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Cinara splendens (Hemiptera: Aphididae: Lachninae)—First Record in Palaearctic Region

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Abstract: Nearctic aphid *Cinara splendens* (Gillette and Palmer, 1924) was collected on ornamental Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) in South Bohemia in 2009. It was the first record of this species in the Palaearctic region. The aim of this research was to study the bionomy of this species in Central Europe and to make descriptions of all available morphs, as previous morphological descriptions of *C. splendens* appeared to be incomplete. Six monitoring sites of this species were established in South Bohemia and were then regularly attended in the period of 2009–2019. The colonies of *C. splendens* were observed; its natural enemies and honeydew users were also registered. Aphids were collected for the microscope slide preparation, followed by the evaluation of thirty of the basic quantitative and seven qualitative morphological characteristics. Partial sequences of mitochondrial COI and nuclear EF-1α were used to confirm morphology-based identification and to compare samples from the Czech Republic with those of North American origin. *Cinara splendens* survived successfully under new ecological conditions, but its population density remained quite low, except for 2009 and 2019, due to a synergistic effect of the dry weather and very high population density of the adelgid *Gilletteella coweni* (Gillette, 1907), which is a key pest of Douglas fir in the Czech Republic. The principle predators were coccinellid beetles, while the aphidophagous hover flies were less abundant. Together with a weak ability to migrate due to a low number of alate viviparous females in population, *C. splendens* cannot be a potential pest of *P. menziesii* in Central Europe.

Keywords: *Cinara splendens*; *Pseudotsuga menziesii*; exotic aphid species; bionomy; natural enemies; mitochondrial COI; nuclear EF-1α

1. Introduction

There have been an increasing number of alien aphid species in Europe in recent decades [1–8], in particular aphids on economic and ornamental plants of foreign origin. Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) attracts the attention of experts as a beautiful decorative plant and as a prospective production tree species in forestry. *Pseudotsuga menziesii* was introduced from North America into Great Britain in 1827. Later (in 1827) it was planted in the Hamburg Arboretum. The species offers tremendous economic potential [9,10], and will likely become even more widely planted in southwestern Germany in the coming decades as a substitute for Norway spruce (*Picea abies* (L.) Karst.), which appears to be seriously threatened by climate change [11]. A series of experiments were set up at the turn of the century (e.g., [12]) to explore the potential value of growing. Currently, Douglas fir is by far the most widely planted exotic tree in Baden-Württemberg, covering about 38,000 hectares and representing approximately 3% of the state's forest area [13]. Similar experiments

have been started in the Czech Republic (in 1971), where Douglas fir is currently cultivated in an area of 5818 hectares [14].

The key pests of North American Douglas fir in Europe are wooly conifer aphids of the genus *Gilletteella*, *G. cooleyi* (Gillette, 1907) and *G. coweni* (Gillette, 1907). In 2009, during the regular sampling of the colonies of *G. coweni*, we found an unknown species of *Cinara* on the model trees *P. menziesii* in park localities in České Budějovice (South Bohemia). These aphids produced small colonies on the two-year-old twigs among the needles. Microscopic and partial DNA sequence analysis of the samples revealed that this aphid species was the strictly monophagous *Cinara splendens* (Gillette and Palmer, 1924) of North American origin. The apterous parthenogenetic females, as well as the larvae of *C. splendens*, are covered with a thick layer of white wax, so in wax-covered colonies of *Gilleteella adelgid* they completely escaped attention. Mimicry of the *C. splendens* enforces the fact that on the infested branches, the ants, which are a characteristic part of the trophic relations in almost all aphids of the family *Lachnidae*, were completely absent.

The aim of this research was to study the bionomy of this species in Central Europe and to make the descriptions of all available morphs, as previous morphological description of *C. splendens* appeared to be incomplete.

2. Materials and Methods

After finding the first colonies of the *C. splendens*, the infestation of *P. menziesii* trees was examined at other localities. Two model localities (Stráž nad Nežárkou and Č. Budějovice) and 1 and 5 groups of model trees, respectively, were established. Furthermore, on the model trees, the life cycle was studied and natural enemies of an invasive species were collected in new conditions. Control of the model trees was carried out regularly, every 3 weeks during the growing season. Aphid material for microscopic analysis was collected only occasionally and samples are listed in Table 1.

Apterous and alate viviparous females were dropped onto a surface of a special plate held beneath an infested plant, and the aphids were put into tubes with 85% ethanol by means of a paintbrush. Moreover, the parts of host plants with aphid colonies were cut off by a trimmer and placed in the plastic bags. Aphids finished their preimaginal development within a few days and then were transferred into storage fluid. Aphids' natural enemies were kept in Petri dishes with plant material to breed adults of predators or parasitoids. Environmental data were registered.

Aphid samples stored in labeled tubes were degreased, macerated, cleared and mounted onto the slide by standard procedure later in the laboratory. Gum-chloral hydrate (Faure-Berlese's fluid) or Canada balsam in xylene was used as mounting fluid. After the aphids were identified, the slides were labeled and used for reference collection.

