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SHORT COMMUNICATION

First record of *Adelges* (*Cholodkovskya*) *viridanus* (Hemiptera: Adelgidae) in North America, with a description of the fundatrix form in Japan

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Abstract

Adelges (*Cholodkovskya*) *viridanus* (Cholodkovsky 1896) (Hemiptera: Adelgidae) is found throughout Eurasia where it is understood to be anholocyclic, feeding on species of larch (*Larix*), without host alternation. For the first time, we report this species in North America, outside of its native range, from specimens collected in an arboretum in Ohio, USA. Molecular phylogenetic analysis was inconclusive as to whether it was introduced from Europe or Asia. In addition, specimens collected from *Picea jezoensis* in Japan were confirmed for the first time as *A. (C.) viridanus* by matching DNA sequences to specimens collected on *Larix*. Therefore, *A.* (*C.*) *viridanus* is either capable of completing a host-alternating holocycle in Japan, or includes very recently diverged anholocyclic populations on *Picea*. Finally, we describe the adult fundatrix form, which was previously unknown.

Key words: complex life cycle, gall, larch, non-native, spruce.

Adelges (*Cholodkovskya*) *viridanus* (Cholodkovsky, 1896) is a species of adelgid (Hemiptera: Adelgidae) found throughout Eurasia, in the Czech Republic, France, Germany, Italy, Lithuania, Poland, Spain, Sweden, Switzerland, United Kingdom, Ukraine, Russia, China, Japan, and Korea (Cholodkovsky 1896, 1902; Inouye 1945; Gaumont 1954; Börner & Heinze 1957; Steffan 1964; Carter 1971; Li & Tsai 1973; Covassi & Binazzi 1981; Pashtshenko 1988; Notario & Baragaño 1992; Albrecht 2017; Havelka *et al.* 2020). All members of *Cholodkovskya* feed on *Larix* (Pinaceae) secondary hosts and have first instar sistentes (wingless overwintering forms) with simple, thin-walled wax pores, and with all nymphal and adult stages with dorsal wax glands present and arranged in six longitudinal lines on the thoracic and first six abdominal segments (Börner & Heinze 1957; Blackman & Eastop 1994). The alate form is distinguished from other subgenera by having anterior and posterior wax glands on each side of the head united (Blackman & Eastop 1994).

Typical adelgid holocycles include host alternation between secondary hosts in the conifer (Pinaceae) genera *Abies*, *Larix*, *Pinus*, *Pseudotsuga,* or *Tsuga* and primary hosts in *Picea*, where there is a sexual generation and the formation of galls. Alternately, adelgid anholocycles include only asexual generations only on either primary or secondary hosts (Havill & Foottit 2007; Sano & Ozaki 2012). *Adelges* (*C.*) *viridanus* is understood to have an anholocycle consisting of up to four parthenogenetic generations per year feeding on species of larch (*Larix decidua* Mill., *L. gmelinii* (Rupr.) Rupr., *L. kaempferi* (Lamb.) Carr., and *L. sibirica* Ledeb.) (Inouye 1945; Gaumont 1954; Steffan 1964; Carter 1971). Overwintering wingless sistentes settle in bark cracks or under bark flakes of larch trees. Their offspring can develop again as sistentes that stay on the bark, or as progredientes that migrate to the stems and needles of young shoots where they develop into alates (Fig. 1). Their offspring develop into either wingless sistentes to overwinter on the bark, or into additional generations of alate

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Figure 1 *Adelges* (*Cholodkovskya*) *viridanus* alate, with the characteristic copious woolly wax, settled on a needle of a Dunkeld larch tree (*Larix* × *marschlinsii*). Photographed on 7 June, 2019 at Dawes Arboretum in Newark, Ohio, USA.

progredientes that can themselves give rise to either sistentes or progredientes (Gaumont 1954).

Specimens of *A.* (*C.*) *viridanus* reproducing on *Picea* could constitute evidence for completion of a host-alternating holocycle. To test whether *A.* (*C.*) *viridanus* could develop on *Picea*, Carter (1971) performed host transfer experiments in England and concluded that they could not reproduce on *Picea abies* (L.) H. Karst. or *P. sitchensis* (Bong.) Carr. We are not aware of tests of its ability to develop on other *Picea* species.

