Zootaxa 5277 (2): 381–387
https://www.mapress.com/zt/

Article

ISSN 1175-5326 (print edition)
ZOOTAXA
ISSN 1175-5334 (online edition)

https://doi.org/10.11646/zootaxa.5277.2.8
http://zoobank.org/urn:lsid:zoobank.org:pub:8B694CC6-ACC3-489A-9F88-2D34AB6BF8C1

A new species of *Karekizo* Morimoto, 1962 (Coleoptera: Curculionidae: Molytinae) from the Alishan Mountains of Taiwan and a first record of *Karekizo impressicollis* outside Japan

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Abstract

Karekizo Morimoto, 1962 represents a rarely collected, mountain-dwelling genus which has hitherto been known only from one species, *K. impressicollis* Morimoto, 1962, in Japan. Here, a second species, *Karekizo depressus* **sp. n.**, is described based on a single specimen collected from Fen-Chi-Hu, Chiayi Hsien, Taiwan. The type species, *K. impressicollis*, is reported for the first time outside of Japan, from South Korea (Jeju Island, Mt. Hallasan). X-ray microtomography is used to non-destructively compare the sub-scale cuticular structure (e.g., density of punctures, presence of tubercles or carinae) of the two species. A photographic key to the species is also presented.

Key words: Weevil, species discovery, Oriental Region, island endemism, micro-CT, Trachodini

Introduction

The little-known genus *Karekizo* Morimoto, 1962 (Curculionidae: Molytinae: Trachodini) was described based on one species, *K. impressicollis* Morimoto, 1962, and has hitherto only been recorded from Japan (from central Japan to Kyushu) (Morimoto 1962; Kojima & Morimoto 2004). *Karekizo* is closely related to *Acicnemis* Fairmaire, 1849 and *Trachodes* Germar, 1823, but has been differentiated from those genera by the combination of possessing a longitudinally ridged pronotum (usually not ridged in *Acicnemis* or *Trachodes*), functional hind wings (reduced in most *Trachodes*), parallel sided elytra, truncate third tarsal segments (distinctly emarginate in most *Acicnemis*; see Lewis in press), and a complete tegmen ring around the aedeagus (open in all examined *Acicnemis*) (Morimoto 1965; Morimoto & Miyakawa 1995). Although there are no reports describing host plant use in *Karekizo*, the genus name is made up of the Japanese word "*Kareki*" (meaning decaying tree) and "*zo*" (part of Japanese word for weevil "*zoumushi*").

Here, a second species, *Karekizo depressus* **sp. n.**, is described based on a single male specimen collected from Fen-Chi-Hu, Chiayi Hsien, Taiwan and the type species, *K. impressicollis*, is reported for the first time outside of Japan, from South Korea (Jeju Is., Mt. Hallasan). In many weevil species, heavy scaling covers the cuticle which may contain informative morphological characters (e.g., punctation density and size, presence of tubercles or carinae) that can serve as evidence of novel taxa. However, when examining type material or rare species physically descaling specimens may not be an option as this results in permanent damage to potentially important scaling patterns and possibly also the cuticle. For this reason, X-ray microtomography is used as a non-destructive alternative method to compare sub-scale cuticular surface structure of the two known species. X-ray microtomography has been widely and successfully used to examine insect internal anatomy (e.g., Nadein & Betz 2018), insects enclosed in amber (e.g., Soriano *et al.* 2013), and non-scaled insects (e.g., Hita-Garcia *et al.* 2019), however, the use of the technology as a means of comparing underlying cuticular structure in heavily scaled weevils for taxonomic purposes is novel and worth exploring. A photographic key is also presented to facilitate identification of the two known species.

Accepted by R. Anderson: 12 Apr. 2023; published: 2 May 2023

Materials and methods

All specimens were procured from the Kyushu University Museum, Fukuoka (KUM) insect collection. Images were taken with a Leica DFC450 C camera (Leica Microsystems, Wetzlar, Germany) and stacked using Helicon Focus ver. 8 (Helicon Soft Ltd., Kharkiv, Ukraine). Distribution maps were created in SimpleMappr (Shorthouse 2010). For specimens which only had city/town/province names associated (i.e., no geographic coordinates) coordinates at the center of the city/town/province were selected using Google Maps (accessed 31 Dec. 2022). All examined specimens were assigned Unique Specimen Identifier (USI) labels that read in the form: JHL_KAR_###. Specimens were dissected using standard procedures and genitalia were cleared in a solution of KOH and water.

