR AAR AAY CA -3'	Folmer et al 1994
Lep F	5'- ATT CAA CCA ATC ATA AAG ATA T -3'	Hebert et al 2004a
Lep R	5'- TAA ACT TCT GGA TGT CCA AAA A -3'	Hebert et al 2004a

The National Science Museum of Korea (NSMK) was designated as the first National Biodiversity Center by the Ministry of Science, ICT, and Future Planning in 2010, and has been constructing infrastructure for effective securement, management, and utilization of natural historic resources, including insect resources. As a part of national biodiversity, the NSMK collected domestic reference specimens, which can be used as source materials in education, exhibition, and various research. Additionally, they have been carrying out research to house genomic DNA and build a DNA-

Table 2. Specimen number, species used in analysis, collecting site and date, and GenBank accession numbers for *COI* sequences.

Specimen number (NARIS)	Species	Collecting site	Collecting date (d-mo-y)	<i>COI</i>	
				GeneBank #	BOLD #
NSMK-IN-0046619~0046621	<i>Thanassimus lewisi</i> Jacobson	Korea, CB, Yeongdong-gun, Chupungnyeong-myeon, Sabu-ri	15-Apr-2014	KU188328~KU188330	ACN3091
NSMK-IN-0046069~0046071	<i>Heterotarsus carinula</i> Marseul	Korea, CB, Yeongdong-gun, Chupungnyeong-myeon, Sabu-ri	10-Oct-2013	KU188331~KU188333	ACW0230
NSMK-IN-0046039~0046041	<i>Gonocephalum pubens</i> Marseul	Korea, GB, Yeongdeok-gun, Byeonggok-myeon	11-Oct-2013	KU188334~KU188336	ACH5388
NSMK-IN-0046059~0046061	<i>Idisia ornata</i> Pascoe	Korea, GB, Yeongdeok-gun, Byeonggok-myeon	11-Oct-2013	KU188337~KU188339	ACV8334
NSMK-IN-0051204~0051206	<i>Caedius marinus</i> Marseul	Korea, CN, Boryeong-si, Ungcheon-eup	21-Jun-2014	KU188340~KU188342	ACV4892
NSMK-IN-0051214~0051216	<i>Anaediulus mroczkowskii</i> Kaszab	Korea, CB, Yeongdong-gun, Hwanggan-myeon, Youngam-ri	29-Jul-2014	KU188343~KU188345	ACD5574
NSMK-IN-0051234~0051236	<i>Cicindela elisae</i> Motschulsky	Korea, CN, Seochon-gun, Janghang-eup, Yubu Island	14-Jul-2014	KU188355~KU188357	ABA8441
NSMK-IN-0046329~0046331	<i>Glycyphana fulvitemma</i> Motschulsky	Korea, CB, Yeongdong-gun, Chupungnyeong-myeon, Sabu-ri	15-Apr-2014	KU188346~KU188348	ACW0254
NSMK-IN-0046319~0046321	<i>Nipponovalgus angusticollis</i> (Waterhouse)	Korea, CB, Yeongdong-gun, Chupungnyeong-myeon, Sabu-ri	15-Apr-2014	KU188349~KU188351	ACV6581
NSMK-IN-0045899~0045901	<i>Gametis jucunda</i> (Faldermann)	Korea, GB, Uiseong-gun, Angye-myeon, Bongam-ri	10-Apr-2014	KU188352~KU188354	ACV8298
NSMK-IN-0051244~0051246	<i>Craspedonotus tibialis</i> Shauman	Korea, CN, Boryeong-si, Ungcheon-eup	21-Jun-2014	KU188358~KU188360	ACQ2621
NSMK-IN-0051254~0051256	<i>Trichiorhyssemus asperulus</i> (Waterhouse)	Korea, CN, Seochon-gun, Janghang-eup, Yubu Island	15-Jul-2014	KU188370~KU188372	ACV5055
NSMK-IN-0046429~0046431	<i>Chlaenius inops</i> Chaudoir	Korea, CN, Seochon-gun, Janghang-eup	19-May-2014	KU188361~KU188363	ACW0207
NSMK-IN-0046259~0046261	<i>Bembidion scopulinum</i> (Kirby)	Korea, CB, Yeongdong-gun, Chupungnyeong-myeon, Jukjeon-ri	2-Apr-2014	KU188364~KU188366	ACV7280
NSMK-IN-0051224~0051226	<i>Bembidion coreanum</i> Jedlička	Korea, GB, Yecheon-gun, Yonggung-myeon, Hyangseok-ri	28-Oct-2014	KU188367~KU188369	ACH4730
NSMK-IN-0046489~0046491	<i>Cephaloon pallens</i> (Motschulsky)	Korea, CB, Yeongdong-gun, Chupungnyeong-myeon, Sabu-ri	1-May-2014	KU188373~KU188375	ACW0563
NSMK-IN-0045919~0045921	<i>Propylea japonica</i> (Thunberg)	Korea, GB, Uiseong-gun, Bian-myeon, Jarak-ri	10-Apr-2014	KU188376~KU188378	AAP7922
NSMK-IN-0045939~0045941	<i>Harmonia axyridis</i> (Pallas)	Korea, GB, Uiseong-gun, Bian-myeon, Jarak-ri	10-Apr-2014	KU188379~KU188381	AAB5640
NSMK-IN-0046209~0046211	<i>Ancylopus pictus asiaticus</i> Strohecker	Korea, GN, Tongyeong-si, Yongnam-myeon, Dongdal-ri	7-Mar-2014	KU188382~KU188384	ACV6599
NSMK-IN-0051264~0051266	<i>Laccobius fragilis</i> Nakane	Korea, CB, Yeongdong-gun, Hwanggan-myeon, Youngam-ri	29-Jul-2014	KU188385~KU188387	ACV4976
NSMK-IN-0051274~0051276	<i>Berosus lewisius</i> Sharp	Korea, CB, Yeongdong-gun, Hwanggan-myeon, Youngam-ri	29-Jul-2014	KU188388~KU188390	ACV5267
NSMK-IN-0051284~0051286	<i>Hydaticus grammicus</i> Germar	Korea, CB, Yeongdong-gun, Chupungnyeong-myeon, Sabu-ri	7-Sep-2014	KU188391~KU188393	ACV5598
NSMK-IN-0051294~0051296	<i>Platambus fimbriatus fimbriatus</i> Sharp	Korea, JB, Muju-gun, Bunam-myeon, Sangguram-ri	25-Jul-2014	KU188394~KU188396	AAJ2741
NSMK-IN-0046369~0046371	<i>Eucryptorrhynchus brandti</i> (Harold)	Korea, CB, Eumseong-gun, Geumwang-eup	8-May-2014	KU188397~KU188399	ACS3792
NSMK-IN-0046499~0046501	<i>Hypera postica</i> Gyllenhal	Korea, CN, Seochon-gun, Janghang-eup, Yubu Island	20-May-2014	KU188400~KU188402	AAN6027
NSMK-IN-0046449~0046451	<i>Scepticus griseus</i> (Roelofs)	Korea, CN, Seochon-gun, Janghang-eup, Yubu Island	19-May-2014	KU188403~KU188405	ACV7816
NSMK-IN-0046559~0046561	<i>Stenus mercator</i> Sharp	Korea, CB, Eumseong-gun, Geumwang-eup	8-May-2014	KU188406~KU188408	ACV9632
NSMK-IN-0046549~0046551	<i>Stenus comma</i> Leconte	Korea, CB, Yeongdong-gun, Chupungnyeong-myeon, Jukjeon-ri	2-Apr-2014	KU188409~KU188411	AAI0092
NSMK-IN-0046249~0046251	<i>Paederus fuscipes</i> Curtis	Korea, CB, Yeongdong-gun, Chupungnyeong-myeon, Jukjeon-ri	2-Apr-2014	KU188412~KU188414	AAX1892
NSMK-IN-0046479~0046481	<i>Platydracus brevicornis</i> (Motschulsky)	Korea, CB, Yeongdong-gun, Chupungnyeong-myeon, Sabu-ri	1-May-2014	KU188415~KU188417	ACV6371