Here, we report the first record of *A.* (*C.*) *viridanus* in North America, outside its native range, and use DNA sequence data to confirm its identity. We also match DNA barcode sequences from *A.* (*C.*) *viridanus* specimens collected from *Larix* to specimens collected from *Picea* in Japan, providing evidence of a possible holocycle or recently diverged anholocycle. Finally, we describe the newly discovered fundatrix form of the species.

DNA sequence data were analyzed from new and previously published specimens (Table 1). For new material, adelgids were removed from host plants and stored in 95% ethanol at −20°C or −80°C. DNA was extracted nondestructively using the Promega DNA IQ Kit (Promega), the Mag-Bind Blood and Tissue Kit (Omega Bio-Tek), or the MagMAX DNA Multi-Sample Ultra Kit (Thermo Fisher Scientific) on a KingFisher Flex automated instrument (Thermo Fisher Scientific), using the manufacturers' protocols. The insect bodies were pierced with a sterile insect pin and the cuticle was removed after the proteinase K incubation step and slide mounted. Additional specimens were also slide mounted from each collection event after clearing overnight with 10% potassium hydroxide. Some specimens were stained with Double Stain (lignin pink and acid fushin; Bioquip). All specimens were mounted in Canada balsam and deposited at the Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Ontario, Canada (CNC), or the Yale Peabody Museum of Natural History, New Haven, Connecticut, USA (YPM).

The mitochondrial cytochrome *c* oxidase I (*COI*) DNA barcoding region was amplified with primers LepF1 and LepR1 (Hebert *et al.* 2004), and a portion of the nuclear elongation factor-1α (*EF1α*) was amplified with primers AdelEF1F1 and AdelEF1R2 (Havill *et al.* 2007). Sequencing was performed at Yale University, New Haven, Connecticut, USA at either the DNA Sequencing Facility on Science Hill or the Keck DNA Sequencing Facility using an Applied Biosystems 3730xL DNA Analyzer. Forward and reverse chromatograms were aligned and edited using the software Geneious Prime version 2022.0.1 (https://www.geneious.com). New sequences produced for this study were deposited in GenBank (Table 1). Additional DNA *COI* and *EF1α* sequences were downloaded from GenBank for specimens collected in Ohio and Japan (Foottit *et al.* 2009), the Czech Republic (Žurovcová *et al.* 2010), and Lithuania (Havelka *et al.* 2019). Sequences were aligned using Muscle (Edgar 2004), as implemented in Geneious Prime. A phylogenetic tree was reconstructed with MrBayes version 3.2.7a (Ronquist *et al.* 2012) using default priors, unlinked GTR + I + G substitution models for each gene region, four incrementally heated Markov chains and two runs of 1,000,000 generations sampled every 10,000 generations. The first 25% trees were discarded. *Adelges* outgroups included: *A.* (*Sacchiphantes*) *abietis* (Linnaeus, 1758), *A.* (*Adelges*) *japonicus* (Monzen, 1929), *A.* (*S.*) *kitamiensis* (Inouye, 1963), *A.* (*Aphrastasia*) *pectinatae* (Cholodkovsky, 1888) and *A.* (*Annandina*) *tsugae* Annand, 1924 (Table 1).

The specimen in Figure 2A was photographed with a DMC4500 digital camera mounted on a DM6 B microscope (Leica Microsystems) with 5× magnification. Symmetrical z-stacking was performed around the mid-point focusing distance as determined by auto-focusing on the whole specimen using the Leica Application Suite X version 5.1.0 software. The specimen in Figure 2B was photographed with a DP28 digital camera mounted on a BX53 microscope (Olympus) with 20× magnification. A series of images covering the X-Y plane of the specimen were obtained using the automatic acquisition

Table 1 Collection and voucher information for *Adelges* (*Cholodkovskya*) *viridanus* and outgroup specimens used in the phylogenetic analysis

