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DESCRIPTION OF LATE-INSTARS OF BRYOTHINUSA KOREANA AHN AND JEON (COLEOPTERA: STAPHYLINIDAE: ALEOCHARINAE) BY ASSOCIATION OF LIFE STAGE BASED ON DNA SEQUENCE DATA

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ABSTRACT

Late-instars of Bryothinusa koreana Ahn & Jeon are described for the first time based upon DNA sequence data of larvae and adults. Twelve larvae were collected on Geoje Island, Korea, in association with adults of B. koreana. The partial cytochrome oxidase II gene (410 bp) was sequenced from the larvae and from several identified adult Bryothinusa specimens, including B. koreana. The intraspecific p-distances of adult B. koreana were from 0 to 4.44%, and interspecific pairwise distances were 10.77% to 13.47%. The sequence results of the larvae were similar to those of adult B. koreana. Based on these results, the larvae were identified as B. koreana and diagnostic characters of the species are provided, with illustrations of features.

Key Words: Bryothinusa koreana, DNA identification, Korea, larval description, Staphylinidae

RESUMEN

Se describen por la primera vez los últimos instares de Bryothinusa koreana Ahn & Jeon basado en los datos de las secuencias de ADN de las larvas y adultos. Se recolectaron doce larvas en la Isla Geoje, Korea, en asociación con adultos de B. koreana. Se hizo una secuencia del gene citocromo-oxidasa II de la larva y de varios especímenes identificados de Bryothinus, incluyendo B. koreana. La distancia p interespecifica de los adultos de B. koreana fue de 0 a 4.44%, y la distancia interespecifica de los pares fue de 10.77% a 13.47%. Los resultados de las secuencias de larvas fueron similares a las de los adultos de B. koreana. Basado en estos resultados, las larvas fueron identificadas como B. koreana y se preveen caracteres diagnósticos de las especies con ilustraciones de sus características.

 MATERIALS AND METHODS

Twelve larvae (identity unknown at time of collection) and many adults of B. koreana were
collected together under stones within a habitat range of about 10 m, on Geoje Island, Korea. A partial sequence of the CO II gene (410 bp) was generated from the unknown larvae and five identified adult specimens of *Bryothinusa* to confirm that the unknown larvae are *B. koreana*. Three *B. koreana* adults, each from different populations (from Gangin, Jindo Is., and Geoje Is.), were included in order to examine intraspecific variation. The sequences of *B. nakanei* (Sawada) from Korea and unidentified *Bryothinusa* species from Philippines were also generated to study interspecific variation among the genus *Bryothinusa*. *Brachyprono- maea esakii* Sawada was included to root the cladogram (Table 1).

Preparation of permanent microscopic slides for late instars was performed with the techniques described by Ashe (1986). Terms and the chaetotaxic system for late-instars follow Ashe & Watrous (1984). Materials for this study were deposited in the Chungnam National University Insect Collection (CNUIC, Daejeon), Korea.

DNA Extraction, Amplification, and Sequencing

For adults, total genomic DNA was extracted from muscles in the head and pronotum to prevent contamination by DNA of parasites or gut contents. Genitalia were preserved to confirm the species identification. After grinding the specimens in liquid nitrogen, we followed the manufacturer’s protocol for the DNeasy Tissue Kit (Qiagen, Hilden, Germany). For larvae, DNA was extracted from muscles in the head and pronotum. The remaining cuticle was used as a voucher specimen of the sample deposited in CNUIC.

The CO II region examined in this study was amplified by a set of primers C2J 3400 (Simon et al. 1994) and TKN 3782 (Brent et al. 1999). PCR was performed in 50 μL with 1-10 μL of the genomic DNA with 1 or 2 units of Taq-polymerase and 3 mmol MgCl₂, 1.5 mmol dNTPs, and 50 pmol of each primer. The amplification involved 2 min of denaturation at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s of primer annealing at 45°C-55°C, and 1 min of extension at 72°C, followed by a final 4-min extension at 72°C. PCR products were cleaned of enzymes and remaining primers with a PCR Product Purification Kit (Roche, Indianapolis, Indiana, USA) and recovered in 20 μL of H₂O.

Amplified DNA was sequenced by a Perkin Elmer ABI377 Automated Sequencer (Applied Biosystems Inc., Foster City, California, USA) and confirmed with both sense and anti-sense strands. Partial CO II sequences (410 bp) of *B. koreana* and related species have been deposited in GenBank under accession numbers EF079108-EF079114 (Table 1).

RESULTS

As the CO II gene is a protein coding region, alignment was performed by SeqPup (Gilbert, 1995). Parsimony analysis was conducted with PAUP* (Swofford 2003) with the Branch and Bound tree search option, and branch support values were estimated by bootstrapping. The analysis resulted in a single most parsimonious cladogram with a length of 126, a consistency index of 0.84 and a retention index of 0.70 (Fig. 1). Average CO II sequence differences among populations of *B. koreana* were 2.38% (0-4.44%). The maximum intraspecific distance in *B. koreana* was 3.9%.

