

## DESCRIPTIONS OF LATE INSTARS OF THREE LITTORAL *CAFIUS* SPECIES (COLEOPTERA: STAPHYLINIDAE) BY ASSOCIATION OF LIFE STAGE WITH DNA SEQUENCES

MI-JEONG JEON AND KEE-JEONG AHN

Department of Biology, Chungnam National University, Daejeon 305-764, Republic of Korea

### ABSTRACT

Late instars of 3 littoral *Cafius* Curtis species [*Cafius fucicola* Curtis, *C. nauticus* (Fairmaire), *C. vestitus* (Sharp)] are described for the first time by using sequences of mitochondrial gene. In total, 13 unknown larvae were collected in association with adults from the Nearctic and Palearctic regions. The partial *cytochrome oxidase* II (CO II) gene was sequenced from the unknown larvae and several identified adult specimens for adults-larvae association. The range of intraspecific p-distance was from 0 to 2.30% and the minimum interspecific p-distance was 14.46% between *Cafius nauticus* and *C. vestitus*. Parsimony and distance analyses were conducted and individuals of a single species were always grouped closely together. Based on these results, the unknown larvae were identified and diagnostic characters of the species are provided, with illustrations of features.

Key Words: DNA identification, larval description, *C. fucicola*, *C. nauticus*, *C. vestitus*

### RESUMEN

Los últimos instares de 3 especies litorales del género *Cafius* Curtis (*Cafius fucicola* Curtis, *C. nauticus* (Fairmaire) y *C. vestitus* (Sharp)) son descritas por primera vez basadas sobre secuencias del gene mitocondrial. En total, 13 larvas desconocidas fueron recolectadas en asociación con adultos de la region neártica y paleártica. El gene citocromo oxidasa II (CO II) fue secuenciado de larvas desconocidas y varios especimenes adultos para relacionar dichos adultos con las larvas. El rango de la distancia-p intra-específico fue de 0 a 2.30% y la distancia-p mínima fue 14.46% entre *Cafius nauticus* y *C. vestitus*. El análisis de parsimonia y distancia fue realizado e individuos de la misma especie siempre fueron agrupadas estrechamente juntas. Basado en estos resultados, las larvas desconocidas fueron identificadas y caracteres diagnósticos de las especies e ilustraciones de las características son proveidas.

The family Staphylinidae includes over 47,744 described species in 3,847 genera and 31 subfamilies in the world (Herman 2001; Thayer 2005). The high diversity of the Staphylinidae is a result of remarkable radiations in diverse habitats. Most of the staphylinids are found in terrestrial habitats such as leaf litter, plant debris, and fungi. However, 442 species in 102 genera and 7 subfamilies are known to be confined to seashore habitats (Ahn & Ashe 1996, 2004; Hammond 2000; Moore & Legner 1976). One of the 7 subfamilies with coastal representatives is Staphylininae.

Most coastal staphylinine larvae live in stranded accumulations of decaying seaweed. The seaweed forms the basis of a food chain. Larvae and adults are predators that feed on amphipods, larvae of seaweed flies (*Fucellia* Robineaux-desvoidy) or enchytraeid worms (*Lumbricillus* Ørsted and *Enchytraeus* Henle) (Moore & Legner 1976; Topp & Ring 1988) that in turn feed on the seaweed.

The immature stages of a few staphylinids were studied early, but taxonomic studies of them remained fragmentary (Smetana 1995). Structures of larvae are diverse and present many sim-

ilarities to those of related families. No single structure characterizes larvae of a family, many genera, and some tribes (Frank 1991). Larvae of staphylinids provide important information for phylogenetic and evolutionary studies. However, very few immature staphylinids have been described because of the difficulty of making larval-adult associations (Ashe & Watrous 1984; Frank 1991). Larvae can be reared through pupae to adults in the laboratory, allowing larval identification and association with adults. However, rearing of larvae is time-intensive, and it is difficult to achieve the appropriate rearing conditions. Recently, DNA sequencing has become straightforward and inexpensive and therefore represents an obvious alternative for the identification of immature insects (Caterino & Tishechkin 2006; Herbert et al. 2003; Tautz et al. 2003; Blaxter 2004). A partial sequence of the *cytochrome oxidase* II (CO II) gene is sufficient to make a confident association between life stages of staphylinid beetles (Jeon & Ahn 2005).

Fifty five species of the genus *Cafius* Curtis are now recognized. It is the most speciose genus of the coastal staphylinid subfamily Staphylininae.

Most are from temperate regions, although several species are widespread in the tropics (Orth & Moore 1980). To date, 9 late instar larvae of *Cafius* species have been identified from the Neotropical and Palearctic regions (Coiffait 1974; James et al. 1971; Jeon & Ahn 2005; Moore 1975; Orth & Moore 1980; Paulian 1941). In spite of the previous studies, most of the *Cafius* still have been insufficiently described. In this study, we first describe larvae of three *Cafius* species [*C. fucicola* Curtis, *C. nauticus* (Fairmaire), *C. vestitus* (Sharp)] through the association of larvae-adults based on DNA sequences. We also provide a key to the *Cafius* larvae and diagnostic characters with illustrations of features and discuss differences between species.

#### MATERIALS AND METHODS

In total, 25 specimens including 13 larval specimens were used in this study (Table 1). Unknown larvae and adult staphylinids were collected together, and we attempted association by gene sequencing of individuals. The partial *cytochrome oxidase II* gene (375 bp) was sequenced from the unknown larvae and identified adult specimens to confirm that the unknown larvae and adult were conspecific. Two or more specimens of several species were sequenced in order to examine intraspecific variation.

