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# Proposal of new specific status for tea-infesting populations of the nominal citrus spiny whitefly *Aleurocanthus spiniferus* (Homoptera: Aleyrodidae)

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## Abstract

The citrus spiny whitefly *Aleurocanthus spiniferus* (Quaintance) is a pest of citrus plants that is native to South-East Asia. Although serious outbreaks of the tea-infesting whitefly in China, Taiwan and Japan have been attributed to this species over the last 20 years, recent research has shown different host preferences between the two whiteflies. Hence, the two pests have tentatively been differentiated as tea-infesting and citrus-infesting populations. We further compared morphological, acoustic and genomic features between the two populations in Japan. Morphological differences were recognised in the arrangement of spines, porettes and papillae on the dorsal disc and number of marginal crenulations and marginal waxy fringe of 4$^{th}$-instar nymphs, as well as wing maculation and genitalic organs of adults. In courtship behaviour, the acoustic properties of male vibratory signals also differed between the two. Furthermore, genetic analysis of mtCOI sequences (759 bp) showed that the tea-infesting population was clearly distinct from the citrus-infesting group, with high bootstrap values. The mtCOI sequence identities were 76.2% between the two populations. Genetic differentiation between the two populations was shown by the high value (0.99650) of pairwise *F*st, indicating the sexual isolation of the two populations. Consequently, these two populations are regarded as different representatives, consisting of a sibling relationship, but clearly distinguished from each other as independent genomic populations. Here, we describe the tea-infesting population and propose a new scientific name, *Aleurocanthus camelliae* Kanmiya & Kasai **sp. nov.**, and a new common name, camellia spiny whitefly, thus distinguishing it from *A. spiniferus* (Quaintance), the citrus spiny whitefly that constitutes the citrus-infesting population.

**Key words:** Aleyrodidae, citrus spiny whitefly, host preference, mating signals, mtCOI sequences, new species, tea pest

## Introduction

The citrus (or orange) spiny whitefly *Aleurocanthus spiniferus* (Quaintance) is among the most serious pests of citrus plants (Byrne *et al*. 1990). It originated in tropical Asia and has spread to Africa, Australia, the Pacific Islands and Italy (Nguyen *et al*. 1993; Gyeltshen *et al*. 2010). *A. spiniferus* was recognised to be an invasive pest of citrus plants planted in Nagasaki, Japan, in 1915; thereafter, it spread rapidly to Kyushu, becoming an exceedingly destructive pest (Clausen 1978). However, *A. spiniferus* was fully controlled on citrus by an introduced parasitoid wasp (*Encarsia smithi*) from China, and heavy infestations decreased to a low level (Kuwana & Ishii 1927; Ohgushi 1969). In addition to being a citrus pest, *A. spiniferus* has also been thought to damage tea plants (*Camel-*

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*lia sinensis*) in temperate China over the past 20 years. Han and Cui (2003) reviewed several prominent outbreaks said to involve *A. spiniferus* in the main tea regions of China since the 1960s.

The distributional range of *A. spiniferus* appeared to have fully expanded to the tea-growing regions of China by 1989. The presence of *A. spiniferus* in tea gardens eventually spread to Taiwan (Suh 1994) and Japan (Yamashita *et al*. 2005), although no records of *A. spiniferus* occurring as a pest of *Ca. sinensis*, *Camellia sasanqua* or *Cleyera japonica* and *Eurya japonica* existed at that time in Japan (Japanese Society of Applied Entomology and Zoology 2006). The tea-infesting *A. spiniferus* population in Japan quickly spread to four prefectures in Kinki district by 2008, and subsequent outbreaks followed in another eight prefectures during 2009–2010 (Kasai *et al*. 2010). Kasai *et al*. (2010) investigated the host plant suitability of some *Camellia* and *Citrus* plant species for tea- and citrus-infesting *A. spiniferus* populations in Japan. They recognised significant differences in host preference between two populations based on oviposition and larval feeding behaviour. The citrus-infesting population laid no eggs on *Camellia* leaves, whereas the tea-infesting population laid a few eggs on *Citrus* but no nymphs settled on *Citrus* leaves. Kasai *et al*. (2010) suggested that the Japanese tea-infesting population was derived from that of China or Taiwan. In this study, we extensively compared all adult and nymphal stages of the tea-infesting population, reared from five theaceous plants, and the citrus-infesting population, reared from three rutaceous plants, to ascertain morphological differences between the two.

In a preliminary investigation of male mating signals of both populations using tea-infesting whiteflies obtained from Uji, Kyoto, and citrus-infesting whiteflies from Okabe, Shizuoka, Japan, we recognised differences in acoustic properties between the host types (Kanmiya *et al*. 2009). In this study, we added acoustic data from specimens obtained from tea plants in four different prefectures and citrus plants from Shizuoka Prefecture, distinguishing them as the tea- and citrus-infesting populations.

## Material and methods

**Taxonomy.** For microscopic examination, nymphs and puparia were mounted using the following method: Live nymphal materials were treated with 10% KOH solution for 1–2 nights at room temperature or heated gently below the boiling point for about 5 min; removed from the KOH solution and placed in 70% acetic acid for 30 min at room temperature; placed in 2.5–3.5% hydrogen peroxide to bleach the black cuticle to a brownish colour; rinsed with 85% ethanol; placed in lactophenol and heated at 70°C for 30 min; removed from lactophenol and placed in 70% acetic acid for 30 min at room temperature; placed in glacial acetic acid for 30 min; removed from glacial acetic acid, treated with acetosalicylate and heated at 70°C for 30 min; transferred to carboxylol for 20 min at room temperature and finally mounted in Canada balsam. The morphological terminology for nymphs and adults was that of Bink-Moenen (1983), Martin (1985) and Gill (1990).

**Morphological observation.** A digital HF microscope ( VH-8000 or VHX-1000, KEYENCE, Tokyo, Japan) with a zoom lens (VH-Z450 or VH-Z75, KEYENCE) and a scanning electron microscope (VE-9800, KEYENCE) were used at the Institute of Comparative Studies of International Cultures and Societies, Kurume University, Kurume, Japan. A scanning electron microscope (JSM-5510LV; JEOL, Tokyo, Japan) and optical stereomicroscope (DM2500; Leica, Wetzlar, Germany) were also used at the Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto, Japan. Body parts were measured using a calibrated ocular micrometer.

**Morphometric analysis.** To compare the quantitative characters of the two pest populations, we collected 4$^{th}$-instar nymphs of tea- and citrus-infesting populations. Table 1 lists the specimens used in this study. In 2010, 45 female 4$^{th}$-instar nymphs of a tea-infesting population were collected in Kyoto City and Yame district, Japan, and 53 female 4$^{th}$-instar nymphs of a citrus-infesting population were collected in Shizuoka City, Shimizu City and Seiyo City, Japan. The live individuals were measured (width of fringe wax) under a stereomicroscope. In 2009, 39 female 4$^{th}$-instar nymphs of a tea-infesting population were collected in Uji City, Ohchi district and Yame district, Japan, and 40 female 4$^{th}$-instar nymphs of a citrus-infesting population were collected in Kyoto City, Chikugo City and Karatsu City, Japan. These individuals (after preservation in 99% ethanol) were measured (number of marginal crenulations, body length and body width) under a stereomicroscope. The width of fringe wax and the number of marginal crenulations were analysed at the $p = 0.05$ level of significance using Holm's sequentially rejective Bonferroni tests after Wilcoxon's rank-sum tests (Holm 1979). The body length and body width data were pooled for each species and analysed at the $p = 0.05$ level of significance using Wilcoxon's rank-sum test.

**Acoustic analysis.** We compared acoustic properties of male mating signals in tea- and citrus-infesting populations. The adult males used in this study originated from the following: Sayama, Saitama Prefecture (recorded Oct. 2009, 3 individuals), Uji, Kyoto Prefecture (Aug. 2008, 3 individuals), Ohchi, Shimane Prefecture (July 2009, 3 individuals), Yame, Fukuoka Prefecture (Sept. 2009, 4 individuals) reared on tea plants, plus Okabe, Fujieda, Shizuoka Prefecture (May 2008, 8 individuals) reared on the citrus plant *Citrus unshiu*. Host plants infested with nymphs were collected from the tea or citrus fields and reared in the laboratory at Kurume University (light:dark, 14:8 h; 25 ± 2°C room temperature) to adult emergence. The emerged adults used for mating in acoustic experiments were separated by sex in the early morning (08:00–09:30) each day, and all males were discarded in the late evening. Virgin male and female adults more than 6 h old were paired and released into a small cylindrical plastic case that contained a piece of live host leaflet. Acoustic recordings were conducted in an anechoic room or an anechoic chamber at 25 ± 2°C. The signal recording and analysing systems were as shown in Kanmiya (1996). The apparatus used to detect substrate transmitted signals in courtship was illustrated in Kanmiya (2006).

**Molecular phylogenetic and population genetic analyses.** *Aleurocanthus* populations for mitochondrial cytochrome oxidase I (mtCOI) gene sequence analysis were collected from 10 regions (7 prefectures) in Japan (Table 2). DNA extraction was performed as described in Ueda and Brown (2006). Whiteflies were ground in extraction buffer (50 mM Tris-HCl, pH 7.0; 100 mM NaCl; 10 mM EDTA; 1% SDS) and treated with a TE-saturated phenol:chloroform:isoamyl alcohol (25:24:1) solution. After centrifugation, the supernatant was mixed with 0.1 volume of 3 M sodium acetate (pH 5.2) and 3 volumes of ethanol. Nucleic acids were precipitated by centrifugation (14,000 RPM, 10 min) at 4°C and dissolved in distilled water. The mtCOI gene sequence was amplified by polymerase chain reaction (PCR) using *Ex Taq* polymerase (Takara, Shiga, Japan) with the primers 5'-TTGATTTTTTGGTCATCCAGAAGT -3' and 5'- TCCAATGCACTAATCTGCCATATTA -3' (Frohlich *et al*. 1999).

The PCR products were cloned into a pGEM-T Easy Vector (Promega, Madison, WI, USA). DNA sequences for deposit in the DDBJ/EMBL/GenBank databases were determined for cloned inserts from independent plasmids using a BigDye Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems, Foster City, CA, USA) and resolved using an ABI PRISM 3100-Avant Genetic Analyser (Applied Biosystems). Phylogenetic relationships were analysed using 759-bp mtCOI sequences from *Aleurocanthus* populations, including *Aleurotrachelus camelliae* Kuwana as an outgroup (Table 2). Its relatedness to genus *Aleurocanthus* is highlighted by features held in common, including similar wing maculation and pulsed mating signals in adult males, black puparium metallic and broadly elliptic, pointed frontal and rounded hind end, adhesive secretion and distinctly sclerotised outer margin and operculum broadly occupying the vasiform orifice. Phylogenetic relationships were determined using the maximum-likelihood (ML) method of PhyML 3.0 (Guindon & Gascuel 2003). Bootstrap values were calculated using the Hasegawa–Kishino–Yano (HKY) model of nucleotide substitution, with 1000 replicates. Genetic distances between mtCOI sequences of *Aleurocanthus* populations were estimated using Kimura's two-parameter model in MEGA 4.0 (Tamura *et al*. 2007). DnaSP v.5.10 (Librado & Rozas 2009) was used to evaluate the number of haplotypes, haplotype diversity values, nucleotide diversity and the average number of differences within populations, along with pairwise estimates of *F*st between populations.

