

DNA Barcoding of Two *Gonioctena* Species (Coleoptera: Chrysomelidae) Described from the Korean Peninsula

Hee-Wook Cho, Sang Ki Kim*

Department of Zoology, Nakdonggang National Institute of Biological Resources, Sangju 37242, Korea

ABSTRACT

Eleven *Gonioctena* species have been recorded in the Korean Peninsula. Most species in the genus have very similar coloration and morphology. The male genitalia is generally used as the only reliable diagnostic character differentiating closely related species, but it is still difficult to identify females to species accurately. Here, we determined the *COI* barcodes of two *Gonioctena* species described from Korea and give morphological descriptions. The interspecific genetic divergence (Kimura 2-parameter) among the *Gonioctena* ranged from 0.073 to 0.138 and averaged 0.108. The pairwise genetic distance between the two Korean species (0.102) is similar to the average interspecific divergence. We predict that these analyses will help to resolve taxonomic issues and find cryptic species.

Keywords: *Gonioctena*, *COI*, DNA barcoding, morphology, Korea

INTRODUCTION

The genus *Gonioctena* Chevrolat, 1836 is one of the most speciose genera in the subfamily Chrysomelinae, with approximately 110 valid species (Cho, 2019). It is widely distributed in the Palearctic and Oriental regions, with four native species in the Nearctic. Members of the genus can be distinguished by the presence of a tooth-like projection near the apex of the tibiae, well-developed hind wings, and appendiculate tarsal claws. Both adults and larvae of the genus feed externally on the foliage of various Betulaceae, Cannabaceae, Fabaceae, Rosaceae, and Salicaceae species, and each species generally has a narrow range of host plants (Jolivet and Hawkeswood, 1995). Since Heyden (1887) reported *Gonioctena fulva*, 11 *Gonioctena* species have been recorded from the Korean Peninsula (e.g., Takizawa, 1985; Cho and An, 2020). Most species of the genus have very similar coloration and morphology. The structure of male genitalia is generally used as the only reliable diagnostic character, and it is still difficult to identify females to species accurately. Recently, molecular methods, particularly DNA barcoding, have proven instrumental in species identification, especially in insects and vertebrates (van Velzen et al., 2012). Here, DNA barcoding of Korean

Gonioctena species was performed as a first step to identify two species described from Korea. Four specimens of *G. coreana* were collected on 13 Apr 2020 by the Yeong River (36°39'13.43"N, 128°7'12.67"E), Mungyeong, South Korea, and three specimens of *G. koryeoensis* were collected on 6 May 2019 on Mt. Cheonhwangsan (35°34'5.38"N, 128°59'29.87"E), Miryang, South Korea. Total genomic DNA was extracted from muscle tissue using a DNeasy Blood and Tissue Kit (QIAGEN, Germany). The specimens examined were deposited in the Nakdonggang National Institute of Biological Resources (NNIBR, voucher nos. IN144235–144241). PCR conditions and primers for the mitochondrial *COI* gene followed Cho and Kim (2021). Pairwise genetic distances among species were calculated using MEGA X (Kumar et al., 2018) with a Kimura 2-parameter (K2P) distance matrix (Kimura, 1980).

RESULTS AND DISCUSSION

Order Coleoptera Linnaeus, 1758
Family Chrysomelidae Latreille, 1802
Genus *Gonioctena* Chevrolat, 1836

© This is an Open Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0/>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

*To whom correspondence should be addressed
Tel: 82-54-530-0812, Fax: 82-54-530-0829
E-mail: ivoice8324@gmail.com