Table 2 (continued)

Specimen number (NARIS)	Species	Collecting site	Collecting date (d-mo-y)	COI	
				GeneBank #	BOLD #
NSMK-IN-0051304~0051306	<i>Onthelostes gracilis</i> (Sharp)	Korea, CB, Yeongdong-gun, Chupungnyeong-myeon, Sabu-ri	26-Jun-2014	KU188418 ~ KU188420	ACD7565
NSMK-IN-0046289~0046291	<i>Aeoloderma agnata</i> (Candéze)	Korea, JB, Gunsan-si, Seongsan-myeon, Seongdeok-ri	11-Mar-2014	KU188421 ~ KU188423	ACI5762
NSMK-IN-0051324~0051326	<i>Meristhus niponensis</i> Lewis	Korea, CN, Seochon-gun, Janghang-eup, Yubu Island	15-Jul-2014	KU188424 ~ KU188426	ACV5668
NSMK-IN-0046179~0046181	<i>Stricticollis valgipes</i> (Marseul)	Korea, GB, Uiseong-gun, Angye-myeon, Yonggi-ri	9-Apr-2014	KU188427 ~ KU188429	ACV7015
NSMK-IN-0051334~0051336	<i>Onthophagus atripennis</i> Waterhouse	Korea, CB, Yeongdong-gun, Chupungnyeong-myeon, Sabu-ri	26-Jun-2014	KU188430 ~ KU188432	ACH8791
NSMK-IN-0046569~0046571	<i>Nicrophorus quadripunctatus</i> Kraatz	Korea, CB, Yeongdong-gun, Chupungnyeong-myeon, Sabu-ri	16-May-2014	KU188433 ~ KU188435	AAE1958
NSMK-IN-0046509~0046511	<i>Nicrophorus maculifrons</i> Kraatz	Korea, CN, Sejong-si, Geumnam-myeon, Donam-ri	30-Apr-2014	KU188436 ~ KU188438	ACV8744
NSMK-IN-0051344~0051346	<i>Ptomascopus morio</i> Kraatz	Korea, CB, Yeongdong-gun, Chupungnyeong-myeon, Sabu-ri	16-May-2014	KU188439 ~ KU188441	ACV5613
NSMK-IN-0046459~0046461	<i>Malachius prolongatus</i> Motschulsky	Korea, CN, Seochon-gun, Janghang-eup	19-May-2014	KU188442 ~ KU188444	ACV6118
NSMK-IN-0046309~0046311	<i>Oomorphoides cupreatus</i> (Baly)	Korea, CB, Yeongdong-gun, Chupungnyeong-myeon, Sabu-ri	15-Apr-2014	KU188445 ~ KU188447	ACP6794
NSMK-IN-0046629~0046631	<i>Smaragdina semiaurantiaca</i> (Fairmaire)	Korea, CB, Eumseong-gun, Geumwang-eup	7-May-2014	KU188448 ~ KU188450	ACV7915
NSMK-IN-0045910~0045911	<i>Chrysomela vigintipunctata</i> (Scopoli)	Korea, GB, Uiseong-gun, Bian-myeon, Jarak-ri	10-Apr-2014	KU188452 ~ KU188453	AAJ5085
NSMK-IN-0046199~0046201	<i>Phyllotreta striolata</i> (Fabricius)	Korea, GN, Tongyeong-si, Yongnam-myeon, Dongdal-ri	7-Mar-2014	KU188454 ~ KU188456	ABX2406
NSMK-IN-0045889~0045891	<i>Chrysomela populi</i> Linnaeus	Korea, GB, Uiseong-gun, Angye-myeon, Bongam-ri	10-Apr-2014	KU188457 ~ KU188459	ACV6390
NSMK-IN-0046399~0046401	<i>Gallerucida bifasciata</i> Motschulsky	Korea, CB, Eumseong-gun, Geumwang-eup	8-May-2014	KU188460 ~ KU188462	ACV9712
NSMK-IN-0046009~0046011	<i>Oides decempunctatus</i> (Billberg)	Korea, GB, Yeongdeok-gun, Yeonghae-myeon, Daejin-ri	11-Oct-2013	KU188463 ~ KU188465	ACV7004
NSMK-IN-0046139~0046141	<i>Agelastica coerulea</i> Baly	Korea, GN, Geochang-gun, Gajo-myeon	1-May-2014	KU188466 ~ KU188468	ACD6431
NSMK-IN-0046279~0046281	<i>Galerucella nipponensis</i> (Laboissiere)	Korea, JB, Gunsan-si, Seongsan-myeon, Seongdeok-ri	11-Mar-2014	KU188469 ~ KU188471	AAG4421
NSMK-IN-0046189~0046191	<i>Gastrophysa atrocyanea</i> Motschulsky	Korea, GN, Tongyeong-si, Yongnam-myeon, Dongdal-ri	7-Mar-2014	KU188472 ~ KU188474	ACH9119
NSMK-IN-0051354~0051356	<i>Basilepta fulvipes</i> (Motschulsky)	Korea, JB, Muju-gun, Bunam-myeon, Sangguram-ri	25-Jul-2014	KU188475 ~ KU188477	ACV6075
NSMK-IN-0051374~0051376	<i>Chrysochus chinensis</i> Baly	Korea, CN, Seochon-gun, Janghang-eup, Yubu Island	14-Jul-2014	KU188478 ~ KU188480	ACV5642
NSMK-IN-0046579~0046581	<i>Cerogria janthinipennis</i> Fairmaire	Korea, CB, Eumseong-gun, Saenggeuk-myeon, Gwanseong-ri	9-May-2014	KU188481 ~ KU188483	ACH9934
NSMK-IN-0051414~0051416	<i>Hister simplicisternus</i> Lewis	Korea, CB, Yeongdong-gun, Chupungnyeong-myeon, Sabu-ri	16-May-2014	KU188484 ~ KU188486	AAW5354
NSMK-IN-0051424~0051426	<i>Margarinotus niponicus</i> (Lewis)	Korea, CB, Yeongdong-gun, Chupungnyeong-myeon, Sabu-ri	16-May-2014	KU188487 ~ KU188489	ACV5614
NSMK-IN-0046609~0046611	<i>Phytoecia rufiventris</i> Gautier	Korea, CB, Yeongdong-gun, Chupungnyeong-myeon, Sabu-ri	9-May-2014	KU188490 ~ KU188492	AAY1332
NSMK-IN-0046339~0046341	<i>Rhaphuma diminuta</i> (Bates)	Korea, CB, Yeongdong-gun, Chupungnyeong-myeon, Sabu-ri	15-Apr-2014	KU188493 ~ KU188495	ACO8984
NSMK-IN-0046379~0046381	<i>Agapanthia pilicornis</i> (Fabricius)	Korea, CB, Eumseong-gun, Geumwang-eup	8-May-2014	KU188496 ~ KU188498	AAJ7170
NSMK-IN-0046589~0046591	<i>Dinoptera minuta</i> (Gebler)	Korea, CB, Eumseong-gun, Geumwang-eup	7-May-2014	KU188499 ~ KU188501	ACV9167
NSMK-IN-0046149~0046151	<i>Oedemeronia lucidicollis</i> (Motschulsky)	Korea, GB, Uiseong-gun, Angye-myeon, Yonggi-ri	9-Apr-2014	KU188505 ~ KU188507	ACH5784
NSMK-IN-0045929~0045931	<i>Moehotypa diphysis</i> (Pascoe)	Korea, GB, Uiseong-gun, Bian-myeon, Jarak-ri	10-Apr-2014	KU188502 ~ KU188504	AAX4575