## Taxonomy

### Genus *Aleurocanthus* Quaintance & Baker, 1914

*Aleurocanthus* Quaintance & Baker, 1914: 102. Type species: *Aleurodes spinifera* Quaintance, 1903: 63–64, by original designation.

*Aleurocanthus* Quaintance and Baker:Quaintance and Baker, 1917 (15 spp. worldwide). —Corbett, 1935 (8 spp. from Malaysia). —Takahashi, 1942 (8 spp. from Thailand). —Takahashi, 1956 (3 spp. from Micronesia). —David and Subramaniam, 1976 (14 spp. from India). —Dubey and Sundararaj, 2005 (31 spp. from India). —Martin, 1987 (5 spp. of worldwide pests). —Martin, 1999, 2005 (generic diagnosis).

**Generic diagnosis.** The genus *Aleurocanthus* Quaintance & Baker is readily recognised in puparium by many stout glandular spines on the dorsal disc and submargin, and the usual carriage of exuviae of earlier instars in a stack on the dorsum, as well as white marginal waxy fringes. Martin (1987) prepared a key to a few species of this

genus which infest economically important plants. The key included detailed figures of five species, including *A. spiniferus*.

Puparium medium in size, subelliptical or oblong in outline, colour usually dark brown to black and often fringed with white waxy secretion marginally. Margin distinctly crenulate or truncate-lobulate; submarginal area not separated from dorsum by suture. Thoracic and caudal tracheal folds and combs not discernible from dorsal view; dorsal disc and/or submargin covered with stout glandular spines of various length with acute or fimbriate apices; cephalic, 8th abdominal and caudal setae present; caudal furrow absent. Vasiform orifice small, subcircular or subcordate in outline, highly elevated as a tubercle-like projection of dorsum; operculum elliptical, almost filling orifice; lingula visible or concealed. Adult forewing usually dusky having several paler maculae, with radius and cubitus, and a prominent flexure present at the branch of R1 (vestigial) and Rs suddenly curving posteriorly at the branch beyond mid-wing length; hind wing with only radius, prominent maculation absent.

**Remarks.** The genus currently contains around 80 described species worldwide (Martin & Mound 2007; Evans 2008). It is well represented in the Oriental region, with about 50 described species. In Japan, only two species, *A. cinnamomi* Takahashi, 1931 and *A. spiniferus* (Q., 1903) are currently distributed (Miyatake 1980). The outbreak population currently infesting tea plants and that on citrus plants, which has been long established, have hitherto both been called *A. spiniferus* in Japan, China and Taiwan. The first observation that the host plant preference of Japanese tea-infesting population differs from that of the citrus-infesting population of *A. spiniferus* (Kasai *et al*. 2010) led to this investigation of possible differences in species recognition between them based on morphometric, bioacoustic and genome analyses, as discussed below.

**TABLE 1.** Geographic origin, collection date and host plants of sample populations used for morphometric analysis.

| Species | Acronym | Geographic origin (prefecture) | Host Plant | Date | n |
|---|---|---|---|---|---|
| *A. camelliae* | Uji† | Uji (Kyoto) | *Ca. sinensis* | 24 July 2009 | 21 |
| | Kyoto* | Kyoto (Kyoto) | *Ca. sasanqua* | 4 Apr. 2010 | 22 |
| | Ohchi† | Ohchi (Shimane) | *Ca. sinensis* | 15 July 2009 | 6 |
| | Yame† | Yame (Fukuoka) | *Ca. sinensis* | 23 Sept. 2009 | 12 |
| | Yame* | Yame (Fukuoka) | *Ca. sinensis* | 18 Dec. 2009 | 23 |
| *A. spiniferus* | Shizuoka* | Shizuoka (Shizuoka) | *Ci. unshiu* | 22 Feb. 2010 | 12 |
| | Shimizu* | Shizuoka (Shizuoka) | *Ci. unshiu* | 16 Apr. 2010 | 24 |
| | Kyoto† | Kyoto (Kyoto) | *Ci. unshiu* | 25 Apr. 2009 | 12 |
| | Seiyo* | Seiyo (Ehime) | *Ci. unshiu* | 29 Apr. 2010 | 17 |
| | Chikugo† | Chikugo (Fukuoka) | *Ci. natsudaidai* | 23 Nov. 2009 | 10 |
| | Karatsu† | Karatsu (Saga) | *Ci. unshiu* | 20 July 2009 | 18 |

*Width of fringe wax measured from live samples. †Number of marginal crenulations, body length and body width measured from samples in ethanol.

## Description of species

### *Aleurocanthus camelliae* Kanmiya & Kasai, sp. nov.

**Puparium.** (Figs. 1F, H, I, 3E, 6A) Length: (female) 1084.8 ± 51.1 µm (mean ± SD), range: 988–1237 µm (n = 42); (male) 796.3 ± 23.2 µm, range: 650–858 (n = 41); width: (female) 751.5 ± 45.9 µm, range: 624–858 µm; (male) 491.3 ± 29.5 µm, range: 390–572 µm. Dorsum highly sclerotised, oval-shaped, convex on submedian areas of cephalothorax and abdomen; middle length of puparium located at abdominal segments II (75%) or III (25%) in the female and abdominal segments I (71%) or II (29%) in the male. Length/width ratio of puparium: (female) 1.45 ± 0.1 µm (n = 18); (male) 1.62 ± 0.1 µm (n = 24). Cephalic eyespot ovoid, clearly defined with a distinct rim, located laterally and close to base of 3rd submarginal spine. Dorsal abdominal sutures distinct on segments III/VIII, especially depressed as a deep suture on VII/VIII. Tergite VIII 49.0 ± 6.9 µm long and 0.74 ± 0.11 times as long as the width of the vasiform orifice (female, n = 10). Vasiform orifice distinctly elevated, obtuse, 1.28 ± 0.08 times

longer than wide, 84.3 ± 4.1 µm long, 65.9 ± 2.3 µm wide (female, n = 10), inset from posterior puparial margin by its own width, fully occupied by the operculum, which obscures lingula unless operculum is raised. Operculum in dorsal view 58.9 ± 8.9 µm long, 56.1 ± 6.4 µm wide (female, n = 10), with posterior margin roundly depressed and fringed by thick, microscopic hairs. Lingula usually not visible in the final pupal stage, but always prominent in 4th nymphal stage, seemingly bi-segmented, with dense microscopic hairs and a pair of long setae apically (Fig. 4B); its length subequal to length of operculum when protruding to excrete.

**Margin.** Outline oblong, widest across abdominal segment II/III in the female, and across abdominal segments I/II in the male; marginal crenulation rather tightly arranged with 1.1–1.3-µm gap between the teeth (Fig. 5E): each tooth 20–22 µm long, 12.5–14 µm wide, total number of marginal crenulations 174.6 ± 10 (n = 21) in female; number of teeth within 0.1 mm: 6–8 in female, 7–10 in male. Microscopic submarginal papillae present roughly in a row outside of submarginal spines that are approximately 2 µm long, 3–5 in number between spines (Figs. 1H, 4D).

**TABLE 2.** Geographic origin, collection years and host plants of sample populations used for molecular phylogenetic and population genetic analysis.

| Code No. | Acronym | Geographic origin (prefecture) | Year | Host plant | Species | Accession no. |
|---|---|---|---|---|---|---|
| 1 | Toshima | Toshima (Kumamoto) | 2008 | *Ci. natsudaidai* | *A. spiniferus* | AB536792 |
| 2 | Ashikita | Ashikita (Kumamoto) | 2008 | *Ci. unshiu* | *A. spiniferus* | AB536793 |
| 3 | Chikugo | Chikugo (Fukuoka) | 2009 | *Ci. natsudaidai* | *A. spiniferus* | AB558172 |
| 4 | Uji | Uji (Kyoto) | 2008 | *Ca. sinensis* | *A. camelliae* | AB536794 |
| 5 | Kameyama | Kameyama (Mie) | 2009 | *Ca. sinensis* | *A. camelliae* | AB536795 |
| 6 | Yagyuu | Yagyuu (Nara) | 2009 | *Ca. sinensis* | *A. camelliae* | AB536796 |
| 7 | Shigaraki | Shigaraki (Shiga) | 2009 | *Ca. sinensis* | *A. camelliae* | AB536797 |
| 8 | Asamiya | Asamiya (Shiga) | 2009 | *Ca. sinensis* | *A. camelliae* | AB536798 |
| 9 | Toyonaka | Toyonaka (Osaka) | 2009 | *Ca. sinensis* | *A. camelliae* | AB536799 |
| 10 | Daito | Daito (Osaka) | 2009 | *Ca. japonica* | *A. camelliae* | AB536800 |
| 11 | Tsubaki | Kurinodake (Kagoshima) | 2009 | *Ca. japonica* | *Aleurotrachelus camelliae* | AB536801 |

**TABLE 3.** Nymphal chaetotaxy of *A. camelliae* **sp. nov.**

| | | Nymphal chaetotaxy | | | |
|---|---|---|---|---|---|
| | | 1st instar | 2nd instar | 3rd instar | 4th instar |
| Anterior marginal setae | | 4 | 0 | 0 | 0 |
| Cephalic setae | | 0 | 1 | 1 | 1 |
| Cephalo-thoratic spines | | 2 | 6 | 7 | 9 |
| Abdominal spines | Submedian | 0 | 0 | 1 | 3 |
| | Subdorsal | 0 | 4 | 6 | 7 |
| Submarginal spines | Cephalothorax | 0 | 0 | 0 | 5 |
| | Abdominal | 0 | 0 | 0 | 6 (5) |
| 8th abdominal setae | | 1 | 1 | 1 | 1 |
| Caudal setae | | 1 | 1 | 1 | 1 |
| Posterior marginal setae | | 1 | 0 | 0 | 0 |
| | Total setae | 7 | 3 | 3 | 3 |
| | Total spines | 2 | 10 | 14 | 30 (29) |
| | In total | 9 | 13 | 17 | 33 (32) |

Numbers in parentheses indicate the number of males.