Preparations of permanent microscopic slides for late-instar larvae were made by the techniques described by Ashe (1986). Specimens pre-

pared for study of microscopic structures were examined under a compound microscope. Terms for larvae follow James et al. (1971) and Paulian (1941). 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 with DNA from parasites in food remains. 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.

The *cytochrome oxidase II* region examined in this study was amplified by primers C2J 3400 (Simon et al. 1994) and TKN 3782 (Brent et al. 1999). PCR was performed in 50  $\mu$ L with 1-10  $\mu$ L of the genomic DNA and 1 or 2 units of *Taq*-polymerase, 3 mmol MgCl<sub>2</sub>, 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 examined by gel electrophoresis. They were cleaned of enzymes and remaining primers with a

TABLE 1. SPECIES AND SPECIMENS, COLLECTION LOCALITIES, AND GENBANK ACCESSION NUMBERS FOR *CYTOCHROME OXIDASE II* (CO II) SEQUENCES USED IN THIS STUDY.

Species	Collection locality	GenBank
<i>Cafius fucicola</i>	[1]* England: Devon, Plymouth, Mount Batten Point	EF450174**
	[2] Same as above	EF450175**
<i>C. nauticus</i>	[1]* USA: Hawaii, Oahu, Honolulu, Kawaihoa beach	EF450198**
	[2]* Same as above	EF450199**
	[3] Same as above	EF450200**
	[4] Australia: Queensland, Daintree N.P., Wonga beach	EF450201**
	[5] Japan: Okinawa, Iriomote Isl., Uibaru	EF450202**
	[6] Philippines: Cebu, Bohol, Panglao Isl., Alona beach	EF450203**
	[7] Vietnam: Hai Phong, Do Son	EF450204**
<i>C. vestitus</i>	[1]* Korea: Gangwon Prov., Donghae City, Daejin beach	EF450210**
	[2]* Same as above	EF450211**
	[3]* Same as above	EF450212**
	[4]* Korea: Jeju Isl., Bukjeju-gun, Gujwa-eup, Hado-ri	EF450213**
	[5] Korea: Jeonnam Prov., Jangheong-gun, Hoejin-myeon	DQ069324
	[6] Korea: Jeju Isl., Moseulpo	EF450214**
	[7] Japan: Hokkaido, Nemuro City, Onneto	EF450215**
	[8] Japan: Honshu, Nagasaki	EF450216**
<i>Philonthus nudus</i>	[1] Korea: Gangwon Prov., Gangreung City, Gyeongpo	EF450227**
	[2] Japan: Hokkaido, Shibetsu-shi, River mouth	EF450228**

\*Indicates larval specimen.

\*\*Indicates new sequence data.

PCR Product Purification Kit (Roche, Indianapolis, Indiana, USA) and recovered in 20  $\mu$ L of H<sub>2</sub>O. Amplified DNA was sequenced with a Perkin Elmer ABI377 Automated Sequencer (Applied Biosystems Inc., Foster City, California, USA) and was confirmed with both sense and anti-sense strands. Partial *cytochrome oxidase* II sequences of larval specimens and related adult species have been deposited in GenBank under accession numbers (Table 1).

#### Parsimony and Distance Analyses for Identification

As the *cytochrome oxidase* II gene is a protein coding region, it is length invariant and easily aligned. Alignment was performed by using SeqPup (Gilbert 1995). Parsimony and distance analyses were carried out in PAUP\* (Swofford 2003). Parsimony analysis was conducted with a branch and bound tree search option. Branch support values were estimated by 100 bootstrap replicates, each comprising 2 heuristic random addition searches. Distance analysis was conducted by Neighbor-Joining method and branch support values were also obtained by bootstrap analysis. *Philonthus nudus* Sharp was selected to root the resulting trees.

#### RESULTS

Sequences for 7 larval specimens were compared with those for 4 adult species collected from the same regions and habitats. The maximum intraspecific pairwise distance was 2.30% between *C. nauticus* Hawaii populations (range 0-2.30%) and the minimum interspecific distance was 14.46% between *C. nauticus*—Hawaii and *C. vestitus*—Donghae, Korea (range 14.46-20.83%). The intraspecific and interspecific congeneric pairwise distances are presented in Table 2.

The analysis resulted in 30 equally parsimonious cladograms with a length of 157, a consistency index of 0.85, a retention index of 0.95, and strict consensus tree as shown in Fig. 1B. The Neighbor-joining analysis showed that specimens of a single species formed cohesive assemblages (Fig. 1A). Every terminal branch at the species level is supported by 100% of branch support values. The unknown larvae grouped unambiguously with the adult specimens of 3 coastal *Cafius* species in both analyses. Individuals of a single species were always grouped closely together, regardless of where they were collected. Therefore, we identified and described the unknown larvae as probable late instars of the genus *Cafius*.

