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Research paper

DNA Barcode Library for Selected Species of Korean Tenebrionidae (Coleoptera)

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Abstract

Members of the family Tenebrionidae (Coleoptera) show varying degrees of morphological differences within a species, as well as between species. In this study, we selected 15 species (in 9 genera, 7 tribes, and 4 subfamilies) of Korean Tenebrionidae belonging to 4 functional groups depending on the feeding habits and habitats, and constructed their DNA barcode library composed of 658-bp partial mitochondrial cytochrome c oxidase subunit I (COI) genes. In addition, we tested the efficiency of DNA barcodes for identifying the intraspecific and interspecific differences within members of the family. Our results indicated that the COI genes are effective in identification of the Tenebrionidae species.

Key words: Tenebrionidae, DNA barcode library, COI gene, habitat preference, Korea

Introduction

The family Tenebrionidae or darkling beetles is one of the most diverse groups in Coleoptera. Approximately 22,000 species belonging to this family have been described worldwide, and 129 species and 66 genera are known in Korea (Jung 2011). Members of the family show extensive variations with respect to body size (1−50 mm), form, and vestiture. Most adults of the Tenebrionidae have elongated oval bodies; heads with horns sometimes, antennae in different shapes such as filiform, moniliform, serrate, and rarely flagellate, mesocoxae with exposed trochantins, tarsal formula are usually 5-5-4, tarsal claws are not pectinate, and intersegmental membranes of abdomen are exposed between ventrites 3−5 (Jung 2011). The larvae of Tenebrionidae are called false wireworms because of the presence of elongated, glabrous, and strongly sclerotized body.

Both adults and larvae of Tenebrionidae inhabit diverse habitats or microhabitats such as grain products, rotten wood, fungi, soil, and sand dunes (Arnett *et al*. 2002, Bouchard *et al*. 2005, Löbl *et al*. 2008, Jung 2011). They show adaptive inhabitation in the roots of plants, under the barks, in rotten wood, in mushrooms, and under leaf debris (Jung 2008). Watt (1974) classified Tenebrionidae into the following 4 groups depending on the feeding habits and habitats:

(A) Cereal group: This group belongs to the tribes Tenebrionini, Triboliini, and genus *Alphitobius* and are pests of stored cereal products. The most important species under this group are *Tribolium confusum* Duval and *Tribolium castaneum* Herbst, which cause considerable damage to stored cereals, especially in temperate climate zones (Cotton 1956, Watt 1974, Grimaldi & Engel 2005).

(B) Forest group associated with rotten wood: Members are the species *Cerogria janthinipennis* (Fairmaire) from the subfamily Lagriinae which live under barks of logs, and some other Lagriini species which live on ground, flowers, and grass (Watt 1974, Hayashi 1964). The species from the tribe Amarygmini are also associated with dead and rotten wood. They are found under the bark and associated subcortical space of logs and are usually nocturnal.

(C) Macrofungi group: Most beetles belonging to the subfamilies of Diaperinae (genera *Platydema*, *Basanus*, and *Scaphidema*) and Stenochiinae (Tribe Cnodalonini) specialize in feeding on macrofungi such as Basidiomycetes, which is the most common food for fungivorous tenebrionids in rotten wood (Marshall 1982, Leschen 1990, Kim & Jung 2005, Jung & Kim 2008, Jung & Lee 2011, Jung & Lee 2012). Both larvae and adults of fungivorous tenebrionids spend their whole lives in the fruiting bodies of bracket fungi.

(D) Sand dune group: Many tenebrionids have developed specific adaptations that allow them to inhabit sand dunes in deserts, riversides, and coastal dunes (Krivolutskaya 1973, Watt 1974, Grimaldi & Engel 2005, Jung *et al*. 2009). In particular, members of the tribe Opatrini (genus *Gonocephalum*) adapted to hot and dry sand (Kim & Kim 2000). Members of this group show a high degree of interspecific variations as well as a substantial level of intraspecific similarities in morphology.

Identification of insect species through traditional taxonomy primarily relies on external morphology. However, phenotypic variation in species with taxonomically similar traits can pose considerable difficulties in species identification (Ball & Armstrong 2006). DNA barcode is a useful tool to solve taxonomic issues, particularly if a comprehensive DNA barcode library is available. Species identification is considered successful when the test DNA sequence matches most closely with its congeners in the library data. However, a DNA barcode reference library is not available for the Korean Tenebrionidae, and misidentified sequences are sometimes existent in the present GenBank.

In this study, we selected 15 species of Korean Tenebrionidae according to feeding habits and habitats shown above, and constructed a DNA barcode library using specific regions of the COI genes. The efficiency of this library was tested for identifying of the Korean Tenebrionidae species.

Materials and methods

Specimens

Specimens used in this study were collected from throughout South Korea during 2008–2012. The specimens were collected from diverse habitats such as rotten trees covered with lichen and sand dunes (Table 1) and were examined and photographed using a microscope (Carl Zeiss Discovery V12) with an image analyzer program (AxioCam I Cc1, Germany).

Adult specimens preserved in 95% ethanol were used for the mitochondrial DNA analysis. We used 50 specimens for DNA extraction, and voucher specimens were deposited in the Entomological Museum of Korea University (KU), Seoul.

The abbreviations used in this study are as follows: SL (Seoul), GG (Gyeonggi-do), CB (Chungcheongbuk-do), CN (Chungcheongnam-do), GB (Gyeongsangbuk-do), GN (Gyeongsangnam-do), GW (Gangweon-do), JN (Jeollanam-do), and JJ (Jeju-do).

