A New Species of the Genus *Eucorydia* (Blattodea: Corydiidae) from the Miyako-jima Island in Southwest Japan

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A new species from the cockroach genus *Eucorydia* Hebard, 1929 from Miyako-jima Island of the Nansei Islands in Southwest Japan was compared to six closely related congeners; *E. yasumatsui* Ashihina, 1971; *E. donanensis* Yanagisawa, Sakamaki, and Shimano, 2020; *E. tokaraensis* Yanagisawa, Sakamaki, and Shimano, 2020; *E. dasytoides* (Walker, 1868); *E. guilinensis* Qiu, Che, and Wang, 2017; and *E. pilosa* Qiu, Che, and Wang, 2017. The new species *Eucorydia miyakoensis* Yanagisawa, Sakamaki, and Shimano, sp. nov. from Miyako-jima Island was characterized by a small overall male body length of 12.5–13.0 mm and tegmina with an uninterrupted orange transversal band in the middle, and a pair of orange pustules at the base. *Eucorydia yasumatsui*, *E. donanensis*, *E. tokaraensis*, the zona population of *E. dasytoides*, and *E. miyakoensis* were divided into five lineages in a maximum likelihood tree generated from a dataset concatenated from five molecular markers (two nuclear: 28SrRNA and histone H3, and three mitochondrial: COII, 12SrRNA, and 16SrRNA). We recognized *E. miyakoensis* as a distinct species, which was also supported by the pairwise genetic distances (3.4%–6.7%, K2P) of the COI sequences to the other Japanese *Eucorydia* species.

**Key Words:** genetic distances, multiple gene loci, genitalia, taxonomy.

**Introduction**

The cockroach genus *Eucorydia* Hebard, 1929 has a metallic greenish blue pronotum and tegmina in adults and some orange markings on the tegmina and/or abdomen of most species. In Japan, *E. yasumatsui* Ashihina, 1971 was described from Iriomote-jima Island and Ishigaki-jima Island (Ashina 1971). Yanagisawa et al. (2020) described two additional species, *E. donanensis* Yanagisawa, Sakamaki, and Shimano, 2020 from Yonaguni-jima Island and *E. tokaraensis* Yanagisawa, Sakamaki, and Shimano, 2020 from Uji-le-jima Island, Ama-mi-Oshima Island, Tokuno-shima Island, and Akuseki-jima Island. Recently, we found another species of *Eucorydia* from Miyako-jima Island in the collection of the Osaka Museum of Natural History, which is thought to be an undescribed species not previously included in the three known Japanese species. To an undescribed species, we gave a new scientific name, *E. miyakoensis* sp. nov. We succeeded in collecting additional specimens from the same Island. The purpose of this study was to clarify the identity of *Eucorydia* species from Miyako-jima Island. We compared the external morphology of four species from Japan and Taiwan [*E. tokaraensis*, *E. yasumatsui*, *E. donanensis*, and *E. dasytoides* (Walker, 1868)] with that of the *E. miyakoensis* sp. nov. from Miyako-jima Island. To confirm the morphological studies, we inferred the phylogenetic relationships between the species from Miyako-jima Island and the other four species from Japan and Taiwan using the DNA sequences from six loci.

**Materials and Methods**

A total of twelve specimens were examined in this study. Nine specimens were collected from Gusukube, Miyakojima Island and three were cultured individuals originating from Gusukube. All of *E. miyakoensis* sp. nov. specimens examined in this study were identified using the unique alpha-numerals eM-001–012 and eMo-001–010, with "eMo" referring to the oothecae. Adults and oothecae were obtained by indoor rearing of the field-collected specimens under natural daylight at 22°C–27°C and 50%–70% humidity on a diet of “mouse food” (Rodents Diets MF; Oriental Yeast Co., Ltd.). The morphological terminology used in this paper mainly follows Qiu et al. (2017) and Yanagisawa et al. (2020). Male genital segments were prepared for dissection by maceration in 10% NaOH to remove the protein and muscles. They were then placed in 75% ethanol and
observed under a stereomicroscope (ST-LED, Kenis) and drawings were made based on these observations. Photographs were taken using a Nikon D5300 camera with a Nikon AF-S VR Micro-Nikkor 105 mm f/2.8G IF-ED lens.

The type series for *E. miyakoensis* sp. nov. was deposited into the Dictyoptera collection (NSMT-I-Dct) of the National Museum of Nature and Science, Tsukuba (NMNS; previous name, the National Science Museum, Tokyo: NSMT).