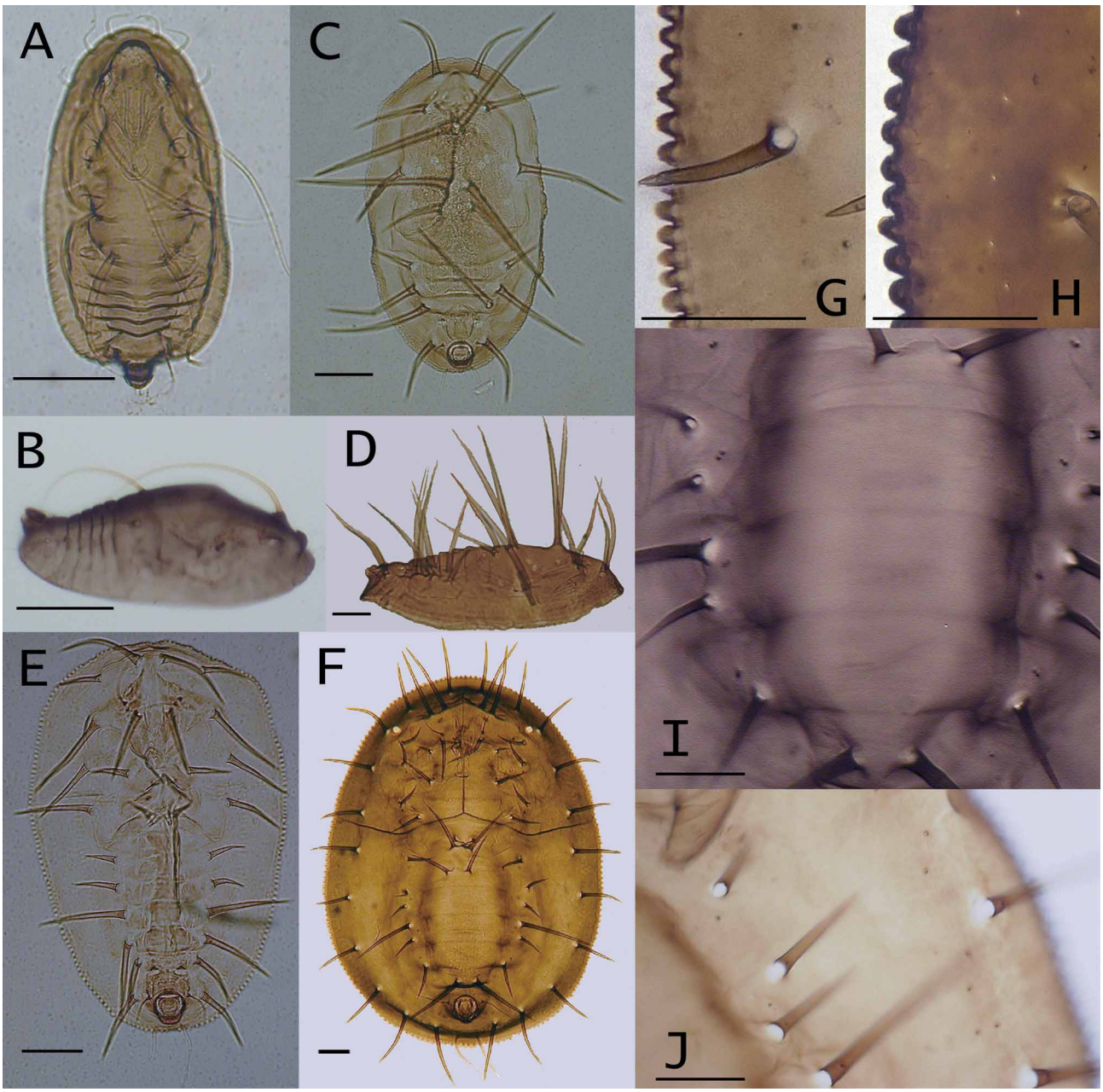


**FIGURE 1.** (A–F): Photomicrographs of slide-mounted *Aleurocanthus camelliae* **sp. nov.** (A) 1st-instar nymph, dorsal view; (B) 1st-instar nymph, lateral view; (C) 2nd-instar nymph, dorsal; (D) 2nd-instar nymph, lateral; (E) 3rd-instar nymph, dorsal; (F) 4th-instar nymph, dorsal; (G) *A. spiniferus*, puparial submarginal area, dorsal; (H, I) *A. camelliae*, puparium; (H) submarginal area; (I) abdominal tergites III–VII, showing spines and paired pores; (J) *A. spiniferus*, showing subdorsal spines, single pores, submarginal spines and porettes. Scale bars show 100 µm.

**Chaetotaxy.** Dorsal surface with 11 (female) or 10 (male) submarginal glandular spines (Figs. 1F, 3E, Table 3) that are about 90–110 µm long. Cephalic setae and 8th abdominal seta subequal in length, about 82 µm; caudal seta much longer, about 134 µm; abdominal submedian spines 3 pairs, located on abdominal tergites I/III, of which the anteriormost is shortest, about 30 µm long, middle is longest, about 130 µm; abdominal subdorsal spines 7 pairs, of which the stout 4th spine is longest and the loci among bases of the 2nd to 5th setae placed roughly linearly. Paired, very closely placed, black microscopic pores present near outside of submedian abdominal spines in vivid puparium (Fig.1I).

**Venter.** (Fig. 4A ): Surface rather smooth; wool-fibre-like, waxy bundle flowing between 1st and 2nd legs, indicating tracheal fold, but no pore and comb observed in the tip; caudal fold absent; antennae often retreated behind foreleg. Rostrum seemingly 3-segmented, 128 µm long in all, basal 1/3 thickened, with 42-µm basal width, distally

narrowed, with a needlelike stylet bundle nearly 56 µm long. Pair of fine ventral abdominal setae 20–23 µm long; spinules scattered around the area of setae. Row of waxy projections produced along inner side of marginal teeth, which line up at slightly wider intervals than marginal teeth, comprising approximately 70% of total marginal teeth; each projection 20–30 µm long, mushroom-like, with basal stalk and flat top, which may serve as larval adhesive to leaf surface.

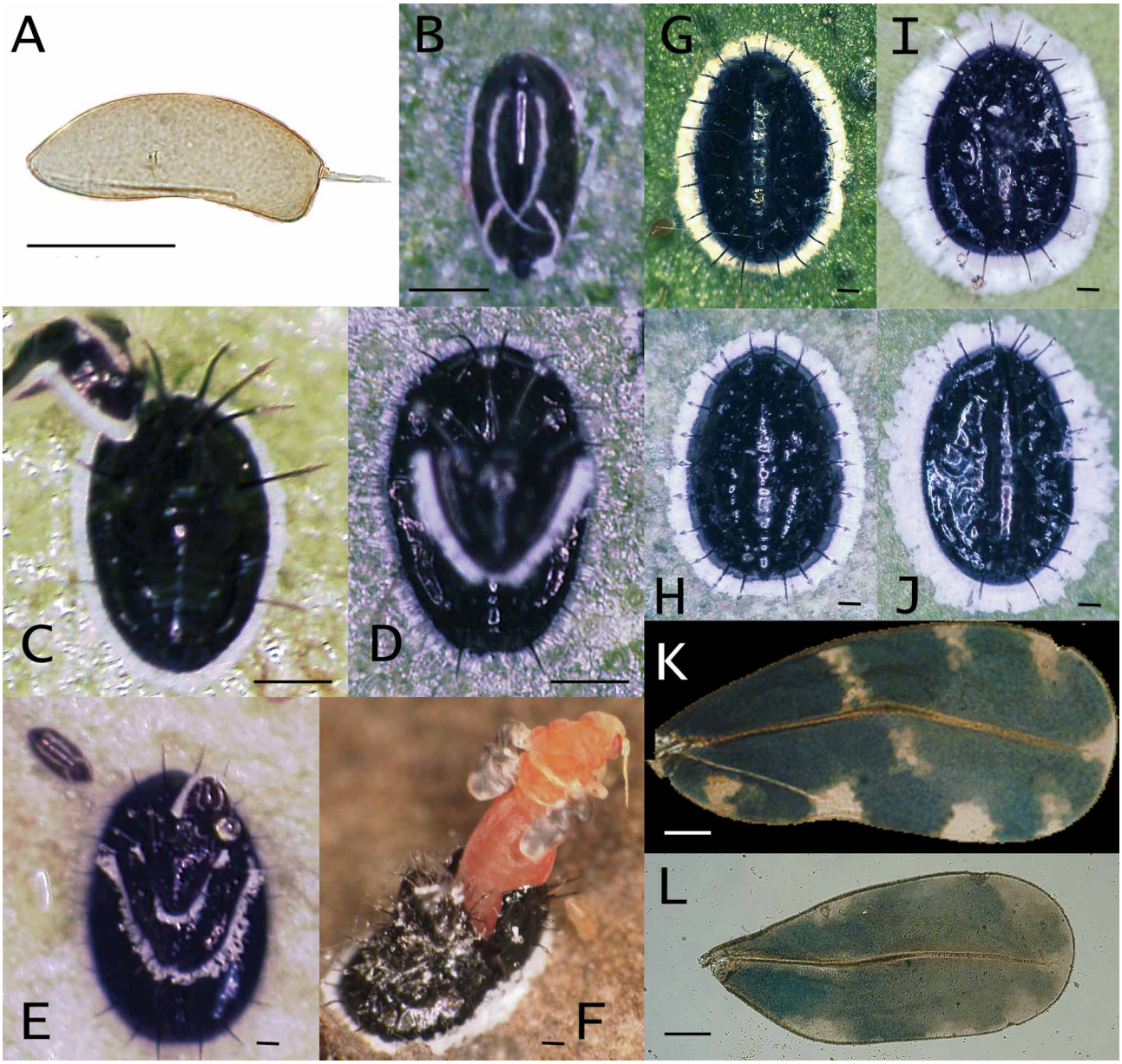


**FIGURE 2.** Habitus photographs. (A–H, K, L) *A. camelliae* **sp. nov.** (A) ovum, lateral view; (B) 1st-instar nymph; (C) 2nd-instar nymph; (D) 3rd-instar nymph; (E) 4th-instar nymph; (F) adult emerging; (G) puparium (Osaka, Daito City, reared from *Ca. japonica*); (H) puparium (Kyoto, Uji City, reared from *Ca. sinensis*); (I, J) *A. spiniferus*; (I) puparium (Fukuoka, Kurume City, reared from *Ci. natsudaidai*); (J) puparium (Shizuoka, Shimada City, reared from *Ci. unshiu*); (K) female forewing; (L) female, hind wing (both Shimane, Ohchi-gun, reared from *Ca. sinensis*). Scale bars show 100 µm.

**Adult (female).** Body length 1.25–1.40 mm. Head in dorsal view 244–272 µm wide, 2.25–2.3 times wider than frons; frons 100–110 µm long, 105–132 µm wide, weakly protruded from eye margin; eye 74–84 µm wide in dorsal view, upper and lower compound eyes connected by 3 ommatidia (Fig. 5A). Antennae (Fig. 6B) 260–320 µm in total length, two basal segments greyish-brown, distal 5 segments yellowish-white, two basal segments thickened, the 2nd nearly 3 times longer than the 1st, the 3rd segment longest, 86.2 ± 4.9 µm long (n = 6), with subapical sensoria and a cone at distal 3/5 of its length, the 7th segment also with a sensorial cone deriving at mid-length.