#### KEY TO THE KNOWN LARVAE OF *CAFIUS*

1. Urogomphus with a single article, spherical; maxillary palpomere 4<sup>th</sup> as long as 3<sup>rd</sup> . . . . . *C. seminitens* Horn
- 1'. Urogomphus with 2 articles, slender or cylindrical; maxillary palpomere 4<sup>th</sup> shorter than 3<sup>rd</sup> . . . . . 2
2. Urogomphus shorter than tergite X, cylindrical; conical ligula much longer than 1<sup>st</sup> labial palpomere; mala with 3 setae on apex. . . . . *C. canescens* (Mäklin)
- 2'. Urogomphus longer than tergite X, slender; conical ligula subequal or shorter than 1<sup>st</sup> labial palpomere; mala with 1 seta on apex. . . . . 3
3. Nasale teeth blunted, central teeth (LT4 and 5) shorter than lateral teeth; tergite X longer than 1<sup>st</sup> article of urogomphi . . . . . *C. mimulus* (Sharp)
- 3'. Nasale teeth stout or acute, central teeth (LT4, 5) subequal or longer than lateral teeth; tergite X shorter or subequal to 1<sup>st</sup> article of urogomphi. . . . . 4
4. Conical ligula as long as 1<sup>st</sup> labial palpomere . . . . . 5
- 4'. Conical ligula shorter than 1<sup>st</sup> labial palpomere. . . . . 8
5. Tergite X longer than 1<sup>st</sup> article of urogomphi; 3<sup>rd</sup> labial palpomere as long as 2<sup>nd</sup> . . . . . *C. rufescens* (Sharp)
- 5'. Tergite X shorter than 1<sup>st</sup> article of urogomphi; 3<sup>rd</sup> labial palpomere much shorter than 2<sup>nd</sup>. . . . . 6
6. Maxillary palpus longer than stipes; mandible with serration . . . . . *C. lithocharinus* (LeConte)
- 6'. Maxillary palpus shorter than stipes; mandible without serration. . . . . 7
7. Mala as long as lacinia; nasale LT4 longer than LT3 . . . . . *C. xantholoma* (Gravenhorst)
- 7'. Mala longer than lacinia; nasale LT4 as long as LT3 (Figs. 2B, C) . . . . . *C. fucicola* Curtis
8. Central three teeth distinct with side teeth, LT5 as long as LT4; 2<sup>nd</sup> article of urogomphi less than 1/3 of 1<sup>st</sup> (Figs. 4B, F) . . . . . *C. vestitus* (Sharp)
- 8'. Central three teeth continued with side teeth, LT5 smallest; 2<sup>nd</sup> article of urogomphi more than 1/2 of 1<sup>st</sup> . . . . . 9
9. Tergite X as long as 1<sup>st</sup> article of urogomphi; mandible without serration . . . . . *C. histrio* (Sharp)
- 9'. Tergite X shorter than 1<sup>st</sup> article of urogomphi; mandible with fine serration (Figs. 3D, F) . . . . . *C. nauticus* (Fairmaire)

TABLE 2. PAIRWISE DISTANCES BETWEEN TAXA IN ANALYSIS EXPRESSED AS A PERCENTAGE OF NUCLEOTIDE DIFFERENCES (P-DISTANCES). INTRASPECIFIC PAIRWISE DISTANCES INDICATED IN BOLD.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
<i>C. fucicola</i> [1]	<b>0.000</b>																		
<i>C. fucicola</i> [2]	0.176	<b>0.176</b>																	
<i>C. nauticus</i> [3]	0.179	0.176	<b>0.021</b>																
<i>C. nauticus</i> [4]	0.179	0.176	<b>0.016</b>	<b>0.011</b>															
<i>C. nauticus</i> [5]	0.171	0.171	<b>0.011</b>	<b>0.021</b>	<b>0.011</b>														
<i>C. nauticus</i> [6]	0.176	0.176	<b>0.019</b>	<b>0.013</b>	<b>0.003</b>	<b>0.013</b>													
<i>C. nauticus</i> [7]	0.168	0.168	<b>0.003</b>	<b>0.023</b>	<b>0.017</b>	<b>0.014</b>	<b>0.017</b>												
<i>C. nauticus</i> [1]	0.170	0.170	<b>0.003</b>	<b>0.023</b>	<b>0.017</b>	<b>0.014</b>	<b>0.017</b>	<b>0.000</b>											
<i>C. nauticus</i> [2]	0.152	0.157	0.152	0.160	0.152	0.158	0.150	0.149	0.148										
<i>C. vestitus</i> [5]	0.152	0.157	0.152	0.160	0.152	0.158	0.150	0.149	0.148	<b>0.000</b>									
<i>C. vestitus</i> [6]	0.152	0.157	0.152	0.160	0.152	0.158	0.150	0.149	0.148	<b>0.000</b>	<b>0.000</b>								
<i>C. vestitus</i> [7]	0.155	0.159	0.152	0.160	0.158	0.163	0.155	0.149	0.148	<b>0.005</b>	<b>0.005</b>	<b>0.005</b>							
<i>C. vestitus</i> [8]	0.163	0.159	0.152	0.160	0.158	0.163	0.155	0.146	0.145	<b>0.003</b>	<b>0.003</b>	<b>0.003</b>	<b>0.003</b>						
<i>C. vestitus</i> [1]	0.159	0.159	0.151	0.162	0.159	0.162	0.159	0.145	0.145	<b>0.006</b>	<b>0.006</b>	<b>0.006</b>	<b>0.006</b>	<b>0.003</b>					
<i>C. vestitus</i> [2]	0.152	0.152	0.155	0.166	0.157	0.155	0.157	0.154	0.156	<b>0.009</b>	<b>0.009</b>	<b>0.009</b>	<b>0.015</b>	<b>0.012</b>	<b>0.012</b>				
<i>C. vestitus</i> [3]	0.158	0.162	0.152	0.160	0.158	0.163	0.155	0.149	0.148	<b>0.005</b>	<b>0.005</b>	<b>0.005</b>	<b>0.005</b>	<b>0.003</b>	<b>0.006</b>	<b>0.009</b>			
<i>C. vestitus</i> [4]	0.163	0.161	0.203	0.200	0.198	0.198	0.200	0.206	0.208	0.174	0.174	0.174	0.176	0.180	0.185	0.178	0.179		
<i>P. nudus</i> [1]	0.163	0.162	0.198	0.195	0.198	0.198	0.200	0.200	0.203	0.174	0.174	0.174	0.171	0.174	0.179	0.178	0.174	0.005	
<i>P. nudus</i> [2]	0.163	0.162	0.198	0.195	0.198	0.198	0.200	0.200	0.203	0.174	0.174	0.174	0.171	0.174	0.179	0.178	0.174	0.005	