A series of individuals were used for measurements: 8 fundatrices, 34 apterous viviparous females, 22 alate viviparous females, 7 males and 21 oviparae. In total, 30 of the basic quantitative and 7 qualitative morphological characters were evaluated at different magnifications under the microscope Olympus BX41 (see Table S1 for details). Species identification was done using keys of Palmer [15] and Blackman and Eastop [16] and its latest internet version on http://www.aphidsonworldsplants.info/ (2018). To check the correctness of the microscopic species determination, partial DNA sequences were analyzed.

Table 1. Microscopic slides of *Cinara splendens* (Gillette and Palmer, 1924) collected in South Bohemia from *Pseudotsuga menziesii* (Mirb.) Franco. Legend: fx = fundatrix; aptvf = apterous viviparous female; alvf = alate viviparous female.

No. of Sample	Locality	GPS	Date	Aphid Morph
09HA3366	Č. Budějovice, Destinové str.	48°58′49.9″ N 14°26′57.0″ E	2009-04-25	fx, aptvf
09HA3367	Č. Budějovice, Destinové str.	48°58′49.9″ N 14°26′57.0″ E	2009-04-25	fx, aptvf
09HA3369	Č. Budějovice, J. Opletala str.	48°58′51.3″ N 14°26′44.1″ E	2009-04-25	fx, aptvf
09HA3377	Stráž nad Nežárkou	49°04′10.7″ N 14°54′08.1″ E	2009-04-27	fx, aptvf
09HA3376	Stráž nad Nežárkou	49°04′10.7″ N 14°54′08.1″ E	2009-04-27	fx, aptvf
09HA3501	Stráž nad Nežárkou	49°04′10.7″ N 14°54′08.1″ E	2009-05-25	aptvf
09HA3502	Stráž nad Nežárkou	49°04′10.7″ N 14°54′08.1″ E	2009-05-25	aptvf
09HA3503	Stráž nad Nežárkou	49°04′10.7″ N 14°54′08.1″ E	2009-05-25	aptvf
09HA3504	Praha 6, Břevnov park	50°05′05.4″ N 14°22′03.4″ E	2009-05-30	aptvf
09HA3546	Stráž nad Nežárkou	49°04′10.7″ N 14°54′08.1″ E	2009-06-15	aptvf
09HA3989	Stráž nad Nežárkou	49°04′10.7″ N 14°54′08.1″ E	2009-10-07	aptvf
09HA4027	Č. Budějovice, Mariánské square	48°58′46.5″ N 14°28′22.0″ E	2009-11-10	male, ovipara
09HA4043	Č. Budějovice, Mariánské square	48°58′46.5″ N 14°28′22.0″ E	2009-11-10	male, ovipara
11HA4255	Č. Budějovice, Mariánské square	48°58′46.5″ N 14°28′22.0″ E	2011-04-28	fx, aptvf
11HA4256	Č. Budějovice, Mariánské square	48°58′46.5″ N 14°28′22.0″ E	2011-04-28	fx, aptvf
12HA4549	Stráž nad Nežárkou	49°04′10.7″ N 14°54′08.1″ E	2012-06-20	aptvf
13HA4682	Č. Budějovice, Stromovka-park	48°58′09.4″ N 14°27′17.7″ E	2013-10-10	aptvf
15HA4669	Č. Budějovice, Destinové str.	48°58′49.9″ N 14°26′57.0″ E	2015-05-25	aptvf, alvf
15HA4670	Č. Budějovice, Destinové str.	48°58′49.9″ N 14°26′57.0″ E	2015-05-25	aptvf, alvf
15HA4671	Č. Budějovice, Destinové str.	48°58′49.9″ N 14°26′57.0″ E	2015-05-25	aptvf, alvf
15HA4775	Č. Budějovice, Destinové str.	48°58′49.9″ N 14°26′57.0″ E	2015-05-25	aptvf, alvf
15HA4781	Č. Budějovice, Destinové str.	48°58′49.9″ N 14°26′57.0″ E	2015-05-25	aptvf, alvf
15HA4782	Č. Budějovice, Destinové str.	48°58′49.9″ N 14°26′57.0″ E	2015-05-25	aptvf, alvf
15HA4783	Č. Budějovice, Destinové str.	48°58′49.9″ N 14°26′57.0″ E	2015-05-25	aptvf, alvf
16HA5106	Č. Budějovice, Stromovka park	48°58′09.4″ N 14°27′17.7″ E	2016-11-11	male
16HA5107	Č. Budějovice, Stromovka park	48°58′09.4″ N 14°27′17.7″ E	2016-11-11	male
16HA5108	Č. Budějovice, Stromovka-park	48°58′09.4″ N 14°27′17.7″ E	2016-11-11	ovipara
16HA5109	Č. Budějovice, Stromovka park	48°58′09.4″ N 14°27′17.7″ E	2016-11-11	ovipara
16HA5110	Č. Budějovice, Stromovka park	48°58′09.4″ N 14°27′17.7″ E	2016-11-11	ovipara
16HA5110	Č. Budějovice, Šumava distr.	48°58′89.3″ N 14°07′66.4″ E	2016-06-16	aptvf, alvf
19HA5939	Č. Budějovice, Stromovka park	48°58′09.4″ N 14°27′17.7″ E	2019-01-25	eggs
19HA5445	Č. Budějovice, Šumava distr.	48°58′89.3″ N 14°07′66.4″ E	2019-06-06	aptvf, alvf
19HA5448	Č. Budějovice, Šumava distr.	48°58′89.3″ N 14°07′66.4″ E	2019-06-06	aptvf, alvf
19HA5450	Č. Budějovice, Šumava distr.	48°58′89.3″ N 14°07′66.4″ E	2019-06-06	aptvf, alvf
19HA5451	Č. Budějovice, Šumava distr.	48°58′89.3″ N 14°07′66.4″ E	2019-06-06	aptvf, alvf
19HA5452	Č. Budějovice, Šumava distr.	48°58′89.3″ N 14°07′66.4″ E	2019-06-06	aptvf, alvf
19HA5454	Č. Budějovice, Šumava distr.	48°58′89.3″ N 14°07′66.4″ E	2019-06-06	aptvf, alvf