Rostrum 136–155 μm total length, seemingly 3-segmented, basal segments 92 μm long, 44 μm wide, distal segments 66 μm long, 24 μm wide, apically browned. Forewing (Figs. 2K, 3A, D): 1.1–1.2 mm long, 413–550 μm wide at widest width (across Fig. 3A, ②－⑦ ) and 320–450 μm wide at middle of wing (across Fig. 3A, ①－⑧ ), with 9 greyish-white maculae (Figs. 2K, 3A), their maculation most distinct soon after emergence (Fig. 3D), then turning largely brownish-green or brownish-blue, with maculae somewhat obscured by waxy powders. Hind wing (Figs. 2L, 3A) 0.95–1.02 mm long, 0.41–0.4 mm wide, evenly greyish-white, or with several blurred maculae depending on age. Abdomen with two ventral wax plates.

**Adult (male).** Body length 0.90–1.10 mm. Wing maculation almost identical to that of female. In dorsal view, tergal sclerite I vestigial, II invisible, III/VIII and subgenital plate distinct (Fig. 6E), highly darkened on tergites VI/VII, subgenital plate and claspers; each III/V tergite subequal in length, about 44 μm; tergite VI longest, 64 μm long; tergite VII reduced, 15 μm long, laterally extended and enclosing 7$^{th}$ spiracle; tergite VIII a small square, distally leaning on subgenital plate. Forewing 0.84–0.9 mm long, about 0.37 mm wide. Vasiform orifice in dorsal view about 45–59 μm long, nearly 1.2–1.3 times longer than wide; operculum rounded with distal incision, 23–35 μm long and wide; lingual 23–28 μm long, 8–11 μm wide. In lateral view, subgenital plate about 85–100 μm deep and 77–85 μm long, widely concave on anterior margin and gently depressed on ventral margin. Four distinct ventral abdominal wax plates (Fig. 6F).

**Genitalia.** (Figs. 4E, F, 6C) Aedeagus 108–120 μm long, gradually broadened basally to 23–27-μm basal width, upcurved toward apex and with slender distal half, apex extending near distal 3/4 length of clasper; clasper in dorsal view 108–123 μm long, weakly incurved and narrowed distally, angulate on outer subbasal corners, with a thin inflatable sac and apical spine.

**Ovum.** (Fig. 2A) Elliptical, the lower surface convex and the upper surface slightly concave, similar to a short banana shape; 219.7 ± 13.2 μm long (n = 11), 95.2 ± 16.5 μm wide (n = 11); stalk 49.4 ± 3.9 μm long (n = 9).

**First-instar nymph.** (Figs. 1A, B, 2B) (male and female): Elongate-oval, normally widest at posterior 3/5 length; 297 ± 18.9 μm long (n = 15), 132.8 ± 17.3 μm wide (n = 10), 93.9 ± 9.9 μm high (n = 9); ratio of length/width 2.12 ± 0.12 (n = 5). Vertex conical, gradually widening posteriorly, suddenly recessed near laterobasal margins of vasiform orifice; prominent protuberance developed at mesial cephalad region and around vasiform orifice; pair of elongate arcing spines behind cephalic protuberance and posterior thoracic margin, with anterior spine 196 ± 25 μm long (n = 15), posterior spine 114 ± 12 μm long (n = 11). Four pairs of fine anterior marginal setae and one pair of fine posterior marginal setae present.

**Second-instar nymph.** (Figs. 1C, D, 2C) More ovate, normally widest at anterior 1/3 length; 442.8 ± 71.2 μm long (n = 10), 277.2 ± 53.5 μm wide (n = 8), about 141–144 μm high; ratio of length/width 1.62 ± 0.08 (n = 8); 6 pairs of cephalothoracic and 4 pairs of abdominal subdorsal spines well developed, of which mesial 2 thoracic spines longest, 175–223 μm.

**Third-instar nymph.** (Figs. 1E, 2D) Elliptical, normally widest at anterior 1/3 length; 611.3 ± 71.6 μm long (n = 10), 403.5 ± 47.4 μm wide (n = 10), ratio of length/width 1.58 ± 0.08 (n = 10); 7 pairs of cephalothoracic and 7 pairs (1 submedian and 6 subdorsal) of abdominal spines present.

**Habitus. Puparium.** Metallic black, medially and peripherally surrounded by white marginal waxy fringe (Fig. 2G, H), width of which (female) 90–160 μm (11–16% width of puparium), (male) 66–150 μm (6–12% width of puparium). Tips of cephalothoracic and abdominal submarginal spines extending to outer edge of white marginal fringe or slightly protruding beyond it. Exuviae of earlier instars (usually 2$^{nd}$ and 3$^{rd}$) often remain stacked up on median area of immature insect (Fig. 2E).

**Adults.** After emergence, eye, thorax and abdomen predominantly reddish-yellow (pinkish), except frons, antennae and legs light yellow, then turning orange to light brown to dark brown, covered by wax powder coating except ruby eye. Wing also pale brown ground colour, with clear white original maculae (Fig. 3D), then totally turning purple–brown to greenish-brown and the maculation somewhat obscured by white waxy powder; forewings bearing 9 white maculae as in Figures 2K and 3A; hind wing pale brown or greyish, without prominent maculae. Ocellus light brown; rostrum darkened at apex. Body and wing surfaces appear white, owing to wax secretions produced from abdominal waxy plates shortly after emergence by manipulating hind legs against glandular pores.

**Ova and nymphs.** Newly deposited eggs pale yellow, then turning brown to darker before hatching; newly emerged nymphs transparent, appearing rather greenish by reflecting colour of leaves, then gradually darkening, finally becoming metallic black; 1$^{st}$-instar nymph starts producing white waxy fringes marginally (Fig. 2B) soon

after sessile state. Width of marginal waxy fringe: 1st-instar nymph (16 ± 3 µm wide, range 12–21 µm) (n = 13); 2nd-instar nymph 29 ± 4.3 µm wide, range 23–48 µm) (n = 5); 3rd-instar nymph 56 ± 7.2 µm wide, range 4–84 µm (n = 6).

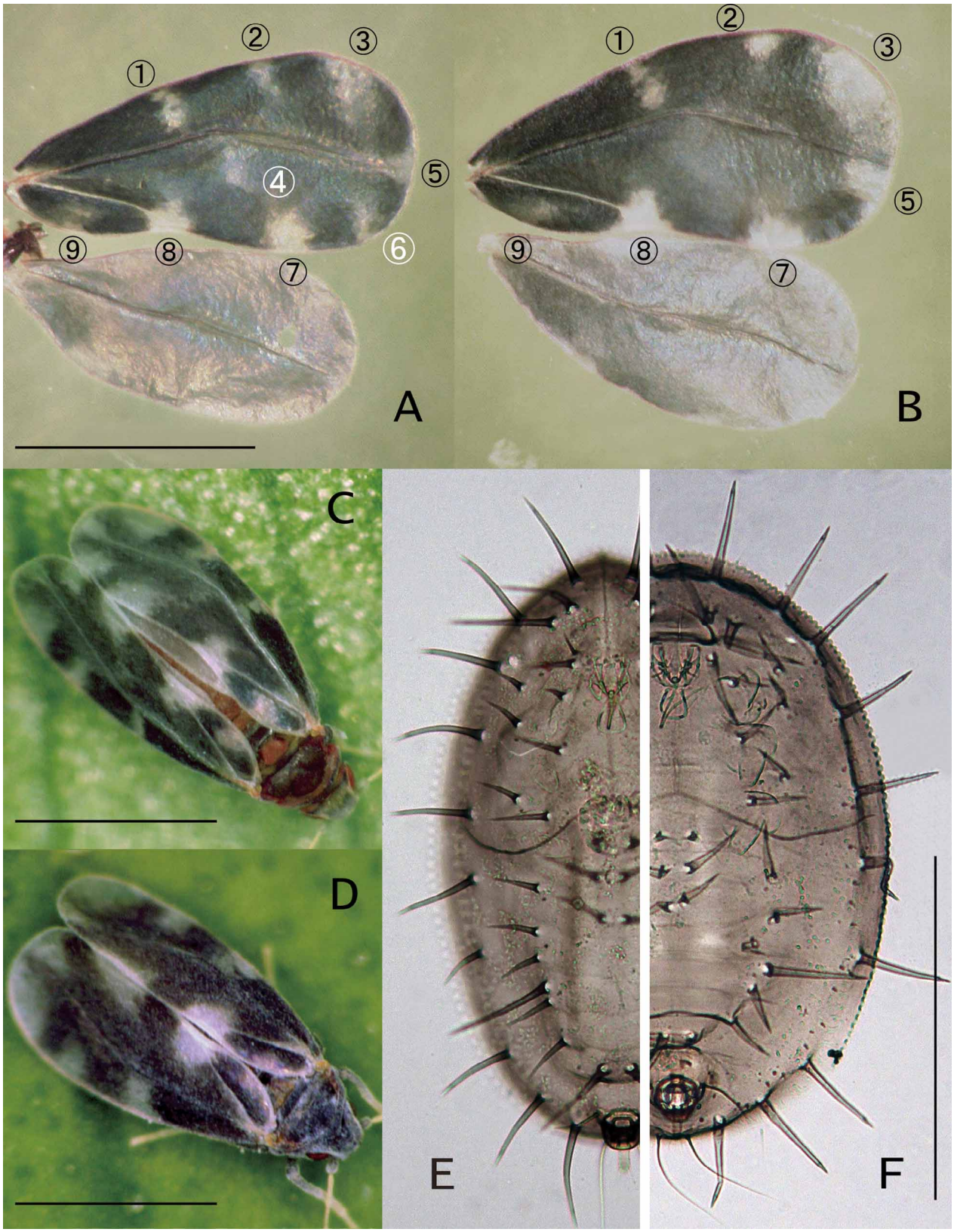


**FIGURE 3.** Habitus photographs. (A, C, E) *A. camelliae* **sp. nov.** (B, D, F) *A. spiniferus*. (A, B) Female right wings (A, Kyoto, Uji City; B, Fukuoka, Chikugo City). (C, D) Adult females, 30 min after emergence (C, Kyoto, Uji City; D, Shizuoka, Shimizu City). (E, F) Female puparia (E, Shiga, Koka City; F, Kumamoto, Toshima City). Scale bars show 500 µm.