COI = cytochrome C oxidase subunit 1; NARIS = National Research Information System; NSMK = National Science Museum of Korea.

barcode database of the collected taxa. Among these, 110 species and 1100 specimens were collected as reference insects (Coleoptera, Lepidoptera, Hemiptera, and Orthoptera), and genomic DNA was extracted from all specimens. All DNA-barcode libraries are housed in the Korean National Research Information System (<http://www.naris.go.kr>) along with their digital images.

Mitochondrial genes have a relatively simple genetic structure, are maternally inherited, and evolve 10-times faster than nuclear DNA. Moreover, they are molecular markers used in various areas, including species identification, phylogenetic classification, and population genetics in the animal kingdom (Doyle and Gaut 2000; Hebert et al 2003, 2004b).

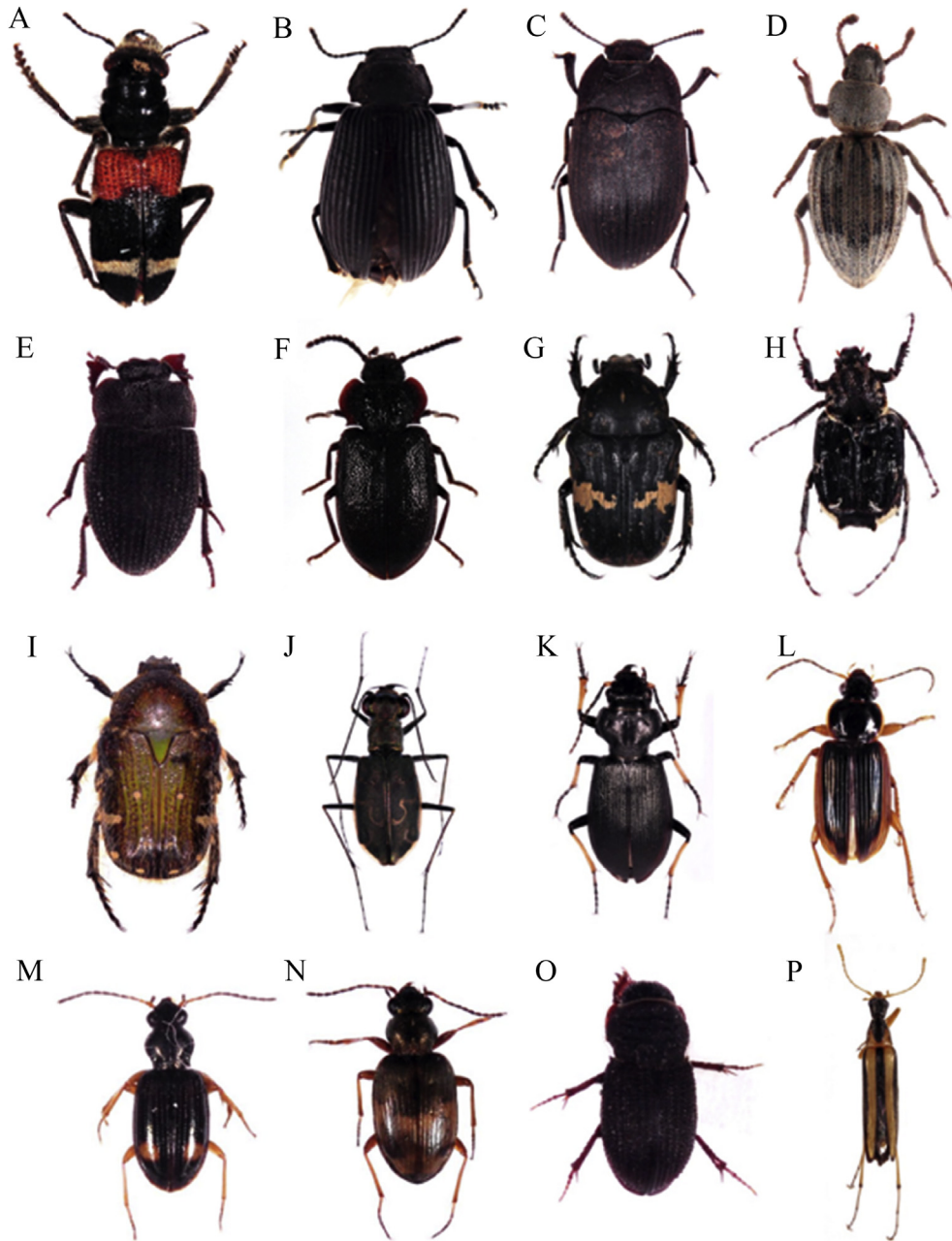