DNA extraction, PCR amplification and DNA sequencing

DNA was extracted from the thorax muscle or legs of adult specimen by using the DNeasy Blood and Tissue Kit (QIAGEN Genomics Inc., Dusseldolf, Germany), according to the manufacturer's protocol. PCR amplification was performed under the following conditions: initial denaturation at 94°C for 3 min; 40 cycles at 94°C for 30 sec (denaturation), 48°C for 1 min (annealing), 72°C for 30 sec (extension), and a final extension at 72°C for 10 min. The PCR products were maintained at 4°C. We used 2 μL of template DNA in a total reaction volume of 20 μL of PCR reaction mixture that contained ultrapure water, 0.5 U Taq DNA polymerase in 1× Taq buffer containing 20 mM Tris-HCI (pH 8.2), 50 mM KCl, 4 mM $MgCl_2$, 200 μM total dNTPs, and 0.5 μM forward and reverse primers. For PCR, we used the universal primer pair LCO1490 (Forward) (5′-GGTCAACAAATCA TAAAGATATTGG-3′) and HCO2198 (Reverse) (5′-TAAA CTTCAGGGTGACCAAAAAATCA-3′) to amplify the 5′-end of the COI gene (Folmer *et al*. 1994). The PCR products were visualized on 1.5% agarose gel stained with GelRed^{TM} (Biotium Inc, Hayward, CA, USA). The PCR products were then sequenced using an ABI 3730 Automated Sequencer (Macrogen Co., Seoul, Korea). The sequences were assembled and aligned using the software, BioEdit (version 7.0.5.3, Hall 1999, http://www.mbio.ncsu.edu/BioEdit/bioedit.html).

Data analysis

Mitochondrial COI sequences were aligned using Clustal X (Version 2.0, Larkin 2007) and edited to a final length of $>$600 bp. Pairwise nucleotide sequence divergences, which was calculated using the Kimura two-parameter (K2P) model (Kimura 1980), and neighbor-joining (NJ) analysis, which was performed using the software Mega 5 (Tamura *et al*. 2011), were used to examine relationships among the taxa. All sequences obtained in this study were deposited in the GenBank.

Results

Taxonomic accounts

Taxonomic accounts dealt in this study are based on previous studies (Jung 2005, 2008, 2011, Jung *et al*. 2009) with modifications and additions of figures and collecting localities. Synonyms are based on previous catalogs (Jung 2011); species authorities and their literatures are therefore not included in the reference.

Family Tenebrionidae

1. *Cerogria janthinipennis* (Fairmaire) (Fig. 1A)

Lagria janthinipennis Fairmaire, 1886: 349.

Cerogria antennata Jacobson, 1913: 1016; ZSK, 1968: 112; ESK & KSAE, 1994: 176; Kwon *et al*., 1996: 162.

Cerogria janthinipennis: Jacobson, 1913: 1016; Kim *et al*., 1994: 101; ESK & KSAE, 1994: 176; Kim, 1995: 129; Kim, 1996: 8; Kim & Kim, 1996: 43; Kim *et al*., 1999: 125; Kim, 2000: 127; Kim, 2002: 225; Kim *et al*., 2002: 115.

Cerogria notability: Park *et al*., 1993: 153 (Misidentification of *Cerogria janthinipennis*).

Table 1. Details of Tenebrionidae specimens used for DNA barcoding and analysis in this study

Species name	Collection locality	Habitat	GenBank accession number (COI)
Cerogria janthinipennis (Fairmaire)	GB	Leaf litter, Rotten wood	KC440128
Tenebrio molitor Linnaeus	Seoul	Cereal	KC407728
Tenebrio molitor Linnaeus	Seoul	Cereal	KC407729
Tenebrio molitor Linnaeus	Seoul	Cereal	KC407730
Tenebrio molitor Linnaeus	Seoul	Cereal	KC407731
Tenebrio molitor Linnaeus	Seoul	Cereal	KC407732
Tenebrio molitor Linnaeus	Seoul	Cereal	KC407733
Tenebrio molitor Linnaeus	Seoul	Cereal	KC407734
Tenebrio molitor Linnaeus	Seoul	Cereal	KC407735
Tenebrio molitor Linnaeus	Seoul	Cereal	KC407736
Tenebrio molitor Linnaeus	Seoul	Cereal	KC407737
Tenebrio molitor Linnaeus	Seoul	Cereal	KC407738
Tenebrio molitor Linnaeus	Seoul	Cereal	KC407739
Tenebrio molitor Linnaeus	Seoul	Cereal	KC407740
Tenebrio molitor Linnaeus	Seoul	Cereal	KC407741
Tribolium castaneum (Herbst)	NCBI	Cereal	NC003081
Tribolium castaneum (Herbst)	NCBI	Cereal	FM877932
Tribolium castaneum (Herbst)	GN	Cereal	KC440129
Tribolium confusum Jacpuelin Du Val.	GN	Cereal	JQ350711
Tribolium confusum Jacpuelin Du Val.	GN	Cereal	KC407742
Tribolium confusum Jacpuelin Du Val.	GN	Cereal	KC407743
Plesiophthalmuns davidis Fairmaire	GW	Dead and rotten wood	KC440130
Plesiophthalmuns davidis Fairmaire	GW	Dead and rotten wood	KC440131
Gonocephalum persimile (Lewis)	GW	Soil, Sand dunes	KC440132
Gonocephalum pubens (Marseul)	CN	Soil, Sand dunes	KC440133
Gonocephalum pubens (Marseul)	CN	Soil, Sand dunes	KC440134
Gonocephalum pubens (Marseul)	GW	Soil, Sand dunes	KC440135
Gonocephalum pubens (Marseul)	GW	Soil, Sand dunes	KC440136
Gonocephalum coenosum Kaszab	GN	Soil, Sand dunes	KC440137
Gonocephalum coenosum Kaszab	GN	Soil, Sand dunes	KC440138
Gonocephalum coriaceum (Motschulsky)	GB	Soil, Sand dunes	KC440139
Basanus tsushimensis M.T. Chûjô	Seoul	Macrofungi in rotten wood	KC440140
Basanus tsushimensis M.T. Chûjô	Seoul	Macrofungi in rotten wood	KC440141
Basanus tsushimensis M.T. Chûjô	Seoul	Macrofungi in rotten wood	KC440142
Platydema recticorne Lewis	GG	Macrofungi in rotten wood	KC440143
Platydema recticorne Lewis	GG	Macrofungi in rotten wood	KC440144
Platydema recticorne Lewis	Seoul	Macrofungi in rotten wood	KC440145
Platydema recticorne Lewis	Seoul	Macrofungi in rotten wood	KC440146
Platydema fumosum Lewis	GG	Macrofungi in rotten wood	KC440147
Platydema fumosum Lewis	GG	Macrofungi in rotten wood	KC440148
Platydema fumosum Lewis	Known	Macrofungi in rotten wood	KC440149
Platydema nigroaeneum Motschulsky	GG	Macrofungi in rotten wood	KC440150
Platydema nigroaeneum Motschulsky	GG	Macrofungi in rotten wood	KC440151
Platydema nigroaeneum Motschulsky	Seoul	Macrofungi in rotten wood	KC440152
Platydema nigroaeneum Motschulsky	Seoul	Macrofungi in rotten wood	KC440153
Diaperis lewisi lewisi Bates	GG	Macrofungi in rotten wood	KC440154
Promethis valgipes valgipes (Marseul)	GG	Macrofungi in rotten wood	KC440155
Promethis valgipes valgipes (Marseul)	GG	Macrofungi in rotten wood	KC440156
Promethis valgipes valgipes (Marseul)	JJ	Macrofungi in rotten wood	KC440157
Promethis valgipes valgipes (Marseul)	JJ	Macrofungi in rotten wood	KC440158