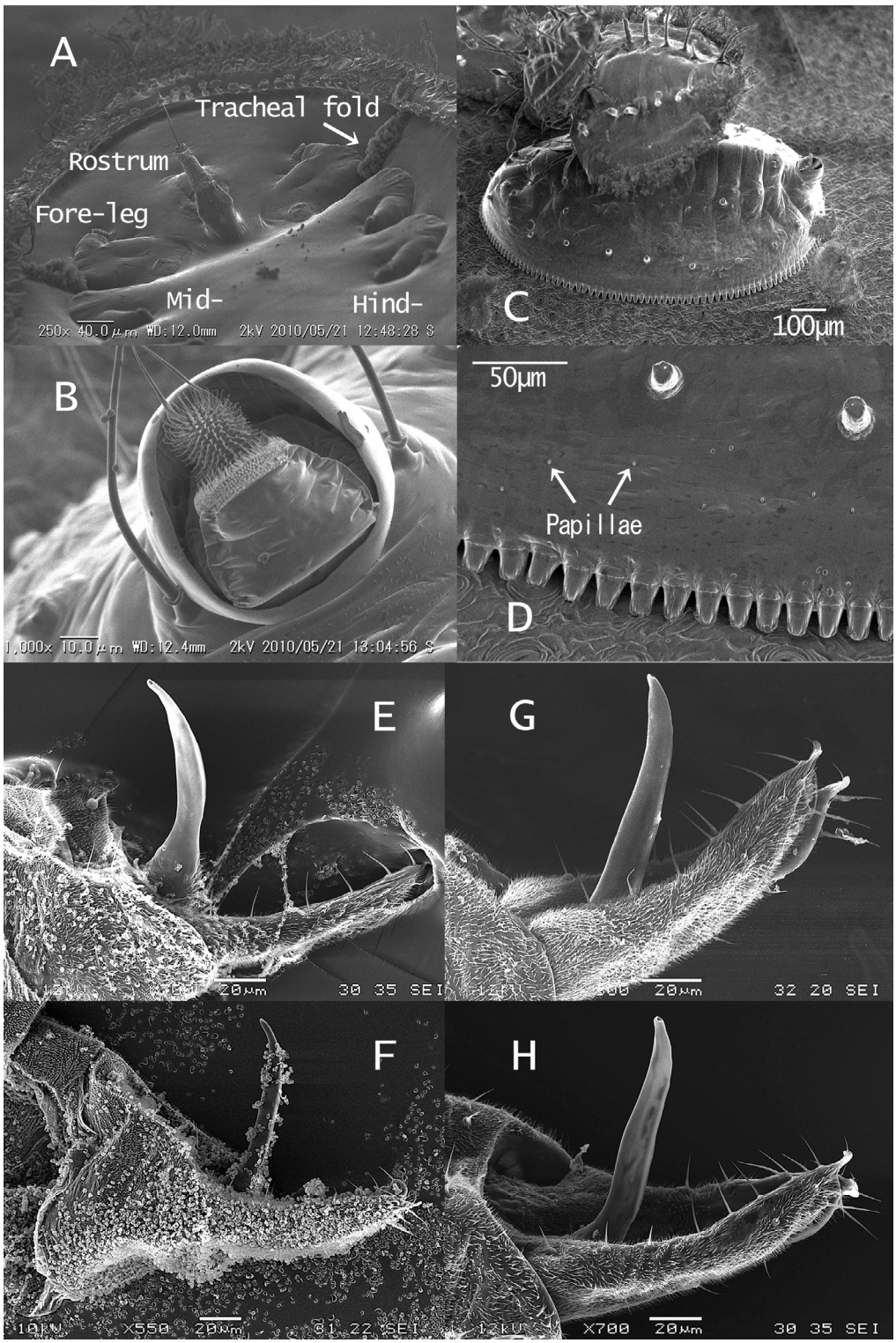


**FIGURE 4.** (A–F): *Aleurocanthus camelliae* **sp. nov.** (Kyoto, Uji City, reared from *Ca. sinensis*). (G, H) *A. spiniferus*. (A) SEM of 4th-instar nymph; (B) SEM of 4th-instar nymph, vasiform orifice; (C) SEM of 4th-instar nymph, with 2nd–3rd exuvia in dorsal; (D) SEM of 4th-instar nymph, marginal teeth and submarginal spines and porettes. (E–H) SEM of male aedeagus (E, Kyoto, Uji City, reared from *Ca. sinensis*; F, Kyoto, Kyoto City, reared from *Ca. sasanqua*; G, Ehime, Seiyo City, H, Shizuoka, Shimizu City, both reared from *Ci. unshiu*).

**Host plants.** Theaceous genera *Camellia*, *Eurya* and *Cleyera* species: *Camellia sinensis*, *Ca. sasanqua*, *Ca. japonica*, *Eurya japonica* and *Cleyera japonica*.

**Material examined.** Holotype puparium (female), Uji, Kyoto Prefecture, on tea, *Camellia sinensis*, 19.iii.2010, A. Kasai leg., deposited in Insect Museum, National Institutes for Agro-Environmental Science, Tsu-

kuba, Japan. Paratypes: 20 puparia, same data as holotype; 16 puparia on tea plants, same locality as holotype, 18.v.2009, K. Kanmiya leg.; 20 puparia on tea plants, same locality as holotype, 10.i.2010, K. Yamashita leg.; 18 puparia on tea plants, Sayama, Saitama Pref., on tea plants, 9.x.2009, Y.Sato leg.; 15 puparia on tea plants, Kami-ishizu-cho, Ohgaki, Gifu Pref., 13.x.2009, Y. Sato leg.; 18 puparia on tea plants, 3.iii.2009, Kameyama, Mie Pref., K. Kanmiya leg.; 15 puparia on tea plants, Tanba, Kyoto Pref., 15.ix.2008. Y. Yoshiyasu leg.; 20 puparia on *Ca. sasanqua*, Nishigyo, Kyoto Pref., 4.iv.2010, Y. Yoshiyasu leg.; 20 puparia on tea plants, Asamiya, Shiga Pref., 4.iv.2009, K. Kanmiya leg.; 20 puparia on tea plants, Asamiya, Shiga Pref., 2.x.2010, A. Kasai leg.; 12 puparia on tea plants, Yagyu, Nara Pref., 4.iv.2009, K. Kanmiya leg.; 10 puparia on *Ca. sasanqua*, Tsukigase, Nara Pref., 4.iv.2009, K. Kanmiya leg.; 7 puparia on tea plants, Kamishinden, Toyonaka, Osaka Pref., 5.v.2009, K. Kanmiya leg.; 20 puparia on tea plants, Ajimaoku, Sasayama, Hyogo Pref., 9.ix.2010, J. Yase leg.; 16 puparia on tea plants, Okayama, Okayama Pref., 7.vii.2010, Y. Sato leg.; 15 puparia on tea plants, 14.vii.2009, Ohchi-gun, Shimane Pref., Y. Sato leg.; 15 puparia on tea plants, 26.xii.2009, Kitsuki, Oita Pref., Y. Sato leg.

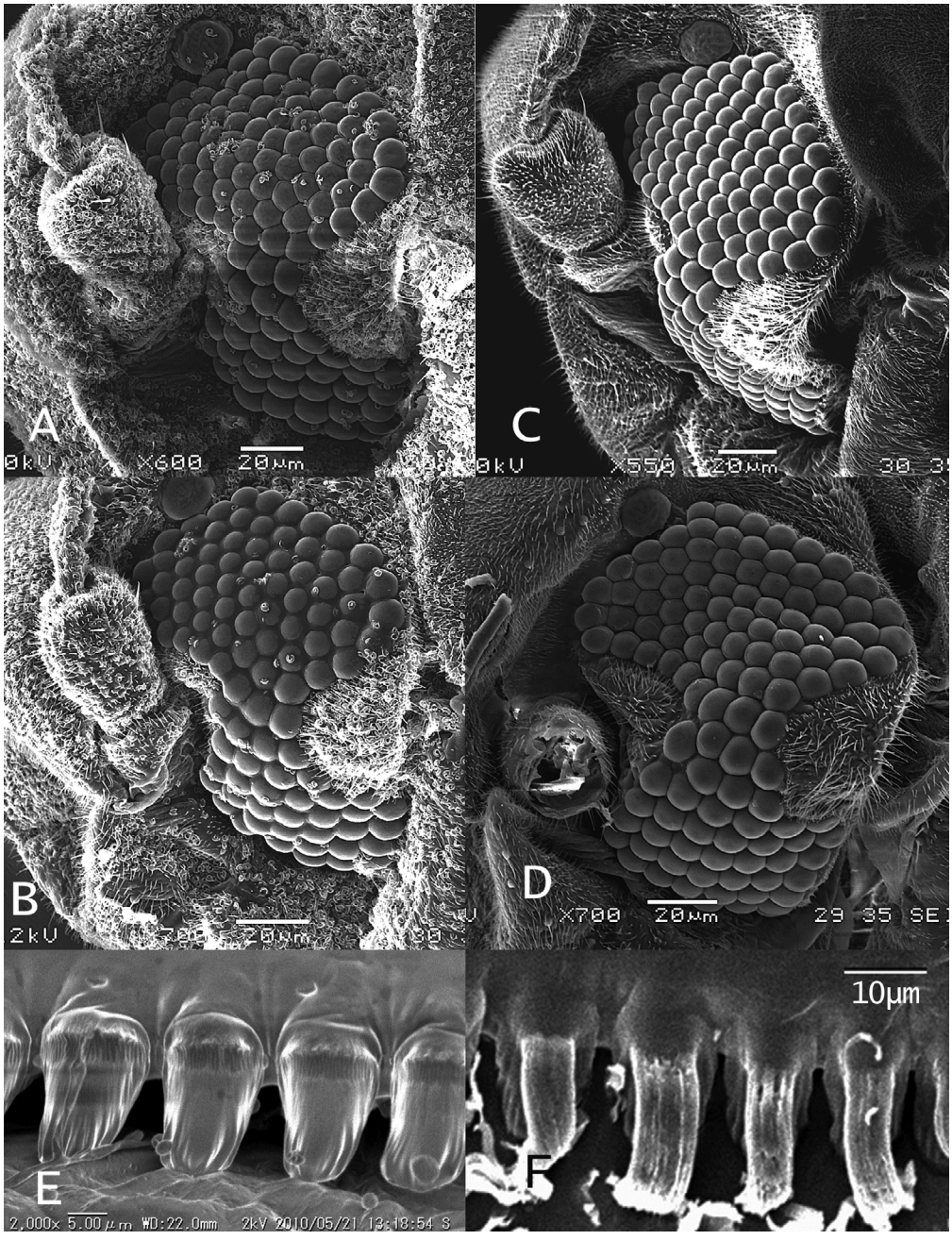


**FIGURE 5.** (A, B, E) *Aleurocanthus camelliae* **sp. nov.** (C, D, F) *A. spiniferus*. (A–D) SEM of compound eyes; (A) Kyoto, Uji City, female; (B) Kyoto, Uji City, male; (C) Shizuoka, Shimizu City, female; (D) Shizuoka, Shimizu City, male); (E, F) SEM of marginal teeth (E, Kyoto, Uji City; F, Shizuoka, Shimizu City).