### Table 1. Species, Collection Information, and GenBank Accession Numbers for Cytochrome Oxidase II Sequences from This Study.

| Species                  | Collection information                                                                 | GenBank accession |
|--------------------------|----------------------------------------------------------------------------------------|-------------------|
| *Bryothinusa koreana*    | Korea: Jeonnam Prov., Gangjin-gun, 19 IV 2004, K.-J. Ahn, S.-M. Choi                   | EF079110          |
| *B. koreana* (adult)     | Korea: Jeonnam Prov., Jindo, Imboe-myeon, Geumgab beach, 21 VIII 2001, K.-J. Ahn      | EF079112          |
| *B. koreana* (adult)     | Korea: Gyeongnam Prov., Geoje Isl., Gabae-ri, 30 VI 2001, C.-W. Shin                    | EF079109          |
| *B. koreana* (larva)     | Korea: Gyeongnam Prov., Geoje Isl., Gabae-ri, 30 VI 2001, C.-W. Shin                    | EF079111          |
| *B. nakanei* (adult)     | Korea: Jeonnam Prov., Gangjin-gun, 19 IV 2004, K.-J. Ahn, S.-M. Choi                   | EF079108          |
| *Bryothinusa* sp. (adult)| Philippines: Camotes Isl., San Francisco, Puertobellu, 14 XII 2003, M.-J. Jeon          | EF079113          |
| *Brachypronomaea esakii* (adult) | Japan: Chatan-cho, Okinawa-honto, Nansei photo, 26 V 2002, M. Moriguchi, K. Miyagi, G. Masaki | EF079114          |
adults was 4.44% between the Gangjin, Jindo, and Geoje populations. The minimum interspecific distance within the genus *Bryothinusa* was 10.7% between *Bryothinusa* sp. from the Philippines and *B. nakanei* (Fig. 1). The CO II sequences from adults and larvae of *B. koreana* were within the range of intraspecific distance (0.7-4.1%). Therefore, we are describing below the larvae as probable late-instar of *B. koreana*.

**Description of Late Instar of *Bryothinusa koreana*** Ahn & Jeon

**DESCRIPTION.** Length 3.5-3.7 mm. General body shape elongate, flattened, nearly parallel sided, pale in color.

HEAD (Figs. 2, 3). About 0.9 times as long as wide, 1 small stemma present on each side. Ecdysial sutures distinct and complete from antennal fossae anteriorly to base of head posteriorly. Antenna (Fig. 4) with 3 articles; article 1 about 0.6 times as long as wide, transverse, 5 campaniform sensilla present around apical margin, 1 campaniform sensillum present on middle of article 1, setae absent; article 2 about 2.0 times as long as article 1; article 3 about 0.4 times as long as article 2; article 2 with 1 solenidium in addition to sensory appendage, length of sensory appendage almost equal to article 2; article 2 and 3 each with 4 and 3 setae; article 2 with 1 long (IIS1) and 1 short (IIS2) so-

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**Fig. 1.** Pairwise distances (A) and single most parsimonious tree (B) estimated from cytochrome oxidase II sequences (Tree length = 126, CI = 0.84, RI = 0.70). Parsimony bootstrap values (>50%) are given above the branches.
Figs. 2-8. *Bryothinusa koreana*. 2, Head, dorsal aspect; 3, head, ventral aspect; 4, antenna, dorsal aspect; 5, Labium, dorsal aspect; 6, mandible, dorsal aspect; 7, maxilla, dorsal aspect; 8, labrum, dorsal aspect. Scales = 0.1 mm.
Figs. 9-15. *Bryothinusa koreana*. 9, Pronotum, dorsal aspect; 10, mesonotum, dorsal aspect; 11, metanotum, dorsal aspect; 12, anterior leg, lateral aspect; 13, sternite VIII, ventral aspect; 14, tergite IX and X, dorsal aspect; 15, urogomphi, dorsal aspect. Scales = 0.1 mm.
### Table 2. Differences between the Late Instars of B. koreana and B. catalinae.

|                  | B. koreana                                                                 | B. catalinae                                                                 |
|------------------|-----------------------------------------------------------------------------|-----------------------------------------------------------------------------|
| Labium           | 2nd palpomere half of the 1st (Fig. 5)                                       | 2nd palpomere as long as 1st (Moore & Roth 1979: Fig. 4)                     |
| Maxilla          | stipes as long as palpus                                                    | stipes longer than palpus                                                   |
| Mandibles        | 4 internal teeth (Fig. 6)                                                    | 5 internal teeth                                                            |
| Urogomphi        | shorter than pseudopod (Fig. 14)                                            | longer than pseudopod                                                       |
| Urogomphi: article 1 | cylindrical (Fig. 15)                                                     | broadly triangular                                                         |

**Remarks.** The differences between late instars of *B. catalinae* and *B. koreana* are presented in Table 2.

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