Micro-CT scanning was performed at the Okinawa Institute of Science and Technology, Tancha, Japan on a ZEISS Xradia 510 Versa using ZEISS Scout and Scan Control System software ver. 14.0.14829 (Carl Zeiss Microscopy, Oberkochen, Germany). Specimens were left attached to their paper points and were fixed to a secure holding stage. Due to the large size of the specimens only the anterior half of the body was scanned, with a focus on the head and pronotum. Scan settings for *K. impressicollis* were as follows: optical magnification (4X), exposure time (1.3 sec.), voltage (50 kV), power (4W), filter (air, no special filter), detector distance (17 mm), source distance (16.5 mm). Scan settings for *K. depressus* were as follows: optical magnification (4X), exposure time (1.1 sec.), voltage (50 kV), power (4W), filter (air, no special filter), detector distance (12 mm), source distance (18 mm). Specimens were rotated 360 degrees throughout the scan run and with 2001 projections. Reconstructions were performed using Zeiss Scout-and-Scan Control System Reconstructor version 14.0.14829 and saved in DICOM format. The DICOM files were loaded into 3D Slicer version 5.0.3 and a 3D model was generated in the Segment Editor module. The 3D weevil models were examined virtually by varying the threshold range (Segment Editor module) to "peer" through the scales covering the cuticle and search for otherwise hidden distinguishing morphological characters. Before producing figures the 3D weevil models were cleaned to remove "noise particles" (i.e., remaining bits of scales) using the islands function (Segment Editor module) and leftover particles were removed using the scissors function (Segment Editor module). Figure production and model shading (Cook-Torrance.gdp) was performed in MeshLab version 2022.02.

Results

Taxonomy

***Karekizo depressus* Lewis, sp. n.**
Figs 1, 2A, C, F, 4A

Diagnosis: Pronotum strongly ridged at lateral edges and in two longitudinal rows along middle (longitudinally). Third elytral interval with several tufts of dark orange, erect scales. Femora with distinct depression dorsally at midpoint. Femora each with large teeth ventrally at apical fourth. Third tarsal segment weakly emarginate. Aedeagus with sclerotized lateral edges convergent in anterior half in dorsal view.

Description: Body length: 4.9 mm. **Head**: Rostrum evenly curved, covered in appressed scales and semi-erect brown scales up to antennal insertion, with antennae inserted at midpoint; cluster of 6–7 brown, erect scales along inner edge of each eye; area between eyes noticeably depressed. **Pronotum**: Covered in light and dark brown appressed scales; lateral edges raised into distinct elevated ridges; also with two distinct ridges running longitudinally along middle of pronotum; anterior edge of pronotum with two tufts of erect, brown scales (one on each side), with two smaller tufts of brown, erect scales posterior to anterior tufts; lateral edge of pronotum each with a tuft of erect, brown scales near middle; anterio-ventral edge of pronotum below eye with row of 4–5 erect, brown scales. **Elytra**: Covered in light to dark brown and dark scales; with distinct longitudinal, curved band of white scales extending across posterior third of elytra; anterior edge of elytra heavily sinuate; scutellum visible, circular; erect, brown scales on odd elytral intervals, with largest clusters of erect scales on third interval, especially near middle. **Abdomen**: Covered in appressed scales (white on first two ventrites, brown on latter ventrites) and also with sparse semi-erect scales; fifth ventrite with fine brown setae at apex. **Legs**: Covered in light to dark brown appressed and semi-erect scales; femora with distinct depression dorsally at midpoint; femora also each bearing a ventral tooth at apical fifth;

tibiae unmodified; third tarsal segment barely emarginate; tarsal claws simple. **Genitalia** (Fig. 2A): Tegmen ring closed around aedeagus, with ventral half expanded into semi-triangular plate; aedeagus with sides parallel-sided in dorsal view; sclerotized edges convergent in anterior half in dorsal view; apex of aedeagus evenly rounded.