Total genomic DNA was extracted from a single aphid using DNeasy Blood and Tissue kit (Qiagen). The following primer pairs were used for the amplification: LCO-1490 and HCO-2198 for COI [17], and EF3 and EF6 for EF-1α [18]. PCR amplification was carried out in a thermal cycler (Eppendorf) in 50 µL volumes containing 2 µL of genomic DNA, 2.5 µL of each primer (0.5 µM), 25 µL of DreamTaq PCR master mix (Thermo Scientific) and 18 µL of nuclease-free water (Thermo Scientific). The cycling parameters were as follows: denaturizing at 95 °C for 5 min (1 cycle), denaturizing at 95 °C for 1 min, annealing at 47 °C (COI) or 50 °C (EF-1α) for 30 s, extension at 72 °C for 2 min (35 cycles in total), and final extension at 72° C for 10 min (1 cycle). PCR products were subjected to electrophoresis on 2% TopVision agarose (Thermo Scientific), stained with ethidium bromide and sized against MassRuler Express Forward DNA ladder (Thermo Scientific) under UV light. PCR products were purified with a Gene Jet PCR purification kit (Thermo Scientific) and sequenced at Macrogen Europe (Amsterdam, the Netherlands). The amplification primers were also used as sequencing primers. DNA sequences were aligned in the BioEdit Sequence Alignment Editor [19]. Additional sequences for comparison were downloaded from GenBank (Table 2). Neighbor-joining (NJ) trees were constructed to examine relationships among taxa and population samples and MEGA 7 [20] was used for this procedure.

Table 2. Partial COI and EF-1α sequences of *Cinara* spp. from *Pseudotsuga* spp.

Voucher [Reference]	Host Species	Location	GenBank Accession no. COI Fragment	GenBank Accession no. EF-1α Fragment
		Cinara pseudotaxifoliae		
2888 [21]	*Pseudotsuga* sp.	Colorado (US)	KF649421	KF693904
2917 [21]	*Pseudotsuga* sp.	New-Mexico (US)	KF649448	KF693924
2919 [21]	*Pseudotsuga* sp.	New-Mexico (US)	KF649450	KF693926
2981 [21]	*Pseudotsuga* sp.	California (US)	KF649478	KF693950
2999 [21]	*Pseudotsuga menziesii*	Oregon (US)	KF649490	KF693964
3001 [21]	*Pseudotsuga menziesii*	Oregon (US)	KF649491	KF693965
3029 [21]	*Pseudotsuga* sp.	Washington (US)	KF649506	KF693978
3047 [21]	*Pseudotsuga* sp.	Oregon (US)	KF649516	KF693988
3056 [21]	*Pseudotsuga* sp.	Oregon (US)	KF649523	KF693995
3076 [21]	*Pseudotsuga* sp.	California (US)	KF649539	KF694010
2921 [21]	*Abies* sp.	New-Mexico (US)	KF649452	KF693928
3407 [22]	*Pseudotsuga menziesii*	–	KY064238	KY064492
3391 [22]	*Pseudotsuga menziesii*	–	KY064227	KY064481
3385 [22]	*Pseudotsuga menziesii*	–	KY064221	KY064476
3384 [22]	*Pseudotsuga menziesii*	–	KY064220	KY064475
3366 [22]	*Pseudotsuga menziesii*	–	KY064205	KY064461
3363 [22]	*Pseudotsuga menziesii*	–	KY064202	KY064458
3343 [22]	*Pseudotsuga menziesii*	–	KY064191	KY064446
		Cinara pseudotsugae		
2881 [21]	*Pseudotsuga* sp.	Colorado (US)	KF649415	–
2889 [21]	*Pseudotsuga* sp.	Colorado (US)	KF649422	KF693905
2918 [21]	*Pseudotsuga* sp.	New-Mexico (US)	KF649449	KF693925
2915bis [21]	*Pseudotsuga* sp.	New-Mexico (US)	KF649446	KF693923
2890 [21]	*Pseudotsuga* sp.	Colorado (US)	KF649423	KF693906
		Cinara splendens		
2987 [21]	*Pseudotsuga* sp.	California (US)	KF649483	KF693954
2996 [21]	*Pseudotsuga menziesii*	California (US)	KF649488	–
3004 [21]	*Pseudotsuga* sp.	Oregon (US)	KF649493	KF693967
3025 [21]	*Pseudotsuga macrocarpa*	Washington (US)	KF649504	KF693976
3077 [21]	*Pseudotsuga* sp.	California (US)	KF649540	KF694011
3093 [21]	*Pseudotsuga* sp.	California (US)	KF649553	KF694025
HA3925	*Pseudotsuga menziesii*	Czech Republic	MT708506	MT708504