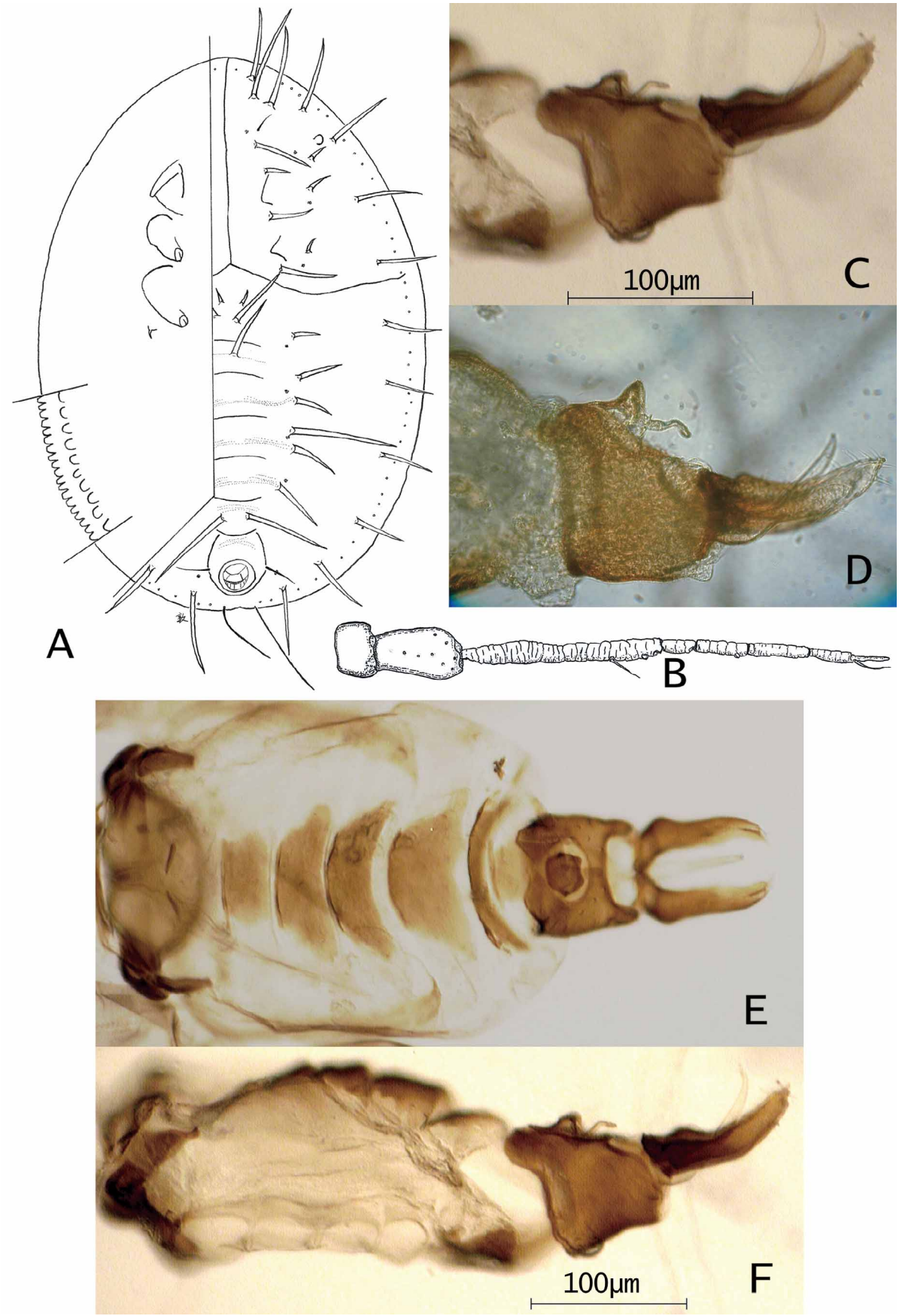


**FIGURE 6.** (A–C, E, F) *Aleurocanthus camelliae* **sp. nov.** (D) *A. spiniferus*. (A) female puparium, dorsal view; (B) female antenna, lateral; (C, D) male genital segments, lateral; (E, F) abdominal segments (E, dorsal view; F, lateral view).

**Specimens depository.** Some paratypes of *A. camelliae* **sp. nov.** will be deposited in the following institutions: The Natural History Museum, London; US National Museum of Natural History, Washington DC; National Taiwan University, Taipei; Institute of Zoology, Chinese Academy of Sciences, Beijing; Yokohama Plant Protection Station, Kanagawa.

**TABLE 4.** Morphological differences in puparial and adult stages between *A. camellinae* **sp. nov.** and *A. spiniferus*.

| Structure | Character | Discernible points | |
|---|---|---|---|
| | | *A. camelliae* | *A. spiniferus* |
| Food habits | Food and oviposition preference | Theaceae, Illiciaceae, Cornaceae, Aquifoliaceae | Rutaceae, Annonaceae, Ebenaceae, Vitaceae, Rosaceae, Flacourtiaceae |
| Wing | Forewing maculation | 9 white maculae | 7 white maculae |
| Male abdomen | Aedeagus lateral view | Distally upcurved on dorsal margin | Distally straight on dorsal margin |
| Ditto | Subgenital plate lateral view | Deeply incised on anterior and ventral margins | Weakly depressed on anterior margin, rather convex on ventral margin |
| Female 4$^{th}$-instar nymph | Marginal wax secretion | Weakly developed, width 11.2–15.8% of puparial width | Well developed, width 17.3–30.0% of puparial width |
| Ditto | Marginal crenulation | Teeth lined with loose gaps, less than 200 total (158–196) | Teeth lined with narrow gaps, more than 200 total (205–242) |
| Ditto | Cephalic eyespot | Clearly difined, very closely placed to 3$^{rd}$ cephalothoracic submarginal spine | Weakly defined, relatively placed close to 3$^{rd}$ submarginal spine than to the 2$^{nd}$ |
| Ditto | Submedian abdominal spines | Sockets of 2$^{nd}$ to 5$^{th}$ spines lined up roughly linearly | Sockets not in line, 2$^{nd}$ & 4$^{th}$ placed distal and 3$^{rd}$ & 5$^{th}$ proximal |
| Ditto | Microscopic papillae near submarginal spines | Lined outside submarginal spines | Situated between submarginal spines |
| Ditto | Abdominal tergite VIII | 49.0 ± 10.1 µm long; length/length of vasiform orifice = 1.60 ± 0.26 µm | 69.3 ± 8.1 µm long; length/length of vasiform orifice = 1.15 ± 0.09 µm |

**Comments.** Despite the almost identical features of the adult and nymphal stages of *A. camellinae* **sp. nov.** and *A. spiniferus* (Q.), we recognised very few, but clearly distinct, morphological differences in the puparial and adult stages, as listed in Table 4. This new species is rather similar to *Aleurocanthus hibisci* Corbett, 1935 distributed in Malaysia, Singapore and Reunion Islands, but its pronounced length of cephalothoracic spines and closely arranged 9$^{th}$ and 10$^{th}$ submarginal spines will be well differentiated from the present new species. *Aleurocanthus gordoniae* Takahashi, 1942 known from Hong Kong is also peculiar in its theaceous host plant, *Gordonia* sp., but is distinguished from the present new species in having vasiform orifice perfectly circular and puparium with reduced spine-chaetotaxy of 8 abdominal pairs (2 submedian + 6 subdorsal) , not 10 pairs.

## Results

**Morphometric analysis.** In each case, the width of fringe wax of *A. camelliae* **sp. nov.** was significantly narrower than that of *A. spiniferus* (Holm's sequentially rejective Bonferroni tests, after Wilcoxon's rank-sum tests, $p < 0.05$; Fig. 7). Likewise, the number of marginal crenulations of *A. camelliae* **sp. nov.** was significantly lower in each case than that of *A. spiniferus* (Holm's sequentially rejective Bonferroni tests, after Wilcoxon's rank-sum tests, $p < 0.05$; Fig. 8). However, body length ($p = 0.0724$) and body width ($p = 0.382$) did not significantly differ between the species (Wilcoxon's rank-sum tests; Fig. 9).

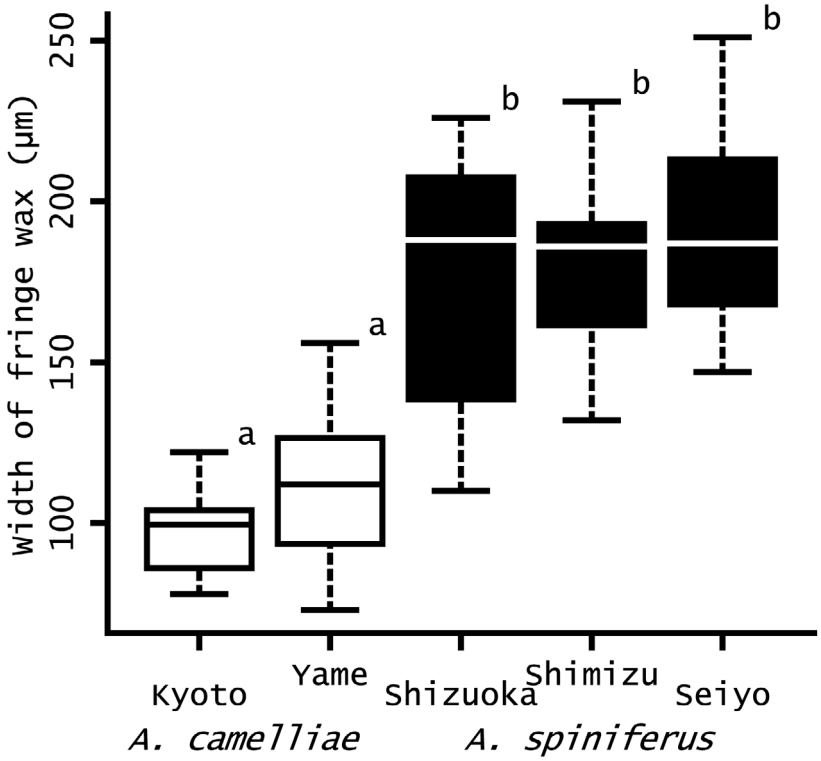


**FIGURE 7.** Width of fringe wax of 4th-instar nymph females of two spiny whitefly species, *A. camelliae* **sp. nov.** and *A. spiniferus*.