Species	Sample no.	Voucher accession	GenBank accession no.		Collection information	Notes
			COI	*EF1*		
Adelges (*Cholodkovskya*) *viridanus*	04-038a	CNC#HEM053372	EF073074	OQ623202	JAPAN; Gifu; Norikura Skyline, Kamitakara-mura; 36.1649, 137.5243; 10 June 2004; Coll. Nathan Havill, Shigehiko Shiyake, G. Yu; ex. *Picea jezoensis*	Adult fundatrix and early instar gallicola nymphs settled at the base of a gall. Reported in Foottit *et al.* (2009)
Adelges (*Cholodkovskya*) *viridanus*	07-021	YPM#ENT996425 to ENT996435	FJ502489	N/A	USA; Ohio, Licking County, Newark, Dawes Arboretum; 39.9803, −82.4117; 23 June 2007; Coll. Nathan Havill; ex. *Larix* x *marschlinsii* (Dunkeld larch)	Alate adults with eggs settled on needles. Reported in Foottit *et al.* (2009)
Adelges (*Cholodkovskya*) *viridanus*	07-064	CNC#HEM058006	FJ502490	N/A	JAPAN; Tochigi, Nikko, University of Tokyo Nikko Botanical Garden; 36.7497, 139.5868; 12 July 2007; Coll. Mitsuru Ayabe; ex. *Picea jezoensis*	Early instar gallicola nymphs dissected from a young gall. Reported in Foottit *et al.* (2009)
Adelges (*Cholodkovskya*) *viridanus*	08-279	CNC#HEM070436	OQ607729	N/A	JAPAN; Yamanashi, Fujiyoshida, Yamanashi Forest Research Institute; 35.237, 138.487; 4 June 2008; Coll. Nathan Havill, Shigehiko Shiyake, Ashley Lamb; ex. *Larix kaempferi*	Late instar exules settled on the stem at the base of needles.
Adelges (*Cholodkovskya*) *viridanus*	19-111	YPM#ENT996436 to ENT996442	OQ607730 and OQ607731	OQ607125 and OQ607126	USA; Ohio, Licking County, Newark, Dawes Arboretum; 39.9803, −82.4117; 7 June 2019; Coll. Nathan Havill; *Larix* × *marschlinsii* (Dunkeld larch)	Alate nymphs and adults with eggs settled on needles
Adelges (*Cholodkovskya*) *viridanus*	22-193	YPM#ENT996678	OQ607732	OQ607127	JAPAN; Gifu, Gero, Nigorigo (upper); 35.9239, 137.4504; 15 June 2022; Coll. Shigehiko Shiyake; ex. *Picea jezoensis*	Adult fundatrix settled at the base of a gall and early instar gallicola nymphs dissected from the gall
Adelges (*Cholodkovskya*) *viridanus*	3326 to 3328	Institute of Entomology, Biology Centre ASCR, ýeské BudČjovice	GU571013 to GU571015	N/A	CZECH REPUBLIC; Nový Dvůr; 16 July 2008; ex. *Larix kaempferi*	Reported in Žurovcová *et al.* (2010)
Adelges (*Cholodkovskya*) *viridanus*	614	Da18-270	MH923979	MK795473	LITHUANIA; Klaipėda; 15 June 2018; ex. *Larix kaempferi*	Reported in Havelka *et al.* (2019)

(*Continues*)

