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SCIENTIFIC NOTE

A new species of *Cinara* (Hemiptera: Aphididae) from Japan, and concomitant specimen data publication

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Abstract
Thirty-one species of *Cinara* Curtis (Hemiptera: Aphididae) are recorded from Japan, including five that are undescribed. We here formally describe one of the five, *Cinara stigmatica* **sp. nov.**, from viviparous apterous females on two species of *Abies* Miller (Pinaceae). Morphological and molecular diagnoses are provided. Specimen data are simultaneously published in an open-access machine-readable format.

Introduction

The genus *Cinara* Curtis is the most speciose of the Lachninae subfamily (Hemiptera: Aphididae), with more than 250 valid species (Favret 2023). Of those, approximately 50 are found in East Asia, but many more may exist that have not yet been described (Chen *et al.* 2016b). Lachninae originally evolved on woody angiosperms, but *Cinara* diversified dramatically when its ancestor adopted a coniferous host (Chen *et al.* 2016a). This genus is characterised by a medium to large body, a short antennal processus terminalis, a rostrum with a distinct joint between the fourth and fifth segments, and pigmented cone-shaped siphunculi (Eastop 1972).

In their 1998 publication, Eastop *et al.* listed 31 species of *Cinara* occurring in Japan and provided an identification key that included five species not formally described. Collections made in Japan in 2010 recovered two populations of their "*Cinara* sp. C" on *Abies homolepis* Siebold and Zuccarini (Pinaceae). Following an evaluation of these new specimens and those used by Eastop *et al.* (1998) from *Abies firma* Siebold and Zuccarini, we confirmed that they do indeed represent a new species and here describe it as such. Along with a morphological description, we provide a molecular diagnosis that satisfies Article 13.1.1 of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 1999).

Aphids were hand-collected in the field, preserved in 95% ethanol, and kept at –80 °C. A nondestructive protocol (Favret 2005) was used to extract DNA from two specimens per population using a DNeasy Blood and Tissue Kit (Qiagen, Düsseldorf, Germany). The cleared aphid cuticles were then slide-mounted in Canada balsam (Favret 2005). The identification keys of Inouye (1970), Eastop *et al.* (1998), and Blackman and Eastop (1994, 2022) were used for specimen determination. When available in the Ouellet-Robert Entomological Collection of the

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Université de Montréal (QMOR; Montréal, Québec, Canada), specimens of Japanese *Cinara* and those found on *Abies* spp. worldwide were compared directly. The original published species descriptions were used as a comparative reference when actual material was unavailable and as a source for specimen measurements.

Specimens are deposited at: (1) the National Agriculture and Food Research Organisation, Tsukuba, Japan (NARO); (2) the aphid collection of the Smithsonian Institution National Museum of Natural History, Beltsville, Maryland, United States of America (USNM-ENT); and (3) the Université de Montréal Ouellet-Robert Entomological Collection (QMOR). Specimen data are published by Hébert *et al.* (2023) and from there are available at the Global Biodiversity Information Facility (GBIF). Catalogue numbers with decimals refer to individual specimens on a slide that has more than one specimen. Specifically, NARO-210102729.001 and NARO-210102729.002 refer to two individual specimens on slide NARO-210102729.

Polymerase chain reaction amplification of the 5′ region of the mitochondrial cytochrome *c* oxidase subunit 1 (*CO*1) gene was realised using LepF and LepR primers (Foottit *et al.* 2008) and standard protocols (Théry *et al.* 2018). The 608- and 660-nucleotide sequences are deposited and available at GenBank (accession numbers: OP536219 and OP536220). To see if any molecular autapomorphies were present in our new species, the alignments of our sequences with those of *Cinara* species known from Japan and those having *Abies* spp. as hosts (but not necessarily restricted to Japan) were realised using the Clustal Omega alignment in Geneious (Sievers *et al.* 2011). Some sequences were taken from GenBank (accession numbers: GU978789, KP339517, KM501334, JX034927, KP339747, KR031213, GU978840, JQ916795, and KC201787), and others come from our personal libraries. We recorded the nucleotide substitutions unique to the two species based on their positions when aligned with a reference sequence from the *Acyrthosiphon pisum* (Harris) (Aphididae: Macrosiphini) mitochondrial genome (accession number: FJ411411).

Z-stacked photographs were taken in brightfield lighting, except for Figure 3A, which was photographed under differential interference contrast conditions, with a Zeiss M2 AxioImager microscope, an AxioCam HRc camera, and Zen 2012 Software, version 1.1.1.0 (Carl Zeiss AG, Oberkochen, Germany).