Specimen examined. 1 adult. GB: Uljin-gun, Jukbyeon-myeon, Bongpyeong-ri, 28.V.2012, HJ Park [KU].

Distribution. Korea, Japan, China, East Siberia.

Habitat preference. Under bark of logs, on the ground on flowers and grass (Watt 1974).

2. *Tenebrio molitor* Linnaeus (Fig. 1B)

Tenebrio molitor Linnaeus, 1758: 417; Kim *et al.*, 1994: 178; Kim & Oh, 1990: 130; Kim, 1995: 407; Kwon & Choi, 1986: 109−110; Kwon *et al.*, 1996: 163; Löbl *et al.*, 2008: 299; Yoon *et al.*, 1989: 112.

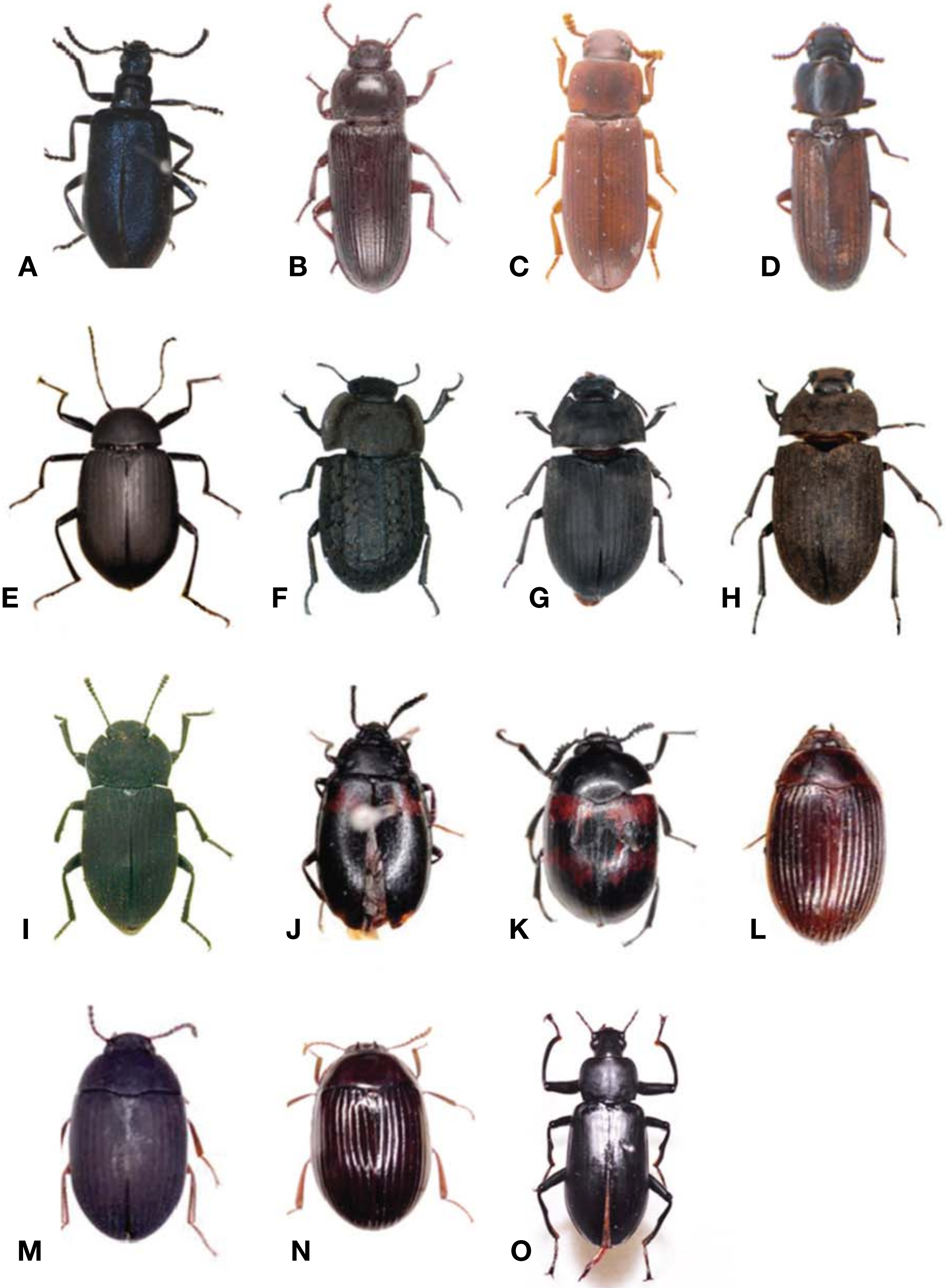

Figure 1. Habitus of Tenebrionidae. A. *Cerogria janthinipennis*; B. *Tenebrio molitor*; C. *Tribolium castaneum*; D. *Tribolium confusum*; E. *Plesiophthalmuns davidis*; F. *Gonocephalum persimile*; G. *Gonocephalum pubens*; H. *Gonocephalum coenosum*; I. *Gonocephalum coriaceum*; J. *Basanus tsushimensis*; K. *Diaperis lewisi lewisi*; L. *Platydema recticorne*; M. *Platydema fumosum*; N. *Platydema nigroaeneum*; O. *Promethis valgipes valgipes*.

Specimen examined. 14 adults. SL: Seongbuk-gu, Anam-dong, 17.IV.2012, SY Cho [KU].
Distribution. Cosmopolitan.
Habitat preference. Stored cereal products.

3. *Tribolium castaneum* (Herbst) (Fig. 1C)

Colydium castaneum Herbst, 1797: 282.
Tribolium ferrugineum Fabricius: Cho *et al.*, 1969: 309; Gu, 1970: 66.
Tribolium castaneum (Herbst): Chûjô & Lee, 1992: 39; Kim *et al.*, 1994: 177; Kim, 1995: 407; Kwon *et al.*, 1996: 163; Masumoto, 1984: 13.

Specimen examined. 1 adult. GN: Yangsan-si, Ugok-dong, 19.III.2012, JH Na [KU].
Distribution. Cosmopolitan.
Habitat preference. Stored cereal products.

4. *Tribolium confusum* Jacpuelin Du Val (Fig. 1D)

Tribolium cafusum Jacpuelin Du Val, 1868: 181; Kim *et al.*, 1994: 177; Kwon *et al.*, 1996: 163; Löbl *et al.*, 2008: 301.

Specimen examined. 3 adults. GN: Yangsan-si, Eogok-dong, 19.III.2012, JH Na [KU].
Distribution. Cosmopolitan.