Material Examined: Holotype (male): TAIWAN: Chiayi Hsien, Fen-Chi-Hu (Fig. 3), 26.VII.1966, H. Sasaji, (KUM), JHL_KAR_001.

Etymology: The specific name *depressus* refers to the femora which are depressed dorsally at the midpoint.

Comments: The current species is placed most appropriately in *Karekizo* as it possesses a longitudinally ridged pronotum (as in *K. impressicollis*), fully developed hind wings, weakly emarginate third tarsal segments, parallel sided elytra, and a complete tegmen ring. As in *Karekizo impressicollis*, this species may also be associated with mountainous regions given the collection locality of the type specimen (Alishan Mountains).

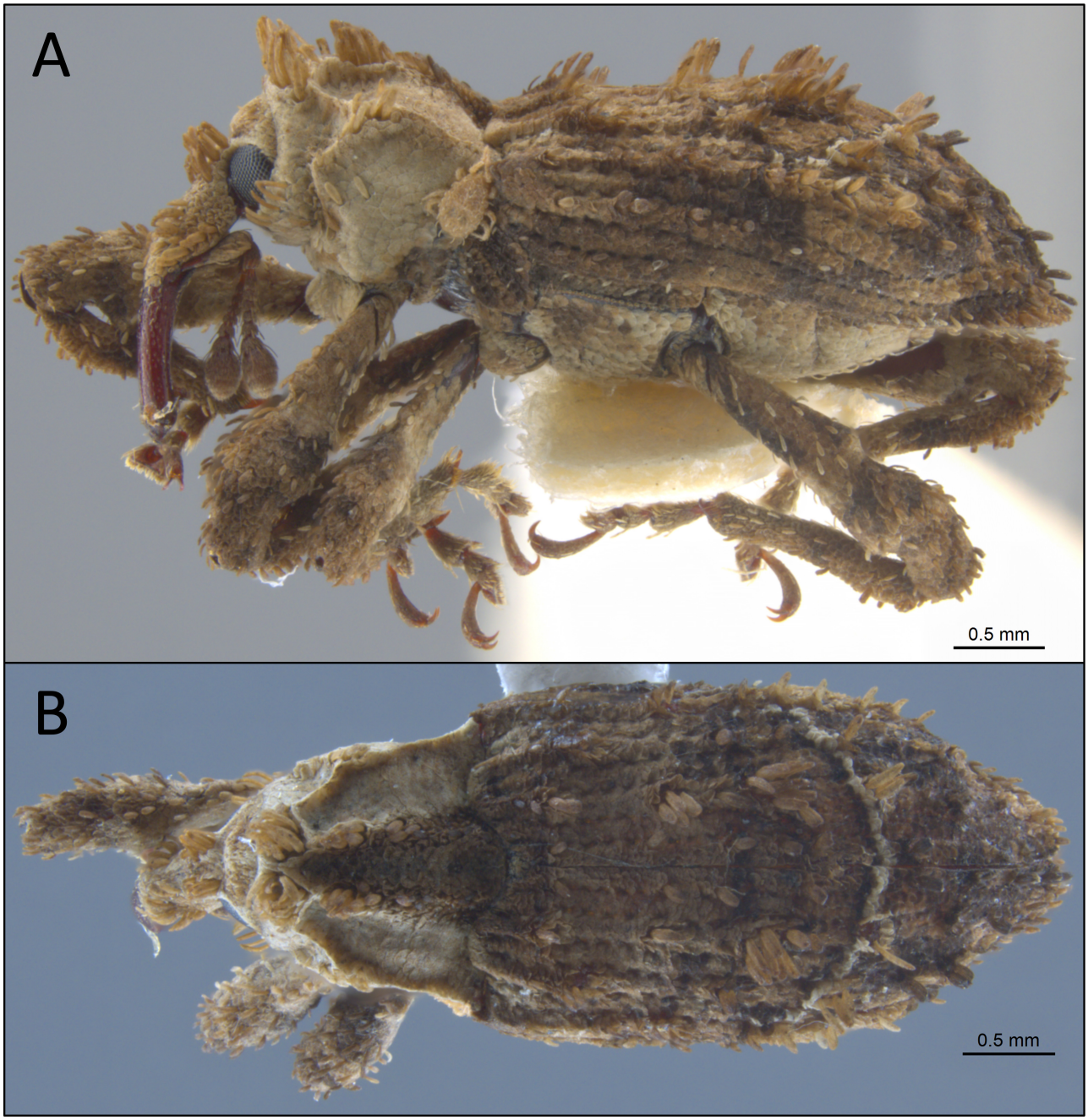

FIGURE 1. *Karekizo depressus* **sp. n.** (pre-dissection holotype male). A) Lateral. B) Dorsal.

