Zootaxa 1740: 15–27 (2008)
www.mapress.com/zootaxa/

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

A new *Wagnerinus* (Coleoptera: Curculionidae) from northern Japan: Description including a DNA barcode

HIRAKU YOSHITAKE, TOSHIHIDE KATO, UTSUGI JINBO & MOTOMI ITO

Department of General Systems Studies, Graduate School of Arts and Sciences, The University of Tokyo, Tokyo 153-8902, Japan. E-mail: ceutorhynchinae@yahoo.co.jp

Abstract

Wagnerinus frugivorus **sp. nov.** (Ceutorhynchinae: Ceutorhynchini) is described from Hokkaido, northern Japan, based on morphological and biological characters. Morphologically, this new species closely resembles *Wagnerinus carinulatus* (Faust) from the Russian Far East and *W. costatus* (Hustache) from Japan and Korea in having an emarginate anterior margin of the rostrum, sternite VIII diminished to a pair of small sclerites, and longitudinal rows of endophallic sclerites. However, it is distinctive enough to be distinguished from *W. carinulatus* and *W. costatus* mainly by the more conspicuous mucrones of mid and hind tibiae, deeper concavities of ventrites I and II, and larger paired prominences of ventrite V. Also, *W. frugivorus* clearly differs from *W. costatus* in terms of host plant utilization, the former feeds on the seed capsules of *Weigela middendorffiana* (Caprifoliaceae) as larvae, while the latter utilizes midge galls on the axillary buds of *Weigela* species. In addition to general taxonomic information, we provide a 1366-bp fragment of the mitochondrial cytochrome oxidase subunit I gene from the holotype as a DNA barcode of *W. frugivorus* and discuss the importance of DNA barcoding combined with species descriptions.

Key words: DNA barcoding, host plant, new species, seed capsule, *Wagnerinus*, *Weigela middendorffiana*

Introduction

The weevil genus *Wagnerinus* Korotyaev, 1980, in the tribe Ceutorhynchini, subfamily Ceutorhynchinae (Colonnelli 1984, 2004), is defined by the slender rostrum, seven-segmented antennal funicle, and sparse vestiture and minute granules on the elytral intervals (Korotyaev 1980). Presently, this genus comprises four species from Northeast Asia: *Wagnerinus carinulatus* (Faust, 1887), *W. costatus* (Hustache, 1916), *W. harmandi* (Hustache, 1916), and *W. shikotanus* Korotyaev, 1981 (Korotyaev 1981, Colonnelli 2004). As Kato *et al.* (2006) suggested, not a few number of undescribed species of *Wagnerinus* occur primarily in Japan. However, species delimitation based only on morphology is often very difficult in this genus due to the presence of morphologically similar species.

Wagnerinus costatus is associated exclusively with galls on the axillary buds of *Weigela hortensis* (Caprifoliaceae) induced by the gall midge *Asphondylia baca* Monzen, 1937 (Diptera: Cecidomyiidae) (Sugiura *et al*. 2004). Obligatory cecidophages (specialist galleaters) are rarely found among herbivorous insects, such as Lepidoptera and Coleoptera. The Ceutorhynchinae is a diverse taxon in terms of host plant utilization, but no other cecidophages than *W. costatus* have been reported from the subfamily. Therefore, *Wagnerinus* is a good target to study the evolution of this unique habit, but no ecological information has hitherto been available for *Wagnerinus* weevils except *W. costatus*.

Through field surveys in Hokkaido, northern Japan, we found an undescribed *Wagnerinus* species, which is distinctive enough not to be confused with any other congeners. This species shows a remarkable difference

Accepted by G. Morse: 12 Feb. 2008; published: 2 Apr. 2008

from *W. costatus* in host plant utilization, feeding on the reproductive organ of a *Weigela* species during larval stage. We regard it as a key taxon for future studies of *Wagnerinus*, because it indicates the presence of an intrageneric variation in host plant utilization and thus the usability of ecological data as a taxonomic character of this genus. Considering the difficulty in morphological discrimination of *Wagnerinus* weevils, this genus should be studied by an integrative approach based on morphological, molecular, and ecological characters.

Recently, DNA barcoding (Hebert *et al*. 2003a, b) has received considerable public attention with the development of international DNA barcoding projects (Consortium for the Barcode of Life [CBOL], http://www.barcoding.si.edu/; Barcode of Life Data Systems [BOLD], http://www.barcodinglife.org/). Although the concept and utility of this standardized tool has been debated (Blaxter 2003, Sperling 2003, Moritz & Cicero 2004, Ebach & Holdrege 2005a, b, Schindel & Miller 2005, Rubinoff 2006, Dasmahapatra & Mallet 2006, DeSalle 2006), it is quite evident that general taxonomic datasets including morphological, ecological, and distributional data can be supplemented effectively with DNA barcodes, which are short gene sequences taken from a standard gene, for species-level identification (Ball *et al.* 2005, Brower 2006, Hajibabaei *et al.* 2006a, b, Scheffer *et al.* 2006, Smith *et al.* 2006). Therefore, providing DNA barcodes as part of the taxonomic information at the level of descriptive taxonomy improves the quality of the fundamental basis of all other biological research, especially in poorly known groups such as Insecta. To our knowledge, only a few insect species descriptions including DNA barcodes have been published to date (Brown *et al.* 2003, Burns *et al.* 2006).

In order to promote further studies of *Wagnerinus*, we describe herein a new species of the genus from northern Japan, including its DNA barcode and ecological characteristics. In addition to discussing the taxonomic position and host plant utilization of the new species, we discuss the importance of DNA barcoding in species descriptions.

Material and methods

This study was based mainly on dry specimens collected in early July 2005 and preserved in the private collection of H. Yoshitake (PCHY). In addition, old specimens in the Entomological Laboratory, Kyushu University, Fukuoka (ELKU) and Museum of Nature and Human Activities, Hyogo (MNHAH) were examined for this study. The holotype of the new species described herein is deposited in ELKU, and its paratypes are deposited in PCHY, ELKU, and MNHAH. Images and primary data of all the specimens used in this study are available at a web-based database system (WIMAGES: http://moth.c.u-tokyo.ac.jp:3001/taxon/view/Family/Curculionidae/).

All descriptive work in this study was completed by H. Yoshitake. External structures were observed under a Nikon SMZ1500 stereoscopic microscope. Measurements of various body parts are coded as follows: LB = length of the body, from the apex of the pronotum to apices of the closed elytra; LR = length of the rostrum, in a lateral view; WP = maximum width across the pronotum; LP = length of the pronotum, from the base to apex along the midline; WE = maximum width across the elytra; LE = length of the elytra, from the level of the basal margins to the apices of the closed elytra. All measurements are in mm. Details of several external structures were examined using a HITACHI-3000N scanning electron microscope under 50x to 400x of magnification. Habitus photographs were taken with a Nikon Coolpix995 attached to the stereoscopic microscope. To examine genitalia, specimens were macerated in hot water and dissected under the stereoscopic microscope. The abdomen was first removed from the body and then cleaned in hot 10% KOH solution for 5 to 10 minutes. Genitalia extracted from the abdomen were mounted on slides with glycerol (males) or pure water (females). Male and female genitalia were studied with a Nikon Eclipse 55i optical microscope and drawn in detail through an attached camera lucida. Scale bars were calibrated using a Nikon objective micrometer. Verbatim label data indicated by quotation marks are provided for the holotype. Label breaks are indicated by a slash ("/").