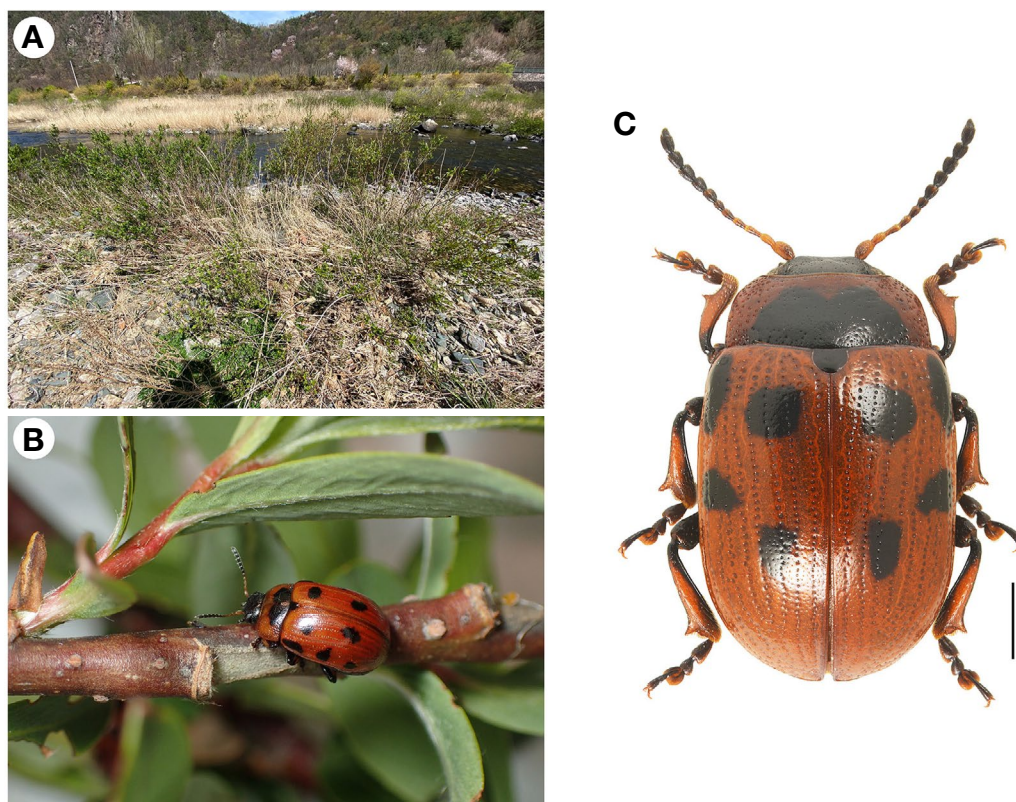


Fig. 1. *Gonioctena coreana* (Bechyně, 1948), habitat (A), live adult (B), and dorsal habitus (C). Scale bar = 1.0 mm.

***Gonioctena coreana* (Bechyně, 1948) (Fig. 1)**

Phytodecta coreanus Bechyně, 1948: 114 (type locality: Korea, Gen-san).

Gonioctena (Gonioctena) coreana: Gressitt and Kimoto, 1963: 358, 361.

Diagnosis. Body length 5.60–6.60 mm, width 3.40–3.80 mm, oblong oval and moderately convex. Head black with antennomeres 1–5 yellowish brown, 6–7 blackish brown, 8–11 black. Pronotum reddish brown, with or without a large black marking. Scutellum black to blackish brown. Elytra reddish brown, generally with 5 pairs of black spots, sometimes spots disappeared or elytra largely black. Venter largely black. Legs black, with tibiae reddish-brown except base and inner margin, tarsi dark brown to blackish brown. Elytral disc covered with 11 regular rows of large punctures; some punctures rather irregular between 7th and 8th striae in apical half. This species can be distinguished from other Korean *Gonioctena* by the leg coloration.

Distribution. Korea (North and South), Russia (Far East), China (Jilin), Mongolia.

Host plant. Salicaceae: *Salix gracilistyla* Miq.

***Gonioctena koryeoensis* Cho and Lee, 2010 (Fig. 2)**

Gonioctena (Gonioctena) koryeoensis Cho and Lee, 2010: 53, 58 (type locality: Korea, Gyeongsangnam-do, Yangsan-si, Mt. Cheonseongsan).

Diagnosis. Body length 4.80–5.65 mm, width 3.20–3.60 mm, oblong oval and moderately convex. Head reddish brown, hind part black, with antennae yellowish brown. Pronotum reddish brown, with 2–3 obscure spots. Scutellum blackish-brown or reddish-brown with basal margin black. Elytra reddish brown, elytral suture except basal 1/3 and apex of elytral epipleura black, with 5 pairs of black spots. Venter almost completely reddish brown. Legs entirely reddish brown. Elytral disc covered with 11 regular rows of large punctures. This species is distinguished from other Korean *Gonioctena* species by the black elytral suture and reddish-brown venter.

Distribution. South Korea.

Host plant. Betulaceae: *Alnus* spp.