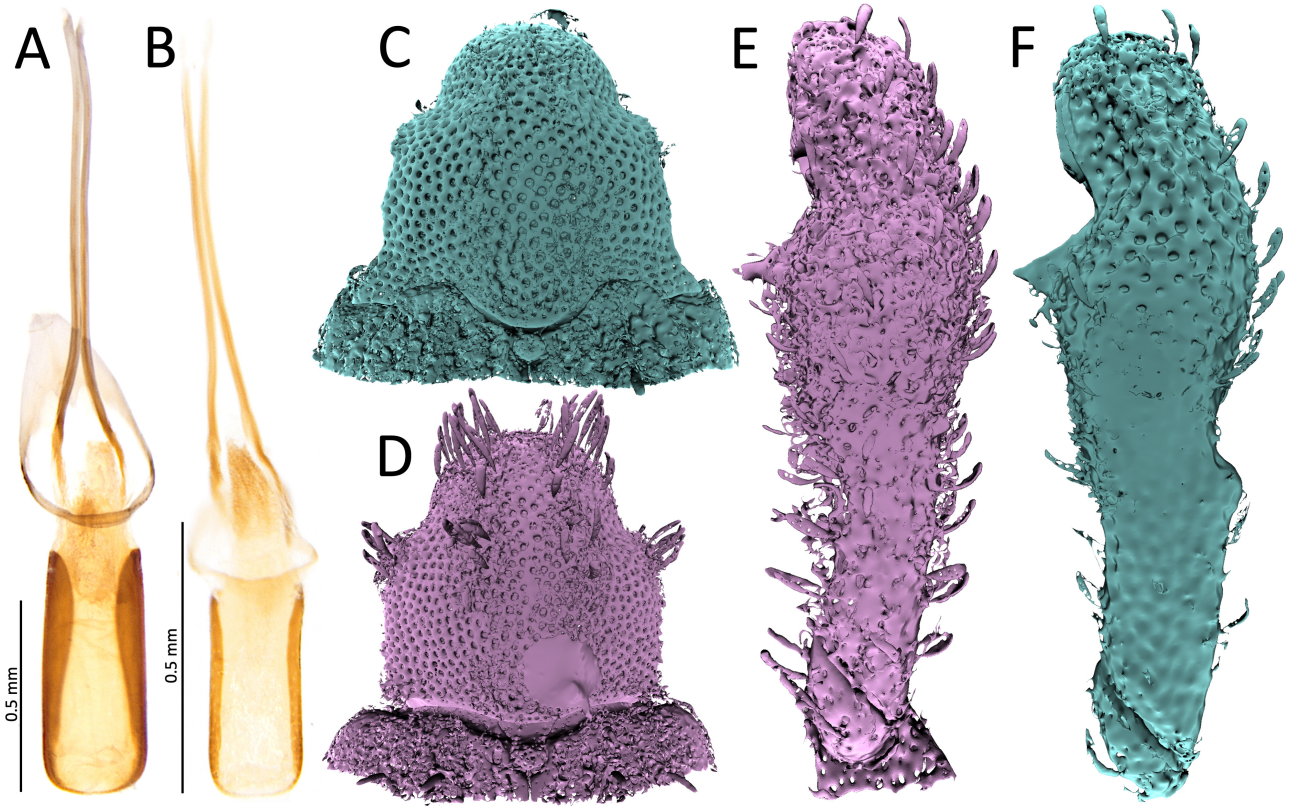

FIGURE 2. A) *Karekizo depressus* **sp. n.** aedeagus. B) *Karekizo impressicollis* Morimoto, 1962 aedeagus. C) *K. depressus* pronotum (micro-ct). D) *K. impressicollis* pronotum. E) *K. impressicollis* forefemur. F) *K. depressus* forefemur.