Habitat preference. Stored cereal products.

5. *Plesiophthalmuns davidis* Fairmaire (Fig. 1E)

Plesiophthalmuns davidis Fairmaire, 1868: 121; Chûjô & Lee, 1993: 119; Kim *et al.*, 1994: 178; Kwon *et al.*, 1996: 163.

Specimen examined. 2 adults. GW: Pyeongchang-gun, Jinbu-myeon, Mt. Odaesan, 18.V.2012, WY Choi [KU].
Distribution. Korea, China.
Habitat preference. Dead and rotten wood or under the bark of dead trees.

6. *Gonocephalum persimile* (Lewis) (Fig. 1F)

Opatrum persimile Lewis, 1894: 381.
Gonocephalum persimile: Chûjô, 1963: 152−153; Chûjô & Baba, 1979: 50; Chûjô & Lee, 1993: 109−110; ESK & KSAE, 1994: 176; Gebien, 1910b: 324, 1939: 447; Hua Li, 2002: 139; Iwan & Löbl, 2008: 265; Kaszab, 1952a: 624; 1952b: 680; 1968: 8; Kim, 1981a: 65; 1995: 141; Kwon & Choi, 1986: 106; Kwon *et al.*, 1996: 163; Masumoto, 1985: 35; Reichardt, 1936: 113; Schuster, 1928: 990.

Specimen examined. 1 adult. GW: Youngwol-gun, Kimsatgat-myeon, Yemil-ri, 5.V.2012, AY Kim [KU].
Distribution. Palaearctic: Korea, Japan, Mongolia, NE China, SE Siberia.
Habitat preference. Sand dune and soil under stones.

7. *Gonocephalum pubens* (Marseul) (Fig. 1G)

Opatrum pubens Marseul, 1876: 97.
Opatrum pubens Mars. Var. *obtusicolle*: Cho, 1957: 52; Heyden, 1887: 259; Kolbe, 1886: 95; Okamoto, 1924: 183.
Gonocephalum pubens: Champion, 1895: 115; Cho, 1963: 50; Chûjô, 1966: 8; Chûjô et Baba, 1979: 50; Chûjô & Lee, 1992: 33−34; 1993: 110; Chûjô, 1994: 152−153; ESK & KSAE, 1994: 176; Gebien, 1910b: 324, 1913: 4, 1939: 447; Gu Gun, 1973: 34−67; Hua Li, 2002: 139; Iwan & Löbl, 2008: 265; Kaszab, 1941a: 51, 1952a: 594, 1952b: 679; Kim & Kim, 1972: 157; Kim, 1995: 406; Kim & Lee, 1979: 83; Kim, 1980: 142, 1981a: 65, 1983: 83, 1995: 141; Kwon & Choi, 1986: 106; Kwon *et al.*, 1996: 163; Lee *et al.*, 1985: 408; Masumoto, 1985: 35−36; Reichardt, 1936: 111; Schuster, 1928: 990.