DNA barcoding of two species of Korean *Gonioctena* was conducted for the first time. The *COI* gene (658 bp) sequences from *G. coreana* (MW960959–62) and *G. koryeoensis* (MW960963–65) were deposited in GenBank. The *COI*

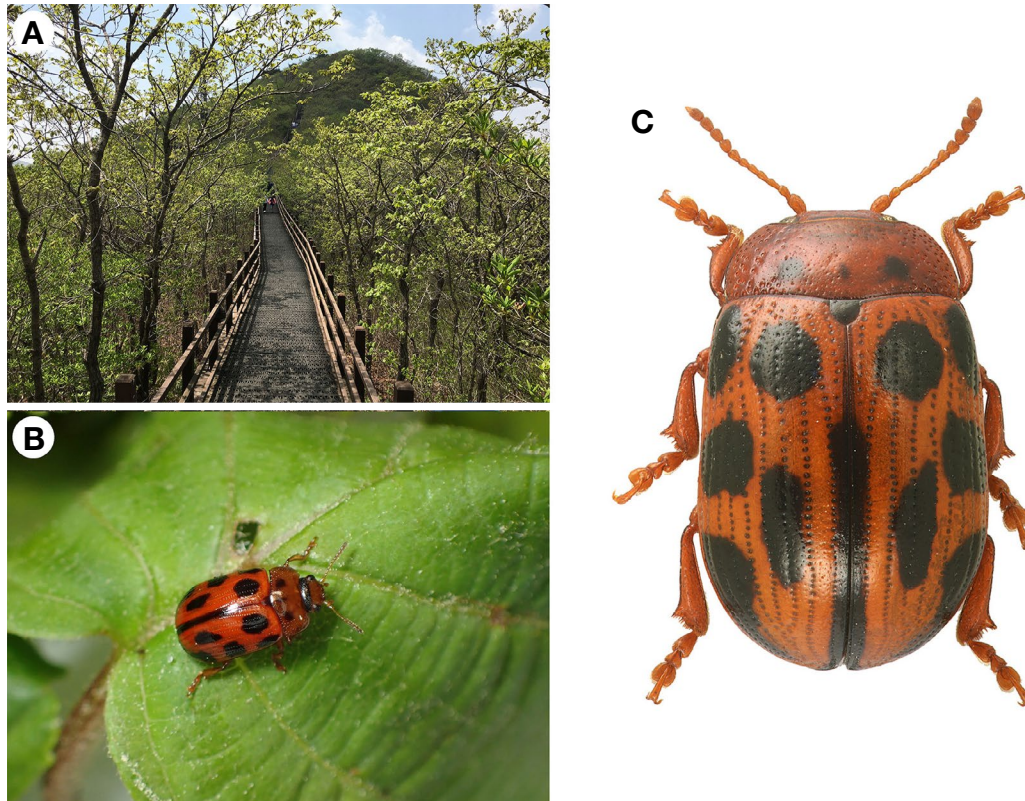


Fig. 2. *Gonioctena koryeoensis* Cho and Lee, 2010, habitat (A), live adult (B), and dorsal habitus (C). Scale bar=1.0 mm.

Table 1. Mean pairwise genetic distance among nine *Gonioctena* species using the Kimura 2-parameter distance

Species	1	2	3	4	5	6	7	8	Accession No.	Reference
1. <i>G. coreana</i>									MW960959–62	This study
2. <i>G. koryeoensis</i>	0.102								MW960963–65	This study
3. <i>G. americana</i>	0.118	0.077							KR480056	Hebert et al. (2016)
4. <i>G. linnaeana</i>	0.096	0.076	0.073						KM443046	Hendrich et al. (2015)
5. <i>G. notmani</i>	0.128	0.091	0.073	0.092					KU875142	Sikes et al. (2017)
6. <i>G. decemnotata</i>	0.121	0.112	0.103	0.101	0.095				MH323136	Magoga et al. (2018)
7. <i>G. nivosa</i>	0.106	0.114	0.127	0.120	0.131	0.138			KM443052	Hendrich et al. (2015)
8. <i>G. viminalis</i>	0.129	0.114	0.093	0.109	0.062	0.107	0.135		KM439485	Hendrich et al. (2015)
9. <i>G. flavicornis</i>	0.132	0.120	0.105	0.114	0.093	0.124	0.151	0.110	KJ964029	Pentinsaari et al. (2014)

sequence dataset consists of nine *Gonioctena* species, with a base composition of A = 30.0%; C = 17.3%; G = 16.1%; T = 36.6%. Table 1 provides the interspecific genetic divergence among *Gonioctena* species. Pairwise genetic distances ranged from 0.073 (*G. americana* vs. *G. linnaeana*) to 0.138 (*G. decemnotata* vs. *G. nivosa*) and averaged 0.108. The pairwise genetic distance between the two Korean species (0.102) is similar to the average interspecific divergence, although it is less than the average interspecific genetic distance of 0.251 for Chrysomelidae (Magoga et al., 2018).

In this study, we determined the *COI* barcodes for two *Gonioctena* species described from the Korean Peninsula and developed morphological descriptions. These results will help to resolve taxonomic issues and find cryptic species.