X-ray microtomography

In addition to morphological differences mentioned in the key below, several characters of taxonomic value were discovered using X-ray microtomography. Additionally, it was found that although the pronotum of *Karekizo* has generally been described as "longitudinally ridged", the micro-CT scans revealed that most of the ridging is actually a product of erect scales piled in rows, and that the underlying cuticle is not prominently ridged in any particular way (Fig. 2C, D).

The micro-CT scan of the *K. depressus* holotype show a pronotum with punctures not dense along the midline (Fig. 2C), however the pronotal scan of *K. impressicollis* show an increase in the interspaces between punctures along the midline in the anterior half (Fig. 2D). The pronotum also differs at the posterior-lateral angle; this extends out forming an acute angle in *K. depressus* (Fig. 2C), but is nearly right-angled in *K. impressicollis* (Fig. 2D). Elytral intervals 3–5 extend forward much more prominently in *K. depressus* covering parts of the pronotum (Fig. 2C), whereas in *K. impressicollis* these intervals are much less prominent (Fig. 2D). The microtomographic scans also show clearly the differences in femur shape; *Karekizo depressus* with a prominent depression at the midpoint along the dorsal edge (Fig. 2F), and *K. impressicollis* with a straight dorsal edge (Fig. 2E).

The present study has demonstrated that X-ray microtomography can be effectively used to examine a cuticle surface which is generally otherwise hidden by a dense cover of scales. Although non-scaled insects often produce clean scans (e.g., see Hita-Garcia *et al.* 2019), the presence of scales in insects such as weevils create a challenge when trying to visualize the cuticular surface as the scales obscure the underlying cuticle during the scanning process and ultimately create noise which results in non-existent bumps, struts, and holes in the final 3D model. Nonetheless, it was found here that by varying the threshold range during segmentation, one can easily determine which elements are remnants of scales and which elements form the underlying cuticle, thereby allowing for comparisons of true underlying cuticle structure.