3. Results

Mounted apterous viviparous females of *Cinara* sp. from *P. menziesii* collected in the Czech Republic were identified as *Cinara splendens* following the key given below (Figures 1 and 2).

The key to the *Cinara* species on *Pseudotsuga menziesii* (Mirb.) Franco in the North America given below is from http://www.aphidsonworldsplants.info/C_HOSTS_Psa_Pyr.htm#Pseudotsuga.

1. Siphunculi pores on broad pigmented conical bases 0.4 mm or more in diameter. (Antennal hairs numerous, 0.08 mm or more long; hind tibial hairs 0.10–0.15 mm long). – *Cinara* (*Cinara*) *commatula*.

– Siphunculi cones very small, with pigmented area less than 0.2 mm in diameter. – 2

2. All tibiae almost wholly pale, except that hind tibiae have contrastingly black apices. – *Cinara* (*Cinara*) *splendens*.

– At least hind tibia dark or shading to dark over distal third or more of length. – 3

3. Longest abdominal hairs more than 0.10 mm long, longer than hairs on dorsal side of hind tibia, which are about 0.08 mm long. – *Cinara* (*Cinara*) *vagabunda*.

– Longest abdominal hairs less than 0.10 mm long or, if longer, then they are shorter than longest hairs on dorsal side of hind tibia. – 4

4. Longest hairs on dorsal side of hind tibia 0.09–0.16 mm. length of sclerotised part of stylet groove 0.8–1.0 mm. – *Cinara* (*Cinara*) *pseudotsugae.*

– Longest hairs on dorsal side of hind tibia 0.050–0.075 mm. length of sclerotised part of stylet groove 1.1–1.5 mm. – *Cinara* (*Cinara*) *pseudotaxifoliae*.