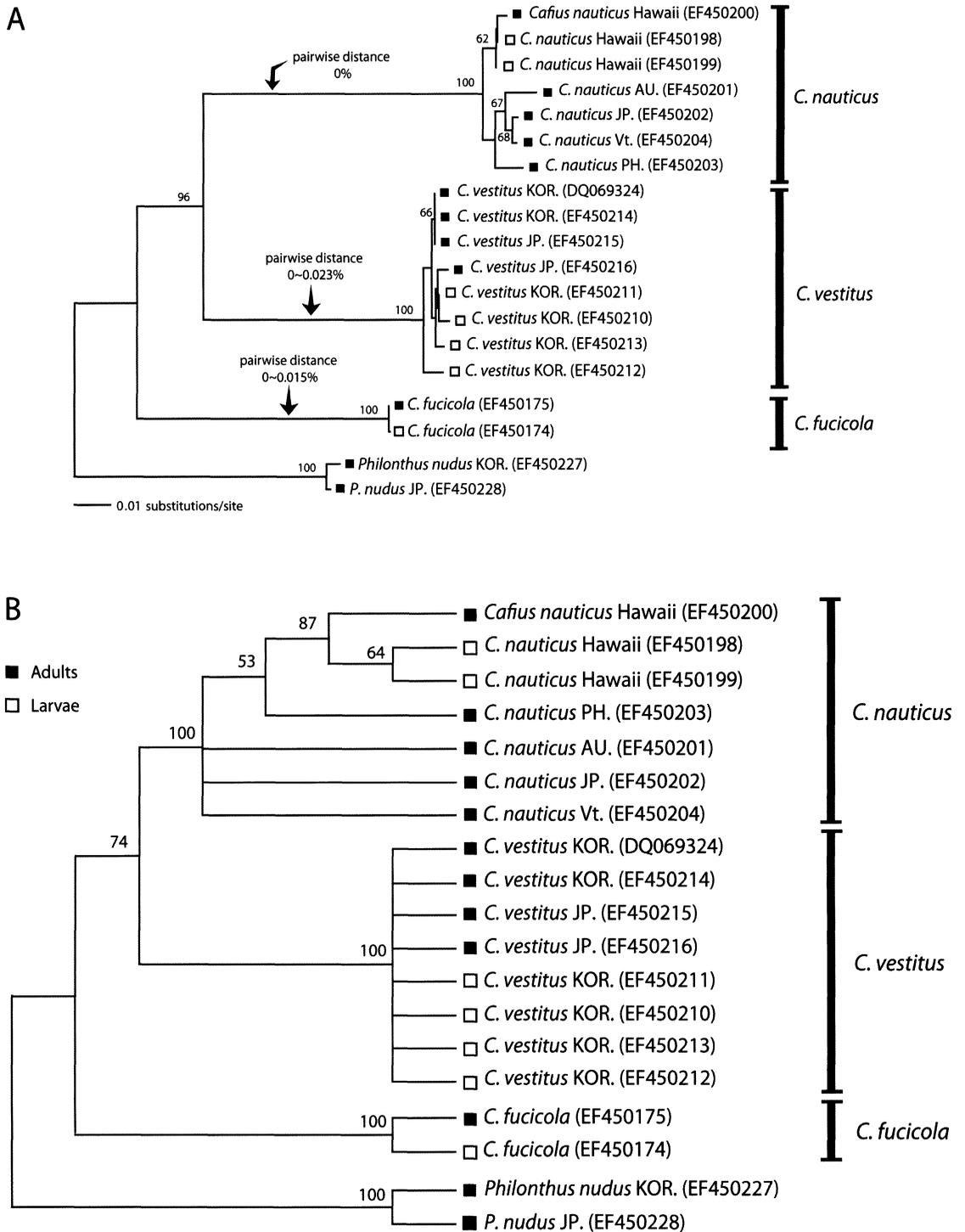


Fig. 1. A, Neighbor-Joining tree based on *cytochrome oxidase* II sequences; B, strict consensus tree of 30 equally parsimonious trees (Tree length = 157, CI = 0.85, RI = 0.95). Every terminal branch at the species level is supported by 100% bootstrap values.

Late Instars of *Cafius fucicola* Curtis

Description: Length 6.5~7.0 mm. General body shape elongate, flattened, parallel-sided. Body pale, head pale ferruginous.

HEAD: Sub-quadrate, almost equally wide from apical to basal margin; about 1.2 times as long as wide. Four stemmata present. Ecdysial sutures distinct and complete from near antennal insertion. Antennae (Fig. 2A): Four-articled. Length of articles 1<sup>st</sup> < 4<sup>th</sup> < 2<sup>nd</sup> = 3<sup>rd</sup>; article 1

longer than wide, transverse; one campaniform sensillum present on middle of article 2; article 3 with 2 solenidia (III S1 and III S2) and 1 campaniform sensillum, 1 corn-type sensory appendage present; article 4 with 4 solenidia (IVS1~IVS4); article 3 and 4 each with 3 setae. Mentum with 3 pairs of setae. Gular suture constricted in middle of head, divergent to apex.

