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ORIGINAL ARTICLE

Taxonomic identity of a galling adelgid (Hemiptera: Adelgidae) from three spruce species in Central Japan

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Abstract

Gall-forming insects are commonly highly host-specific, and galling species once thought to be oligo- or polyphagous are often found to represent a complex of host-specific races or cryptic species. A recent DNA barcoding study documented that an unidentified species of the genus *Adelges* is a gall-former associated with four spruce species (*Picea bicolor*, *P. koyamai*, *P. maximowiczii*, *P. polita*) as the primary hosts, with little genetic differentiation among insects on different host species. In this study, we investigated the morphology of this galling adelgid to determine its taxonomic identity. Morphological inspection of insects collected from three of the spruce species confirmed that this adelgid is a single galling species, and is identified as *Adelges* (*Sacchiphantes*) *kitamiensis*, which was previously known only from the secondary host. We described the gallicola adults of this species, as well as the first-instar exules which are the offspring of gallicolae. Finally, we verified the taxonomic identity of this species and discuss its life cycle and host distribution.

Key words: *Adelges*, host alternation, *Larix*, *Picea*, *Sacchiphantes kitamiensis*.

INTRODUCTION

Gall-forming insects are commonly highly host-specific (Dreger-Jauffret & Shorthouse 1992), and recent molecular studies have revealed that apparently oligo- or polyphagous galling species can represent a complex of host-specific lineages, cryptic species, or host races (Waring *et al*. 1990; Nyman 2002; Scheffer *et al*. 2004; Stireman *et al*. 2010). However, a few molecular studies have documented that polyphagous non-galling species exhibit little genetic differentiation among insects that feed on different host species (Uechi *et al*. 2003; Hulcr *et al*. 2007).

The Adelgidae is a small group of insects within the Aphidoidea (Hemiptera), containing about 70 described species distributed in temperate and boreal regions of the Northern Hemisphere (reviewed by Havill & Foottit 2007). Adelgids typically induce pineapple-shaped galls on shoots of *Picea* spp. (Pinaceae), and most adelgid species are associated with a few species for galling (Blackman & Eastop 1994; Holman 2009). For example, galls of *Adelges japonicus* (Monzen, 1929) are very common in plantations of *P. jezoensis* in Hokkaido, northern Japan, but have never been found in *P. glehnii* plantations.

Although adelgids include destructive pest species in forest ecosystems (e.g. *Adelges tsugae* Annand, 1924, see Havill *et al*. 2006), taxonomy of the Adelgidae is still problematic partly due to their complex life cycles involving up to five distinct morphs within a species. Recently, Foottit *et al*. (2009) showed that DNA barcodes using the 5′-end of the mitochondrial cytochrome-*c* oxidase subunit 1 gene (*COI*) are a useful tool for species identification in adelgids, and found several unidentified species. However, it is uncertain whether the unidentified species represent undescribed species or unknown morphs of named species that are not included in the DNA barcodes.

"*Adelges* sp. A" is one of the unidentified species reported in Foottit *et al*. (2009), and its samples were

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collected from galls on four spruce species (*Picea bicolor*, *P. koyamai*, *P. maximowiczii*, *P. polita*) in Central Japan. The samples from each host species shared an almost identical *COI* haplotype (0.002% *COI* sequence divergence) (Foottit *et al.* 2009). This lack of host-specific genetic differentiation is surprising, since the galls are induced on the four spruce species, not all of which are phylogenetically closely related (Ran *et al.* 2006). Nevertheless, the taxonomic identity of this unidentified species has not yet determined. In the present study, we inspected voucher specimens of *Adelges* sp. A, from which DNA was extracted in Foottit *et al.* (2009), and additional specimens including first-instar exules obtained through a laboratory rearing to determine its taxonomic identity. We described the gallicola adults and first-instar exules borne by gallicolae of this species, and evaluated the taxonomic identity of this species in relation to the host distribution.