DNA barcoding was conducted chiefly by T. Kato. All legs on the right side of the body were removed from specimens preserved in 99.5% ethanol, and DNA was extracted using a DNeasy Tissue Kit (Qiagen, Hilden, Germany). The legs were homogenized in 180μl PBS with 20μl proteinase K and incubated at 72°C for 24 h. After incubation, total genomic DNA was extracted following the manufacturer's instructions. A DNA fragment of the mitochondrial cytochrome oxidase subunit I (COI) gene was amplified using the polymerase chain reaction (PCR) with the primers COI-F3 and COI-R1 (Table 1). The template profile was as follows: 94.0°C for 5 min; 35 cycles at 94.0°C for 45 sec, 48.0°C for 45 sec, and 72.0°C for 90 sec; with a final extension at 72.0°C for 8 min. PCR was performed in a reaction volume of 40 μl using 10× EX *Taq* Buffer (Takara Bio, Tokyo, Japan), 0.2 mM of each dNTP, 0.5 μM of each primer, 0.5 U/μl of EX *Taq* DNA polymerase (Takara Bio), and 0.8 μl of temperate DNA. The PCR product was purified using Montage PCR (Millipore, Billerica, MA, USA) and served as template for cycle sequencing reactions with CEQ quick start mix (Beckman Coulter, Fullerton, CA, USA) following the manufacturer's instructions. The internal primers used are listed in Table 1. After ethanol precipitation, the cycle sequencing products were sequenced using the CEQ8000 Genetic Analysis System (Beckman Coulter). DNA sequences obtained in both directions were assembled and edited using ATGC version 4.0 (Genetyx, Tokyo, Japan).

Field surveys to gather fundamental ecological information were carried out by H. Yoshitake from 5 to 7 July 2005 at the following three sites in northern Japan: 1) Aizankei, 930–1035 m elevation, 43°43'28.4"N, 142°48'32.4"E to 43°42'51.2"N, 142°48'15.8"E, Kamikawa, Hokkaido; 2) Yukomanbetsu, 1035–1100 m elevation, 43°38'50.7"N, 142°47'24.3"E to 43°39'0.81"N, 142°47'53.0"E, Higashikawa, Hokkaido; and 3) Horoshikatouge Pass, 1050–1085 m elevation, 43°19'52.4"N, 143°10'35.7"E to 43°20'13.9"N, 143°9'45.8"E, Kamishihoro, Hokkaido (Fig. 1). Weevil larvae were collected and reared under laboratory conditions to confirm the larval-adult association. The host plant of the newly described species was identified by M. Ito, and voucher specimens were deposited at the herbarium of the University of Tokyo (TI). Plant nomenclature follows Ohba (1993) and Yonekura & Kajita (2003).

TABLE 1. List of primers used in this study.

Primer	Sequence	Source
COI-F3[a]	5'-ACT AAC CAT AAA GAT ATT GG-3'	This study
C1J-1751ceu[a]	5'-TAG GAG CAC CAG ATA TGG CAT TTC-3'	Modified from Simon *et al.* (1994)
COI2-1[b]	5'-CTT TAT CAA CAT TTA TTT TGA TTT TTT-3'	Tuda *et al.* (1995)
COI-iF1[b]	5'-GAT ACA TAT TAT GTA GTA GC-3'	This study
COI-iR2[b]	5'-GCT ATT ATA GCA TAA ATT ATT C-3'	This study
COI-iR3[b]	5'-CCC TTA TCT ACA ATT CTT CTT A-3'	This study
COI2-2ceu[b]	5'-ACT CCT ATA AAT ATA GTT AAA AAT T-3'	Modified from Tuda *et al.* (2004)
COI-R1[a]	5'-TCC ATT GCA CTA TTC TGC C-3'	This study

a: PCR primers. b: Internal primers.

Wagnerinus frugivorus **Yoshitake, sp. nov.**
(Figs. 2–28)

Diagnosis. This species is distinguished from other congeners mainly by the following characteristics in males: mucrones of mid and hind tibiae larger, more acute (Figs. 12, 13); concavity on ventrites I and II deeper (Fig. 14); paired prominences on ventrite V larger (Figs. 15, 16).

Description. Male. LB: 2.25–2.68 (mean 2.48). LR: 1.11–1.36 (mean 1.23). WP: 0.81–1.00 (mean 0.91). LP: 0.63–0.76 (mean 0.70). WE: 1.40–1.69 (mean 1.56). LE: 1.68–2.03 (mean 1.86). N = 5 for all measurements. Habitus as shown in Figs. 2 and 3.

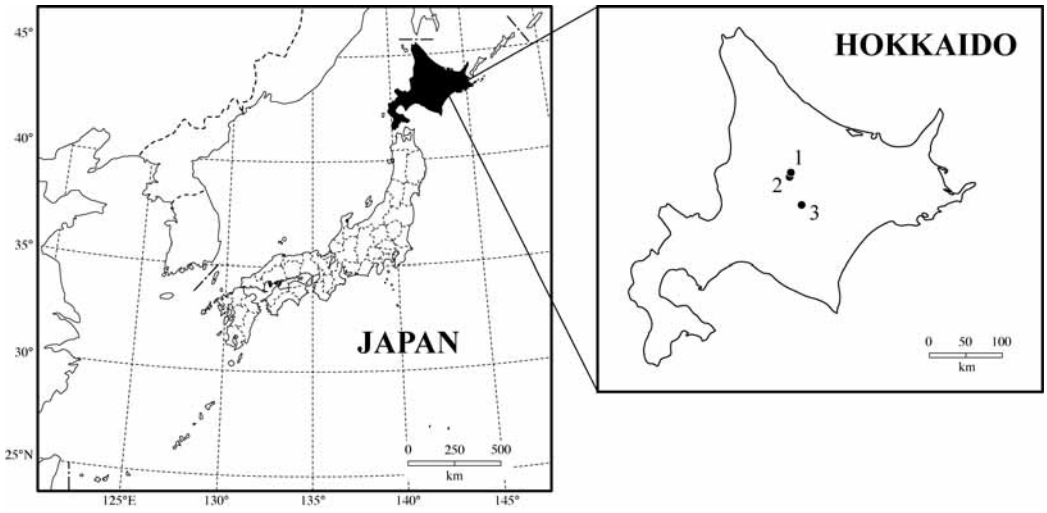


FIGURE 1. Study sites (filled circles).

Black in general appearance; apex of rostrum, antennae, and legs tinged with red.