Figure 2. Habitus. A, *Thanassimus lewisi* Jacobson; B, *Heterotarsus carinula* Marseul; C, *Gonocephalum pubens* Marseul; D, *Idisia ornata* Pascoe; E, *Caedius marinus* Marseul; F, *Anaedius mroczkowskii* Kaszab; G, *Glycyphana fulvistemma* Motschulsky; H, *Nipponovalgus angusticollis* (Waterhouse); I, *Gametis jucunda* (Faldermann); J, *Cicindela elisae* Motschulsky; K, *Craspedonotus tibialis* Shauman; L, *Chlaenius inops* Chaudoir; M, *Bembidion scopulinum* (Kirby); N, *Bembidion coreanum* Jedlicka; O, *Trichiorhyssemus asperulus* (Waterhouse); P, *Cephaloon pallens* (Motschulsky).

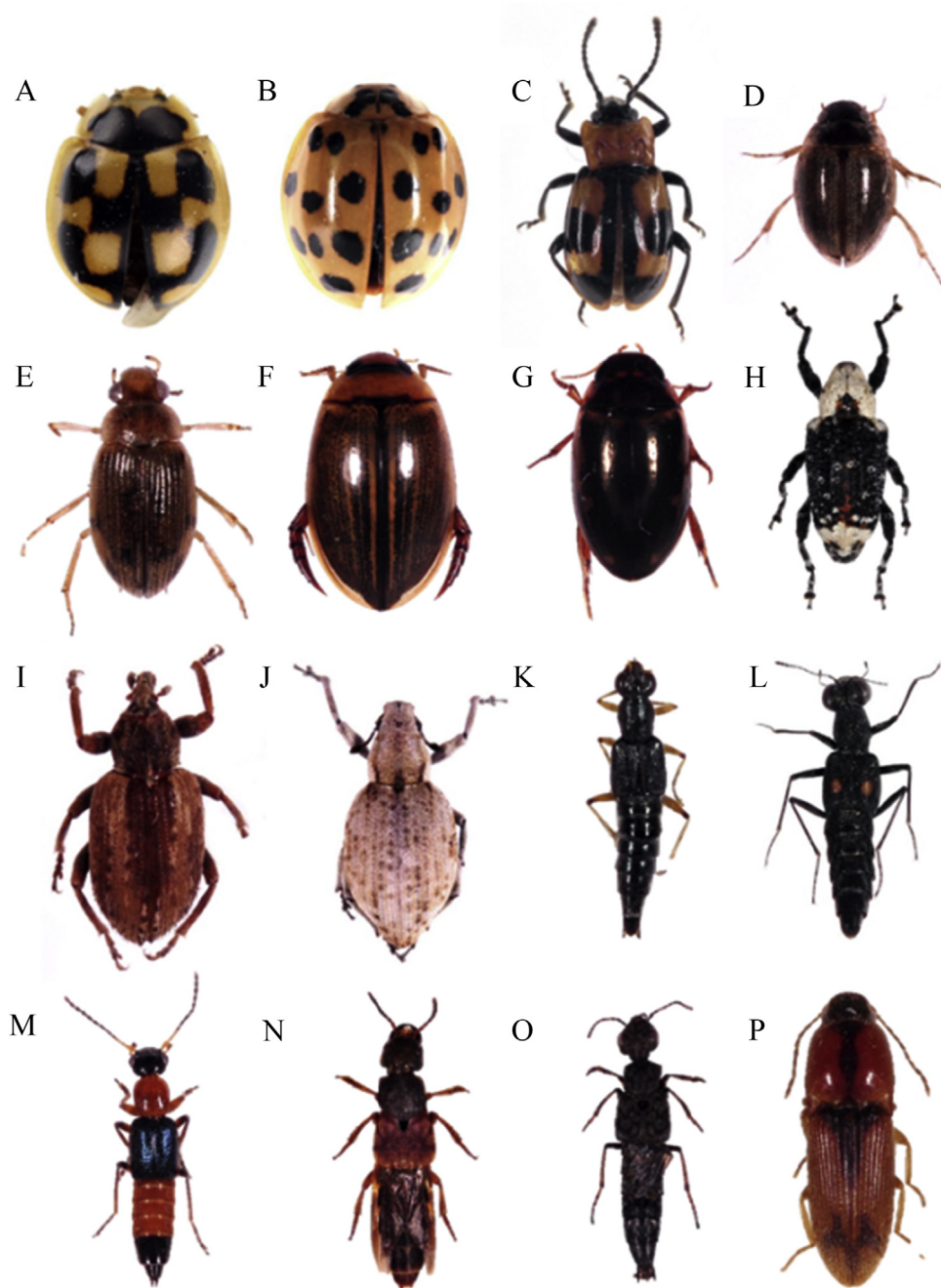


Figure 3. Habitus. A, *Propylea japonica* (Thunberg); B, *Harmonia axyridis* (Pallas); C, *Ancylopus pictus asiaticus* Strohecker; D, *Laccobius fragilis* Nakane; E, *Berosus lewisius* Sharp; F, *Hydaticus grammicus* Germar; G, *Platambus fimbriatus* Sharp; H, *Eucryptorrhynchus brandti* (Harold); I, *Hypera postica* Gyllenhal; J, *Scepticus griseus* (Roelofs); K, *Stenus mercator* Sharp; L, *Stenus comma* Leconte; M, *Paederus fuscipes* Curtis; N, *Platydracus brevicornis* (Motschulsky); O, *Onthelostes gracilis* (Sharp); P, *Aeoloderma agnata* (Candéze).