MATERIALS AND METHODS

We used slide-mounted voucher specimens of fundatrix and gallicola adults of *Adelges* sp. A, as designated by Foottit *et al.* (2009), which were deposited in the Canadian National Collection of Insects (Agriculture and Agri-Food Canada, Ottawa). To obtain first-instar offspring of gallicolae, mature galls of *Adelges* sp. A were collected from *P. bicolor*, *P. maximowiczii* and *P. polita* in several localities of Nagano and Yamanashi Prefectures, Central Japan, and reared in the laboratory. Gallicola adults that emerged from each gall were transferred to a plastic cage with shoots of the host spruce and Japanese larch (*Larix kaempferi*; Pinaceae), a possible secondary host. After gallicola adults deposited egg masses on spruce or larch needles, a needle containing a gallicola adult and an egg mass was confined in a glass vial (6 cm^3). We observed some hatched offspring under a binocular microscope, and then poured 80% ethanol into the vials. The gallicola adults and hatched offspring were mounted on microscope slides in balsam or gum–chloral mountant, and deposited in the Hokkaido Research Center, Forestry and Forest Products Research Institute, and Systematic Entomology, Hokkaido University (Sapporo, Japan).

The slide-mounted specimens were inspected under optical microscopes. Measurements of morphological characters were indicated as ranges with means in parentheses, based on 10 individuals. Drawings were based on specimens collected from *P. maximowiczii* in Mibu-gawa River, Ina-shi, Nagano Prefecture.

RESULTS

No obvious morphological differences were observed among the gallicola adults and its first-instar offspring from the three spruce species (*P. bicolor*, *P. maximowiczii*, *P. polita*), suggesting that these adelgids belong to the same species, as documented by Foottit *et al.* (2009) based on DNA sequence data. It was not possible to compare the morphology of gallicola adults and its first-instar offspring from *P. koyamai* with those from the other three spruce species, because the voucher specimens include only early-instar gallicolae from *P. koyamai*. Fundatrix adults were collected only from *P. maximowiczii*; thus, we can not compare the morphology of fundatrix adults among host species.

Morphological inspection showed that the first-instar offspring of gallicolae were exules, not fundatrices, suggesting that this species has a holocyclic life cycle with host-alternation between the primary host (*Picea* spp.) and the secondary host. The morphology of the first-instar exulis agrees well with the syntypes and original description of *Sacchiphantes kitamiensis* Inouye, 1963, which is known only from the secondary host, except for body color: *Sacchiphantes kitamiensis* has a dark blue to dirty blue body color in life (Inouye 1963), while the body color of *Adelges* sp. A is yellow in life. The body color of adelgid exules vary among generations in some species (e.g. Binazzi 2004; Sano *et al.* 2008). Therefore, we concluded that *Adelges* sp. A is conspecific with *Adelges* (*Sacchiphantes*) *kitamiensis*.

Description

Adelges (*Sacchiphantes*) *kitamiensis* (Inouye, 1963)

Sacchiphantes kitamiensis Inouye, 1963.
Adelges (*Sacchiphantes*) *kitamiensis*: Carter, 1971; Blackman & Eastop, 1994; Havill & Foottit, 2007.
Adelges sp. A: Havill *et al.*, 2007; Foottit *et al.*, 2009.

Adult gallicola (Fig. 1a)

Body 1.55–2.53 (1.89) mm long, with wax gland plates scattered over the abdomen.

Antennae (Fig. 1a) 5-segmented, 0.40–0.48 (0.43) mm long, 0.19–0.27 (0.23) times as long as body. Antennal segment III as long as segment IV, segment V longer than segment III or IV. Length of each segment as follows: III 0.09–0.11 (0.10) mm, IV 0.08–0.11 (0.10) mm long, V 0.11–0.14 (0.12) mm long. Segments IV and V tapering below rhinarium towards the base, with an articulation. Rhinaria square-shaped, sometimes with sinuate margins, covering more than half the circumference from the ventral side. Rhinarium on