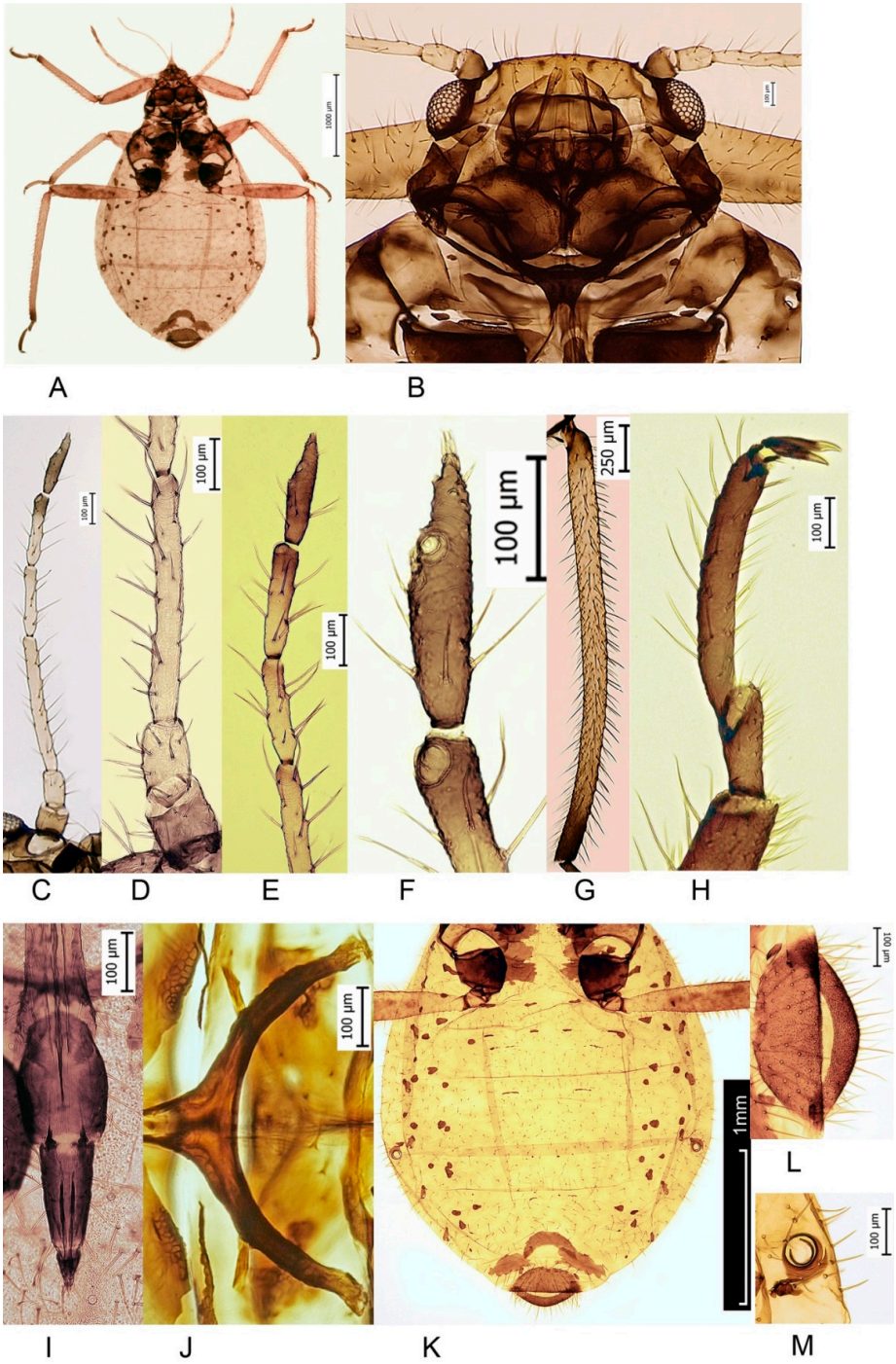

Figure 1. *Cinara splendens* (Gillette and Palmer, 1924) apterous viviparous female. (**A**) Whole body; (**B**) head; (**C**) antenna; (**D**) 1st–3rd segments of antenna; (**E**) 4th–6th segments of antenna; (**F**) 6th segment of antenna; (**G**) hind tibia; (**H**) hind tarsus; (**I**) 4th + 5th segments of rostrum; (**J**) furca of the mesothotax; (**K**) abdomen; (**L**) cauda; (**M**) siphunculus.