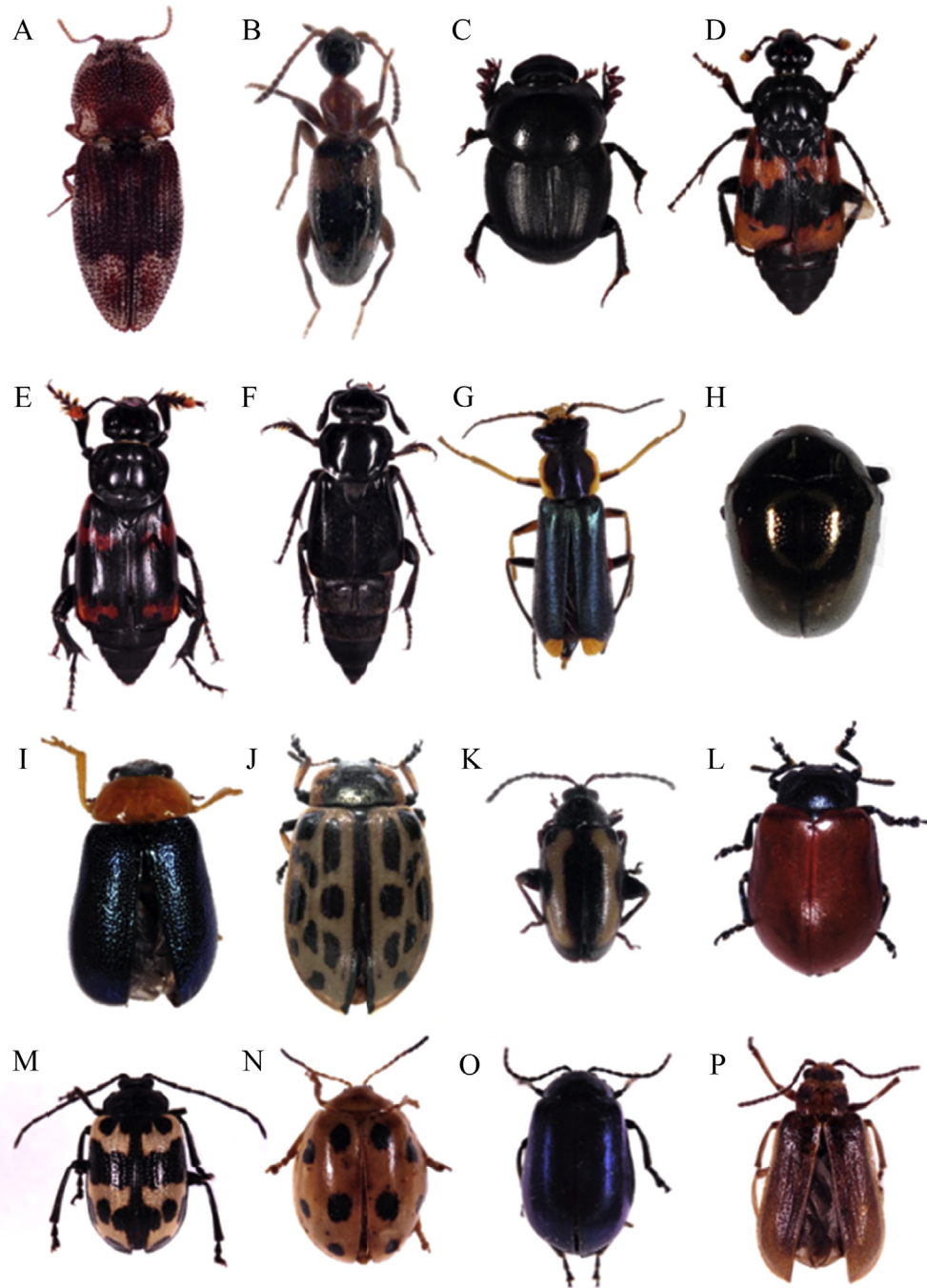


Figure 4. Habitus. A, *Meristhus niponensis* Lewis; B, *Stricticollis valgipes* (Marseul); C, *Onthophagus atripennis* Waterhouse; D, *Nicrophorus quadripunctatus* Kraatz; E, *Nicrophorus maculifrons* Kraatz; F, *Ptomascopus morio* Kraatz; G, *Malachius prolongatus* Motschulsky; H, *Oomorphoides cupreatus* (Baly); I, *Smaragdina semiaurantiaca* (Fairmaire); J, *Chrysomela vigintipunctata* (Scopoli); K, *Phyllotreta striolata* (Fabricius); L, *Chrysomela populi* Linnaeus; M, *Gallerucida bifasciata* Motschulsky; N, *Oides decempunctatus* (Billberg); O, *Agelastica coerulea* Baly; P, *Galerucella nipponensis* (Laboissiere).