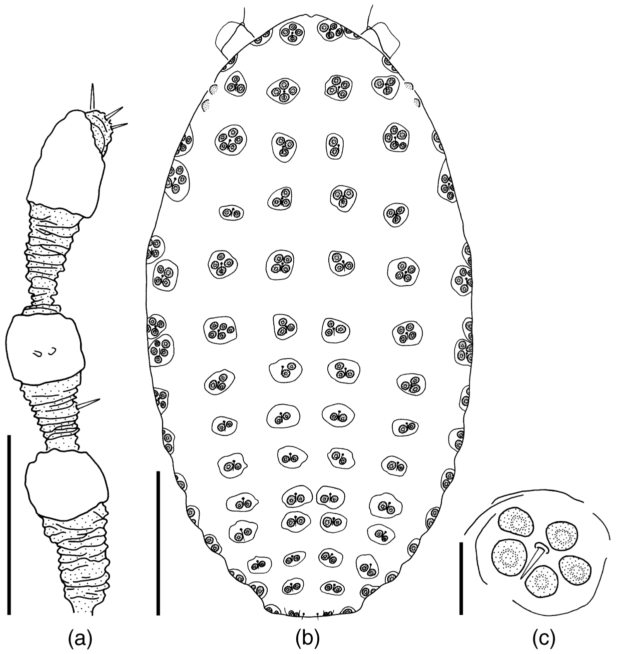

Figure 1 *Adelges kitamiensis*. (a) Antennal segments III–V of adult gallicola in ventral view, (b) first-instar exulis in dorsal view, (c) wax gland plate of first-instar exulis at the lateral position on abdominal segment IV. Scales: (a,b) 0.1 mm, (c) 0.01 mm.

segment III occupying less than half the length of segment, 0.40–0.49 (0.45) times as long as segment III. Rhinarium on segments IV and V usually occupying about half of, or more than half the length of each segment, 0.42–0.61 (0.52) and 0.40–0.59 (0.51) times as long as each segment, respectively. Some small rhinaria present dorsally on segment V. Setae on the apex of segment V stout, spine-like. Ultimate rostral segment without accessory seta, 0.06–0.07 (0.06) mm long.

Head without distinct faceted wax gland plates, bearing wax gland areas of granular appearance with a few short setae. Cephalic anterior and posterior wax gland areas united. Prothorax with wax gland areas of granular appearance with short setae. Spinal and pleural wax gland areas of prothorax without faceted wax gland plates. One or two small wax gland plates present on anterior lateral area. A pair of transversely extended wax gland plates present on posterior lateral area along the posterior margin. Mesothorax with a spinal pair of indistinct wax gland plates between mesothoracic lobes. Metathorax with a spino–pleural pair of transversely extended wax gland plates. Abdominal segments I with 1 spinal and 1 lateral pairs of wax gland plates. Segments II–VI each with 3 pairs at spinal, pleural, and lateral positions. Segments VII and VIII each with a lateral pair. Spinal wax gland plates on segment I large, transversely extended. Pleural wax gland plates on segment II small, sometimes absent. Spinal and pleural wax gland plates sometimes fragmented into a few small ones or fused to next ones to form an irregular transverse row between lateral ones. Lateral wax gland plates on anterior abdominal segments crescent-shaped with sinuate margins, but those on posterior segments transversely extended.

Forewings with medial vein slightly curved outward; cubital vein slightly curved outward or almost straight; anal vein strongly arched towards the posterior margin. Hindwings with oblique vein arising at almost right or obtuse angles from longitudinal vein, slightly curved back or almost straight.

Hind femorotrochanter 0.28–0.40 (0.33) mm long. Second tarsal segment 0.10–0.14 (0.12) mm long in hind legs. Empodial pair of setae reaching the points of claws.

First-instar exulis borne by gallicola (Fig. 1b,c)

Color in life yellow. Body (Fig. 1b) elliptical, 0.36–0.41 (0.38) mm long, 0.54–0.60 (0.57) times as wide as long, with wax gland plates scattered over.