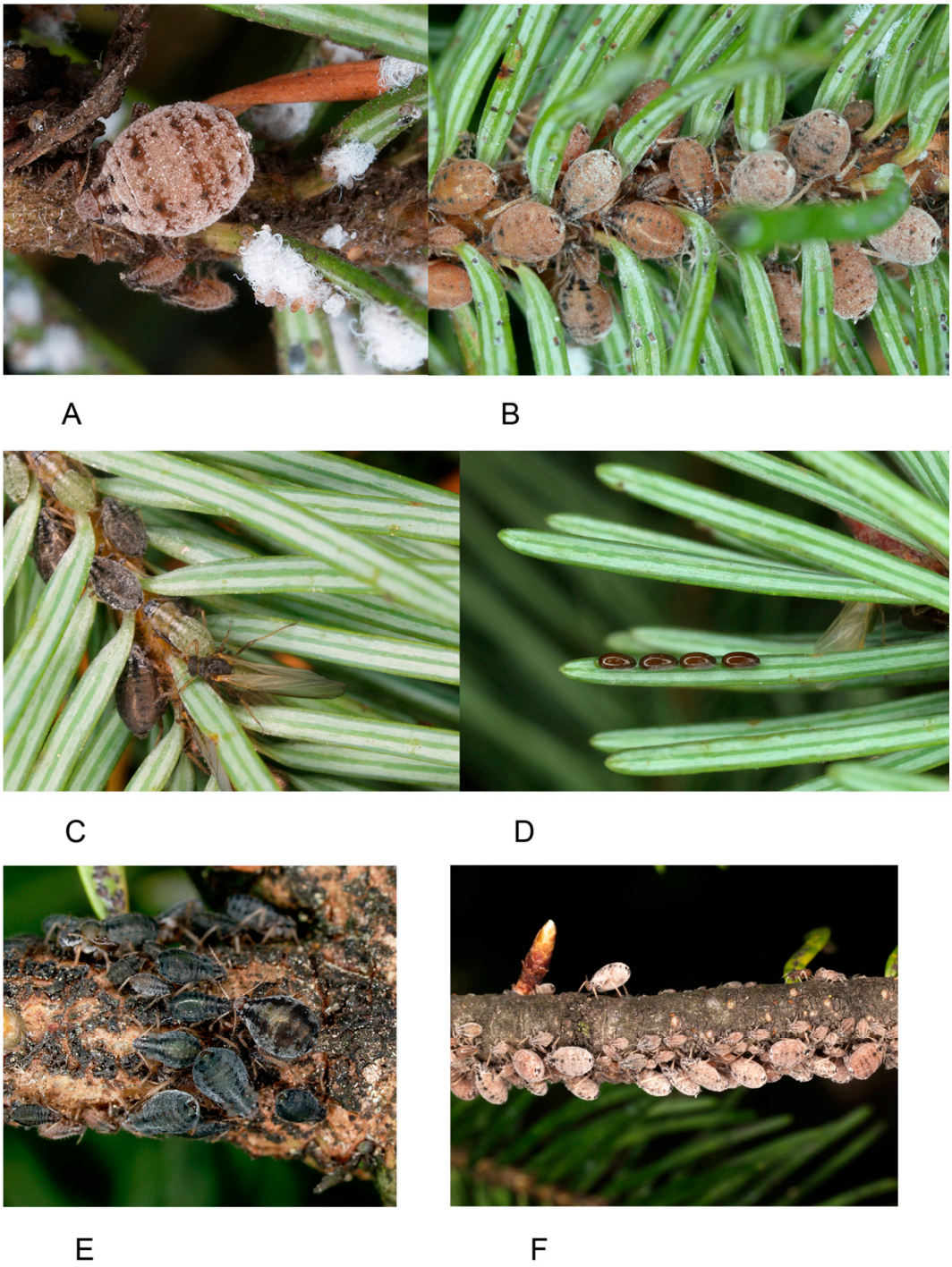

Figure 2. *Cinara splendens* (Gillette and Palmer, 1924). (**A**) Apterous viviparous female; (**B**) colony of apterous viviparous females; (**C**) oviparae, alate males and male nymphs; (**D**) eggs on needles of the *Pseudotsuga mensiesii* (Mirb.) Franko; (**E**) fundatrix; (**F**) damage of Douglas fir by *Cinara* and *Gilletteella.*

The original description of *C. splendens* as well as bionomy appeared to be incomplete. Therefore, here we present the main data concerning the morphology and average values of some quantitative characters of this species, based on samples collected in the Czech Republic (see the Table S1 in Supplementary Material for details).

A fundatrix is a wingless viviparous female. It differs from the apterous viviparous females of the summer generations only by its slightly larger abdomen, less intensive wax powder, darker color and shorter appendages. The body length is 3.7 mm.

An apterous viviparous female is light brown or yellowish brown, strongly powdered with wax making a pattern of transverse stripes, with two darker longitudinal dorsal stripes without wax diverging from thorax posteriorly (Figure 2). The abdomen has two more weakly distinct longitudinal bands on the abdominal segments; the siphuncular cones are black, very small; legs yellowish, with dark brown distal ends of tibiae. The antenna is six segmented, 1.16 mm (0.10:0.10:0.38:0.19:0.21:0.18). The basal part of the last antennal segment VI (VIa) is about twice as long as processus terminalis (VIb). The processus terminalis has four subapical hairs, while VIa has six hairs confined to the basal half. The primary rhinarium on segment VI has a ring of small sclerites. The rostrum is short (0.98 mm) and the ultimate rostrum segments are 0.2 mm, with 4–5 accessory hairs. The ventral side of the first segment of hind tarsus is 0.14 mm, the dorsal side is 0.07 mm, the inter-tarsal side is 0.06 mm, and the basal side is 0.05 mm. The second segment of hind tarsus (hts2) is 0.32 mm. The diameter of siphuncular sclerite (cone) is about 0.09 mm. Hairs on the abdomen are numerous, fine, up to about 0.09 mm. The body length is 3.4 mm.

An alate viviparous female is much like the apterous viviparous female. The third antennal segment is 0.5 mm long with 5–7 secondary rhinaria. Body length is 3.0 mm.

An oviparous female is similar to the apterous viviparous female and is brown, with a weak wax-covering. The pericaudal wax ring is absent (Figure 2). The hind tibia is slightly thickened from base to apex, with more than 100 small scent plaques. The hairs on the hind tibia are 0.12 mm, while the hairs on third abdominal segment are 0.07 mm long. The ventral side of the first segment of hind tarsus is 0.14 mm, dorsal side is 0.07 mm, intertarsal side is 0.06 mm, basal side is 0.05 mm. Body length is 3.3 mm.

An alate male's body is oblong-oval, light green color (Figure 2), with two darker longitudinal dorsal stripes. The body length is 2.5 mm. The antenna is 1.4 mm, light brown in color with long hairs (0.1 mm). The third antennal segment is 0.5 mm long with 42–52 secondary rhinaria, the fourth antennal segment is 0.2 mm long with 11–16 secondary rhinaria, and the fifth antennal segment is 0.3 mm long with 9–12 secondary rhinaria. The hind tibia is 1.6 mm long, light brown, with long (0.14 mm), dense hairs. The hairs on the third abdominal segment are 0.08 mm long. The ventral side of the first segment of hind tarsus is 0.11 mm, the dorsal side is 0.07 mm, the inter-tarsal side is 0.05 mm, and the basal side is 0.07 mm.

The whole life cycle of *C. splendens* in the Czech Republic is presented in Figure 3. There were probably seven generations during the season, depending mainly on mean temperature. *Cinara splendens* overwinters as eggs, usually in groups of four on needles. Fundatrices hatch in the middle of April in the Czech Republic. The second generation consists of apterous viviparous females occurring from the end of May until the end of June. The percentage of alate viviparous females in the colonies depends on the host plant's physiological condition, influenced primarily by dry weather and the presence of dense *G. coweni* colonies. At those stress conditions, about 50% of winged partenogentic females appear already in the third generation between June and early July. Around mid-July until the end of September, *C. splendens* practically disappears from the branches of *P. menziesii*, probably due to migration to the roots of its host tree. This phenomenon is also common in other *Cinara* species, such as *Cinara confinis* (Koch, 1856), *Cinara kochiana* (Börner, 1939), *Cinara mordvilkoi* (Pašek, 1954) and *Cinara curvipes* (Patch, 1912). Therefore, except from four generations observed on the branches of *P. menziesii*, there may be at least three generations during the summer hiding under the ground or next to the ground.