Table 1 Continued

Species	Sample no.	Voucher accession	GenBank accession no.		Collection information	Notes
			COI	*EF1*		
Adelges (*Cholodkovskya*) *viridanus*	615 and 616	Da18-250 and Da18-256	MH923980 and MH923981	MK795474 and MK795475	LITHUANIA; Neringa, Juodkrantė; 13–14 June 2018; ex. *Larix* sp.	Reported in Havelka *et al.* (2019)
Adelges (*Cholodkovskya*) *viridanus*	617	Da18-188	MH923982	MK795476	LITHUANIA; Kaunas; 6 June 2018; ex. *Larix* sp	Reported in Havelka *et al.* (2019)
Adelges (*Sacchiphantes*) *abietis*	03-016	CNC# HEM053176	FJ502368	EF073223	USA; Massachusetts, Suffolk County, Jamaica Plain, Arnold Arboretum; 42.296, −71.126; 23 June 2003; Coll. Nathan Havill; ex. *Picea abies*	Reported in Havill *et al.* (2007) and Foottit *et al.* (2009)
Adelges (*Adelges*) *japonicus*	05-076	CNC# HEM054839	FJ502414	OQ607124	JAPAN; Hokkaido, Lake Horokayantô, Bansei; 42.527, 143.479; 7 July 2005; Coll. Shigehiko Shiyake; ex. *Larix kaempferi*	Reported in Foottit *et al.* (2009)
Adelges (*Sacchiphantes*) *kitamiensis*	04-068	CNC#HEM053415	EF073102	EF073250	JAPAN; Yamanashi, Yamanaka, Yamanakako; 35.44541, 138.85217; 11 June 2004; Coll. Shigehiko Shiyake; ex. *Picea torano*	Reported in Havill *et al.* (2007)
Adelges (*Aphrastasia*) *pectinatae*	04-040	CNC#HEM053377	EF073081	EF073236	JAPAN; Gifu; Norikura Skyline, Kamitakara-mura; 36.16653, 137.52198; 10 June 2004; Coll. Nathan Havill, Guoyue Yu, Shigehiko Shiyake; ex. *Abies veitchii*	Reported in Havill *et al.* (2007)
Adelges (*Annandina*) *tsugae*	04-036	CNC#HEM053369	EF073094	EF073245	JAPAN; Gifu; Norikura Skyline, Kamitakara-mura; 36.16313, 137.52752; 10 June 2004; Coll. Nathan Havill, Guoyue Yu, Shigehiko Shiyake; ex. *Tsuga diversifolia*	Reported in Havill *et al.* (2007)

N/A, not available.

z-stack function in the cellSens version 3.2 software (Olympus), and the resulting images were stitched with the photomerge function in Adobe Photoshop version 23.3.2. The inset image of a wax gland in Figure 2B was acquired as a z-stack at 40× magnification with the same equipment.

Three of the samples (04-038a, 07-021 and 07-064; Table 1) were previously reported as undetermined "*Adelges* sp. D" in Foottit *et al.* (2009). For our study, we determined the alate specimens on *Larix* from Ohio (samples 07-021 and 19-111) as *A.* (*C.*) *viridanus* using the keys in Pashtshenko (1988) and Binazzi (2000). Exules on *Larix* from Japan (sample 08-279) were determined as *A.* (*C.*) *viridanus* by comparison to the description and illustrations in Inouye (1945). Fundatrices (samples 04-038a and 22-193) and gallicolae (sample 07-064) on *Picea* from Japan were determined as *A.* (*C.*) *viridanus* using similarity (<1.0% sequence divergence) of DNA barcodes to our morphologically determined samples and to those reported in Žurovcová *et al.* (2010) and Havelka *et al.* (2019).

We obtained *COI* sequences for all samples, and *EF1α* for all samples except one of the collections of alates from *Larix* in Ohio (sample 07-021), gallicolae from *Picea* in Tochigi, Japan (sample 07-064), exules from *Larix* in Yamanashi, Japan (sample 08-279) and the samples from the Czech Republic (samples 3326–3328). The DNA sequence alignments were 658 and 689 bp long for *COI* and *EF1α*, respectively. *EF1α* contained an intron that varied in length from 67 to 84 bp, with most of the variation among different species. For *COI*, the uncorrected *p*-distance from *A.* (*C.*) *viridanus* to the outgroups ranged from 6.3% to 11.7%. Within *A.* (*C.*) *viridanus*, the mean distance between Ohio and Japan was 0.8%, between Ohio and Lithuania was 0.5%, and between Ohio and the Czech Republic was 0.3%. For *EF1α*, the uncorrected *p*-distance from *A.* (*C.*) *viridanus* to the outgroups ranged from 3.6% to 4.8%. Within *A.* (*C.*) *viridanus*, the *EF1α* sequences were identical, except for two