Specimen examined. 4 adults. CN: Taean-gun, Changfi-ri, Gijipo beach, 5.V.2012, SY Cho & SH Han [KU]; KW: Kangreung-si, Anhyun-dong, Sungeut beach. 3−4.VIII. 2010. HG Min & GS Oh [KU].
Distribution. Palaearctic: Korea, China, India, Japan, Taiwan.
Habitat preference. Sand dune.

8. *Gonocephalum coenosum* Kaszab (Fig. 1H)

Gonocephalum coenosum Kaszab, 1952: 643−646; Chûjô, 1963: 152−153; Chûjô, 1966: 6, 1978b: 1; Chûjô & Imsaka, 1982: 59; Chûjô & Lee, 1992: 32; ESK & KSAE, 1994: 176; Hua Li, 2002: 138; Iwan & Löbl, 2008: 263; Kim, 1983: 83; Kim, 1995: 405; Kim & Oh, 1991: 155; Kwon & Choi, 1986: 106; Kwon *et al.*, 1996: 163; Lee & Kwon, 1974: 43; Lee *et al.*, 1985: 408; Masumoto, 1985: 35−36; Masumoto & Kondo, 1984: 3; Matsumoto & Akita, 2008: 121.

Specimen examined. 2 adults. JN: Shinan-gun, Bigeum-myeon, Duksan-ri, Bigeum-do, 28.VII.2011, BH Jung & JB Seung [KU].
Distribution. Palaearctic: Korea, China, Japan, Taiwan.
Habitat preference. Sand dune.

9. *Gonocephalum coriaceum* (Motschulsky) (Fig. 1I)

Opatrum coriaceum Motschulsky, 1858: 34; Cho, 1957: 52; Kolbe, 1886: 201; Lewis, 1894: 381.
Gonocephalum coriaceum: Chûjô, 1963: 152−153; Chûjô, 1994: 247; Chûjô & Baba, 1979: 49−50; Chûjô & Lee, 1992: 33; 1994: 189; ESK & KSAE, 1994: 176; Gebien,

1910b: 322, 1913: 6, 1939: 447; Harold, 1876: 130; Hua Li, 2002: 138; Iwan & Löbl, 2008: 263; Kaszab, 1941a: 51, 1952a: 667, 1952b: 451, 1954b: 248; Kim *et al*., 1972: 222; Kim & Lee, 1979: 83; Kim, 1981b: 344; 1983: 83; Kim, 1995: 405; Kwon & Choi, 1986: 106; Kwon *et al*., 1996: 163; Marseul, 1876: 96; Masumoto, 1985: 35-36; Masumoto & Kondo, 1984: 4; Motschulsky, 1866: 173; Reichardt, 1936: 112; Reitter, 1904: 143; Schuster, 1928: 989; Yoon & Nam, 1978: 83.

Specimen examined. 1 adult. GB: Ulgin-gun, Seo-myeon, Hawon-ri, Bulyoungsa, 02.VIII.2006, BH Jung [KU].
Distribution. Korea, China, Japan, NE.China, Taiwan.
Habitat preference. Sand dune and soil under stones.

10. *Basanus tsushimensis* Chûjô (Fig. 1J)

Basanus tsushimensis M.T. Chûjô, 1963: 17; Kwon & Choi, 1986: 105; Chûjô & Lee, 1992: 31; Chûjô & Lee, 1993: 109; Paik *et al*., 1995: 405; Kwon *et al*., 1996: 162; Kim *et al*., 2004: 111; Kim & Jung, 2005: 95.
Basanus tsushimensis kompancevi Kaszab & Medvedev, 1984: 75; ESK & KSAE, 1994: 176.

Specimen examined. 3 adults. SL: Gangbuk-gu, Ui-dong, Dosunsa, 22.VI.2006, AY Kim [KU].
Distribution. Korea, Japan.
Habitat preference. Fruiting bodes of bracket fungi.

11. *Diaperis lewisi lewisi* Bates (Fig. 1K)

Diaperis lewisi lewisi Bates, 1873: 14; Kolbe, 1886: 202; Kim *et al*., 1972: 103; Kwon & Choi, 1986: 108; Chûjô & Lee, 1993: 109; ESK & KSAE, 1994: 176; Kwon *et al*., 1996: 163; Kim & Jung, 2005: 95.

Specimen examined. 1 adult. SL: Gangbuk-gu, Ui-dong, Dosunsa, 22.VI.2006, AY Kim [KU].
Distribution. Korea, Japan, China (Manchuria), Russia (Southern Primorye, Siberia).
Habitat preference. Fruiting bodes of bracket fungi.

12. *Platydema recticorne* Lewis (Fig. 1L)

Platydema recticorne Lewis, 1894: 394; Chûjô & Lee, 1993: 109; Chûjô & Lee, 1994: 189; Kwon *et al*., 1996: 162; Kim & Jung, 2005: 9.

Specimen examined. 4 adults. GG: Namyangju-si, Jinjeop-myeon, Bupyeong-ri, Gwangreung, 18.IX.2009, BH Jung [KU]; SL: Gangdong-gu, Gil-dong, Gildong Eco-park, 22.V.2010, BH Jung [KU].
Distribution. Korea, Japan.
Habitat preference. Fruiting bodes of bracket fungi with rotten tree.