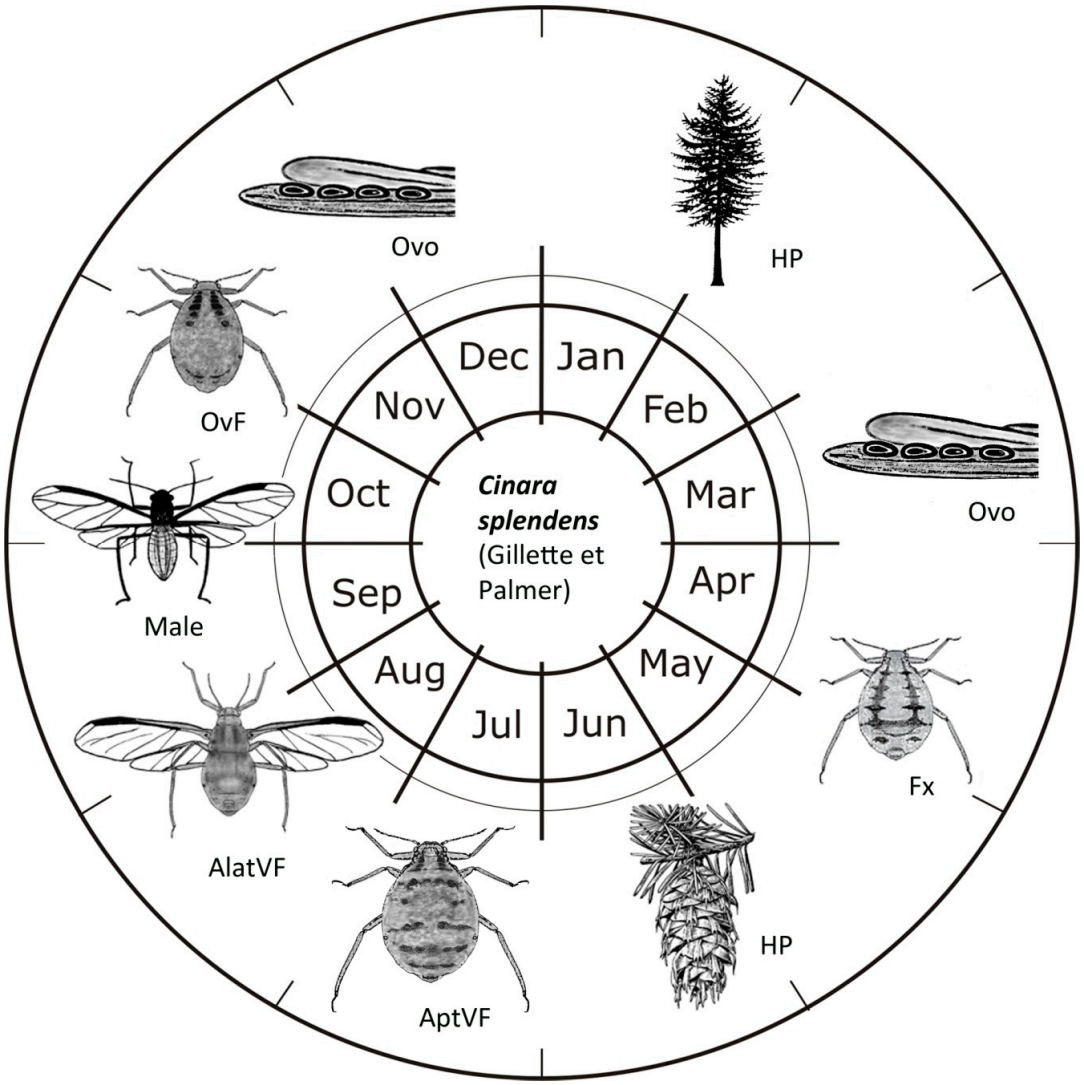

Figure 3. *Cinara splendens* (Gillette and Palmer, 1924) life-cycle in Central Europe. (HP = host plant; Fx = fundatrix; AptVF = apterous viviparous female; AlatVF = Alate viviparous female; OvF = ovipara).

Morphology-based identification was confirmed by the analysis of two DNA fragments, mitochondrial COI and nuclear EF-1α, of the collected samples. Additional partial sequences of COI (n = 29) and EF-1α (n = 27) genes were downloaded from GenBank for comparison (Table 2). Neighbor-joining (NJ) trees for both fragments were constructed to examine the position of the *Cinara* sample collected on *Pseudotsuga* in the Czech Republic among available different taxa of *Cinara*, feeding on the same host plant. In both cases, the sample from the Czech Republic clustered together with partial COI or EF-1α sequences obtained from samples identified as *C. splendens*. The most similar COI haplotype to that detected in the Czech Republic was from the sample collected in Washington (USA). In the case of the EF-1α fragment, the closest sequence was that originating from Oregon (USA). NJ trees are presented in Figures 4 and 5.