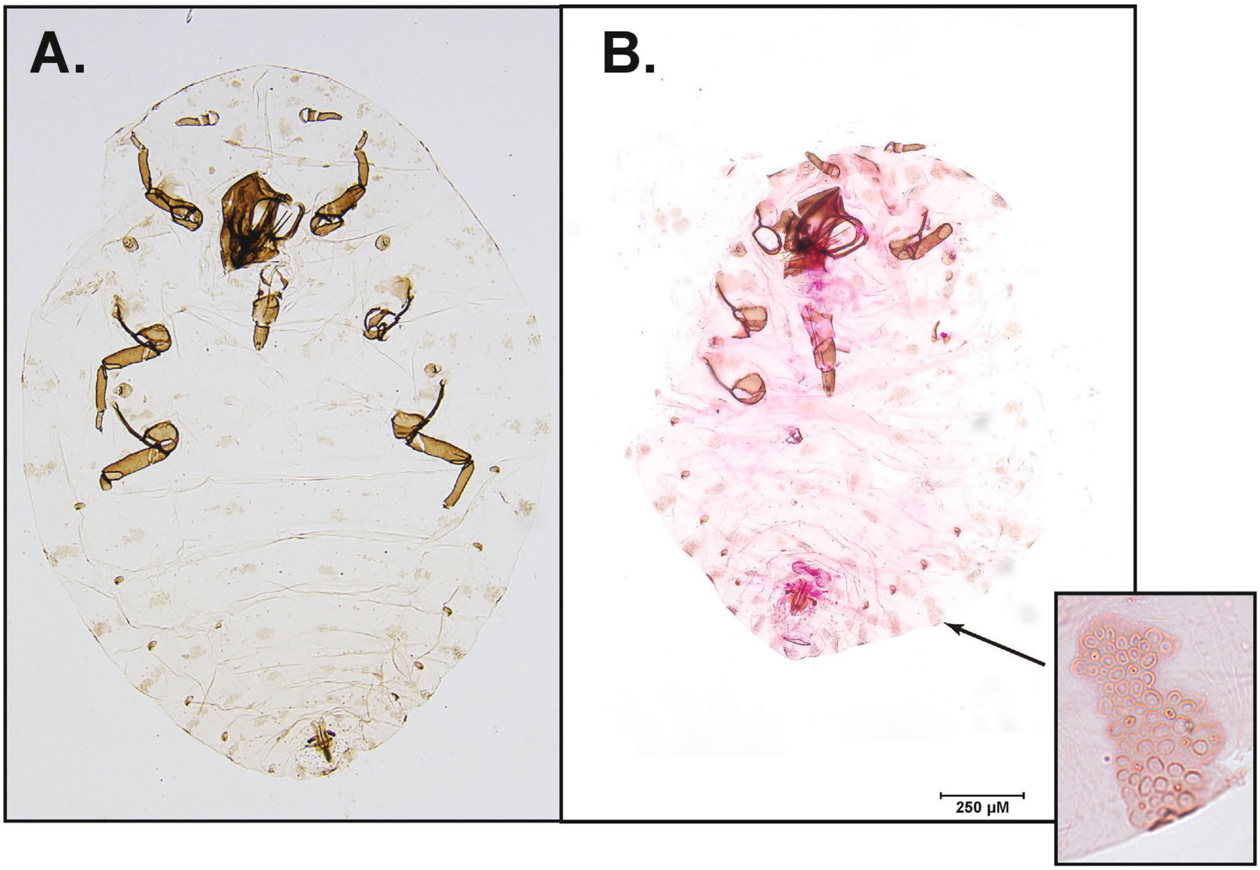

Figure 2 *Adelges* (*Cholodkovskya*) *viridanus* fundatrices collected from *Picea jezoensis* in Gifu Prefecture, Japan. (A) Sample 04-038a collected on 10 June, 2004. (B) Sample 22-193-01 collected on 15 June, 2022. The inset shows a close-up of an abdominal wax gland.

substitutions in the exons in one of the fundatrices from Japan (sample 04-038a; 0.3% divergence), and a 1 bp deletion in the intron from one of the sequences from Lithuania (sample 614).

The specimens from Ohio (samples 07-021 and 19-111) represent the first report, to our knowledge, of *A.* (*C.*) *viridanus* outside of its Eurasian native range. This population is likely the result of a human introduction, rather than an undiscovered native lineage, because it had not been reported previously despite a long history of adelgid surveys in North America (e.g. Patch 1909; Annand 1928; Foottit *et al.* 2009) and because the *COI* distance to the Eurasian samples is shorter than would be expected from intercontinental divergence. In contrast, the closest known intercontinental sister relationship between native adelgid lineages is between *A.* (*An.*) *tsugae* in Japan and western North America (Havill *et al.* 2016a). Using population genetic data, these groups were inferred to have diverged from each other approximately 10,200–47,600 years ago after trans-Beringian colonization from Asia to North America. *COI* sequences diverged between these groups by a minimum of 0.9% (six substitutions) (Havill *et al.* 2016a), while *A.* (*C.*) *viridanus* in North America to Eurasia had a minimum divergence of 0.3% (two substitutions).