13. *Platydema fumosum* Lewis (Fig. 1M)

Platydema fumosum Lewis, 1894: 395; Chûjô & Lee, 1993: 109; Kwon *et al*., 1996: 162; Kim & Jung, 2005: 9.

Specimen examined. 3 adults. GG: Guri-si, Donggu-dong, Donggureung, 30.VII.2012, BH Jung [KU].
Distribution. Korea, Japan (Honshu, Tsushima Island).
Habitat preference. Fruiting bodes of bracket fungi with rotten tree.

14. *Platydema nigroaeneum* Motschulsky (Fig. 1N)

Platydema nigroaeneum Motschulsky, 1860: 18; Heyden, 1887: 243; Kim *et al*., 1971: 157; Kim & Nam, 1981: 126; Kwon & Choi, 1986: 105; ESK & KSAE, 1994: 176; Kim *et al*., 1994: 127; Kwon *et al*., 1996: 162; Kim *et al*., 1998: 163; Kim *et al*., 2002: 115; Kim & Jung, 2005: 9; Kim *et al*., 2005: 223.

Specimen examined. 4 adults. GG: Gwangju-si, Chowol-eup, Mt. Mugapsan, 27.V.2010, BH Jung [KU]; Guri-si, Donggu-dong, Donggureung, 30.VII.2012, BH Jung [KU]; SL: Gangdong-gu, Gil-dong, Gildong Eco-park, 22.V.2010, BH Jung [KU].
Distribution. Korea, Japan.
Habitat preference. Fruiting bodes of bracket fungi.

15. *Promethis valgipes valgipes* (Marseul) (Fig. 1O)

Nyctobates valgipes Marseul, 1876: 117.
Setenis valgipes: Lewis, 1894: 473; Kwon & Choi, 1986: 106.

Table 2. Summary of intraspecific Kimura 2-parameter distances in the Korean Tenebrionidae species

Scientific name	Mean	Range	S.E.	No. of specimens
Subfamily Lagriinae				
Cerogria janthinipennis	n/c	n/c	n/c	1
Subfamily Tenebrioninae				
Tenebrio molitor	1.17	0.00-2.39	0.38	14
Tribolium castaneum	0.72	0.72-0.72	0.34	3
Tribolium confusum	0.00	0.00-0.00	0.00	3
Plesiophthalmuns davidis	0.00	0.00-0.00	0.00	2
Gonocephalum persimile	n/c	n/c	n/c	1
Gonocephalum pubens	0.18	0.00-0.36	0.15	5
Gonocephalum coenosum	0.00	0.00-0.00	0.00	2
Gonocephalum coriaceum	n/c	n/c	n/c	1
Subfamily Diaperinae				
Basanus tsushimensis	0.00	0.00-0.00	0.00	3
Platydema recticorne	0.18	0.00-0.36	0.16	4
Platydema fumosum	0.36	0.36-0.36	0.25	3
Platydema nigroaeneum	0.36	0.00-0.72	0.22	4
Diaperis lewisi lewisi	n/c	n/c	n/c	1
Subfamily Stenochiinae				
Promethis valgipes valgipes	0.09	0.00-0.18	0.08	4

Table 3. Pairwise comparison of COI gene sequences in Tenebrionidae. The values were calculated using the Kimura 2-parameter method (Kimura, 1980). All results are based on the gene divergence analysis of 15 Korean species of Tenebrionidae according to habitat preference*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1. *Cerogria janthinipennis*															
2. *Tenebrio molitor*	0.299														
3. *Tribolium castaneum*	0.300	0.222													
4. *Tribolium confusum*	0.249	0.231	0.208												
5. *Plesiophthalmuns davidis*	0.291	0.226	0.200	0.190											
6. *Gonocephalum persimile*	0.257	0.224	0.203	0.186	0.193										
7. *Gonocephalum pubens*	0.239	0.238	0.167	0.208	0.204	0.154									
8. *Gonocephalum coenosum*	0.239	0.238	0.167	0.208	0.204	0.154	0.001								
9. *Gonocephalum coriaceum*	0.226	0.219	0.187	0.188	0.213	0.150	0.122	0.122							
10. *Basanus tsushimensis*	0.302	0.290	0.271	0.262	0.238	0.259	0.225	0.225	0.246						
11. *Platydema recticorne*	0.274	0.210	0.201	0.218	0.201	0.186	0.167	0.167	0.171	0.255					
12. *Platydema fumosum*	0.239	0.219	0.180	0.212	0.229	0.214	0.176	0.176	0.190	0.265	0.171				
13. *Platydema nigroaeneum*	0.247	0.219	0.185	0.180	0.194	0.158	0.167	0.167	0.175	0.234	0.155	0.168			
14. *Diaperis lewisi lewisi*	0.286	0.242	0.207	0.218	0.211	0.197	0.190	0.190	0.204	0.228	0.211	0.207	0.195		
15. *Promethis valgipes valgipes*	0.270	0.207	0.203	0.225	0.221	0.210	0.215	0.216	0.212	0.263	0.217	0.217	0.243	0.226	

*Habitat preference: in cereal (genera *Tenebrio* and *Tribolium* in tribe Tenebrionini); in sand dune (genus *Gonocephalum* in tribe Opatrini); in macrofungi (genera *Basanus* and *Platydema* in tribe Diaperinae, *Promethis valgipes valgipes* in tribe Stenochiinae); in rotten wood (*Cerogria janthinipennis* in tribe Lagriinae and *Plesiophthalmuns* in tribe Amarygmini)

Table 4. Base composition and GC percentage of COI gene sequences in the Korean Tenebrionidae specie determined in this study