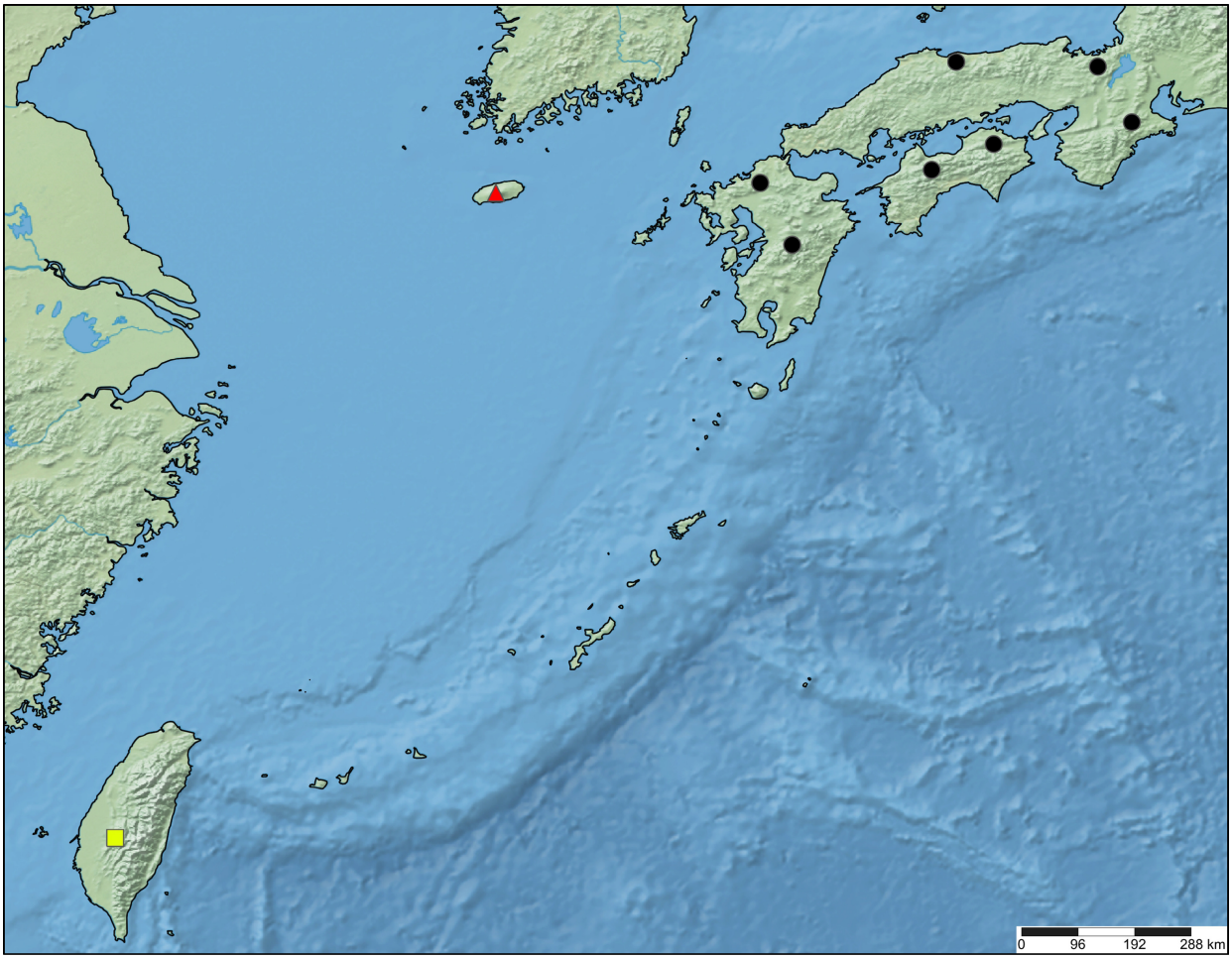

FIGURE 3. Map showing the collection locality of *Karekizo depressus* **sp. n.** (yellow square; Taiwan, Chiayi Hsien, Fen-Chi-Hu) and new country record for *K. impressicollis* Morimoto, 1962 (red triangle; South Korea, Jeju Island, Yonshil, Mt. Hallasan). The black circles represent collection localities of examined specimens of *K. impressicollis*.