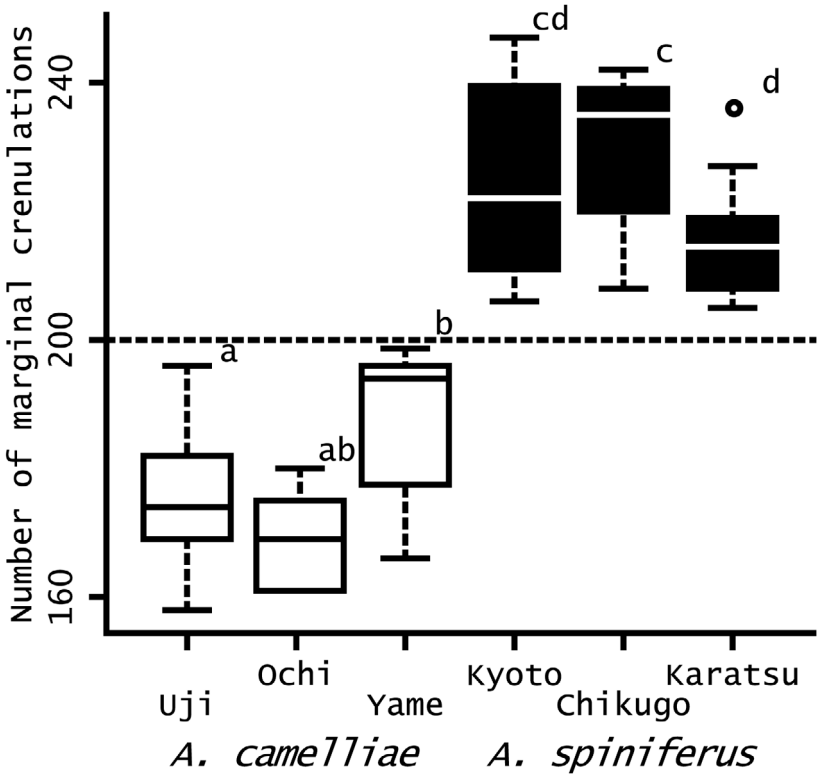


**FIGURE 8.** Number of marginal crenulations of 4th-instar nymph females of two spiny whitefly species, *A. camelliae* **sp. nov.** and *A. spiniferus*.

**Acoustic analysis.** Newly emerged (after 1/8–1/4 day) adult males and females of both *A. camelliae* **sp. nov.** and *A. spiniferus* gather on young shoots of their host plants as a mating site. The courting males engage in competitive mating behaviours and continuously emit vibratory signals to females on sunny mornings from about 10:00–12:00 h. Receptive females return response signals using similar vibratory sounds. We found that the signal components of *A. camelliae* **sp. nov.** and *A. spiniferus* were quite different. Males of *A. camelliae* **sp. nov.** produced 1–5 series of compact 11–25 pulses lasting 30–52 ms, with very constant long intervals of 157–271 ms (Fig. 10). Males of *A. spiniferus* produced a train of continuous pulse series lasting 27.6.9 ± 5.6 ms, with very short intervals of 60.5 ± 12.5 ms. In *A. camelliae* **sp. nov.**, the fundamental frequency of the male mating signals was 250–311 Hz, whereas it was 190–205 Hz in *A. spiniferus*. Figure 11 shows the most significant difference between *A. camelliae* **sp. nov.** and *A. spiniferus* pulse intervals.

**Molecular phylogenetic and population genetic analyses.** Of the mtCOI sequence, 759 bp was used in phylogenetic and population genetic analyses. The phylogenetic relationships analysis of the mtCOI sequences showed a clear distinction between seven *A. camelliae* **sp. nov.** individuals (Table 2, nos. 4–10) and three *A. spiniferus* individuals (Table 2, nos. 1–3), with high bootstrap values (Fig. 12). mtCOI sequence identities were 76.2% between *A. camelliae* **sp. nov.** and *A. spiniferus*. In population genetic analysis (Table 2), seven *A. camelliae* **sp. nov.** samples showed two haplotypes (haplotype diversity: Hd = 0.28571; Table 5), with the average number of differences (K) equaling 0.57143 and nucleotide diversity ($\pi$) being 0.00075. These values were lower than those of *A. spiniferus* (Hd = 0.66667, K = 0.66667, $\pi$ = 0.00088; Table 5). Genetic differentiation between *A. camelliae* **sp. nov.** and *A. spiniferus* was estimated using pairwise *F*st. The high *F*st value (0.99650) of pairwise comparisons indicated genetic differentiation between the two species (Table 5). These results strongly supported the results of the morphological analysis.

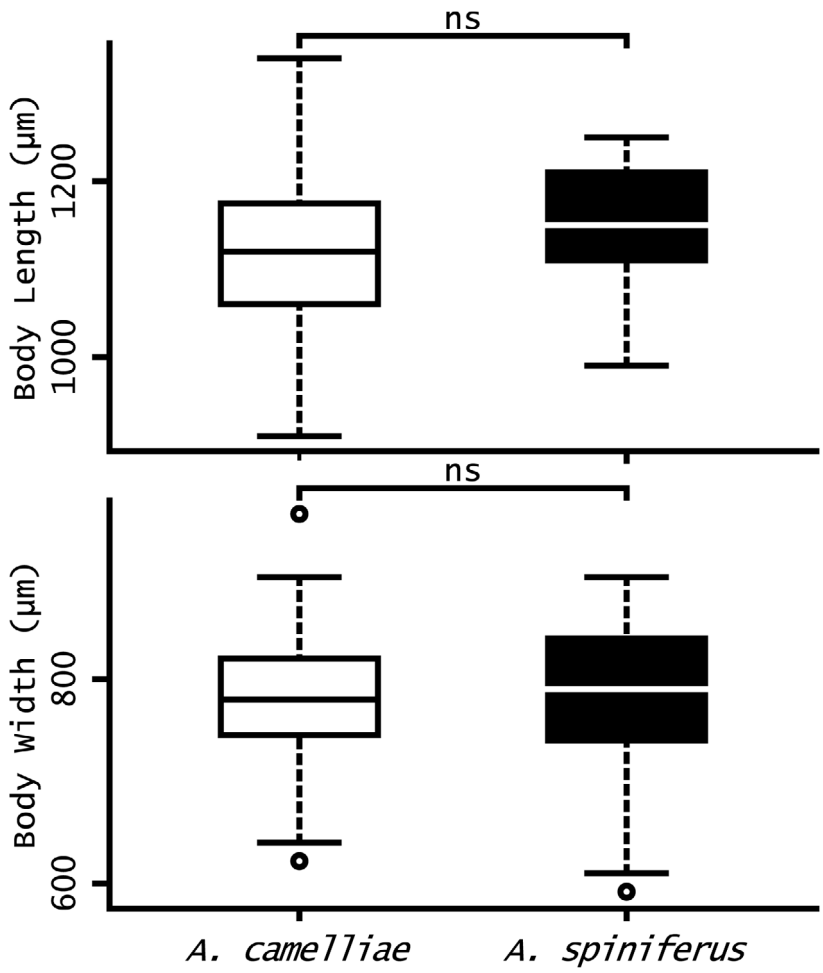


**FIGURE 9.** Body length (upper) and body width (lower) of 4th-instar nymph females of two spiny whitefly species, *A. camelliae* **sp. nov.** and *A. spiniferus.*

**TABLE 5.** Genetic variation and pairwise estimates of *F*st based on mtCOI sequences on populations of *A. camellinae* sp. nov. and *A. spiniferus*.

| Population (n) | Species | Number of haplotype (h) | Haplotype diversity (Hd) | Average number of differences (K) | Nucleotide diversity (π) | *F*st |
|---|---|---|---|---|---|---|
| Camellia (7) | *A. camelliae* | 2 | 0.28571 | 0.57143 | 0.00075 | 0.99650* |
| Citrus (3) | *A. spiniferus* | 2 | 0.66667 | 0.66667 | 0.00088 | |
| Total (10) | | 4 | 0.64444 | 82.73333 | 0.10900 | |

* P < 0.01

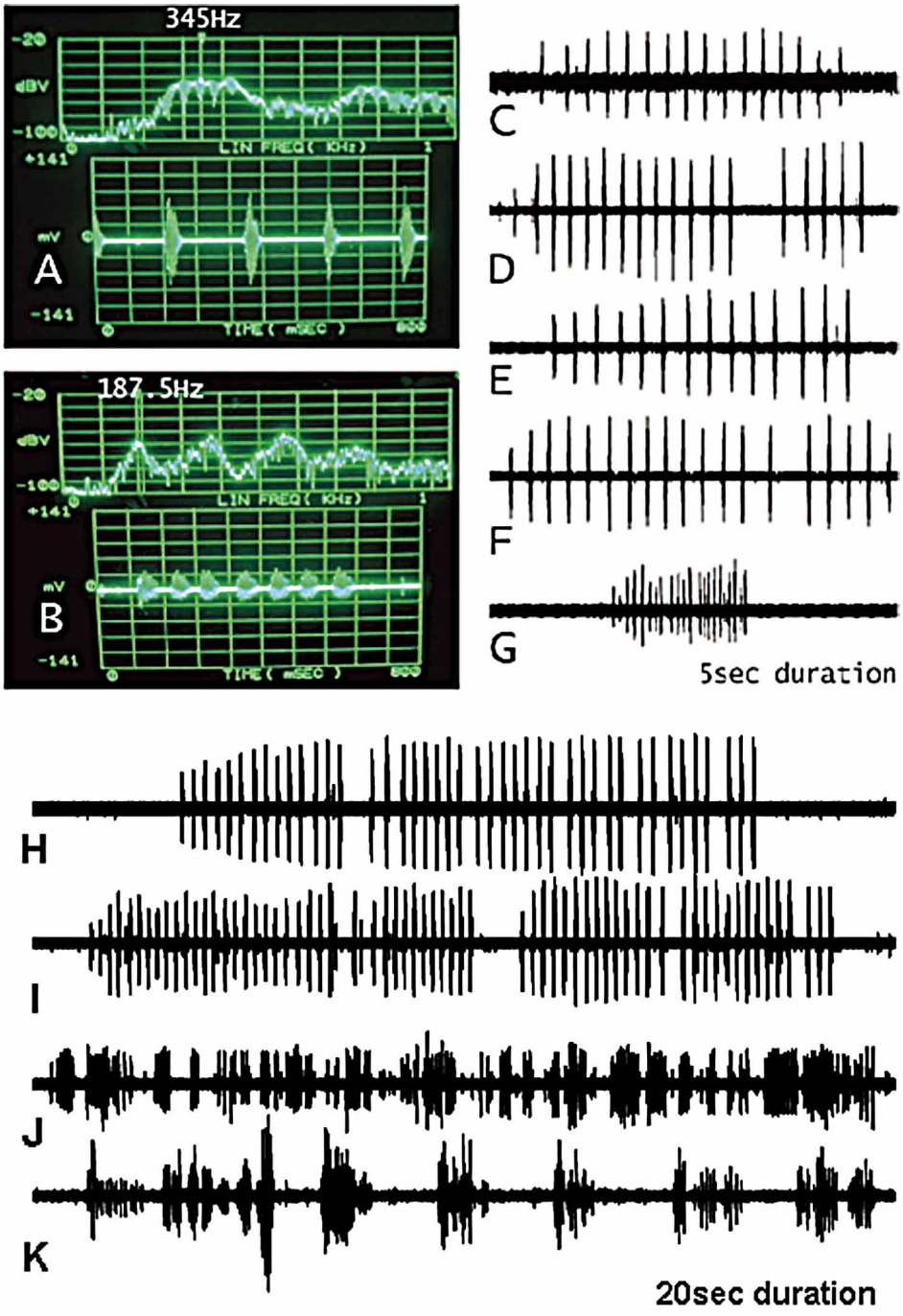


**FIGURE 10.** (A, C–F) *Aleurocanthus camelliae* **sp. nov.** from *Ca. sinensis*; (B, G) *A. spiniferus* from *Ci. unshiu*. (A, B) upper, 1-kHz instantaneous power spectra; lower, 800-ms oscillograms; (C–G) oscillograms of male mating sounds within 5-s durations; (C) Saitama, Sayama City, (D) Kyoto, Uji City; (E) Shimane, Ohchi-gun, (F) Fukuoka, Yame City; (G) Shizuoka, Okabe-cho.