The morphological abbreviations are as follows: BL – body length (measured from the frontal margin of the head to the end of the cauda); BW – greatest body width; HWE – head width at eyes; ANT I, II, III, IV, V, VI – antennal segments I, II, III, IV, V, VI, or their length; PT – processus terminalis of the last antennal segment or its length; BASE – base of the last antennal segment or its length; LSANT III – length of the longest setae on antennal segment III; ROS – rostrum or the length of the sclerotised stylet groove; ROS IV, V – fourth or fifth segment of the rostrum or their length; URS – ultimate rostral segments (IV + V) or their length; HCOX – width of the hind coxa; LMF – length of the metafemur; WMF – largest width of the metafemur; LMT – length of the metatibia; WMT – largest width of the metatibia; LDMTS – length of the longest dorsal setae of the metatibia; LVMTS – length of the longest ventral setae of the metatibia; HT I – first segment of the hind tarsus or its length (measured on the ventral side); HT II – second segment of the hind tarsus or its length; ABD III – third segment of the abdomen; SIPH – siphunculus or its diameter at base; and SPIR – spiracular sclerites or their diameters.

A single measurement per character was made on each specimen. It may have been of either the left or right of the specimen, depending on which side had the more visible and easily measured character. The mean diameter of the spiracular sclerites did not statistically differ between each abdominal segment ($F_{6,63}$, $P = 0.249$); therefore, the measures given represent the mean and range of the seven pairs of abdominal spiracles of the combined specimens. All measurements are in micrometres (μm).

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Figure 1. Holotype of *Cinara stigmatica* sp. nov. (NARO-210104343): **A**, holotype slide; and **B**, habitus of apterous vivipara holotype specimen.

Cinara stigmatica sp. nov

ZooBank Nomenclature Act: urn:lsid:zoobank.org:act:3BA802A2-E7F4–46C9-AC44-A992C5F0B802

Holotype. NARO-210104343 (Fig. 1): apterous vivipara, Japan, Nagano Prefecture, Fujimi-kôgen Highland, 35.9370° N, 138.3176° E ± 10m, 2010–07–03, on *Abies homolepis*, Colin FAVRET and Masakazu SANO *leg*. **Paratypes.** NARO-210102729, 2 apterous viviparae, Japan, Miyagi Prefecture, Kinkasan, [38.294° N, 141.565° E ± 2500 m], 1966–08–05, on *Abies firma*, Zenpei YAMASHITA *leg*.; USNM-ENT760076, QMOR71646, QMOR71649, QMOR71650, QMOR71636, five apterous viviparae, Japan, Yamanashi Prefecture, Yamanaka-ko Lake, 35.4469° N, 138.8547° E ± 10 m, 2010–07–02, on *Abies homolepis*, Colin FAVRET and Masakazu SANO *leg*.; QMOR71647, QMOR71648, 2 apterous viviparae, Japan, Nagano Prefecture, Fujimi-kôgen Highland, 35.9370° N, 138.3176° E, 2010–07–03, on *Abies homolepis*, Colin FAVRET and Masakazu SANO *leg*. See also Hébert *et al.* (2023).

Diagnosis. The new species can be distinguished by a combination of characters: (1) the size and colour of the SPIR, (2) the colour of the tibiae, (3) the length of the tibial setae, and (4) the appearance of the muscle attachment plates. It shares some of those characters and its host associations with *C. matsumurana* Hille Ris Lambers, 1966 (sensu Inouye 1970), *C. todocola* (Inouye, 1936) (sensu Inouye 1970), and *C. longipennis* (Matsumura, 1917) (sensu Inouye 1970). It differs in that the length of the dorsal tibial setae is much shorter – 54.82 µm (208.62 µm in *C. longipennis*, 171.74 µm in *C. matsumurana*, and 118.43 µm in *C. todocola*) – and all tibiae are all black (lighter apical region in *C. matsumurana* and basal region in *C. longipennis*). The main difference between this new species and *C. todocola* is the presence of large spinal and marginal sclerites on the dorsum of the abdomen in the latter. It is also morphologically close to *C. formosana* (Takahashi, 1924) (sensu Inouye 1970) in having short and thick setae, prominent muscle attachment plates surrounded by sclerites, and in the size and colour of the SPIR, but it differs in that HT II is 2.57–2.98 × HT I (1.6–1.9 in *C. formosana*). There are also no scleroites at

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Table 1. Diagnostic nucleotide differences in *CO*1 gene between *Cinara stigmatica* sp. nov., 23 Japanese *Cinara* species, and 21 *Cinara* species that have *Abies* spp. as hosts.