Scientific name	Sequence length	%A	%C	%G	%T	%G+C	%A+T	No. of specimens
Cerogria janthinipennis	658.0	41.3	14.7	28.0	16.0	42.7	57.3	1
Tenebrio molitor	658.0	28.9	22.2	30.9	18.0	53.1	46.9	14
Tribolium castaneum	652.2	31.6	21.9	29.9	16.6	51.8	48.2	3
Tribolium confusum Jacpuelin.	658.0	33.0	19.6	30.9	16.6	50.5	49.5	3
Plesiophthalmuns davidis	658.0	30.4	21.6	32.5	15.5	54.1	45.9	2
Gonocephalum persimile	658.0	33.6	19.8	29.8	16.9	49.5	50.5	1
Gonocephalum pubens	658.0	34.5	19.4	29.8	16.3	49.2	50.8	4
Gonocephalum coenosum	653.0	34.5	19.5	29.8	16.3	49.3	50.7	2
Gonocephalum coriaceum	658.0	35.9	18.1	30.1	16.0	48.2	51.8	1
Basanus tsushimensis	658.0	32.1	23.6	29.3	15.0	52.9	47.1	3
Platydema recticorne	620.9	31.1	22.0	30.6	16.3	52.6	47.4	4
Platydema fumosum	652.7	32.9	20.9	29.9	16.3	50.8	49.2	3
Platydema nigroaeneum	636.5	33.0	20.2	30.6	16.1	50.8	49.2	4
Diaperis lewisi lewisi	658.0	33.0	22.6	29.2	15.2	51.8	48.2	1
Promethis valgipes valgipes	658.0	29.6	23.3	29.4	17.7	52.6	47.4	4
Average	652.1	31.6	21.4	30.3	16.8	51.7	48.3	50

Promethis valgipes: Kaszab, 1988: 85; Chûjô & Lee, 1992: 31; ESK & KSAE, 1994: 176; Kwon *et al.*, 1996: 162.

Specimen examined. 4 adults. JJ: Seogwipo-si, Seongsan-eup, 5.V.2012, BH Jung & JB Seung [KU]; Jeji-si, Gujwa-eup, Pyeongdae-ri, Bijarim, 18.VIII.2012, BH Jung & JB Seung [KU].

Distribution. Korea, Japan, China.

Habitat preference. Fruiting bodes of bracket fungi.

Construction of DNA barcode library

The 50 COI sequences ranged from 600 to 658 bp (mean= 652 bp with <1% ambiguity) in length from 50 specimens belonging to 15 species, 9 genera, and 4 subfamilies.

The pairwise genetic distances among the individuals within one species are shown in Table 2. The mean of intraspecific divergence variation was 0.9% and ranged between 0.0–2.39%. The result matched the range for the species *Tenebrio molitor*.

The mean of interspecific genetic divergence was 19.5% (SE=1.3%), whereas divergence variation widely ranged from a minimum of 0.1% to a maximum of 30.2% (Table 3). Majority of the species (65%) exceeded a value of 20% genetic divergence between the species. When the sequences of 2 species, *Cerogria janthinipennis* (Lagriinae) and *Basanus tsushimensis* (Diaperinae), were compared for genetic divergence, their DNA barcode gaps were far larger (mean=