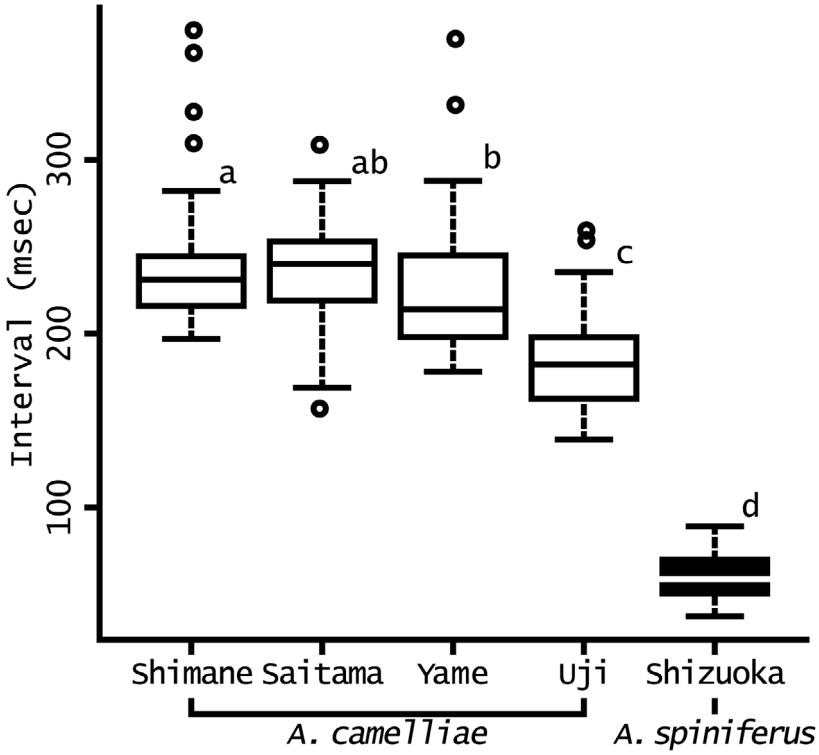


**FIGURE 11.** Pulse intervals within a train of male vibratory sounds of two spiny whitefly species, *A. camelliae* **sp. nov.** and *A. spiniferus*. Different letters in the figure indicate significant differences at $p = 5\%$ (Wilcoxon's rank-sum test).

## Discussion

Many whitefly species have become serious pests upon introduction into new geographical areas, where they may outcompete other pests (Martin 1996, 2001, 2004), usually in the absence of natural control agents such as parasitoids. The occurrence of *A. camelliae* **sp. nov.** in Japan appears to be such an introduction, even though it is likely to have come from elsewhere in the Oriental or eastern Palaearctic area. Tokihiro (2005) recorded 14 whitefly species from freshly cut twigs of *Cl. japonica* and *E. japonica* imported from China and intercepted at Japanese plant quarantine. Both theaceous trees are widely used for religious purposes, and large quantities of cut twigs are imported from China. Among the intercepted whiteflies was *A. spiniferus*, found on both *E. japonica* and *Cl. japonica*, with photo evidence of habitus puparium. According to our diagnostic criteria, the narrow waxy fringe shown in the puparium photo (width of the fringe observable was about 12% of the puparium width) clearly corresponds to the characteristics of *A. camelliae* **sp. nov.** Fortunately, we were able to examine puparial specimens obtained from live twigs of Chinese *E. japonica* intercepted at Japan plant quarantine offices, courtesy of the Yokohama Plant Protection Station. These specimens were in good accordance with the description of the present new species. These results suggest that the Japanese population of *A. camelliae* **sp. nov.** is recently derived from the reported foreign population imported with freshly cut twigs of theaceous plants.

Adult females of *A. camelliae* **sp. nov.** lay a few eggs on the citrus leaves, but nymphs do not settle on these leaves (Kasai *et al*. 2010). In contrast, adult *A. spiniferus* females do not lay eggs on tea leaves (Kasai *et al*. 2010). This difference in host plant acceptance should have reduced mating opportunities between the species, which require no host alternations, as both tea and citrus plants are evergreen. In addition, we found significant differences in waveform and pulse intervals of male mating signals between *A. camelliae* **sp. nov.** and *A. spiniferus* when analyzing the acoustic properties of the mating sounds produced by courting males. The vibratory sounds of each whitefly were species-specific, and courtship signals can be important and useful traits in specific identification (Kanmiya 2006). These acoustic property differences between *A. camelliae* **sp. nov.** and *A. spiniferus* could play a role in maintaining reproductive isolation between them. Notably, in 2009–2010, we observed three sympatric populations of these two species within a range of 100 m in Senri, Osaka Prefecture, with *A. camelliae* **sp. nov.** on two

*Ca. sinensis* trees and one *Ca. sasanqua* tree, and *A. spiniferus* on two *Citrus natsudaidai* trees. This example demonstrates the presence of two independent species, which are maintained by different host preferences and courtship behaviours. Furthermore, genetic analysis of mtCOI sequences (759 bp) showed that *A. camelliae* **sp. nov.** was clearly distinct from *A. spiniferus*, with high bootstrap values. mtCOI sequence identities between the two species were 76.2%. The genetic differentiation between *A. camelliae* **sp. nov.** and *A. spiniferus* was indicated by the high (0.99650) pairwise *F*st value, showing the reproductive isolation of the two species.

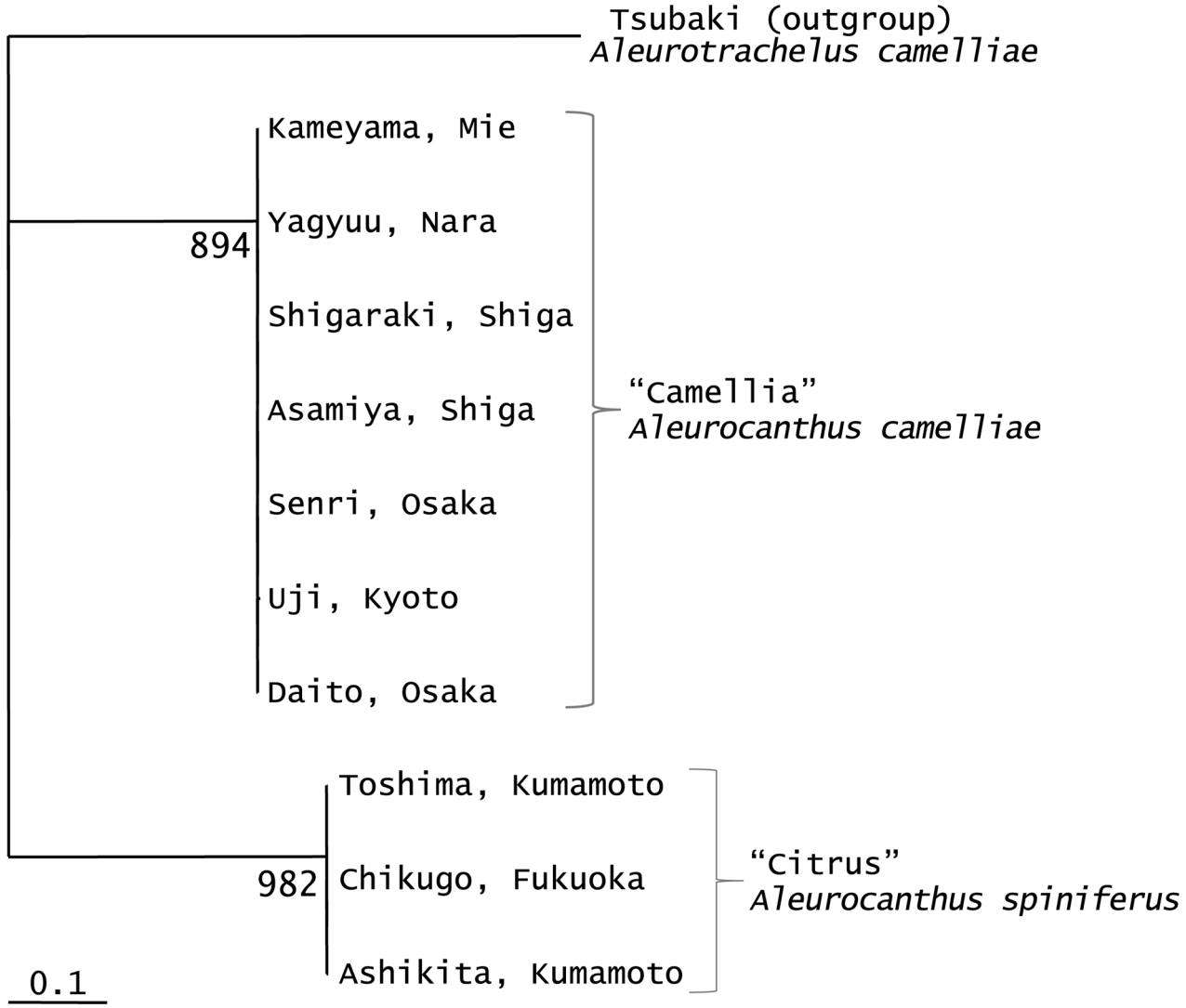


**FIGURE 12.** Phylogenetic tree of mtCOI sequences of *Aleurocanthus camelliae* **sp. nov.** based on the maximum-likelihood method using PhyML 3.0. Numbers at each node indicate bootstrap value. Horizontal branch lengths are drawn to scale, with the bar indicating 0.1-nt replacements per site. The outgroup was *Aleurotrachelus camelliae.*

Mound and Halsey (1978) reported 13 families that serve as host plants of *A. spiniferus*: Annonaceae, Convolvulaceae, Ebenaceae, Elaeocarpaceae, Euphorbiaceae, Flacourtiaceae, Hamamelidaceae, Lardizabalaceae, Rosaceae, Rutaceae, Sabiaceae, Salicaceae and Vitaceae. However, some of the *"A. spiniferus"* populations they recorded may be not be *A. spiniferus*, but rather *A. camelliae* **sp. nov.** Further identifications and investigations of *"A. spiniferus"* populations infesting other plant families are required to judge the risk of this new species and determine whether the speciation of these spiny whiteflies resulted from coevolutionary processes with host plants.

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