MOUTHPARTS: Nasale (Fig. 2B): Nine teeth present; central 3 teeth pointed (LT4 and LT5), central tooth (LT5) smallest, penultimate teeth

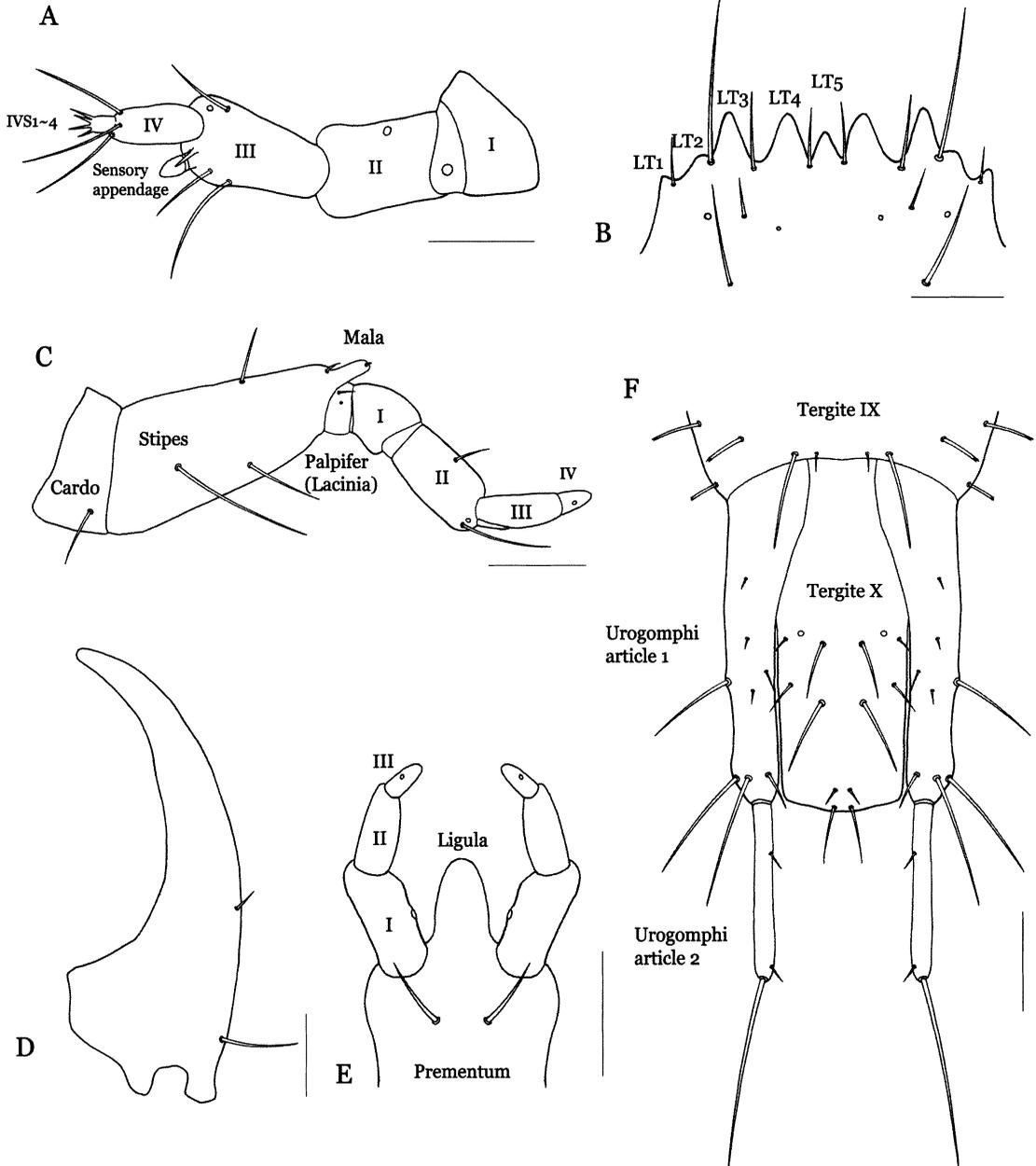


Fig. 2. *Cafius fucicola*. A, Antenna; B, nasale; C, maxilla; D, mandible; E, labium; F, urogomphi, dorsal view. Scale = 0.1 mm.

(LT2) very weak. Eight setae present along outer line. Maxilla (Fig. 2C): Stipes almost as long as maxillary palpus; articulated mala with small seta at apex, small, elongate-ovoid; maxillary palpus with 4 articles, a separate sclerotization forming a short ring at base in form of an extra segment present, 1 seta and 1 campaniform sensillum present; length ratio of palpomeres 1<sup>st</sup> : 2<sup>nd</sup> : 3<sup>rd</sup> : 4<sup>th</sup> = 1:2.8:2.8:0.6; width of palpomeres 1<sup>st</sup> = 2<sup>nd</sup> > 3<sup>rd</sup> = 4<sup>th</sup>. Mandible (Fig. 2D): Two macrosetae present along outer surface, falciform, undivided at acute apex, almost symmetrical. Labium (Fig. 2E): Labial palpus with 3 articles; length ratio of palpomeres 1<sup>st</sup> : 2<sup>nd</sup> : 3<sup>rd</sup> = 1:0.91:0.49, conical ligula reaching end of labial palpomere 1, pubescent at base.

THORAX: Pronotum: Transverse, lightly sclerotized, setae scattered at sides and on disc. Length of pronotum about 2× length of mesonotum. Mesonotum length subequal to metanotum, both slightly broader than posterior margin of pronotum. Legs: Coxa, trochanter, femur, tibia and tarsungulus distinguishable, tarsungulus with 3 articulated spines.