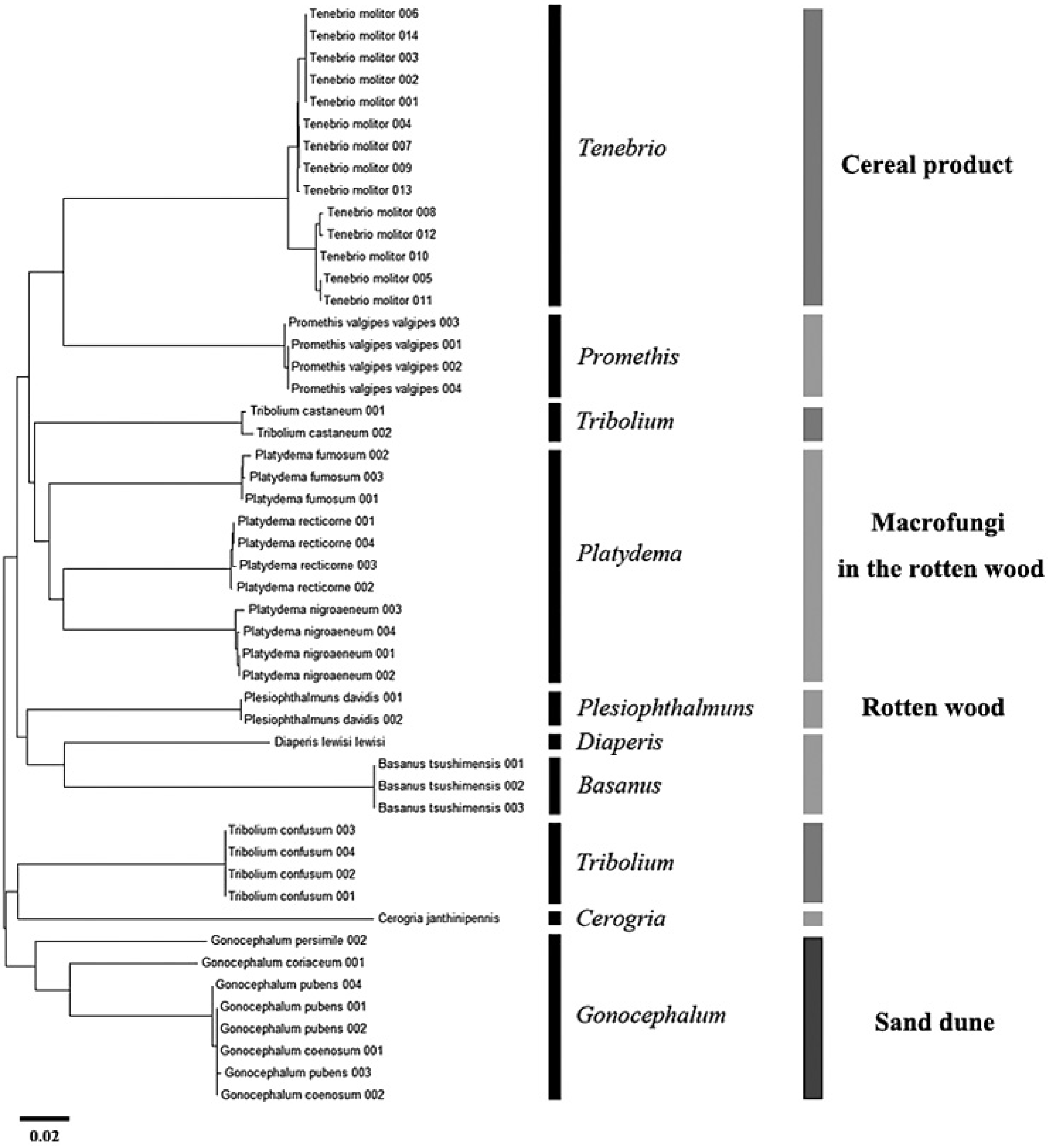

Figure 2. Phylogenetic trees using neighbor-joining analyses for COI divergence in the selected Korean Tenebrionidae species.

30.2%, SE=2.7%) as compared to that between other species (mean=19.5%, SE=1.3%).

The nucleotide content of the COI gene sequences determined in this study, covering the Tenebrionidae, is summarized in Table 4. The COI sequences from the sampled species showed a strong A+T bias in the nucleotide content (mean=58.3%) relative to the C+G content (mean=41.7%), which is typical of arthropods. Only *Cerogria janthinipennis* showed a low G+C content of 42.7%. The G+C content of the genus *Gonocephalum*, which inhabits sand dunes, was 49.5% (range 48.2–49.5%). The mean G+C content of genus *Platydema* was 51.4% (range 50.8–52.6%), while the G+C content of 2 other species *Basanus tsushimensis* and *Diaperis lewisi lewisi*, which occupy similar habitats were 52.9% and 51.8%, respectively, similar to that of the genus *Platydema*. All the above genera showed atypically high

G+C content compared to the usually occurring G+C content of 30–50% in insects.

Discussion

Efficiency of DNA barcodes in identification of Tenebrionidae species

The results suggest that DNA barcodes are effective in identification of the Tenebrionidae species. For example, a large barcode gap (interspecific variation=20.8%, range 20.7–20.9%, SE=2.1%) was observed between *Tribolium castaneum* and *Tribolium confusum* despite morphological similarities between the two species. Taxonomical identification of the genus *Gonocephalum* belonging to the tribe Opatrini is difficult due to morphological similarities between the species (Kim & Kim 2000). Four species, *G. persimile*, *G. pubens*, *G. coenosum*, and *G. coriaceum*, were sequenced for the COI gene, and the mean interspecific divergences between *G. persimile* and *G. pubens* and between *G. persimile* and *G. coenosum* were 15.4%, whereas those between *G. pubens* and *G. coriaceum* and between *G. coenosum* and *G. coriaceum* were 12.2%. On the other hand, those between *G. pubens* and *G. coenosum* was 0.1%. This result suggests that the classification of the Korean species of *Gonocephalum*, in particular, those of *G. pubens* and *G. coenosum* needs to be reviewed.

Kim (2003) discussed that the genus *Gonocephalum* belonging to the tribe Opatrini is a monophyletic group. However, the genus *Idisia* (*Idisia ornata*) belonging to the same tribe shows a paraphyletic cluster with another genus *Tenebrio*. The DNA barcodes generated in this study also indicate that *Idisia ornate* has a closer genetic similarity with *Tenebrio molitor* than with the genus *Gonocephalum* (data not shown). Similarly, while it is known that both *Phaleromela subhumeralis* in the tribe Diaperinae and the tribe Opatrini inhabit sand dunes, it is also known that their respective subfamilies Diaperinae and Tenebrioninae are genetically closely related (Watt 1974, Doyen 1982).

Relationship between tenebrionid species and habitat preference

The NJ tree according to habitat preference of the Korean Tenebrionidae species is given in Figure 2. Tenebrionidae species inhabit diverse habitats and the species have adapted to their unique habitat environment. After grouping the species based on habitat preference, the genetic differences and the NJ tree were analyzed (Table 3, Fig. 2).

The results indicated that members of the Korean Tenebrionidae formed a coherent cluster by taxa and habitat preference such as cereals, rotten wood, and macrofungi. The tree shows that the genus *Tribolium* is not monophyletic. The genus *Gonocephalum* in sand dune habitat formed a monophyletic cluster. The mean of pairwise divergences between interspecies within a group was 22.6% (range 20.7–24.1%, SE=2.2%) for cereal group, 29.0% (range 29.0–29.0%, SE=2.7%) for rotten wood group, 21.6% (range 15.2–26.8%, SE=2.2%) for fungi group, and 8.6% (range 0.0–15.6%, SE=1.1%) for sand dune group. As a conclusion, DNA barcode data obtained from the mitochondrial COI gene could effectively discriminate the species and groups of the Korean Tenebrionidae, which is based on habitat preference, despite of some exceptional cases.

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