**DNA extraction.** Each specimen used for DNA analysis was dissected under the stereomicroscope and their appendages were used for DNA extraction. The total genomic DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen), with modifications per Johnson et al. (2004). The samples were incubated for at least 48 h to lyse the tissue. The exoskeletons were retrieved from the appendages before each lysed mixture was pipetted into the spin column, and preserved with the rest of the body in 100% ethanol as voucher specimens.

**PCR amplification and sequencing.** The partial sequences of six genes were amplified (two nuclear: 28S rRNA and histone H3, and four mitochondrial: COI, COII, 12S rRNA, and 16S rRNA). Amplification of each target gene was conducted using the primers listed in Table 1. The PCR reactions were performed in a TaKaRa PCR Thermal Cycler Dice Touch (TaKaRa) in 10 µL volumes, which each contained 1 µL of the template solution, 2 mM MgCl₂, 2.5 mM dNTP, 10 pmol of each primer, and 0.25 U Ex Taq polymerase (TaKaRa) in 1× buffer provided by the manufacturer. The amplification conditions were 95°C for 2 min; 35 cycles at 98°C for 10 s, 50°C for 30 s, 72°C for 1 min (for histone H3, COI, COII, 12S rRNA, and 16S rRNA) or 2 min (for 28S rRNA); and 72°C for 7 min. The amplified products were purified using ExoSAP-IT Express PCR Cleanup Reagents (Thermo Fisher Scientific, Waltham, MA, USA). The nucleotide sequences were determined using direct sequencing via a BigDye Terminator Cycle Sequencing Kit (for 28S rRNA); and 72°C for 7 min. The amplified products consisted of five gene data sets for both analyses (Table 2). Bootstrap analyses (Felsenstein 1985) of 1000 pseudo-replicates were performed for ML tree. For BI analyses, the Markov-chain Monte-Carlo process used random starting trees and involved two runs of four chains each (three hot and one cold) for 20 million generations. Trees were sampled every 100th generation; the first 25% of trees were discarded as burn-in. Convergence was inferred when the standard de-

### Table 1. List of primers used in this study.

| Genes | Primer name | Sequence (5′−3′) | Source | used for PCR |
|-------|-------------|-----------------|--------|--------------|
| 28S rRNA | 28S-01 | GAC TAC CCC CTG AAT TTA AGC AT | Kim et al. (2000) | ○ |
|       | 28SR-01 | GAC TCC TTG GTC GTG TTG TCA AG | Kim et al. (2000) | ○ |
|       | 28f | TGG GAC CCG AAA GAT GGT G | Luan et al. (2005) | ○ |
|       | 28S-Euc11F | ACG GAC CAA GGA GTC TAA CWT | Yanagisawa et al. (2020) | ○ |
|       | 28S-Euc16R | TAA AGT TTG AGAATA GGA GTG TGA GTG C | Yanagisawa et al. (2020) | ○ |
|       | 28sr | ACA CAC TCC TTA GGG GA | Luan et al. (2005) | ○ |
|       | 28S_2KF | TGT GAA TCC TGT AAG GAG TG | Hiruta et al. (2016) | ○ |
|       | 28S_3KR | CCA ATC CTT TTC CCG AAG TG | Hiruta et al. (2016) | ○ |
| H3 | H3 AF | ATG GCT GGT ACC AAG CAG ACV GC | Inward et al. (2007) | ○ |
|    | H3 AR | ATA GCC CCA TCC ACC AAT ATG GC | Inward et al. (2007) | ○ |
| COI | LCO1490 | GGT CAA CAA ATC ATA AAG AAT TTG G | Folmer et al. (1994) | ○ |
|     | HCO2198 | TAA ACT TCA GGG TGA CCA AAT CA | ○ |
| COII | COII-F | AGA GCW TCA CCT ATT ATA GAA C | Park et al. (2004) | ○ |
|      | COII-R | GTA RWA CRT CTG CTG CTG TTA C | ○ |
| 12S rRNA | 12S forward | ATC TAT GGT AGC ACT TAT | Inward et al. (2007) | ○ |
|       | 12S reverse | AAA CTA GGA TTA GAT ACC C | Kambhampati (1995) | ○ |
| 16S rRNA | 16S Forward | CCG CTG TTT AAC AAA AAC AT | Simon et al. (1994) | ○ |
|       | 16S Reverse | TTT AAT CCA ACA TCG AGG | Cognato and Vogler (2001) | ○ |
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Table 2. Substitution models used in this study as determined by Partition Finder 2.1.1 (Lanfear et al. 2017)