ABDOMEN: Abdominal tergite I-VIII transverse, parallel sided, slightly narrowed to apex. Tergites and sternites divided by midlongitudinal line; Tergite X (Fig. 2F) about 3 times longer than wide. Urogomphi (Fig. 2B): Two-articled, much longer than tergite X; article 1 slender and with 4 pairs of macrosetae, as long as tergite X; article 2 with 2 small setae and 1 large seta arising from apex, much narrower than article 1.

SPECIMENS EXAMINED: England: Devon, Plymouth, Mount Batten point, 7 VIII 2004, K.-J. Ahn and J.-S. Park, *ex* under seaweeds (CNUIC, 1).

REMARKS: This species can be distinguished from other *Cafius* species by the combination of the following features: 2<sup>nd</sup> antennomere as long as 3<sup>rd</sup>, mala longer than lacinia, 2 articled urogomphi, 1<sup>st</sup> article of urogomphi as long as tergite X, conical ligula subequal to 1<sup>st</sup> labial palpomere, and mandible without serration.

#### Late Instars of *Cafius nauticus* (Fairmaire)

Description: Length 8.3~9.0 mm. General body shape elongate, flattened, parallel-sided. Body pale, head pale ferruginous.

HEAD: Sub-quadrate, almost equally wide from apical to basal margin. About 0.8 times as long as wide. Four stemmata present. Ecdysial sutures distinct and complete from near antennal insertion. Antennae (Fig. 3A): Four-articled. Length of articles 1<sup>st</sup> = 4<sup>th</sup> < 2<sup>nd</sup> = 3<sup>rd</sup>; article 1 longer than wide, transverse; 1 campaniform sensillum present on middle of article 2; article 3 with 2 solenidia (III S1 and III S2) and 2 campaniform sensilla, 1 corn-type sensory appendage present; article 4 with 4 solenidia (IVS1~IVS4); article 3 and 4 each with 3 setae. Mentum with 3

pairs of setae. Gular suture converged in middle of head, divergent to apex.

MOUHPARTS: Nasale (Fig. 3B): Nine teeth present; central 3 teeth pointed (LT4 and LT5), central tooth (LT5) smallest, penultimate teeth (LT2) very weak. Eight setae present along outer line. Maxilla (Fig. 3C): Stipes almost as long as maxillary palpus; articulated mala with small seta at apex, small, elongate-ovoid; maxillary palpus with 4 articles, a separate sclerotization forming a short ring at base in form of an extra segment present, 1 seta and 1 campaniform sensillum present; length ratio of palpomeres 1<sup>st</sup> : 2<sup>nd</sup> : 3<sup>rd</sup> : 4<sup>th</sup> = 1:2.6:2.6:1.1; width of palpomeres 1<sup>st</sup> = 2<sup>nd</sup> > 3<sup>rd</sup> > 4<sup>th</sup>. Mandible (Fig. 3D): Two macrosetae present along outer surface, falciform, undivided at acute apex, almost symmetrical and molar area saw-like. Labium (Fig. 3E): Labial palpus with 3 articles; length ratio of palpomeres 1<sup>st</sup> : 2<sup>nd</sup> : 3<sup>rd</sup> = 1:0.8:0.4, conical ligula a little shorter than palpomere 1, pubescent at base.

THORAX: Pronotum: Transverse, lightly sclerotized, setae scattered at sides and on disc. Pronotum about 1.6 times longer than mesonotum. Mesonotum length subequal to metanotum, both as long as posterior margin of pronotum. Legs: Coxa, trochanter, femur, tibia and tarsungulus distinguishable, tarsungulus with 3 articulated spines.

ABDOMEN: Abdominal tergite I-VIII transverse, parallel sided, slightly narrowed to apex. Tergites and sternites divided by midlongitudinal line; Tergite X (Fig. 3F) about 3 times longer than wide. Urogomphi (Fig. 3F): Two-articled, much longer than tergite X; article 1 slender, as long as tergite X; article 2 with 2 small setae and 1 large seta arising from apex, much narrower than article 1.

SPECIMENS EXAMINED: USA: Hawaii, Oahu, Honolulu, Kawailoa beach, 1 VIII 1992, K.-J. Ahn, *ex* under seaweeds (CNUIC, 8).

REMARKS: This species can be distinguished from other *Cafius* species by combination of the following features: 2<sup>nd</sup> antennomere as long as 3<sup>rd</sup>, mala as long as lacinia, 2 articled urogomphi, 1<sup>st</sup> article of urogomphi as long as tergite X, conical ligula subequal to 1<sup>st</sup> labial palpomere, and mandible with serration.

#### Late Instars of *Cafius vestitus* (Sharp)

Description: Length 7.0~9.0 mm. General body shape elongate, flattened, parallel-sided. Body pale, head pale ferruginous.