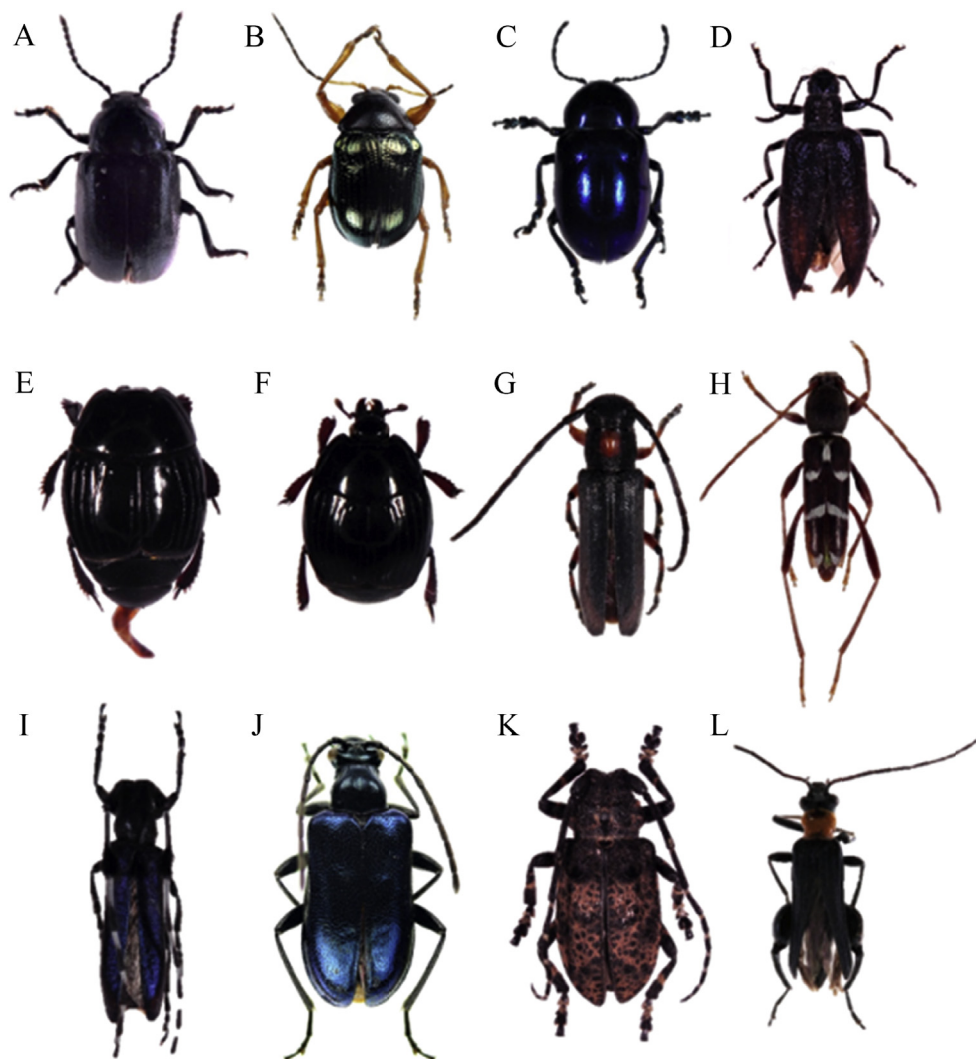


Figure 5. Habitus. A, *Gastrophysa atrocyanea* Motschulsky; B, *Basilepta fulvipes* (Motschulsky); C, *Chrysochus chinensis* Baly; D, *Cerogria janthinipennis* Fairmaire; E, *Hister simplicisternus* Lewis; F, *Margarinotus niponicus* (Lewis); G, *Phytoecia rufiventris* Gautier; H, *Rhapsoma diminuta* (Bates); I, *Agapanthia pilicornis* (Fabricius); J, *Dinoptera minuta* (Gebler); K, *Moechotypa diphysis* (Pascoe); L, *Oedemeronia lucidicollis* (Motschulsky).

This study performed swift and accurate species identification for mega-diverse insects order Coleoptera by building a standard database for the DNA. Furthermore, the accumulated DNA information is expected to be utilized as important basic data for a molecular phylogenetic analysis and in-depth biogeographic study of Coleoptera.

Materials and methods

Field investigation and sampling

All materials were collected using flight-interception traps (170 cm × 120 cm; mesh size: 1 mm), sweeping, beating, pitfall traps, and light traps from 21 sites (Figure 1) between March 2013 and October 2014. The collected specimens were preserved in 96% ethanol and kept in a freezer at −30°C for DNA extraction. Before DNA extraction, genitalia of some specimens were extracted for species identification and were glued together with the specimens. All the specimens are deposited in NSMK.

DNA extraction and sequencing

Total genomic DNA was extracted from muscles in the legs and wings. After grinding the specimens, DNA extraction was performed using PrimePrep Genomic DNA Isolation Kit (Genetbio, Seoul, Korea) and DNeasy Blood & Tissue Kits (Qiagen, Hilden, Germany). Template DNA [1 μL (10 ng/μL)] and three pairs of primers (Table 1) were used to amplify the DNA-barcoding region of the *COI* gene (658 bp).

Each 20 μL polymerase chain reaction (PCR) contained 1 μL of template DNA and each primer at 1 μL (5 pmol/μL), with 10 μL Prime Taq Premix (2×) [Prime Taq DNA polymerase 1 U/10 μL, 25 mM Tris-HCl (pH 9.0), 50 mM KCl, 4 mM MgCl₂, 2 mM each of dNTP mix, loading dye] and sterilized distilled water. For PCR amplification, initial denaturation was conducted for 5 min at 95°C, denaturation for 30 s at 95°C, annealing for 40 s at 47°C, extension for 30 rounds at 1 min at 72°C, and a final extension for 5 min at 72°C. PCR products were confirmed through electrophoresis using a 1.0% agarose gel in 1×TBE buffer. Sequences (658 bp) were aligned using BioEdit Sequence Alignment Editor version 7.2.5 (Hall 1999)

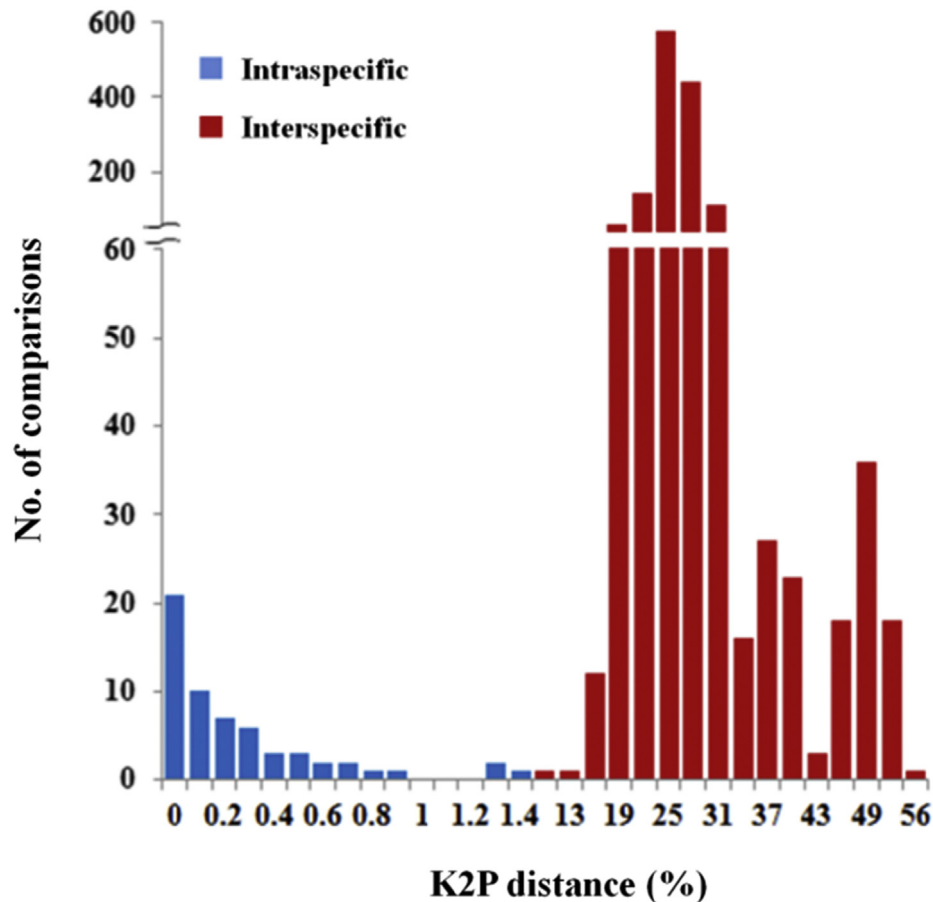


Figure 6. Frequency distribution of intraspecific and interspecific genetic divergences in 60 species of Coleoptera. Distances were generated after multiple sequence alignment (<http://www.ebi.ac.uk/Tools/msa/muscle/>) and calculated with MEGA 6.0 (pairwise deletion) using a Kimura two-parameter substitution model.

and Clustal X version 1.8 (Thompson et al 1997). All sequences were submitted to GenBank (accession numbers: KU188328–KU188450 and KU188452–KU188507) and BOLD systems (<http://www.boldsystems.org>), where voucher data can be accessed in the public dataset “NSMK reference specimens” (Table 2).