Eyes represented only by triommatidia. Antennae 3-segmented, 0.09–0.10 (0.09) mm long, 0.21–0.28 (0.24) times as long as body, 1.6–1.9 (1.7) times the length of hind femorotrochanter. Antennal segments I and II smooth, segment III slightly imbricated. Segment III a little longer than segments I and II combined, 0.05–0.06 (0.05) mm long. Arrangement of antennal setae on each segment as follows: I 2 (one of which minute), II 2 and III 9. The apical seta on segment III longest and thickest, about 0.04 mm long, with a large socket; the five subapical setae rather long and thick; the other setae minute. Two primary rhinaria present posteriorly on segment III, one on the subapical position and the other on apical 1/3 of the segment, each not surrounded by cilia, with a semitransparent tonguelet projecting from an opening. The subapical tonguelet larger than the other. A few small accessory rhinaria present close to the subapical primary one, not surrounded by cilia, without a tonguelet. Rostrum reaching hind coxae. Ultimate segment smooth on the surface, with 3 pairs of accessory setae, 0.03–0.04 (0.04) mm long, 0.60–0.72 (0.66) times the length of hind femorotrochanter.

Femorotrochanter short, smooth, 0.05–0.06 (0.05) mm long. Tibia as long as femorotrochanter, smooth. Tarsus 2-segmented, first segment with 2 spine-like setae, second segment imbricated, about 0.02 mm long in hind legs, with 7 setae; 1 pair dorso-apically, 1 pair ventro-apically, 1 pair latero-apically, 1 seta dorso-basally and 1 pair on empodium. In fore legs one of the two dorso-apical setae capitate and longer, but in middle and hind legs the two setae capitate and equal in length. Empodial setae simple, exceeding the point of claws.

Wax gland plates (Fig. 1c) circular or elliptical, consisting of one or a few, same-sized circular facets, with a stout seta. Facets double-walled except for those of facial and coxal indistinct wax gland plates. Head (Fig. 1b) with 7 pairs of wax gland plates; on the dorsum 1 pair medio-posteriorly, 1 pair latero-posteriorly and 1 pair medio-anteriorly; on the ventrum 2 pairs just anteriorly and posteriorly to antennal bases, 1 pair medio-anteriorly and 1 pair medio-facially. Pronotum with 2 pairs of wax gland plates spinally, pleurally and laterally. Meso- and metanotum each with 1 spinal, 1 pleural and 2 lateral pairs. Abdominal tergites I–VI each with 3 pairs at spinal, pleural and lateral positions. Abdominal tergite VII with 1 spino-pleural and 1 lateral pairs. Abdominal tergite VIII with 1 lateral pair. Abdominal tergite IX with 1 transversely extended wax gland plate at central position. Lateral pair rather located on the ventral side. Middle and hind coxae basally with wax gland plates. Wax gland plates on head and thorax usually consisting of 3 to 5 facets; spinal and pleural plates on abdomen usually consisting of 2 or 3 facets; lateral ones on abdomen large, usually consisting of 4 or 5 facets.

Body setae all simple and fine. Head with 1 pair of setae just outside facial wax gland plates. Abdominal tergite IX with 2 pairs of rather long setae. Cauda with 1 pair of minute setae.

Gall (Fig. 2)

Pineapple shape, with needles, without hairs and a waxy bloom, green turning red before opening. Galls are usually induced on terminal and lateral buds of young shoots, but sometimes also on dormant buds of old branches. In the latter case, galls are small and disk-like.

Figure 2 Gall of *Adelges kitamiensis* on *P. maximowiczii* (Mitsumine-gawa River, Ina-shi, Nagano Pref., Japan).