| Information | Partition scheme | Character set | Substitution models |
|-------------|------------------|---------------|---------------------|
| AICc        | 4                | 12S, 16S      | GTR + I             |
|             |                  | COI           | GTR + I             |
|             |                  | 28S           | TRN + G             |
|             |                  | H3            | SYM + G             |
| BIC         | 4                | 12S, 16S      | GTR + I             |
|             |                  | COI           | HKY + I             |
|             |                  | 28S           | HKY                |
|             |                  | H3            | K80 + G             |

Results

Taxonomy

*Eucorydia miyakoensis* Yanagisawa, Sakamaki, and Shimano, sp. nov.

[New Japanese name: Benieri-rurigokiburi]

(Figs 1A–P, 2)

Material examined. Holotype: male (NMNS, NSMT-I-Dct-542), Gusukube, Miyako-jima Island, Okinawa, Japan, 12 November 2019, S. Yanagisawa leg. (eM-001). Paratypes: 2 males and 5 females (NMNS, NSMT-I-Dct-543–549), Gusukube, Miyako-jima Island, Okinawa, Japan, 12 November 2019, S. Yanagisawa leg. (eM-002–eM-008).

Differential diagnosis. This new species resembles *E. guilinensis* Qiu, Che, and Wang, 2017, *E. dasytoides*, *E. yasumatsui*, *E. tokaraensis*, and *E. donanensis*, and *E. pilosa* (Figs 1A–P, 2). *Eucorydia miyakoensis* sp. nov. is distinguishable from the new species in that their males have an overall length >18 mm (Qiu et al. 2017), whereas overall length of the male *E. miyakoensis* sp. nov. is <13 mm. Both the size and shape of genitalia are similar to those of the three Japanese species, namely *E. yasumatsui*, *E. tokaraensis*, and *E. donanensis*, but it can be distinguished from them by orange pubescence at the base of the tegmina.

Description. Male (n=3): Body length 11.5 mm; overall length 12.5–13.0 mm; pronotum length 3.4–3.5 mm, width 5.6–5.9 mm; tegmen length 10.0–10.8 mm (Fig. 1A, B). Head shiny, black. Antenna black, consisting of 36–38 segments with 6–7 whitish subapical segments. Numbers of whitish segments sometimes different between the left and right sides. Pronotum metallic blue to metallic bluish green. Tegmina metallic blue to metallic bluish green, similar to pronotum color with a pair of orange pubescent blotches at the base near scutellum. Distal half with distinct, uninterrupted orange band. Hindwings hyaline, pale brown, becoming darker toward the apex with an orange blotch at middle of costa. Sc single; RA+RP with 5–8 branches; M single, but with one cell and some crossoveins in middle part; CuA with 8 branches; CuP single; and AA+AP with 11–14 branches (Fig. 1I). Legs shiny, black.

Dorsum dark purple with yellowish area occupied from caudal half of 2nd segment to 5th segment; 6th segment of tergite dark purple in middle and yellow on lateral sides (Fig. 1M). Ventrum black, with yellowish area occupied from 2nd to 6th segments. Supra-anal plate black, widely lobed; cercus consisting of 8 segments (Fig. 1E); subgenital plate black, rounded, with styli.

Genitalia (Fig. 1F–I). Left phallomere: L3 slender and curved, gradually narrowing toward the apex with a distinct hook (Fig. 1I); L7 round in the basal half, spatulate in the distal half, with beak-like apex (Fig. 1H). Right phallomere: R2 slightly elongated, round; basal left with a lobated protrusion (Fig. 1G).

Female (n=5): Body length 13.0–13.7 mm; pronotum length 3.5–3.9 mm, width 6.1–6.5 mm; tegmen length 8.7–9.0 mm (Fig. 1C, D). Head, pronotum, and tegmen similar to male in color (Fig. 1K). Dorsum brownish black with yellow area occupied from 2nd to 5th segments and lateral sides of 6th segment. Supra-anal plate semicircular in shape and brownish black. Ventral side of abdomen brownish black with yellow area occupied from 1st through 6th segments; subgenital plate brownish black.