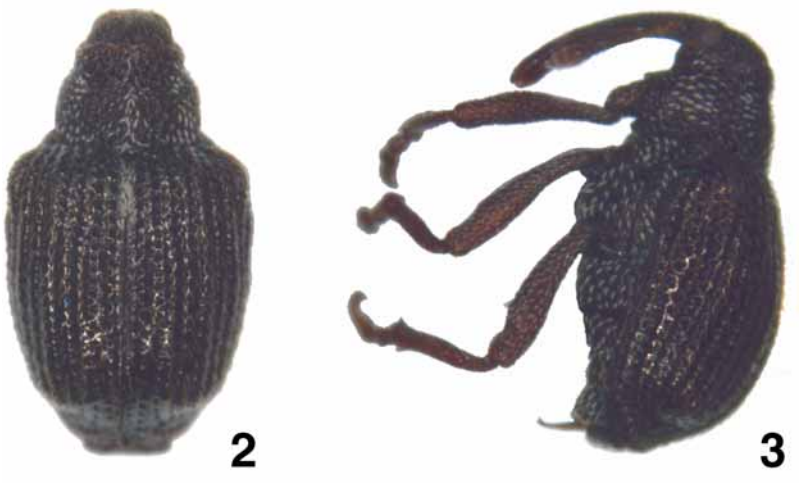
Body surface very shiny, but evenly covered with ochraceous secretions in life (Fig. 26). Head moderately covered with dark hair-like scales; scales becoming paler on frons. Rostrum moderately covered with dark hair-like scales at base; scales becoming sparser and minute apically. Prothorax moderately covered with dark hair-like scales, mingled with light-colored scales; dorsum with 3 stripes of white elliptic to lanceolate scales; basal margin fringed with row of minute ovate scales; latero-ventral parts with stripes of white elliptic to lanceolate scales; ocular lobes fringed with short vibrissae. Elytra sparsely covered with dark hair-like scales; interval I with longitudinal postscutellar patch of dense elliptic to lanceolate white scales. Legs moderately covered with white hair-like to linear scales; scales replaced with brown setae in apical part of each tibia. Lateral pieces of meso- and metasterna sparsely covered with white elliptic to lanceolate scales, except upper part of mesepimera and posterior part of metepisterna densely covered with white lanceolate scales. Sterna moderately covered with light-colored elliptic to lanceolate scales, sparsely mingled with hairs and linear scales; scales becoming sparser on the periphery. Venter moderately covered with elliptic to lanceolate white scales, sparsely mingled with hairs and linear scales; scales becoming sparser on the periphery; ventrites III and IV with scales in line on disc; ventrite V covered with dark hairs and white linear scales on prominences; posterior margins of prominences fringed with dense white acicular to lanceolate scales. Tergite VIII mostly covered with dark fine incurved hairs, mingled with white linear scales.

Head (Figs. 4, 5) finely reticulately punctured, with long glossy median carina extending from vertex to base of forehead; forehead faintly depressed, slightly wider than base of rostrum. Eyes (Figs. 4, 5) moderately prominent from outline of head, not approximated anteriorly. Rostrum (Figs. 4, 5) slender, 1.70–1.79 times as long as prothorax, evenly moderately curved; dorsum with 3 glossy carinae extending from base to middle, minutely punctured along carinae, and shallowly emarginate at apex; punctures becoming minute and sparser apically; sides slightly dilated from constricted base, subparallel in basal 1/3, slightly dilated to antennal insertions, subparallel to apical 1/4, then gradually expanded toward apex; antennal scrobes well-separated along entire length. Antennae (Figs. 6, 7) inserted at middle of rostrum; scape moderate in length, nearly as long as funicular segments I, II, III, IV, and V combined, bluntly produced into short lamina at apex; funicle 7-segmented, with segment I as long as II, II nearly twice as long as III, III nearly as long as IV, IV slightly longer

than V, V as long as VI, VI as long as VII, and VII evidently longer than wide; club lanceolate, finely pubescent except basal 1/4.

Prothorax (Figs. 8, 9) 1.28–1.33 times as wide as long, closely punctured; dorsum densely finely punctured along midline; apical margin slightly anteriorly produced, widely shallowly emarginated in middle, nearly flat in profile; basal margin bisinuate, nearly flat, not raised to basal margin of elytra, smooth, not crenulate; sides slightly dilated from constricted base, widest at basal 1/3, slightly narrowed to apical 1/3, then rapidly convergent toward subapical constriction.

Elytra (Fig. 10) 1.18–1.21 times as long as wide, very shiny, widest just behind humeri, subparallel-sided in basal half, then gently convergent toward subapical calli; suture nearly straight; each interval with 1–2 rows of small squamate granules; odd-numbered intervals more prominent than even-numbered ones; striae shallow; each puncture in striae oval, bearing minute hair, flanked by granules, separated by distance greater than its diameter; basal margin fringed with row of minute hairs.

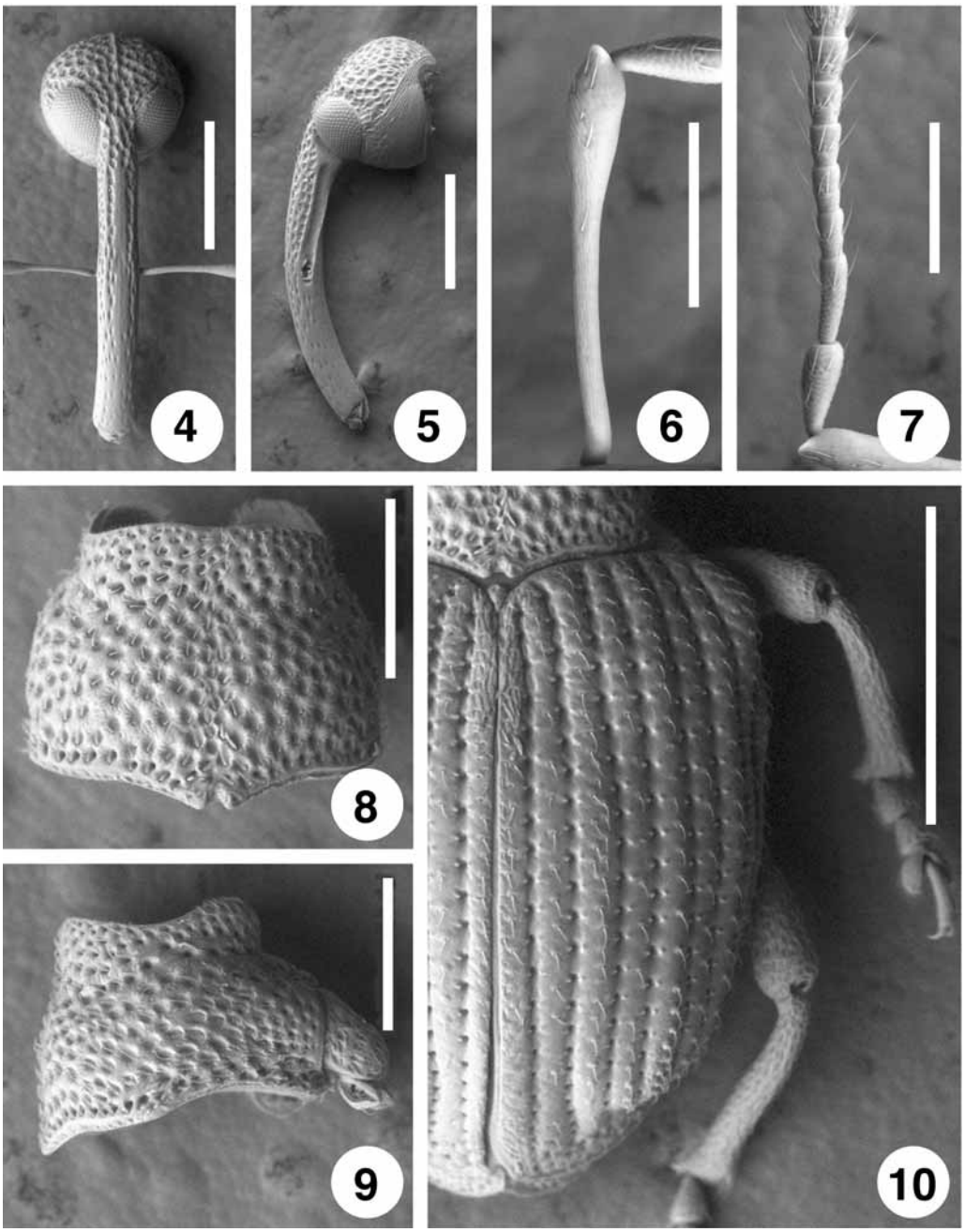


FIGURES 2, 3. *Wagnerinus frugivorus* Yoshitake. 2. Dorsal habitus. 3. Lateral habitus.