Data analysis and photos

The genetic distance was calculated using the Kimura two-parameter model (Kimura 1980), and a neighbor-joining tree (Saitou and Nei 1987) was constructed in MEGA 6.0 (Tamura et al 2013). Additionally, the sequences of all 60 species were compared to the data registered in the National Center for Biotechnology Information Genbank and BOLD systems.

Dorsal habitus were taken using a digital camera (Nikon D90, Tokyo, Japan), and the images were edited using Adobe Photoshop CS6 [Adobe Systems, San Jose, CA, USA (Figures 2–5)].

Results and discussion

A total of 60 species (179 specimens) in 57 genera belonging to 22 families were investigated in this study (Table 2). Based on species identification, 179 specimens of beetles were sequenced successfully. As a result of amplifying the *COI* gene (658 bp), there was no insertions or deletions from each sequence. The average percentages of A, C, G, and T were 29.5% (21.0–33.4%), 17.7% (13.4–25.5%), 16.3% (12.0–20.4%) and 36.6% (28.7–41.5%), respectively. Intraspecific and interspecific variations were clearly

distinguishable from each other, with averages of $0.70 \pm 0.45\%$ and $26.34 \pm 6.01\%$, respectively. Furthermore, intraspecific and interspecific variation rates were in the ranges of 0% to 1.45% and 9.83% to 56.23%, respectively (Figure 6). The genetic divergences (< 2.0%) of *COI* sequences within species were proposed in previous studies of insects groups (Hebert et al 2004a; Lin et al 2015). Therefore, our results of DNA sequences will be expected to be used for molecular identification not only of different life stages, such as larvae and pupae, but also of damaged specimens.

All analyzed species were more than 99% identical to species registered in GenBank and BOLD systems. Also, two species of *Gonocephalum pubens* Marseul and *Anaedioides mroczkowskii* Kaszab (family Tenebrionidae) were newly submitted in BOLD systems. However, the similarity of the genetic distance of *Stenus comma* Leconte (Staphylinidae) was 99% identical to that of *Stenus tenuipes* Sharp, which inhabits China. These two species have been confused with one another due to their highly similar general morphology. *S. comma* differs from *S. tenuipes* by the following diagnostic characters: head with distinct and dense punctures, width of elytra pattern more than half of the elytra width, male sternite VII with weak impression, both sides of the impressions are round, and the apex of the median lobe is somewhat triangle-shaped. (Puthz 1981; Naomi 1988). To confirm these two species, more extensive sampling with other molecular markers is necessary to determine whether *S. tenuipes* registered in GenBank is misidentified. All species had unique *COI* gene sequences at each genus in the neighbor-joining tree (Figure 7); however, genetic relationship at genus and family levels based on the *COI* gene was too low.

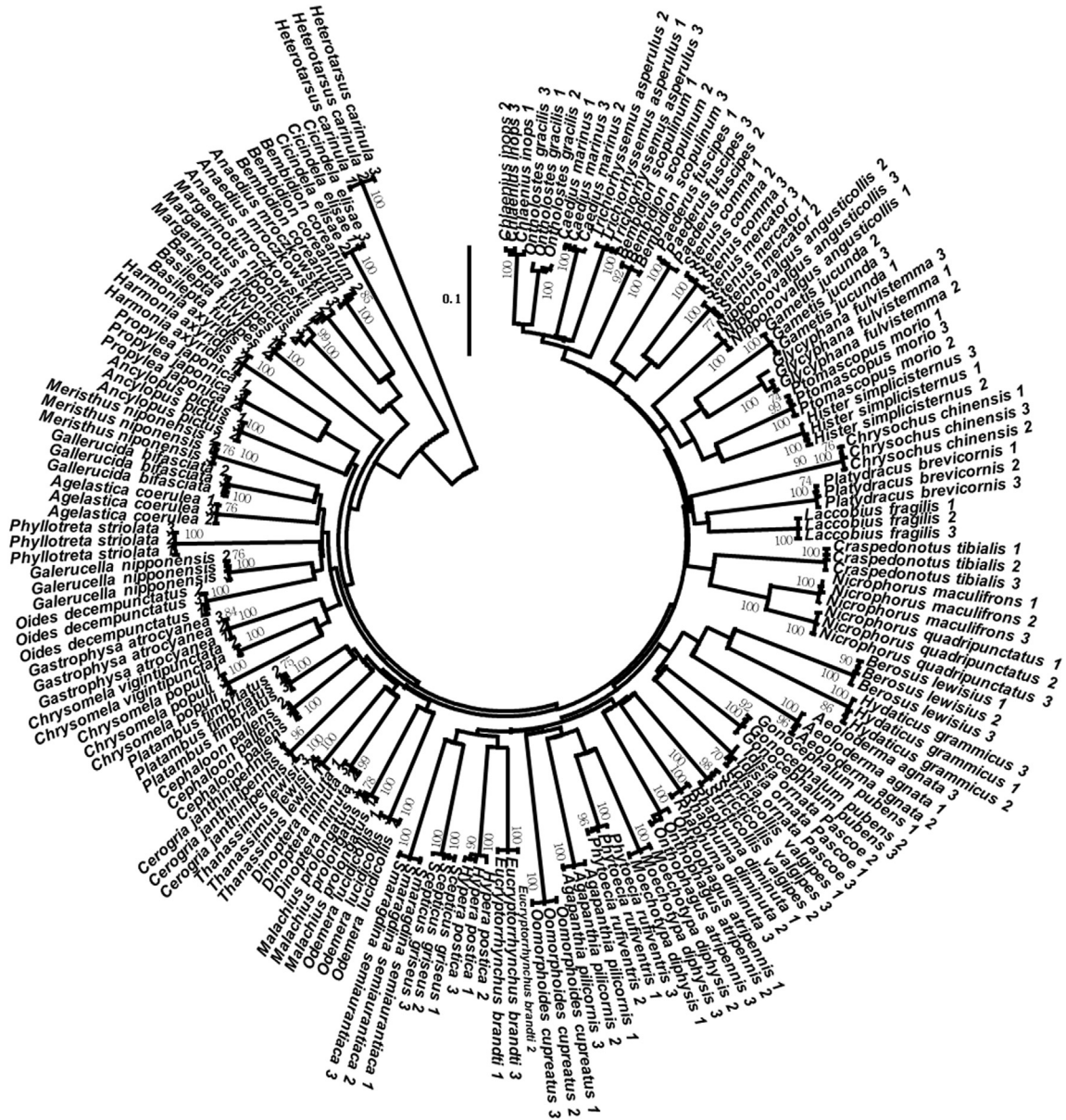


Figure 7. Neighbor-joining tree based on Kimura two-parameter distance with pairwise deletion of gaps and missing data using *COI* sequences (658 bp). Numbers at branches are indicated by the bootstrap values (2000 replications). Scale bar represents 0.1 nucleotide changes.