The North American specimens were collected at Dawes Arboretum in Newark, Ohio on the needles (Fig. 1) of a Dunkeld larch tree, *Larix* × *marschlinsii* Coaz, which is a hybrid of European larch, *L. decidua*, and Japanese larch, *L. kaempferi*. The tree (Accession D1964-0013.002) was obtained from Schroth's Nursery in Indiana County, Pennsylvania and planted in 1964 (G. A. Payton, pers. comm., 2021). It is not

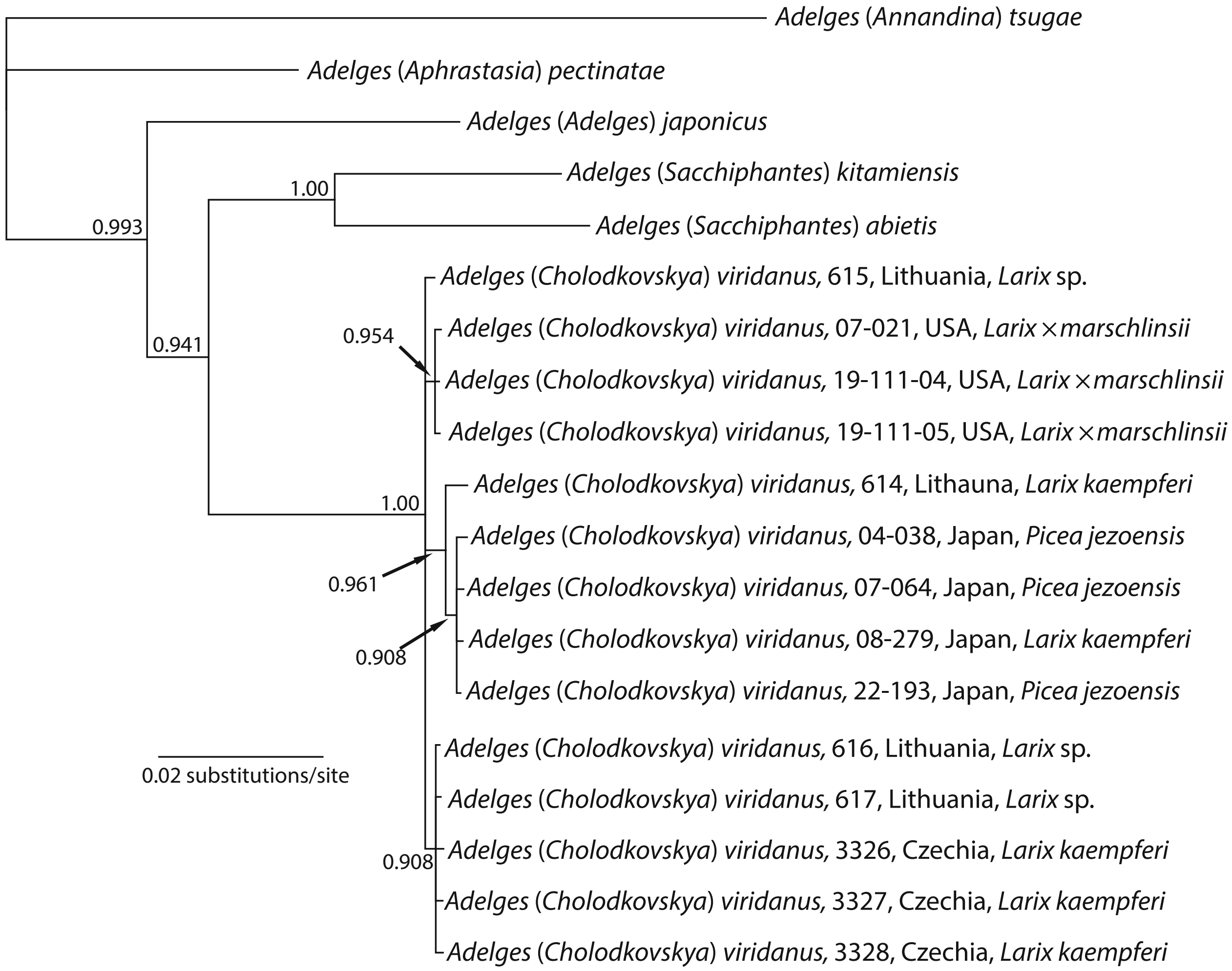

Figure 3 Bayesian phylogeny reconstructed using mitochondrial DNA barcode and nuclear *EF1α* sequences. For each specimen, the species name is followed by sample name, country of origin and host plant, corresponding to Table 1. Posterior probabilities are shown for each clade.