Legs relatively slender; femora clavate, toothed; each tooth small, obtuse, concealed by bundle of suberect white scales; hind femora simple, lacking jumping organ; tibiae (Figs. 11–13) mucronate on mid and hind legs; each mucro acute, conspicuous; tarsi simple, bearing no projection; claws without setae, appendiculate; appendages large, acute.

Sterna moderately punctured; prosternum densely punctured in intercoxal area, with deep rostral groove before coxae; groove limited laterally by keels; mesosternum densely punctured in anterior part, with shallow circular depression in middle for reception of rostrum; metasternum coarsely punctured on sides, with shallow longitudinal depression in middle for reception of rostrum. Venter (Figs. 14, 15) moderately punctured, opaque; ventrite I widely concave on disc, with strong luster in concavity; ventrite II narrowly concave in middle; ventrites III and IV with punctures in line; ventrite V with large semicircular concavity on disc; concavity deep, finely punctured, bearing large acute prominence on each side. Tergite VII with pair of minute plectral tubercles; each tubercle bearing hair. Pygidium (Fig. 16) transverse-pentagonal, nearly twice as wide as long, opaque, densely punctured; upper flange smooth, lacking projection, arcuate downward on each side. Sternite IX (Fig. 19) diminished to pair of small oblong-ovate sclerites; spiculum gastrale (Fig. 19) slightly longer than aedeagal body, nearly as long as aedeagal apodemes, faintly curved leftward. Tegmen (Fig. 20) with slender apodeme; apodeme slightly longer than diameter of tegminal ring. Aedeagal body (Figs. 17, 18) slender, weakly curved, with blunt apical projection on ventral side; sides moderately expanded from base, slightly constricted at basal 1/4, gently narrowed to apical 1/4, then straightly narrowed to subapical part, and

finally more strongly straightly convergent to apex; apodeme slender, nearly as long as body. Endophallus (Fig. 17) moderate in length, slightly longer than aedeagal body, with 2 plate-like sclerites at base, with 4 longitudinal rows of dentiform sclerites in middle; plate-like sclerites followed by obtuse triangular spicules; rows of dentiform sclerites followed by acicular spicules; triangular spicules dense, forming spiculate field in basal part; acicular spicules rather sparse, forming indistinct spiculate field in subapical part.

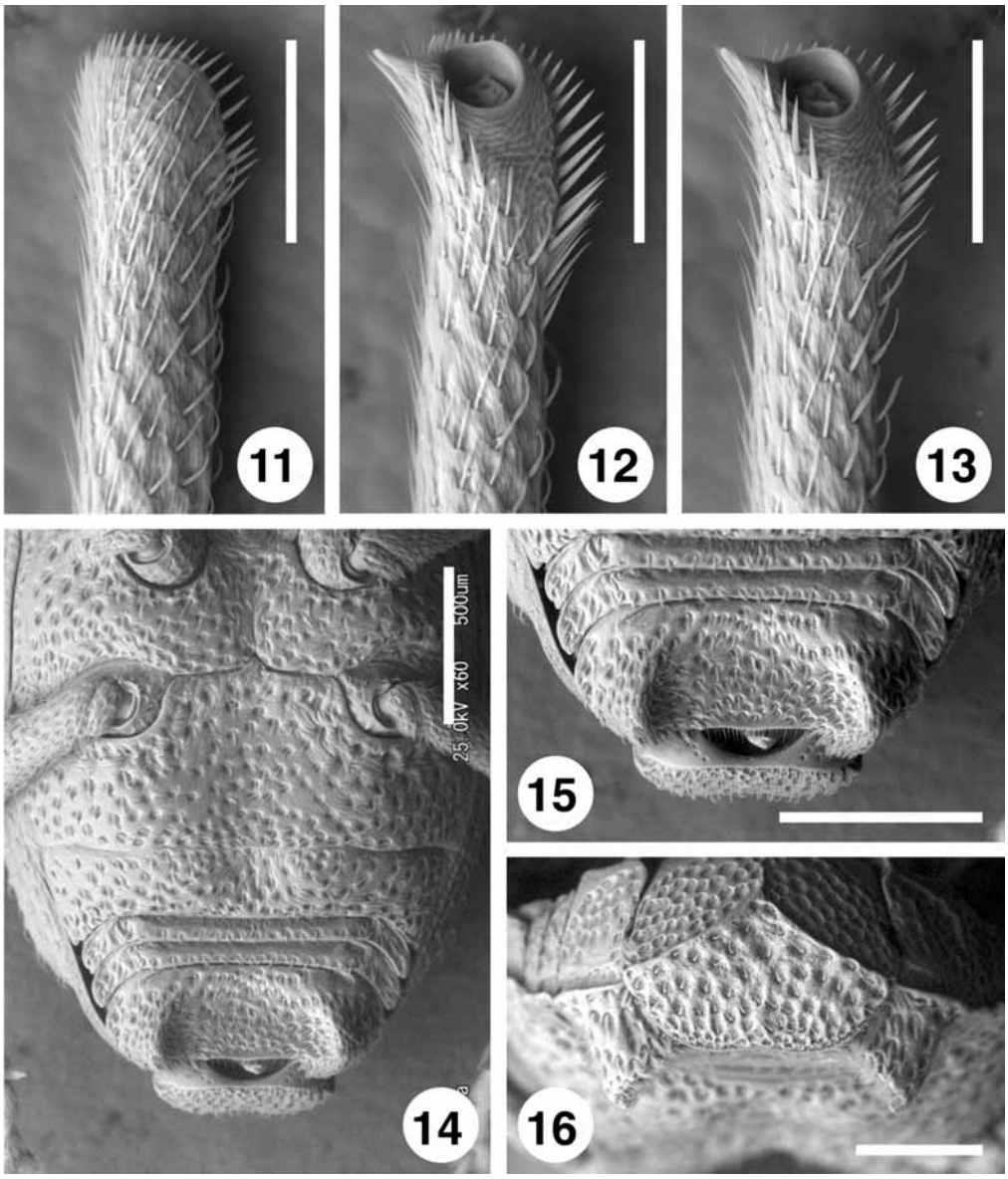


FIGURES 4–10. *Wagnerinus frugivorus* Yoshitake. 4. Dorsal view of head, male. 5. Lateral view of head, male. 6. Antennal space. 7. Antennal funicle. 8. Dorsal view of prothorax. 9. Lateral view of prothorax. 10. Elytron. Scale: 0.50 mm for 4, 5, 8, 9; 0.20 mm for 6, 7; 1.00 mm for 10.

Female. LB: 2.35–2.68 (mean 2.57). LR: 1.15–1.33 (mean 1.28). WP: 0.88–0.99 (mean 0.95). LP: 0.65–0.75 (mean 0.72). WE: 1.49–1.68 (mean 1.62). LE: 1.80–2.00 (mean 1.93). N = 5 for all measurements.