ORCID

Hee-Wook Cho: <https://orcid.org/0000-0003-3771-865X>

Sang Ki Kim: <https://orcid.org/0000-0003-2387-4264>

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

ACKNOWLEDGMENTS

This work was supported by the Nakdonggang National Institute of Biological Resources (NNIBR) under project No. NNIBR202101101.

REFERENCES

- Bechyně J, 1948. Příspěvek k poznání rodu *Phytodecta* Kirby. Additamenta ad cognitionem specierum generis *Phytodecta* Kirby (Col. Phytoph. Chrysomelidae). Sborník Národního Musea v Praze, 3B:89-158.
- Cho HW, 2019. Redescription of mature larva and biological notes on the nominotypical subgenus *Gonioctena* Chevrolat (Coleoptera: Chrysomelidae: Chrysomelinae) from South Korea. *Zootaxa*, 4544:557-571. <https://doi.org/10.11646/zootaxa.4544.4.6>
- Cho HW, An SL, 2020. An annotated checklist of leaf beetles (Coleoptera: Chrysomelidae) in the Korean Peninsula, with comments and new records. *Far Eastern Entomologist*, 404:1-36. <https://doi.org/10.25221/fee.404.1>
- Cho HW, Kim SK, 2021. An integrative approach reveals a new species of flightless leaf beetle (Chrysomelidae: *Suinzona*) from South Korea. *Scientific Reports*, 11:8595. <https://doi.org/10.1038/s41598-021-88011-2>
- Cho HW, Lee JE, 2010. *Gonioctena koryeoensis* (Coleoptera: Chrysomelidae: Chrysomelinae), a new species from Korea, with a description of immature stages. *Zootaxa*, 2438:52-60. <https://doi.org/10.11646/zootaxa.2438.1.3>
- Gressitt JL, Kimoto S, 1963. The Chrysomelidae (Coleopt.) of China and Korea, Part 2. *Pacific Insects Monograph*, 1B:300-1026.
- Hebert PD, Ratnasingham S, Zakharov EV, Telfer AC, Levesque-Beaudin V, Milton MA, Pedersen S, Jannetta P, DeWaard JR, 2016. Counting animal species with DNA barcodes: Canadian insects. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371:20150333. <https://doi.org/10.1098/rstb.2015.0333>
- Hendrich L, Morinière J, Haszprunar G, Hebert PDN, Hausmann A, Köhler F, Balke M, 2015. A comprehensive DNA barcode database for Central European beetles with a focus on Germany: adding more than 3500 identified species to BOLD. *Molecular Ecology Resources*, 15:795-818. <https://doi.org/10.1111/1755-0998.12354>
- Heyden L, 1887. Verzeichniss der von Herrn Otto Herz aur der chinesischen Halbinsel Korea gesammelten Coleopteren. *Horae Societatis Entomologicae Rossicae*, 21:243-273.
- Jolivet P, Hawkeswood TJ, 1995. Host-plants of Chrysomelidae of the world: an essay about the relationships between the leaf-beetles and their food-plants. Backhuys, Leiden, pp. 1-281.
- Kimura M, 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, 16:111-120. <https://doi.org/10.1007/BF01731581>
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K, 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35:1547-1549. <https://doi.org/10.1093/molbev/msy096>
- Magoga G, Sahin DC, Fontaneto D, Montagna M, 2018. Barcoding of Chrysomelidae of Euro-Mediterranean area: efficiency and problematic species. *Scientific Reports*, 8:13398. <https://doi.org/10.1038/s41598-018-31545-9>
- Pentinsaari M, Hebert PDN, Mutanen M, 2014. Barcoding beetles: a regional survey of 1872 species reveals high identification success and unusually deep interspecific divergences. *PLoS ONE*, 9:e108651. <https://doi.org/10.1371/journal.pone.0108651>
- Sikes DS, Bowser M, Morton JM, Bickford C, Meierotto S, Hildebrandt K, 2017. Building a DNA barcode library of Alaska's non-marine arthropods. *Genome*, 60:248-259. <https://doi.org/10.1139/gen-2015-0203>
- Takizawa H, 1985. Notes on Korean Chrysomelidae, part 2. *Nature and Life (Kyungpook Journal of Biological Sciences)*, 15:1-18.
- van Velzen R, Weitschek E, Felici G, Bakker FT, 2012. DNA barcoding of recently diverged species: relative performance of matching methods. *PLoS ONE*, 7:e30490. <https://doi.org/10.1371/journal.pone.0030490>

Received April 26, 2021
Revised May 3, 2021
Accepted May 28, 2021