known whether *A.* (*C.*) *viridanus* arrived at the Arboretum with this accession.

Reconstruction of phylogenetic relationships (Fig. 3) did not conclusively show the origin of the *A.* (*C.*) *viridanus* population in Ohio because they did not form an exclusive clade with specimens from Europe or Japan. A shorter sequence divergence between Ohio and Europe *versus* between Ohio and Japan suggests a European origin, but data from additional specimens collected in other parts of its range, such as the rest of Europe, Korea and China, would better pinpoint its origin.

While other adelgid species, such as *A.* (*An.*) *tsugae* and *A.* (*Dreyfusia*) *piceae* (Ratzeburg, 1844), have become devastating pests in North America since their introduction (Montgomery & Havill 2014; Havill *et al.* 2016b), it does not seem likely that *A.* (*C.*) *viridanus* will be a serious pest in North America. *Adelges* (*C.*) *viridanus* is reported to be rare and relatively harmless in its native range (Steffan 1964), and no damage from this species has been reported at Dawes Arboretum on the collection tree or on specimens of the other *Larix* species planted there (*L. decidua*, *L. kaempferi*, *L. gmelinii*, *L. laricina* (Du Roi) K. Koch; G. A. Payton, pers. comm., 2021). These results reinforce the value of botanic gardens as resources for predicting the impact of nonnative species (Barham *et al.* 2016).

In Japan, the matching DNA sequences from specimens collected on *Larix* and *Picea* provide the first evidence that *A.* (*C.*) *viridanus* might complete a holocycle in that part of its range. We collected young galls on *Picea jezoensis* (Siebold & Zucc.) Carr. in Tochigi (sample 07-064) and Gifu (sample 04-038a). These young galls were of the typical closed strobile type for *Adelges*, but as the galls were early in development, it is not known how their morphology might differ at maturity from galls of other *Adelges* species on *P. jezoensis*: *A.* (*Ad.*) *isedakii* Eichhorn, 1978, *Adelges* (*Ad.*) *japonicus*, *A.* (*S.*) *karafutonis* Kono & Inouye, 1938, *A.* (*Aph.*) *pectinatae* or *A.* (*S.*) *torii* (Eichhorn, 1978) (Inouye 1945; Eichhorn & Carter 1978; Shiyake 2018). From the galls, we dissected early instar gallicola nymphs and removed the fundatrix settled at each of their bases (Table 1). The *COI* DNA barcode sequences from these specimens were identical to *A.* (*C.*) *viridanus* collected from *L. kaempferi* in Yamanashi (sample 08-279). This could be evidence of a holocycle in Japan; however, some other adelgid species complexes include holocyclic and anholocyclic forms with little genetic difference between them (e.g. *A.* (*Ad.*) *laricis* Vallot, 1836 plus *A.* (*Ad.*) *tardus* (Dreyfus, 1888), and *A.* (*S.*) *viridis* (Ratzeburg, 1843) plus *A.* (*S.*) *abietis*; Žurovcová *et al.* 2010). Indeed, Havill and Foottit (2007) suggested that adelgids associated with *Larix* are more likely to give rise to anholocyclic lineages on *Picea* than species associated with other secondary host genera. Thus, it is possible that the *A.* (*C.*) *viridanus* collected from *P. jezoensis* are not part of a holocycle but are instead very recently diverged anholocyclic populations. Confirmation of a holocycle will require host transplant experiments. Regardless, the evidence supports a holocycle in the recent evolutionary history of this species.