Rostrum 1.74–1.79 times as long as prothorax, slightly thinner, polished in apical half. Antennae inserted just behind middle of rostrum. Prothorax 1.30–1.35 times as wide as long. Elytra 1.18–1.21 times as long as

wide. All tibiae simple, not mucronate. Ventrite I faintly narrowly concave in middle. Ventrite II simple, lacking concavity. Ventrite V slightly inflated on disc. Pygidium smaller, narrower, fan-shaped. Sternite VIII (Fig. 21) with slender arms nearly as long as apodemes, apically furnished with pair of patches of several long setae; apodemes moderate in length. Ovipositor (Fig. 22) with coxites robust, nearly half as long as plate of sternite VIII, nearly twice as long as styli, partially sclerotized, furnished with several minute setae; each coxite followed by plate-like sclerite; styli cylindrical, nearly three times as long as broad, inserted apicolaterally, apically furnished with several short setae. Spermatheca (Fig. 23) larger than apex of ovipositor; cornu long, attenuate, with small projection at apex; collum moderately developed; ramus indistinct, almost uniformly continuous with body; gland short, slightly longer than body; insertions of duct and gland close to each another. Otherwise, essentially as in males.

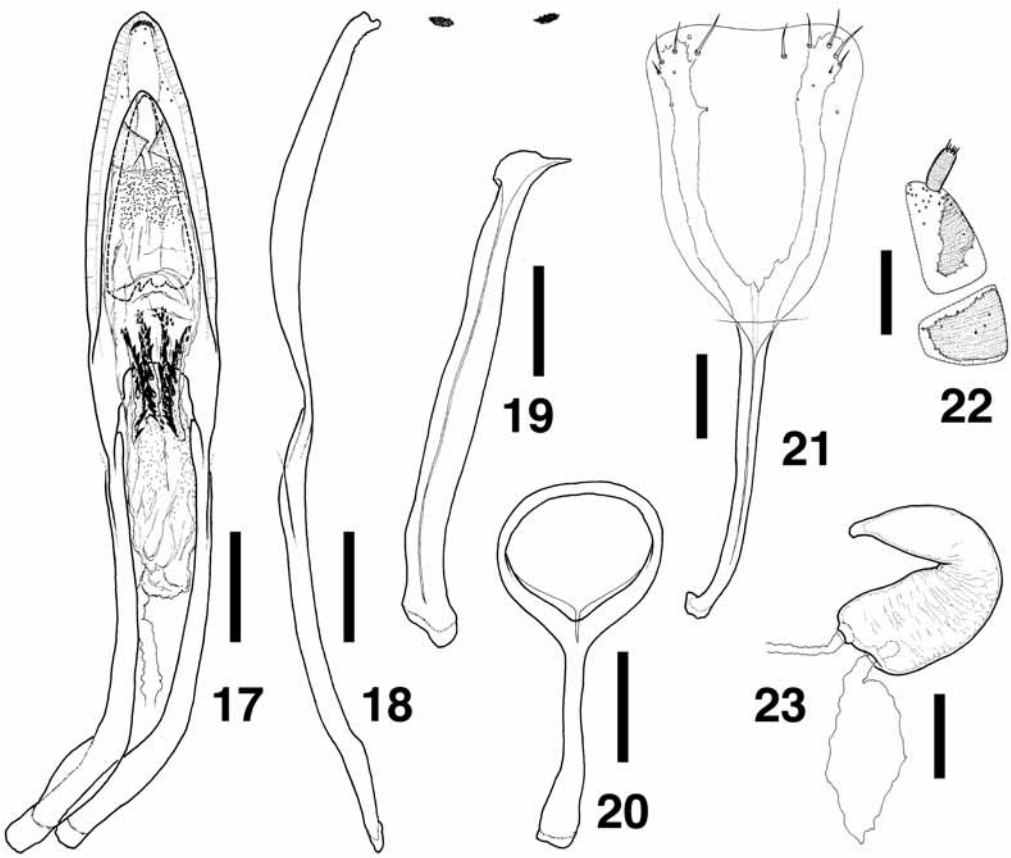


FIGURES 11–16. *Wagnerinus frugivorus* Yoshitake. 11. Front tibia, male. 12. Mid tibia, male. 13. Hind tibia, male. 14. Venter, male. 15. Ventrites III–V, male. 16. Pygidium, male. Scale: 0.20 mm for 11–13; 0.50 mm for 14, 15; 0.25 mm for 16.

DNA barcode. A 1366-bp fragment of the COI gene from the holotype was determined. The sequence data including the standard barcoding region for animal species has been deposited as a DNA barcode of *W. frugivorus* in the DDBJ Nucleotide Sequence Database under accession number AB250208.

Type series. HOLOTYPE male (Type No. 3238, Voucher No. 00000001, ELKU), "[JAPAN: Hokkaido] Kamikawa / Aizankei (930–1,035 m) / N 43°43'28.4"E142°48'32.4"– / N43°42'51.2"E142°48'15.8" / 5. VII. 2005 Hiraku Yoshitake" (typed on a white card), "On *Weigela middendorffiana* / (Carr.) K. Koch / Caprifoliaceae) / [JN: *Ukon-utsugi*] / Det. Motomi Ito, 2005" (typed on a white card), "male" (typed on white card), "Right legs away / for DNA extraction" (typed on a yellow card), "[HOLOTYPE] / *Wagnerinus frugivorus* / Yoshitake, 2006" (typed on a red card); "ELKU Voucher Specimen / No. 00000001" (typed on a red card). PARATYPES (46 males and 35 females). JAPAN: HOKKAIDO. Aizankei, Kamikawa: 17 males and 10 females, same data as for the holotype (PCHY); 2 males and 2 females, 2. vii. 1982, Y. Sawada, on *W. middendorffiana*. Yukomanbetsu, Higashikawa (MNHAH): 22 males and 19 females, 1035–1100 m, 6. vii. 2005, H. Yoshitake, on *W. middendorffiana* (PCHY); 1 female, 1000 m, 6–8. vii. 1980, H. Takemoto (ELKU). 5 males and 3 females, Horoshikatouge Pass, 1050–1085 m, Kamishihoro, 7. vii. 2005, H. Yoshitake, on *W. middendorffiana* (PCHY).

Distribution. The type series were collected from mountainous areas (930–1100 m) in Daisetsuzan National Park, which is located in the center of Hokkaido, northern Japan (Fig. 1).