Position	159	171	228	372	423	450	492	566	609
Cinara stigmatica sp. nov.	G	C	T	T	C	C	T	C	C
Japanese species	A	T	A/G	A	T/C	A	A	T/A	T
Species with *Abies* hosts	A	T/C	A/G/T	A	T	A/G	A/T/C	C	T/C

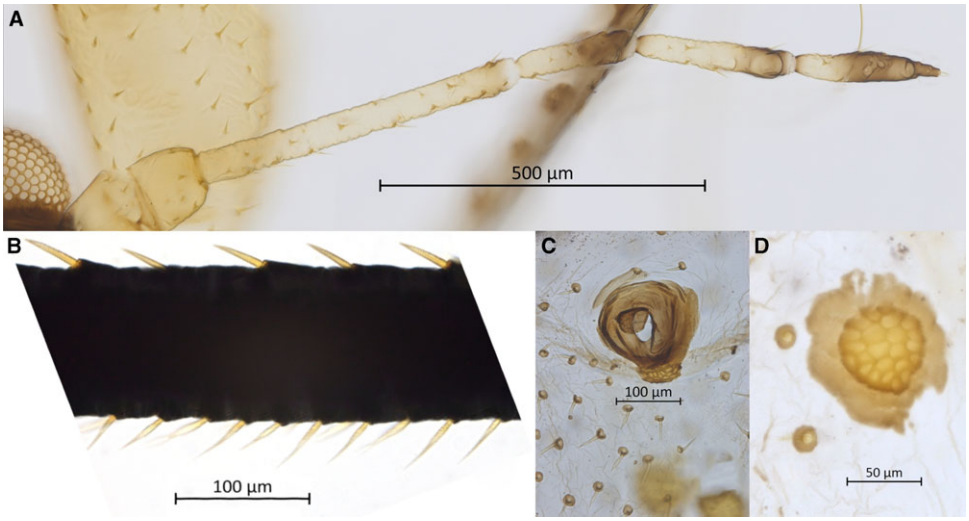

Figure 2. Morphological features of apterous vivipara of *Cinara stigmatica* sp. nov.: **A**, antenna (NARO-210104343); **B**, dorsal (upper) and ventral (lower) hind tibial setae. (NARO-210104343); **C**, large spiracular sclerite with adjoined muscle attachment plate and ventral setae with scleroites (QMOR71647); **D**, large sclerotised muscle insertion plate and dorsal setae with scleroites (NARO-210104343).

the base of the setae in *C. formosana*, their tibiae are lighter in colour, and it is known to feed on *Pinus* spp. The keys to the *Cinara* spp. on *Abies* spp. of Blackman and Eastop (1994, 2022) lead to *C. kiusa* Hottes, 1957 (couplets 25 and 31 in the 1994 and 2022 versions, respectively), but the ROS is much shorter in our species, reaching only to the HCOX (reaching past the SIPH in *C. kiusa*). For DNA characters, eight diagnostic nucleotides were found when compared with species known from Japan and four were found when compared with those that have *Abies* spp. as a plant host (Table 1).

Colour. In life: green. In slide-mounted specimens: head and body beige (Fig. 1B). Antennae pale (Fig. 2A), with darker apical half on ANT IV–VI. Coxae, SIPH, abdominal sclerotisation, genital plate, anal plate, and cauda yellowish to light brown. Femora yellowish brown, with hind femur dark brown on distal half to three-quarters and front and middle femur sometimes with a darker line on the posterior face. Tibiae all black (Figs. 1B and 2B). SPIR dark brown (Fig. 2C). Muscle attachment plates light to dark brown (Fig. 2D).

Body. Large aphid (BL 3969–4685 µm) with an oval-shaped body (Fig. 1B). HWE 0.42–0.54 × ANT. ANT 0.28–0.32 × BL. **Head**. Head covered by pointed setae. Triommatidium distinct. ANT III longest, 0.52–0.75 × SIPH and without secondary sensoria. ANT IV shortest and without secondary sensoria. ANT V 0.94–1.33 × ANT VI and 0.44–0.56 × HT II, with one rounded secondary sensorium. ANT VI with a cluster of approximately four small secondary sensoria laterad to the primary one, with PT