Table 2 Morphometric characteristics of *Adelges* (*Cholodkovskya*) *viridanus* fundatrices

Measurement	*n*	Mean ± SD (μm)	Range (μm)
Bl	2	1890.5 ± 410.8	1600–2181
Bw	2	1459 ± 224.9	1300–1618
A1	4	28.2 ± 2.3	26–31.5
A2	4	29.5 ± 3.9	27.3–35.3
A3	4	72.4 ± 3.2	68.4–76.1
A1w	2	46.8 ± 3.1	44.6–48.9
A3bw	2	27.3 ± 0.2	27.2–27.4
R3mw	2	76.9 ± 2.6	75.1–78.8
R4bw	2	42.5 ± 2.9	40.5–44.6
R4l	2	76.8 ± 0.2	76.7–76.9
F1	2	138.7 ± 1.2	137.9–139.5
F1w	2	46.8 ± 1.4	45.8–47.8
Tb1	2	126.6 ± 10.1	119.4–133.7
Ts1	2	50.6 ± 1.1	49.9–51.4
F2	1	151.5	N/A
F2w	1	51.4	N/A
Tb2	1	142	N/A
Ts2	1	54.8	N/A
F3	2	178.7 ± 1.8	177.4–180
F3w	2	52.3 ± 3.1	50.1–54.4
Tb3	1	159.4	N/A
Ov	2	125.1 ± 14.6	114.7–135.4

Note: Ts3 is missing because neither specimen had intact hind tarsi.

A[1–3], antennal segments I–III length; A1w, antennal segment I width; A3bw, antennal segment III basal width; Bl, body length; Bw, body width; F[1–3], femur length on fore-, mid-, and hind-legs; F[1–3] w, femur width; N/A, not available; Ov, ovipositor length; R3mw, rostral segment III width at mid-point; R4bw, ultimate rostral segment basal width; R4l, ultimate rostral segment length; Tb[1–3], tibia length; Ts[1–3], tarsus II length.

Description of *Adelges* (*Cholodkovskya*) *viridanus* fundatrix (Fig. 2, Table 2): adult 2.1 mm in length and 1.6 mm in width. Sclerotized plates covering head and prothorax lacking. Antennae dark brown in color. Antennal segment III (mean = 72.4 μm, range 68.4–76.1, *n* = 4) longer than segments I (mean = 28.22 μm, range 26.0–31.46, *n* = 4) and II (mean = 29.5 μm, range 27.3–35.3, *n* = 4) combined. Color of rostrum and legs matching antennae. Rostrum reaching to mid-coxae with ultimate segment as long (mean = 76.8 μm, range 76.7–76.9, $n = 2$) as width of segment III at midpoint (mean = 76.9 μm, range 75.1–78.8, $n = 2$). Width of base of ultimate rostral segment approximately two-thirds its length (mean = 42.5 μm, range 40.5–44.6, $n = 2$). Femur approximately 1.1× longer than tibia on each thoracic segment. Hind femur length-to-width ratio approximately 1.2× that on fore- and mid-legs. Wax glands on all coxae. Small dorsal wax gland plates in mesial, pleural, and marginal positions on all thoracic segments and abdominal segments I–VI. Wax gland plates circular or elliptical, bearing wax glands with tightly packed, sometimes overlapping pores with thickened walls (Fig. 2B, inset). Sharply pointed setae abundant on abdominal segment IX. Ovipositor about as long (mean = 125.1 μm, range 114.7–135.4, $n = 2$) as fore-femur or antennal segments I to III combined. Differs from fundatrix of *A.* (*Aph.*) *pectinatae* in lacking plates covering the head and prothorax (Inouye 1945), and from *A.* (*Ad.*) *japonicus* in having smaller dorsal wax glands (Inouye 1945). To our knowledge, adult fundatrices have not been described for the other species that are known to form galls on *P. jezoensis* in Japan: *A.* (*Ad.*) *isedakii*, *A.* (*S.*) *karafutonis,* and *A.* (*S.*) *torii.*

Specimens examined. JAPAN: Gifu, Norikura Skyline; 36.165, 137.524; 10 June 2004; *ex Picea jezoensis*; coll. N. P. Havill, S. Shiyake, G. Yu; deposited in CNC [#HEM053372]. JAPAN: Gifu, Gero, Nigorigo (upper); 35.9239, 137.4504; 15 June 2022; *ex. Picea jezoensis*; coll. S. Shiyake; deposited in YPM [#ENT996678].

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