HEAD: Sub-quadrate, almost equally wide from apical to basal margin. About 0.74 times as long as wide. Four stemmata present. Ecdysial sutures distinct and complete from near antennal insertion. Antennae (Fig. 4A): Four-articled. Length of articles 1<sup>st</sup> = 4<sup>th</sup> < 3<sup>rd</sup> < 2<sup>nd</sup>; article 1 longer than wide, transverse; 1 campaniform sen-

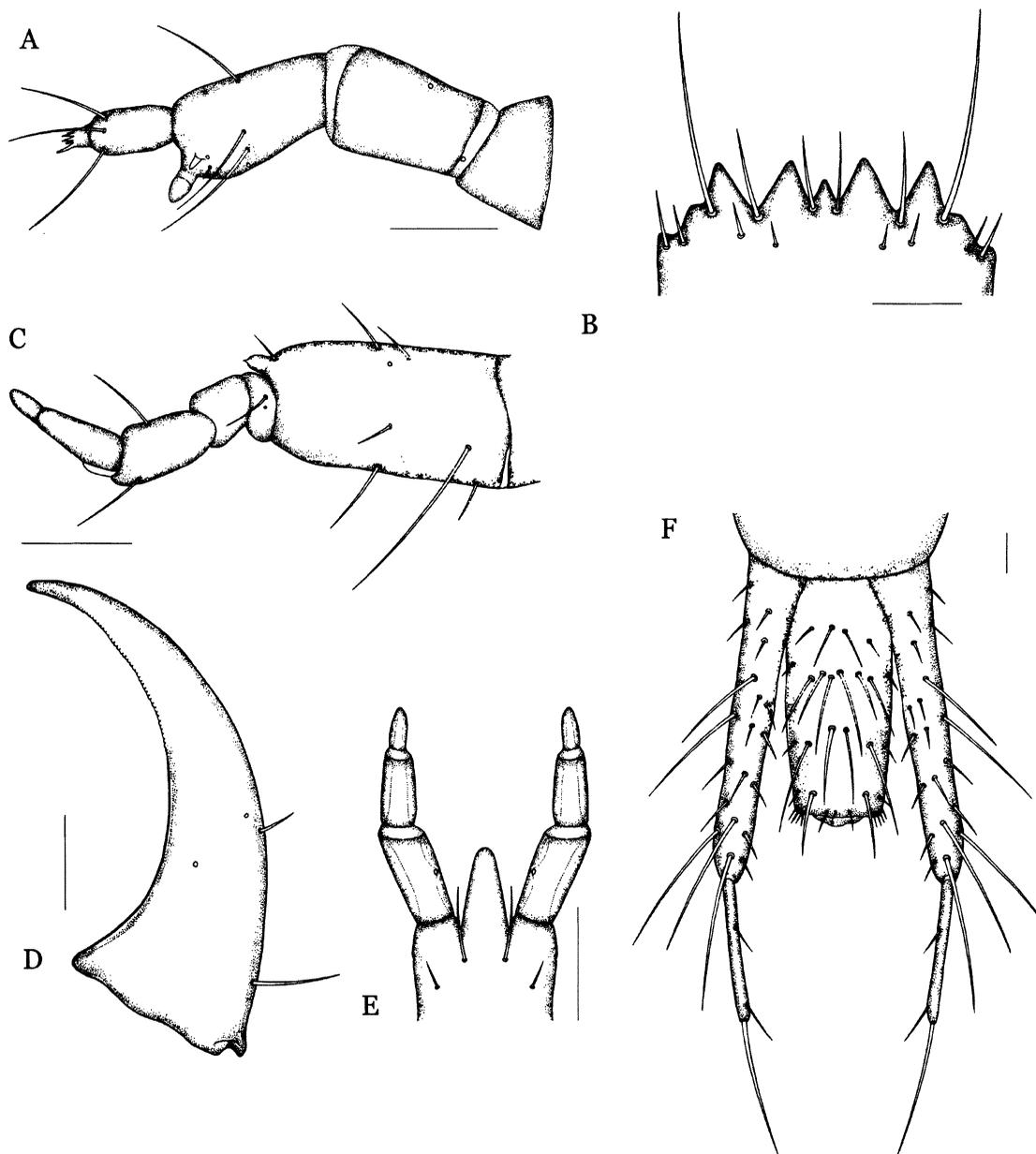


Fig. 3. *Cafius nauticus*. A, Antenna; B, nasale; C, maxilla; D, mandible; E, labium; F, urogomphi, dorsal view. Scale = 0.1 mm.

sillum present on middle of article 2; article 3 with 2 solenidia (III S1 and III S2) and 2 campaniform sensillia, 1 corn-type sensory appendage present; article 4 with 4 solenidia (IVS1~IVS4); article 3 and 4 each with 3 setae. Mentum with 3 pairs of setae. Gular suture converged in middle of head, divergent to apex.

**MOUTHPARTS:** Nasale (Fig. 4B): Nine teeth present; central 3 teeth pointed (LT4 and LT5), central tooth (LT5) smallest, penultimate teeth (LT2) very weak. Eight setae present along outer

line. Maxilla (Fig. 4C): Stipes a little shorter than maxillary palpus; articulated mala with small seta at apex, elongate-ovoid; maxillary palpus with 4 articles, a separate sclerotization forming a short ring at base in form of an extra segment present, 1 seta and 1 campaniform sensillum present; length ratio of palpomeres 1<sup>st</sup> : 2<sup>nd</sup> : 3<sup>rd</sup> : 4<sup>th</sup> = 1:2:2:1; width of palpomeres 1<sup>st</sup> = 2<sup>nd</sup> > 3<sup>rd</sup> > 4<sup>th</sup>. Mandible (Fig. 4D): Two macrosetae present along outer surface, falciform, undivided at acute apex, almost symmetrical. Labium (Fig. 4E): La-