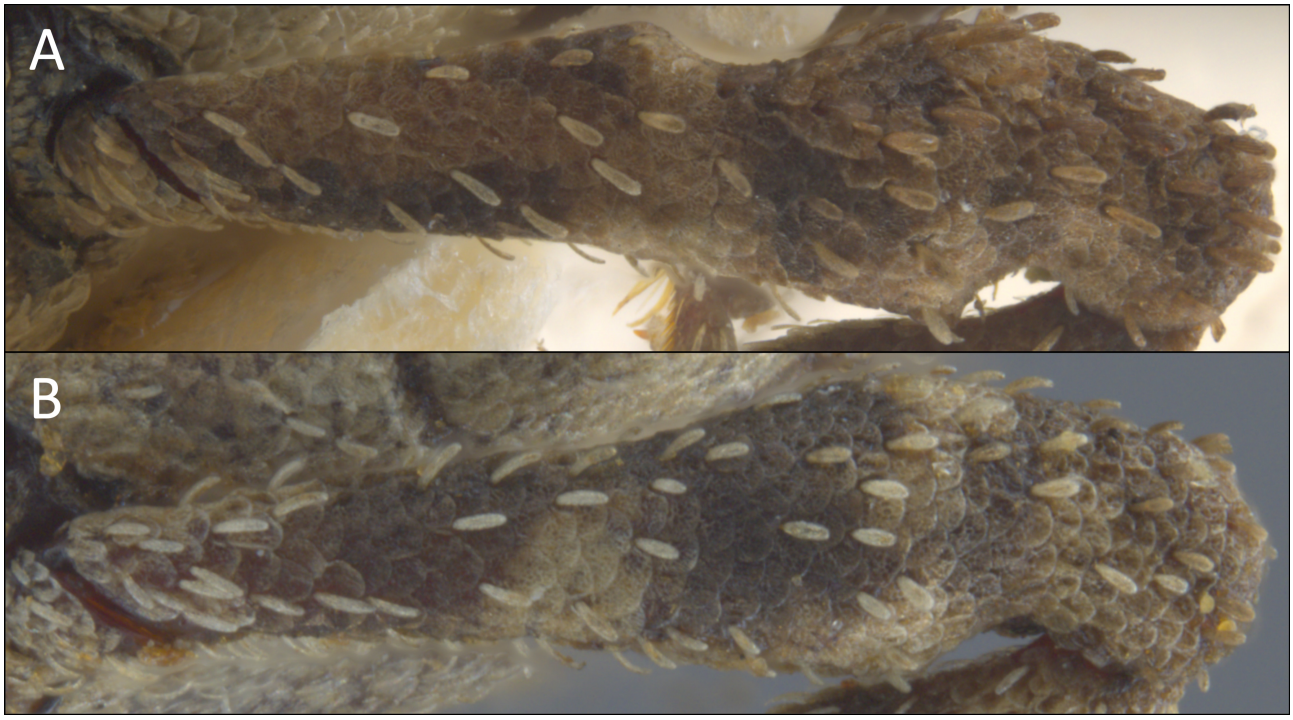

FIGURE 4. A) *Karekizo depressus* **sp. n.** forefemur. B) *Karekizo impressicollis* Morimoto, 1962 forefemur.

A key to the species of *Karekizo*

1 Tufts of erect scales along third elytral intervals unicolorous (dark orange) (Fig. 1); femora with distinct depression dorsally near mid-point (Fig. 2F, 4A); tooth on mid- and hind-femora large, distinct; sclerotized edges of aedeagus convergent in anterior half in dorsal view (Fig. 2A). . . . . .*K. depressus* **sp. n.**

- Tufts of erect scales along third elytral interval alternating in color (dark and yellow) (Fig. 5); femora without depression dorsally at mid-point (Fig. 2E, 4B); tooth on mid- and hind-femora small to absent; sclerotized edges of aedeagus parallel in dorsal view (Fig. 2B) . . . . . .*K. impressicollis* Morimoto, 1962

A first record of *K. impressicollis* from South Korea

In the process of comparing specimens of the type species *K. impressicollis* (listed below) with the newly described *K. depressus*, one specimen of *K. impressicollis* from South Korea (Fig. 3) was discovered in the Kyushu University Museum Collection. This represents the first record of *K. impressicollis* outside of Japan and from South Korea (based on lack of records in Hong *et al.* 2011 and Alonso-Zarazaga *et al.* 2017). Notably, this specimen was collected at Mt. Hallasan, further supporting the association of the genus with mountainous regions.