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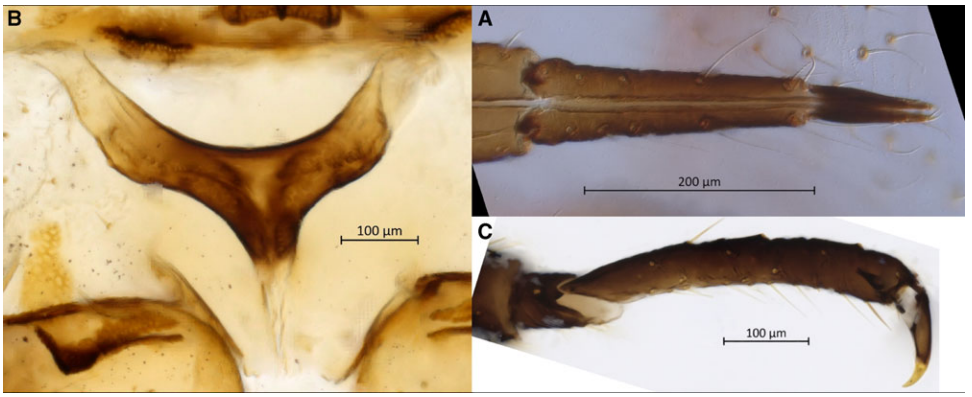

Figure 3. Morphological features of apterous vivipara of *Cinara stigmatica* sp. nov.: **A**, ultimate rostral segments bearing seven accessory setae (NARO-210102729.002); **B**, mesosternal furca (QMOR71647); **C**, hind tarsus (USNM-ENT760076).

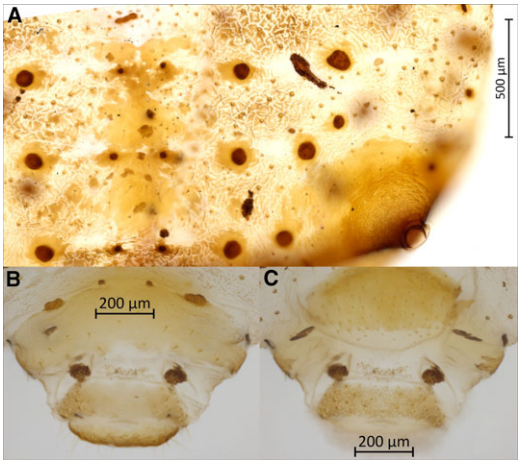

Figure 4. Morphological features of apterous vivipara of *Cinara stigmatica* sp. nov.: **A**, dorsal abdominal segments IV, V, and VI, bearing spinal sclerites, three rows of muscle attachment plates, pigmented patterning, setae with scleroites, and siphunculus with broad sclerotic base and numerous setae visible as pale spots throughout its surface (NARO-210102729.001); **B**, dorsum of terminal abdominal segments; the scale bar is placed over the eighth abdominal tergite; below that are two muscle attachment plates on either side of the rudimentary gonapophyses, followed by the anal plate and cauda (QMOR71647); **C**, venter of terminal abdominal segments; the scale bar is placed over the cauda; above that is the anal plate, followed by two muscle attachment plates on either side of the rudimentary gonapophyses, and above these the genital plate (QMOR71647).

0.21–0.39 × BASE and bearing four subapical setae. Antennae with short and spinelike setae. LSANT III 0.73–0.90 × the width of ANT III. ROS reaching the HCOX, 0.33–0.42 × BL. ROS IV 1.96–2.28 × ROS V and 1.51–1.77 × HT I, bearing four to eight accessory setae (Fig. 3A). **Thorax**. Mesosternal furca fused, Y-shaped with short arms and a well-developed stem (Fig. 3B). Prosternal tubercle distinct. Femora and coxae large. HCOX 1.59–2.06 × WMF. Femora and tibiae with short and spinelike setae (Fig. 2B). LMT 39.58–51.76 × LDMTS. HT II with few dorsal and more numerous ventral short spine-like setae (Fig. 3C), 1.66–1.82 × ROS IV. **Abdomen**. Dorsal and ventral setae with small scleroites (Figs. 2C and D). Ventral setae on ABD III finer and longer (44.82–68.14 µm) than the dorsal ones (30.42–46.68 µm). Dorsum, especially posteriorly, sometimes textured with irregularly shaped darkened patterns (lighter or darker in different specimens; Fig. 4A). Small and irregular spinal sclerites on tergites (III) IV–VI

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Table 2. Measurements (in µm) of apterous viviparous females of *Cinara stigmatica* sp. nov.