Specimens examined

Seven slides containing 7 gallicolae, Yamanakako, Yamanaka, YAMANASHI PREF., Japan (35.44541N, 138.85217E). 11.vi.2004, ex *Picea polita*, S. Shiyake leg (HEM53415-4–10); 3 slides containing 4 gallicolae and 12 first-instar exules, the lake side of Yamanaka-ko Lake, Yamanakako-mura, Yamanashi Pref., Japan. 22.vi.2009, ex *Picea polita*, M. Sano leg; 5 slides containing 8 gallicolae and 24 first-instar exules, Fujiyoshida Branch of Yamanashi Forest Research Institute, Fujiyoshida-shi, Yamanashi Pref., Japan. 23.vi.2009, ex *Picea maximowiczii* and *Picea polita*, M. Sano leg; 5 slides containing 5 gallicolae and 6 first-instar exules, Kiyosato, Hokuto-shi, Yamanashi Pref., Japan. 28.viii.2008, ex *Picea bicolor*, K. Ozaki leg; 3 slides containing 3 gallicolae, Mibu River, Ina, NAGANO PREF., Japan (35.62131N, 138.14844E). 22.vi.2006, ex *Picea maximowiczii*, S. Shiyake leg (HEM56319-2, 6 & 10); 2 slides containing 2 gallicolae and 10 first-instar exules, Mibu-gawa River, Ina-shi, Nagano Pref., Japan. 25.vi.2009, ex *Picea maximowiczii*, M. Sano leg.

DNA barcode

GenBank accession numbers EF073102, FJ502480–FJ502486. See Foottit *et al.* (2009), as *Adelges* sp. A.

Distribution

Northern Japan (eastern Hokkaido), Central Japan (Nagano and Yamanashi Prefectures).

Host plant

Primary host: *Picea bicolor*, *P. koyamai*, *P. maximowiczii* and *P. polita* (Pinaceae). Secondary host: *Larix kaempferi* (=*L. leptolepis*; Pinaceae).

Life cycle

It is most likely that this species is host-alternating between *Picea* primary and *Larix* secondary hosts. Fundatrix adults deposited egg masses in early May on *P. maximowiczii*. Galls on *P. maximowiczii* and *P. polita* began to dehisce from late June to early July, but those on *P. bicolor* from late August to early September. For galls on *P. koyamai*, we have no data on when galls begin to dehisce, but it is possible that dehiscence begins in late August or early September, because gallicolae were early instars on *P. koyamai* in late June as in those on *P. bicolor*.

Remarks

Adelges kitamiensis can be distinguished from the other species of the subgenus *Sacchiphantes* by the following characters: first-instar exules have distinct faceted wax gland plates, and spinal and pleural wax gland plates on the abdomen usually consist of only two or three facets; in adult gallicolae, antennal segment V is longer than segment III or IV, rhinaria on antennal segments IV and V occupy about half of or more than half the length of each segment, and anal veins of the hindwings are sometimes curved back toward the body.

DISCUSSION

"*Adelges* sp. A" is well-differentiated by DNA barcodes from nine identified congeneric species included in Foottit *et al.* (2009). However, this species was represented only by fundatrix adults, and gallicola nymphs and adults on the primary hosts. It was unknown whether this unidentified species represented an undescribed species or primary-host morphs of an already named species that are not included in previous DNA analyses, because many species of adelgids are known only from one or a few morphs (Blackman & Eastop 1994; Havill & Foottit 2007). In the present study, we collected gallicola adults of *Adelges* sp. A from the primary host, and obtained first-instar exules, which live on the secondary host, through laboratory rearing. Based on the morphology of first-instar exules, we identified *Adelges* sp. A as *A. kitamiensis*, which was known only from first-instar and adult exules, and sexuparae on *Larix kaempferi*, the secondary host, in Hokkaido, Northern Japan. *Larix kaempferi* is not native to Hokkaido (Kisanuki 2000), and have been artificially introduced to Hokkaido from Central Japan, where *Adelges* sp. A was collected from the primary host. Thus, it is reasonable that *Adelges* sp. A and *A. kitamiensis* are conspecific, with a holocyclic life cycle alternating between *Picea* spp. and *L. kaempferi*. To reconfirm the taxonomic identity and life cycle of this species, it will be necessary to conduct transfer experiments or DNA barcoding of *A. kitamiensis* from *L. kaempferi*.

The present morphological inspection confirmed that *A. kitamiensis* does not have any differentiation among host spruce species. However, gall-forming insects are commonly highly host-specific, and only a few studies have reported polyphagous species for non-galling insects (Uechi *et al.* 2003; Hulcr *et al.* 2007). Therefore, molecular analysis using rapidly evolving nuclear marker such as microsatellites might be needed to further evaluate whether there is host-specific genetic differentiation within this species.

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