Ootheca (n=4): Length 3.0–3.1 mm, width 5.0–6.0 mm. Light brown, trapezoidal pouch, with fine serration on one side of the margin with five longitudinal ridges on each side (Fig. 1P).

Larva (last instar): Male (n=1) body length 11.5 mm, mesonotum width 7.0 mm (Fig. 1N). Female (n=1) body length 12.1 mm, mesonotum width 7.0 mm. Body brown, with many fine bristles (Fig. 1O).

Distribution. Miyako-jima Island, Southwest Japan (Fig. 2).
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Fig. 1. A new species of *Eucorydia miyakoensis* Yanagisawa, Sakamaki, and Shimano, sp. nov. A–B, male (eM-001 holotype); C–D, female (eM-004 paratype); E, supra-anal plate; F, right phallomere with L7; G, R2; H, L7; I, genital hook/L3; J, basal tegmina of male (eM-001 holotype); K, basal tegmina of female (eM-004 paratype); L, hind wing of male (eM-001 holotype); M, dorsal view of the abdominal segments of male (eM-001 holotype); N, larva (last instar) male from Gusukube, Miyako-jima Island, 12 November 2019, S. Yanagisawa leg. (no voucher specimen left); O, larva (last instar) female from Gusukube, Miyako-jima Island, 12 November 2019, S. Yanagisawa leg. (no voucher specimen left); P, ootheca (eMo-001). Scale bars: 5 mm.
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**Etymology.** The new species was named after the sampling site Miyako-jima Island.

**Sequences.** LC565385–LC565406, including two nuclear markers (28S and H3) and four mitochondrial markers (12S, 16S, COI, COII) from four specimens (eM-009–eM-012).

**Phylogeny and genetic distance.** Table 3 lists the sequences used for molecular phylogenetic reconstruction, including five molecular markers obtained from four individuals of *E. miyakoensis* sp. nov. ML tree inferred from the five concatenated markers is shown in Fig. 3 with nodal support values. Trees resulting from ML and BI had identical topologies; therefore, only ML tree is presented. The Japanese *Eucorydia* populations formed a sister clade with the *E. dasytoides* population of Taiwan Island. The Japanese populations were also monophyletic and divided into four lineages. New species from Miyako-jima Island formed a clade with *E. donanensis* from Yonaguni-jima Island.

Table 4 lists pairwise genetic distances of COI sequences in Japanese *Eucorydia* species and related species. Although only a small number of individuals were determined to have the COI sequence, Japanese *Eucorydia* species are genetically homologous on each island, and almost no intraspecific variation was detected for the COI gene. Only *E. yasumatsui*, distributed in Iriomote-jima and Ishigaki-jima Islands, has three haplotypes in COI sequences. Differences between these haplotypes include one or two substitutions (K2P 0.2%–0.3%). The new species were separated by genetic distances ranging from 3.4% to 6.7% (K2P) (Table 3). Similarly, genetic distances between the Japanese *Eucorydia* species and the *zonata* population of *E. dasytoides* were relatively large (K2P 8.7%–10.3%).
Remarks. This species was found only on Miyako-jima Island (Fig. 2). The presence of this new species was also suggested in the remarks section which discussed the Euco-
rydia (E. tokaraensis) in Asahi et al. (2016), but it was not described as a valid species since there were an insuffi-
cient number of specimens at that time. Obscureness of the orange band on the tegmina varied among individuals. The oothecae of this species are similar to the ootheca of E. yasu-
matsui described by Fujita and Machida (2014).

Keys to Japanese Eucorydia species with similar conge-
ners from adjacent areas; based on Qiu et al. (2017) (E. gui-
linensis, the zonata and purpuralis populations of E. dasy-
toides, and E. yunnanensis Woo, Guo, and Feng, 1986) and Yanagisawa et al. (2020) (E. yasumatsui, E. donanensis and
E. tokaraensis).

1. Tegmina with pubescence at the basal portion ....... 2 — Tegmina without pubescence on the surface .......... 3
2. L7 of male genitalia round in the basal half; pubescence on tegmina orange; overall length less than 13 mm in male ................. E. miyakoensis sp. nov. — L7 of male genitalia curved toward left-posterior with an elongate and sharp process in left base; pubescence on tegmina yellowish white; overall length more than 13 mm ......................... E. guilinensis
3. Overall length more than 18 mm in male .......... 4 — Overall length less than 16 mm ...................... 5
4. Orange band of tegmina twice interrupted .........
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