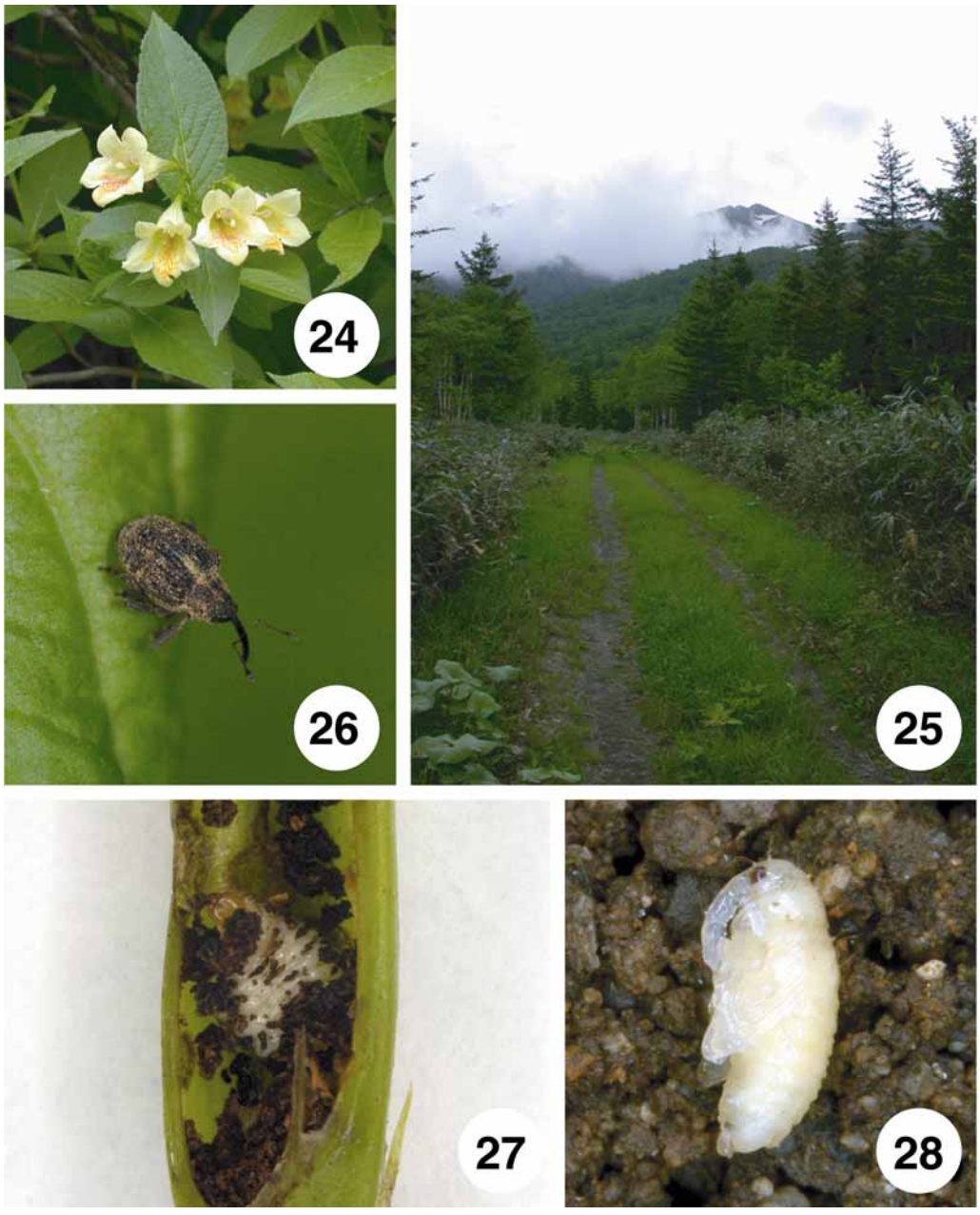


FIGURES 17–23. *Wagnerinus frugivorus* Yoshitake. 17–20. Male genitalia. 17. Dorsal view of aedeagus. 18. Lateral view of aedeagus. 19. Sternite IX. 20. Tegmen. 21–23. Female genitalia. 21. Sternite VIII. 22. Ovipositor. 23. Spermatheca. Scale: 0.20 mm for 17–20; 0.10 mm for 21–23.

Natural history. This species is associated with *Weigela middendorffiana* (Caprifoliaceae) (Fig. 24). The host plant is a deciduous shrub distributed in Sakhalin, the Kuriles, Hokkaido, northern Honshu, Ussuri, Amur, and Okhotsk, and in subalpine scrub and on exposed slopes (Ohba 1993). In all study sites, many adults

were found on *W. middendorffiana* growing along margins of subalpine forests dominated by *Picea jezoensis* (Pinaceae), *Picea glehnii* (Pinaceae), and *Betula ermanii* (Betulaceae) (Figs. 25, 26). They were diurnal and were observed feeding on flowers and leaves of *W. middendorffiana*. Female adults laid their eggs singly in the unripe seed capsules of *W. middendorffiana*. Hatched larvae grew in and fed on the capsules (Fig. 27) and pupated in the soil when they fully matured (Fig. 28). About one month elapsed between hatching and emergence. The voltinism of *W. frugivorus* is not clear at this time, although this species may be univoltine, as are most other ceutorhynchine weevils from temperate regions.

Etymology. This species was named after its larval feeding habit.



FIGURES 24–28. *Wagnerinus frugivorus* Yoshitake. 24. Host plant, *Weigela middendorffiana*. 25. Habitat in Aizankei, Kamikawa, Hokkaido. 26. Adult on a leaf of *W. middendorffiana*. 27. Third-instar larva in a seed capsule of *W. middendorffiana*. 28. Pupa in the soil.

Discussion

Taxonomic position of W. frugivorus

Currently, the genus *Wagnerinus* comprises four East Asian species: *W. carinulatus* from the Russian Far East, *W. costatus* from Japan and Korea, *W. harmandi* from Japan, and *W. shikotanus* from Shikotan Island, Japan (cf. Colonnelli 2004). These species can be divided into two distinct groups based on the structures of the elytra, as shown in the identification key provided by Korotyaev (1981). One of these groups consists of *W. carinulatus* and *W. costatus*, and the other of *W. harmandi* and *W. shikotanus*.

Wagnerinus frugivorus is similar to *W. carinulatus* and *W. costatus* in having the elytra with scarcely rounded sides and angular humeral and subapical calli. These three species can be grouped together based on several other shared characteristics, such as the rostrum (Fig. 4) with a shallowly emarginate apex, sternite IX (Fig. 19) diminished to a pair of small oblong-ovate sclerites, and endophallus (Fig. 17) with four longitudinal rows of dentiform sclerites in the middle. Therefore, based on their morphological similarities, *W. frugivorus* may have some affinity with *W. carinulatus* and *W. costatus*.

Our preliminary study suggests that more than ten undescribed species of this genus occur primarily in Japan (Kato *et al*. 2006). A systematic revision is needed to shed light on the phylogenetic relationships among *Wagnerinus* weevils.

Host plant utilization of W. frugivorus

To date, host plant utilization of *Wagnerinus* weevils has never been satisfactorily studied, except for *W. costatus*, which is an obligatory cecidophage (specialist galleater) whose larvae feed exclusively on galls induced by the gall midge *Asphondylia baca* on the axillary buds of *Weigela hortensis* (Caprifoliaceae) (Sugiura *et al*. 2004).

Wagnerinus frugivorus is associated with *Weigela middendorffiana* (Fig. 24), belonging to the same genus as the host plant of *W. costatus*, but shows a considerable difference from *W. costatus* in terms of host plant utilization. This new species utilizes the unripe seed capsules of *W. middendorffiana* as its larval food resource (Fig. 27). Although no midge galls were found on the host plant of *W. frugivorus* at any of the study sites in central Hokkaido, Uechi *et al.* (2004) recorded *W. middendorffiana* as one of the host plants of *A. baca* from Mt. Tarumaezan, northwestern Hokkaido. An intensive survey including the habitat of the gall midge is necessary to confirm whether *W. frugivorus* is a specialist seed-capsule eater.

At present, fundamental ecological traits of three other congeners, *W. carinulatus*, *W. harmandi*, *W. shikotanus*, are unknown, and as previously mentioned, there are several *Wagnerinus* species that still require descriptions. Further studies of ecological traits combined with a systematic revision will elucidate host utilization patterns within *Wagnerinus* weevils.

Importance of species descriptions with DNA barcodes

DNA barcoding is a standardized approach to identifying animals and plants by a short section of DNA sequence, the so-called DNA barcode. For animal taxa, Hebert *et al*. (2003a, b) have proposed establishing a DNA-based identification system using a partial sequence of the mitochondrial COI gene. Since then, many authors have emphasized the remarkable advantages of COI-based DNA barcoding, demonstrating their great success in identification and delimitation of various animal species (Hebert *et al*. 2004, Janzen 2004, Ball *et al.* 2005, Greenstone *et al.* 2005, Monaghan *et al.* 2005, Hajibabaei *et al*. 2006a, b, c, Scheffer *et al.* 2006, Smith *et al.* 2006).