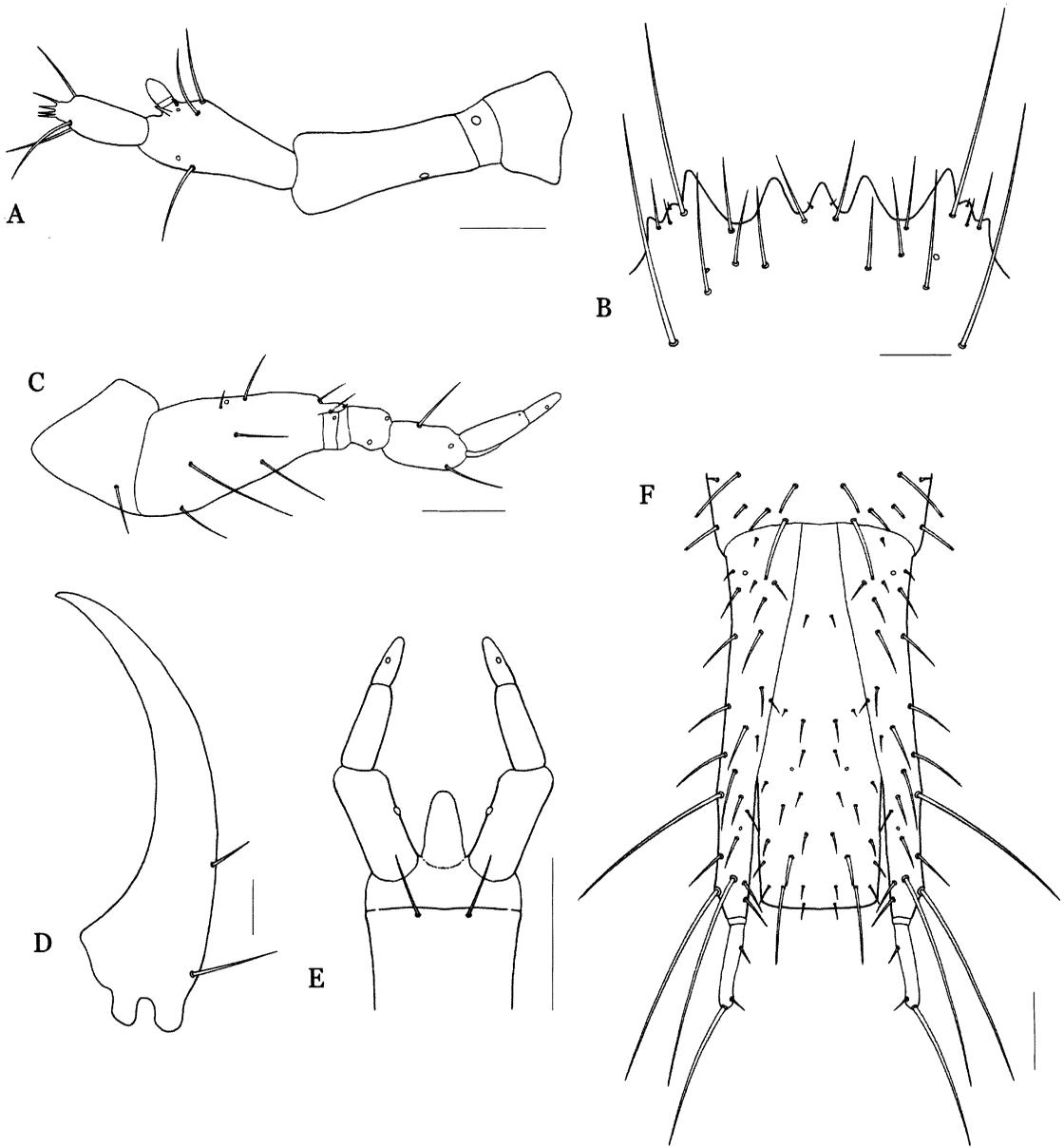


Fig. 4. *Cafius vestitus*. A, Antenna; B, nasale; C, maxilla; D, mandible; E, labium; F, urogomphi, dorsal view. Scale = 0.1 mm.

bial palpus with 3 articles; length ratio of palpomeres 1<sup>st</sup> : 2<sup>nd</sup> : 3<sup>rd</sup> = 1:0.8:0.5, conical ligula a little shorter than palpomere 1, pubescent at base.

**THORAX:** Pronotum: Transverse, lightly sclerotized, setae scattered at sides and on disc. Pronotum about 1.5 times longer than mesonotum. Mesonotum length subequal to metanotum, both as long as posterior margin of pronotum. Legs: Coxa, trochanter, femur, tibia and tarsungulus distinguishable, tarsungulus with 3 articulated spines.

**ABDOMEN:** Abdominal tergite I-VIII transverse, parallel sided, slightly narrowed to apex. Tergites and sternites divided by midlongitudinal line; Tergite X (Fig. 4F) about 3 times longer than wide. Urogomphi (Fig. 4F): Two-articled, longer than tergite X; article 1 slender, longer than tergite X; article 2 with 2 small setae and 1 large seta arising from apex, much narrower than article 1.

**SPECIMENS EXAMINED:** Korea: Gangwon Prov., Donghae City, Daejin beach, 21 VII 2004, M.-J. Jeon, *ex* under seaweeds (CNUIC, 3); Jeju

Prov., Bukjeju-gun, Gujwa-eup, Hado-ri, 11 VI 2005, M.-J. Jeon and K.-J. Ahn, S.-J. Park, *ex* under seaweeds (CNUIC, 1).

REMARKS: This species can be distinguished from other *Cafius* species by a combination of the following features: 2<sup>nd</sup> antennomere longer than 3<sup>rd</sup>, mala as long as lacinia, 2 articulated urogomphi, 1<sup>st</sup> article of urogomphi longer than tergite X, conical ligula shorter than 1<sup>st</sup> labial palpomere, and mandible without serration.

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