Length	No. of specimens	Mean	Range
BL	10	4312	3969–4685
BW	10	2759	2349–3013
HWE	10	622	586–654
ANT	5	1311	1206–1406
ANT I	10	151	111–167
ANT II	10	110	92.8–127
ANT III	6	445	368–508
ANT IV	6	171	159–200
ANT V	6	222	193–239
ANT VI	5	201	145–226
BASE	5	158	104–187
PT	5	42.3	39.3–45.8
LSANT III	6	40.0	35.9–44.4
ROS	9	1605	1464–1756
ROS IV	8	250	245–257
URS	9	372	358–383
HCOX	10	527	493–604
LMF	10	1621	1471–1779
WMF	10	286	262–322
LMT	10	2463	2225–2643
WMT	10	138	122–150
LDMTS	10	54.8	47.7–62.9
LVMTS	10	53.4	43.2–62.7
HT I	10	156	139–166
HT II	10	434	407–455
SIPH	10	683	543–747
SIPH pore	7	95.1	79.1–118
SPIR	10	161	130–191

BL, body length; BW, greatest body width; HWE, head width at eyes; ANT I, II, III, IV, V, VI, antennal segments I, II, III, IV, V, VI or their length; PT, processus terminalis of the last antennal segment or its length; BASE, base of the last antennal segment or its length; LSANT III, length of the longest setae on antennal segment III; ROS, rostrum or the length of the sclerotised stylet groove; ROS IV, V, fourth or fifth segment of the rostrum or its length; URS, ultimate rostral segments (IV + V) or their length; HCOX, width of the hind coxa; LMF, length of the metafemur; WMF, largest width of the metafemur; LMT, length of the metatibia; WMT, largest width of the metatibia; LDMTS, length of the longest dorsal setae of the metatibia; LVMTS, length of the longest ventral setae of the metatibia; HT I, first segment of the hind tarsus or its length; HT II, second segment of the hind tarsus or its length; ABD III, third segment of the abdomen; SIPH, siphunculi or its diameter at base; SPIR, spiracular sclerites or their diameters.

(Figs. 1B and 4A). Large rounded SPIR with adjoined muscle insertion plate (Fig. 2C), 0.21–0.30 × SIPH. Muscle insertion plates distinct and often surrounded by a lighter sclerotic region on the dorsum (Figs. 2D and 4A). SIPH broadly conical with a large, pigmented area at the base (Fig. 4A), 4.59–8.89 × SIPH pore. Eighth abdominal tergite bearing 14–24 setae (Fig. 4B).

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Genital plate with 59–77 setae (Fig. 4C). Cauda and anal plate spinulose, bearing long and fine setae. Cauda semicircular (Fig. 4B). Measurements are given in Table 2.

Etymology. The species is named for the sclerotic spiracles (from "stigmate", the French term for spiracle). The feminine ending for this adjective accords with the grammatically feminine *Cinara*; the masculine and neuter forms are *stigmaticus* and *stigmaticum*, respectively.

Molecular analysis. A BLAST search on GenBank with our *CO*1 sequences returned a closest match of 94% similarity, much farther than the 2% dissimilarity threshold that is commonly used to discriminate animal species (Hebert *et al.* 2003) or the 2% found to be the average intraspecific divergence in *Cinara* species (Chen *et al.* 2012). The single close genetic match in the Barcode of Life Data System (BOLD) was 98.6% (BOLD 2023). The single specimen (BIOUG00939-C10), from Changbai Mountain in Jilin Province, China, likely is *Cinara stigmatica* sp. nov., but we are unable to evaluate it properly as it is an immature unassociated with any host.

Specimen data publication. Data accessibility is lacking in classical taxonomy. Although specimen metadata are always presented, most of the information about the material examined is in simple text format within the article. Specimen data availability in machine-readable format on open-access repositories such as GBIF can contribute to subsequent research (Favret 2014; Gemeinholzer *et al.* 2020). According to Heberling *et al.* (2021), the use of data from GBIF in scientific research has increased, whereas specimen-based data deposited at GBIF from explicitly taxonomic resources have decreased. There is great potential for taxonomists to help the field and others make use of the valuable information linked to every specimen observed in their work: the "material examined" specimen data published in taxonomic works are typically of the highest quality and, in our opinion, their accessibility in a machine-readable format should be a matter of course. Sheffield and Heron (2018) and Nelson and Moffat (2022) published, at Canadensys.net, downloadable Darwin Core archives (Sheffield 2018; Nelson 2023) to accompany their respective regular articles. We added to their model by publishing a data paper that is as citable as any other journal article (Costello *et al.* 2013; Hébert *et al.* 2023). We hope this new model will incentivise taxonomists to better share their valuable specimen data.

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Competing interests. Apart from their current and future employment and funding, the authors declare no conflicts of interest.

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