However, the utility of the animal DNA barcoding concept has met with a number of criticisms (Sperling 2003, Moritz & Cicero 2004, Will & Rubinoff 2004, Ebach & Holdrege 2005a, b, Hurst & Jiggins 2005, Will *et al.* 2005, Cognato 2006, Dasmahapatra & Mallet 2006, DeSalle 2006, Rubinoff 2006), and recent studies have revealed obvious limitations in this single-character approach, as pointed out by some critics (Brower

2006, Gompert *et al.* 2006, Meier *et al.* 2006, Scheffer *et al.* 2006). Briefly, COI-based barcoding is only a part of integrative taxonomy based on multiple datasets including morphological, ecological, distributional, and nuclear and mitochondrial DNA sequence data.

However, this does not deny the value of animal DNA barcoding based on the COI gene as the first step in developing a multiple-component barcoding system, because it is a very useful and convenient tool, when applied in conjunction with traditional approaches, for improving species-level taxonomy and flagging candidates for new species (e.g. Savolainen *et al.* 2005). Also, DNA barcodes will play an important role in the near future as an entry point to access various data on biological species through an integrative species information database, such as the SpeciesBank of the Global Biodiversity Information Facility (GBIF, http://www.gbif.org/).

Insects are the most diverse group in the animal kingdom, with nearly one million species described from a wide variety of habitats (Grimaldi & Engel 2005). Besides their prominent diversity, the presence of many pests and benefactors of humans and the difficulty identifying most taxa has necessitated identification and inventories of insect species using DNA barcodes (e.g. All-Leps:: Barcode of Life, http://www.lepbarcoding.org/). In the barcoding approach, all barcodes must link with taxonomic vouchers identified accurately by experts on the respective taxa. Considering that thousands of new insect species are described every year, providing barcodes in species descriptions together with other taxonomic data is the best way to develop DNA barcode databases for insects.

Brown *et al.* (2003) described a new tortricid species in a series of studies on New Guinea moths, providing DNA barcodes as part of the taxonomic dataset for the first time. More recently, a new skipper butterfly was described by Burns *et al.* (2006) from Costa Rica with DNA barcode of the holotype. Here, we provided a barcode in a description of a new weevil species and designated the voucher specimen as its holotype. The utility of the barcoding region is problematic with respect to intra- and interspecific variation (Dasmahapatra & Mallet 2006). We believe that the type specimen of a species is the most certain source of DNA data to link it with other species information.

In large beetles, enough DNA can be extracted from a portion of the thoracic muscle, whereas DNA extraction from small beetles is a more or less destructive procedure. In this study, we removed all right legs from the holotype for DNA extraction, preserving the greater part of the specimen including all left legs for morphological observations. If possible, however, preservation of the whole insect body is desirable even after DNA extraction. At present, a method described by Gilbert *et al.* (2007) for DNA extraction from dry beetle specimens without causing external morphological damage is the most prospective way to obtain enough DNA in good quality from important specimens, such as holotypes. Besides, two different methods both for DNA extraction and morphological observation have been used in Phthiraptera (Cruickshank *et al.* 2001) and in Lepidoptera (Knölke *et al.* 2005). Development of better techniques for extraction of DNA from small species is required to facilitate the utility of DNA barcoding in Coleoptera.

Hajibabaei *et al.* (2006b) demonstrated that DNA barcodes of standard length do not contain enough information to assess phylogenetic relationships between species. For this reason, we determined a longer sequence of the COI gene than the standard barcode region to promote molecular phylogenetic studies in the future. Our preliminary study suggests that *W. frugivorus* and *W. costatus* have a 32-bp (2.34%) sequence difference in the 1366-bp region of their COI gene and a 25-bp (3.76%) difference in the 665-bp standard barcode region (Yoshitake *et al.* unpublished data). Continuous efforts to provide DNA sequence data in species descriptions will not only improve the quality but will also expand the utility of descriptive works.

Acknowledgments

We thank Y. Sawada (MNHAH) for the loan of specimens and E. Colonnelli (Rome) for his suggestion on the scientific name of the new species. We appreciate the helpful comments of reviewers.

References

Ball, S.L., Hebert, P.D.N., Burian, S.K. & Webb, J.M. (2005) Biological identifications of mayflies (Ephemeroptera) using DNA barcodes. *Journal of the North American Benthological Society*, 24, 508–524.

Blaxter, M. (2003) Counting angels with DNA. *Nature*, 421, 122–124.

Brower, A.V.Z. (2006) Problems with DNA barcodes for species delimitation: 'ten species' of *Astraptes fulgerator* reassessed (Lepidoptera: Hesperiidae). *Systematics and Biodiversity*, 4, 127–132.

Brown, J.W., Miller, S.E. & Horak, M. (2003) Studies on New Guinea moths. 2. Description of a new species of *Xenothictis* Meyrick (Lepidoptera: Tortricidae: Archipini). *Proceedings of the Entomological Society Washington*, 105, 1043–1050.

Burns, J.M., Janzen, D.H., Hajibabaei, M., Hallwachs, W. & Hebert, P.D.N. (2006) DNA barcodes of closely related (but morphologically and ecologically distinct) species of Skipper Butterflies (Hesperiidae) can differ by only one to three nucleotides. *Journal of the Lepidopterists' Society*, 61, 138–153.

Cognato, A.I. (2006) Standard percent DNA sequence difference for insects does not predict species boundaries. *Journal of Economic Entomology*, 99, 1037–1045.

Colonnelli, E. (1984) Notes sur quelques Ceutorhynchinae de l'Afrique tropicale (Coleoptera, Curculionidae). *Annales Historico-naturales Musei Nationalis Hungarici*, 76, 208–238.

Colonnelli, E. (2004) *Catalogue of Ceutorhynchinae of the World, with a Key to Genera (Insecta: Coleoptera: Curculionidae)*. Argania editio, Barcelona.

Cruickshank, R.H., Johnson, K.P., Smith, V.S., Adams, R.J., Clayton, D.H. & Page, R.D.M. (2001) Phylogenetic analysis of partial sequences of elongation factor 1α identifies major groups of lice (Insecta: Phthiraptera). *Molecular Phylogenetics and Evolution*, 19, 202–215.

Dasmahapatra, K.K. & Mallet, J. (2006) DNA barcodes: recent successes and future prospects. *Heredity*, 97**,** 254–55.

DeSalle, R. (2006) Species discovery versus species identification in DNA barcoding efforts: response to Rubinoff. *Conservation Biology*, 20, 1545–1547.

Ebach, M.C. & Holdrege, C. (2005a) DNA barcoding is no substitute for taxonomy. *Nature*, 434, 697.

Ebach, M.C. & Holdrege, C. (2005b) More taxonomy, not DNA barcoding. *BioScience*, 55, 823–824.

Faust, J. (1887) Curculioniden aus dem Amur-Gebiet. *Deutsche entomologische Zeitschrift*, 31, 161–180.

Gilbert, M.T.P., Moore, W., Melchior, L. & Worobey, M. (2007) DNA extraction from dry museum beetles without conferring external morphological damage. *PLoS ONE* 2(3), e272. doi:10.1371/journal.pone.0000272

Greenstone, M.H., Rowley, D.L., Heimbach, U., Lundgren, J.G., Pfannenstiel, R.S. & Rehner, S.A. (2005) Barcoding generalist predators by polymerase chain reaction: carabids and spiders. *Molecular Ecology*, 14, 3247–3266.