According to current phylogenetic studies, two mitochondrial (*rml* and *COI*) and two nuclear (*18S* and *28S* rRNA) genes are used to strongly support inter-relationship among Coleoptera (Bocak et al 2014). Also, the analysis of the *COI* gene sequence was assessed to have a relatively low reliability, except for species identification, given that it exhibits rapid evolution.

Currently, molecular studies of insects, including Coleoptera, are being conducted on various taxa (Chang et al 2014; Etzler et al 2014; Oba et al 2015). Most domestic museums, public institutions and university laboratories, and Korean Ministry of Environment have specimens that can be morphologically identified with DNA extraction, because they can be utilized as bioresources in biological industries. However, as a system that incorporates all such data does not exist, it is difficult to determine how many useful organisms have been deposited. Although BioBarcode (<http://www.asianbarcode.org>), with mapping using barcodes, and the Korea Barcode of Life ([\[koreabarcode.org\]\(http://koreabarcode.org\)\) system were established in 2007 and 2009, respectively \(Kim et al 2012; Lim et al 2009\), their services are no longer available in Korea.](http://</p>
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Therefore, now is the time to build a systematic information network enabling use of the massive information on biodiversity and biological resources in managing and utilizing national biological resources. Additionally, along with bio-information construction, a national policy that supports the fostering of taxonomists is also necessary. Thus, sample securement, digital image collection, and DNA-barcode construction systems associated with NSMK and this study are expected to constitute core data that can maximize the effective preservation and utilization of organisms by managing international biodiversity studies and biological genetic resource information. Moreover, the genomic DNA and specimens of the reference specimens were provided to researchers through an online system, enabling their application in various research areas.

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References

- Bocak L, Barton C, Crampton-Platt A, et al. 2014. Building the Coleoptera tree-of-life for >8000 species: composition of public DNA data and fit with Linnaean classification. *Systematic Entomology* 39:97–110.
- Chang H, Liu Q, Hao D, et al. 2014. DNA barcodes and molecular diagnostics for distinguishing introduced *Xleborus* (Coleoptera: Scolytinae) species in China. *Mitochondrial DNA* 25:63–69.
- Chapman AD. 2009. *Numbers of Living Species in Australia and the World*. 2nd ed. Toowoomba, Australia: Australian Biodiversity Information Services. pp. 1–80.
- Cloyd RA, Timmons NR, Goebel JM, et al. 2009. Effect of pesticides on adult rove beetle *Atheta coriaria* (Coleoptera: Staphylinidae) survival in growing medium. *Journal of Economic Entomology* 102:1750–1758.
- DeWalt RE. 2011. DNA barcoding: a taxonomic point of view. *Journal of the North American Benthological Society* 30:174–181.
- Doyle JJ, Gaut BS. 2000. Evolution of genes and taxa: a primer. *Plant Molecular Biology and Biotechnology* 3:294–299.
- Etzler FE, Wanner KW, Morales-Rodriguez A, et al. 2014. DNA Barcoding to Improve the Species-Level Management of Wireworms (Coleoptera: Elateridae). *Journal of Economic Entomology* 107:1476–1485.
- Folmer O, Black M, Hoeh W, et al. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3:294–299.
- Hall TA. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95–98.
- Hebert PDN, Cywinska A, Ball SL, et al. 2003. Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences* 270:313–321.
- Hebert PDN, Penton EH, Burns JM, et al. 2004a. Ten species in one: DNA barcoding reveals cryptic species in the Neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences* 101:14812–14817.
- Hebert PDN, Stoeckle MY, Zemplak TS, et al. 2004b. Identification of birds through DNA barcodes. *PLoS Biology* 2:1657–1663.
- Jäch MA. 2003. Fried water beetles Cantonese style. *American Entomologist* 49:34–37.
- Kim S, Kim CB, Min GS, et al. 2012. Korea barcode of life database system (KBOL). *Animal Cells and Systems* 16:11–19.
- Kimura M. 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution* 16:111–120.
- Lim J, Kim SY, Kim S, et al. 2009. BioBarcode: a general DNA barcoding database and server platform for Asian biodiversity resources. *BMC Genomics* 10:58.
- Lin X, Stur E, Ekrem T. 2015. Exploring genetic divergence in a species-rich insect genus using 2790 DNA barcodes. *PLoS ONE* 10:e0138993.
- Naomi SI. 1988. Studies on the Subfamily Steninae from Japan IV. New or little known species of the subgenus *Stenus s. str.* of the genus *Stenus* Latreille (Coleoptera: Oxyporidae). *Transactions of the Shikoku Entomological Society* 19: 55–62.
- Oba Y, Ōhira H, Murase Y, et al. 2015. DNA barcoding of Japanese click beetles (Coleoptera, Elateridae). *PLoS ONE* 10:e0116612.
- Otavo SE, Parrado-Rosselli A, Noriega JA. 2013. Scarabaeoidea superfamily (Insecta: Coleoptera) as a bioindicator element of anthropogenic disturbance in an amazon national park. *Revista de Biología Tropical* 61:735–752.
- Paek MK, Hawng JM, Jung KS, et al. 2010. Checklist of Korean Insects. In: Paek MK, Cho YK, editors. *Nature & Ecology Academic Series 2*. Seoul: Nature & Ecology. pp. 1–598 [in Korean].
- Puthz V. 1981. On some species of the genus *Stenus* Latreille from Taiwan, including descriptions of new species, a key to the East Asiatic representatives of the *comma*-group, and a check list of species known from Taiwan (Coleoptera, Staphylinidae). *Fragmenta Coleopterologica* 29:115–124.
- Saitou N, Nei M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4:406–425.
- Tamura K, Stecher G, Peterson D, et al. 2013. MEGA 6: molecular evolutionary genetics analysis version 6.0. *Molecular Biology and Evolution* 30:2725–2729.
- Thompson JD, Gibson TJ, Plewniak F, et al. 1997. The CLUSTAL X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* 25:4876–4882.
- Yeates DK, Harvet MS, Austin AD. 2003. New estimates for terrestrial arthropod species-richness in Australia. *Records of the South Australian Museum Monograph Series Number 7*:231–242.
- Zhang ZQ. 2011. Animal biodiversity: an introduction to higher-level classification and taxonomic richness. *Zootaxa* 3148:7–12.