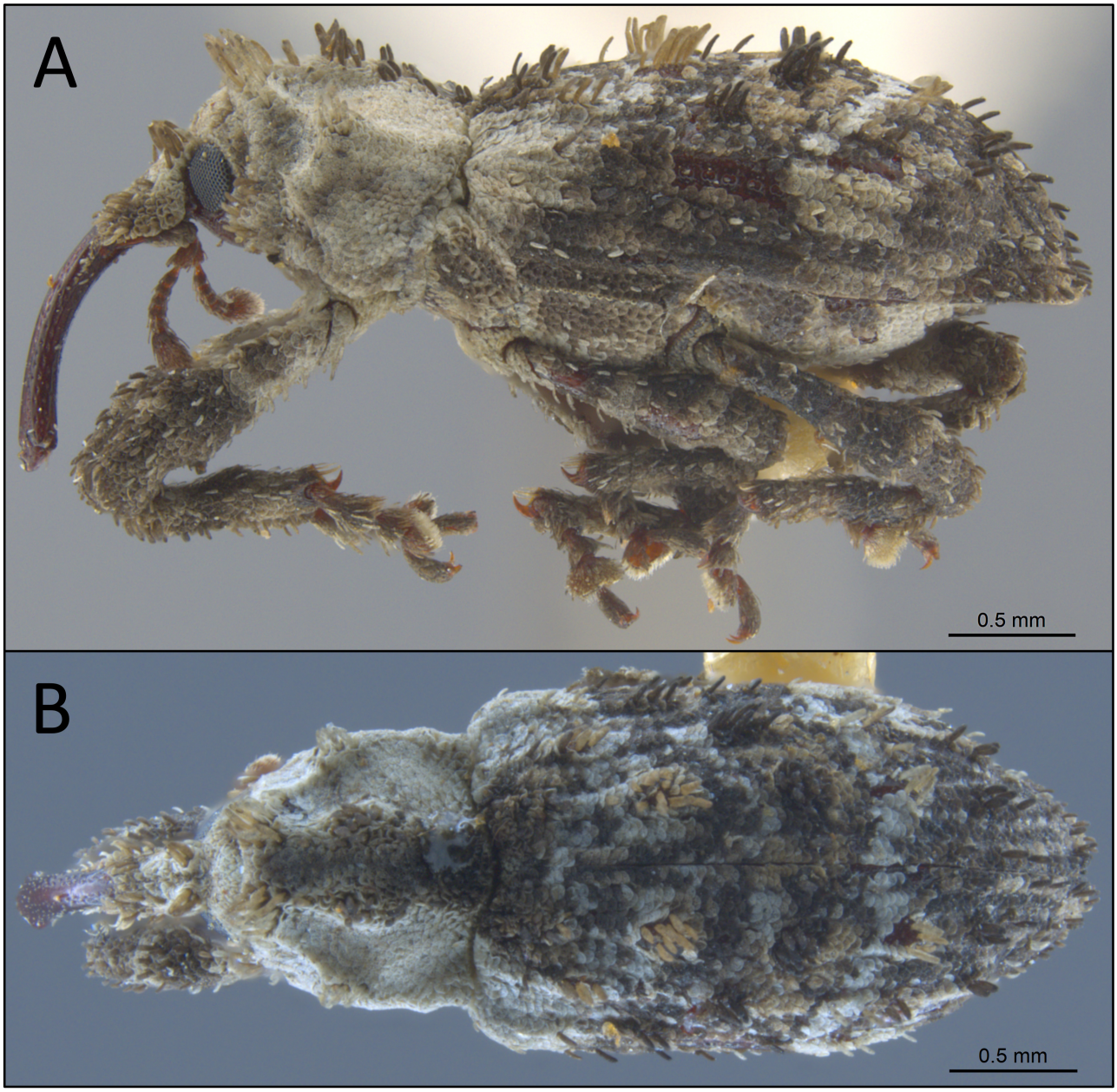

FIGURE 5. *Karekizo impressicollis* Morimoto, 1962 (JHL_KAR_016). A) Lateral. B) Dorsal.

Material examined (*Karekizo impressicollis*). JAPAN: Fukuoka Prefecture: Mt. Kumado, 3.V.1959, (1, KUM), JHL_KAR_016; **Kagawa Prefecture:** summit of Mt. Ohtaki, 4.VI.1995, on fallen wood of *Carpinus*, S. Hisamatsu, (4, KUM), JHL_KAR_018, JHL_KAR_019; **Kochi Prefecture:** Mt. Tebako, 7–10.VIII.1957, K. Morimoto, (1, KUM), JHL_KAR_015; **Kumamoto Prefecture:** Shiiya-toge, 13.VI.1976, H. Irie, (1, KUM), JHL_KAR_010; same locality, 24.VII.1986, H. Irie, (4, KUM), JHL_KAR_011 – JHL_KAR_014; **Kyoto Prefecture:** Kyoto University Experimental Forest, 31.V.2010, Aoki Junichi, (1, KUM), JHL_KAR_020; **Mie Prefecture:** Ichishi-gun, Mie University Forest, 11.VI.1956, H. Ichihashi, (1, KUM), paratype, JHL_KAR_005; **Tottori Prefecture:** Mt. Daisen, 21.VII.1962, K. Shirai, (2, KUM), JHL_KAR_002, JHL_KAR_003; same locality, 10.VII.1951, S. Shibanai, (1, KUM), paratype, JHL_KAR_004; **SOUTH KOREA:** Jeju Island, Yonshil, Mt. Hallasan (Fig. 3), 24.VII.1990, S. Miyamoto, (1, KUM), JHL_KAR_017.

Acknowledgements

I thank Dr. Naomichi Tsuji, Dr. Shunsuke Imada, Dr. Munetoshi Maruyama, and Dr. Toshiharu Mita (Kyushu University) for their assistance in preparing a loan of specimens for this project.

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