Grimaldi, D. & Engel, M.S. (2005) *Evolution of the Insects*, Cambridge University Press, New York.

Gompert, Z., Nice, C.C., Fordyce J.A., Forister M.L. & Shapiro A.M. (2006) Identifying units for conservation using molecular systematics: the cautionary tale of the Karner blue butterfly. *Molecular Ecology*, 15, 1759–1768.

Hajibabaei, M., Janzen, D.H., Burns, J.M., Hallwachs, W. & Hebert, P.D.N. (2006a) DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences of the United States of America*, 103, 968–971.

Hajibabaei, M., Singer, G.A.C. & Hickey, D.A. (2006b) Benchmarking DNA barcodes: an assessment using available primate sequences. *Genome*, 49, 851–854.

Hajibabaei, M., Smith, M.S., Janzen, F.H., Rodriguez, J.J., Whitfield, J.B. & Hebert, P.D.N. (2006c) A minimalist barcode can identify a specimen whose DNA is degraded. *Molecular Ecology Notes*, 6, 959–964.

Hebert, P.D.N., Cywinska, A., Ball, S.L. & DeWaard, J.R. (2003a) Biological identifications through DNA barcodes. *Proceedings of the Royal Society London B*, 270, 313–321.

Hebert, P.D.N., Ratnasingham, S. & deWaard, J.R. (2003b) Barcoding animal life: cytochrome *c* oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society London B*, 270 (Supplement), S96–S99.

Hebert, P.D.N., Penton, E.H., Burns, J.M., Janzen, D.H. & Hallwachs, W. (2004) Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator. Proceedings of the National Academy of Sciences of the United States of America*, 101, 14812–14817.

Hustache, A. (1916) Synopsis des Ceuthorrhynchini du Japon. *Annales de la Société Entomologique de France*, 85, 107–144.

Hurst, G.D.D. & Jiggins, F.M. (2005) Problems with mitochondrial DNA as a marker in population, phylogeographic and phylogenetic studies: the effects of inherited symbionts. *Proceedings of the Royal Society London B*, 272, 1525–1534.

Janzen, D.H. (2004) Now is the time. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 359, 731–732.

Kato, T., Yoshitake, H. & Ito, M. (2006) Phylogenetic position of the genus *Wagnerinus* Korotyaev (Coleoptera: Curculionidae) associated with galls induced by *Asphondylia baca* Monzen (Diptera: Cecidomyiidae). *In*: Ozaki, K.,

Yukawa, J., Ohgushi, T. & Price, P. W. (Eds), *Galling Arthropods and Their Associates: Ecology and Evolution*. Springer-Verlag, Tokyo, pp. 297–305.
Knölke, S., Erlacher, S., Hausmann, A., Miller, M.A. & Segerer, A.H. (2005) A procedure for combined genitalia dissection and DNA extraction in Lepidoptera. *Insect Systematics and Evolution*, 35, 401–409.
Korotyaev, B.A. (1980) Materials to the knowledge of Ceutorhynchinae (Coleoptera, Curculionidae) of Mongolia and the USSR. *Nasekomie Mongolii*, 7, 107–282. (In Russian)
Korotyaev, B.A. (1981) New and little-known weevils of the subfamily Ceutorhynchinae (Coleoptera, Curculionidae) from the Palearctic, Indo-Malayan, and Australian regions. *Entomologicheskoe obozrenie*, 60, 126–159. (In Russian)
Meier, R., Shiyang, K., Vaidya, G. & Ng, P. K. L (2006) DNA barcoding and taxonomy in Diptera: a tale of high intraspecific variability and low identification success. *Systematic Biology*, 55, 715–728.
Monaghan, M.T., Balke, M., Gregory T.R. & Vogler, A.P. (2005) DNA-based species delineation in tropical beetles using mitochondrial and nuclear markers. *Philosophical Transactions of the Royal Society B*, 360, 1925–1933.
Monzen, K. (1937) On some new gall midges. *Kontyu*, 11, 180–194. (In Japanese)
Moritz, C. & Cicero, C. (2004) DNA barcoding: promise and pitfalls. *PLoS Biology*, 2, 1529–1531.
Ohba, H. (1993) Caprifoliaceae. *In*: Iwatsuki, K., Boufford, D. E. & Ohba, H. (Eds.), *Flora of Japan IIIa*. Kodansha, Tokyo, pp. 420–448.
Rubinoff, D. (2006) Utility of mitochondrial DNA barcodes in species conservation. *Conservation Biology*, 20, 1026–1033.
Savolainen, V., Cowan, R.S., Vogler, A.P., Roderick, G.K. & Lane, R. (2005) Towards writing the encyclopaedia of life: an introduction to DNA barcoding. *Philosophical Transactions of the Royal Society B*, 360**,** 1805–1811.
Scheffer, S.J., Lewis, M.L. & Joshi, R.C. (2006) DNA barcoding applied to invasive leafminers (Diptera: Agromyzidae) in the Philippines. *Annals of the Entomological Society of America*, 99, 204–210.
Schindel, D.E. & Miller, S.E. (2005) DNA barcoding a useful tool for taxonomists. *Nature*, 435, 17.
Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. (1994) Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Annals of the Entomological Society of America, 87, 651-701.
Smith, M.A., Woodley, N.E., Janzen, D.H., Hallwachs, W. & Hebert, P.D.N. (2006) DNA barcodes reveal cryptic host-specificity within the presumed polyphagous members of a genus of parasitoid flies (Diptera: Tachinidae). *Proceedings of the National Academy of Sciences of the United States of America*, 103, 3657–3662.
Sperling, F. (2003) DNA barcoding: Deus ex Machina. *Newsletter of the Biological Survey of Canada (Terrestrial Arthropods), Opinion Page*, 22, 50–53.
Sugiura, S., Yamazaki, K. & Fukasawa, Y. (2004) Weevil parasitism of ambrosia galls. *Annals of the Entomological Society of America*, 97, 184–193.
Tuda, M., Fukatsu, T. & Shimada, M. (1995) Species differentiation of bruchid beetles (Coleoptera: Bruchidae) analyzed by mitochondrial DNA polymorphism. Applied Entomology and Zoology, 30, 377-380.
Tuda, M., Wasano, N., Kondo, N., Horng, S-B., Chou, L-Y. & Tateishi, Y. (2004) Habitat-related mtDNA polymorphism in the stored-bean pest Callosobruchus chinensis (Coleoptera: Bruchidae). Bulletin of Entomological Research, 94, 75-80.
Uechi, N., Yukawa, J. & Yamaguchi, D. (2004) Host alternation by gall midges of the genus *Asphondylia* (Diptera: Cecidomyiidae). *Bishop Museum Bulletin in Entomology*, 12, 53–66.
Yonekura, K. & Kajita, T. (2003) *BG Plants*. Available from: http://bean.bio.chiba-u.jp/bgplants/ylist_main.html (10 January 2007).
Will, K.W. & Rubinoff, D. (2004) Myth of the molecule: DNA barcodes for species cannot replace morphology for identification and classification. *Cladistics*, 20, 47–55.
Will, K.W., Mishler, B.D. & Wheeler, Q.D. (2005) The perils of DNA barcoding and the need for integrative taxonomy. *Systematic Biology*, 54, 844–851.