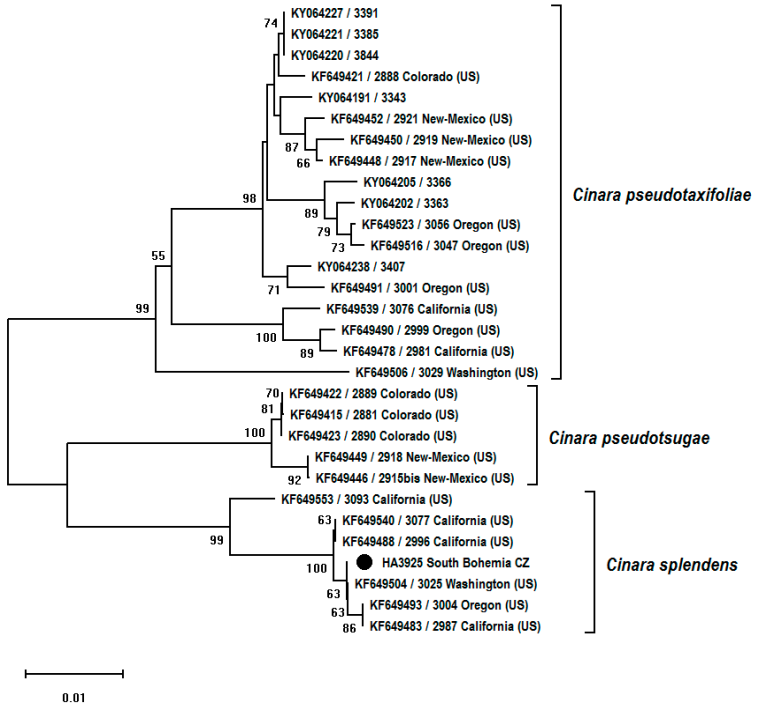

Figure 4. Neighbor-joining tree (p-distances, 1000 bootstrap replications) based on COI fragment (658 bp) of species of the genus *Cinara* collected from *Pseudotsuga* spp. Bootstrap values over 50% are shown next to branches. The sample from the Czech Republic is marked with a black circle.

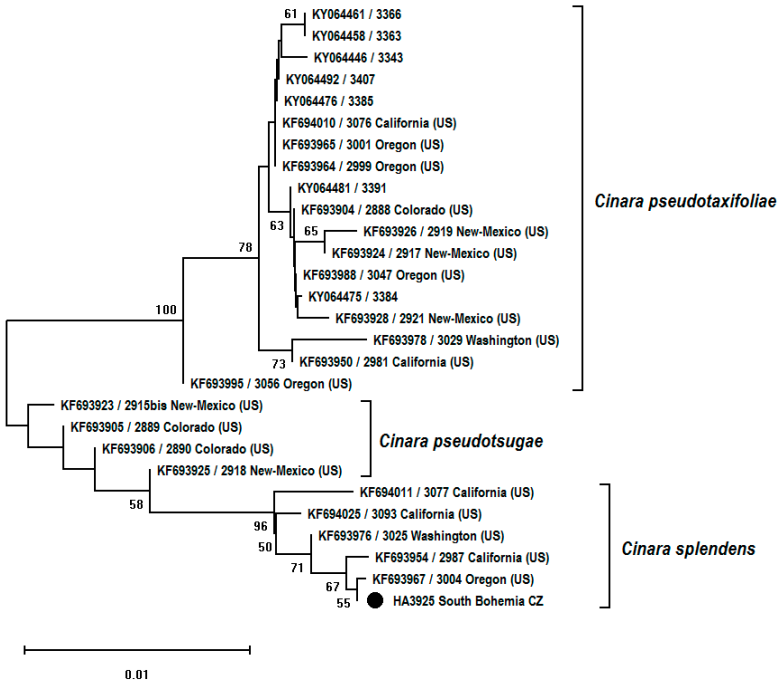

Figure 5. Neighbor-joining tree (p-distances, 1000 bootstrap replications) based on EF-1α fragment (1024 bp) of species of the genus *Cinara* collected from *Pseudotsuga* spp. Bootstrap values over 50% are shown next to branches. The sample from the Czech Republic is marked with black circle.

The native range of *C. splendens* is the Nearctic region, USA (Colorado), and Canada (British Columbia). In Central Europe, fundatrices occur in the April–May period. It is a monophagous species on *P.*

menziesii, feeding mostly on the bark of 1-year-old twigs at the tips of the branches just below the buds. In the summer, it produces differently sized colonies between the needles on young twigs, often in the community with adelgids of the genus *Gilletteella*. They are not attended by ants. Alatae males and oviparae appear on the young twigs in late October and during November (Figure 3). After mating, oviparous females lay their glossy, coffee-dark brown eggs in small groups on the needles of host plants (Figure 2).

The principle species of predators in the Czech Republic were those from the family Coccinellidae (Coleoptera): *Harmonia axyridis* (Pallas, 1773), *Coccinella septempunctata* Linnaeus, 1758 and *Aphidecta obliterata* (Linnaeus, 1758). The hover flies (Diptera: Syrphidae), *Syrphus ribesii* (Linnaeus, 1758) and *Episyrphus balteatus* (De Geer, 1776) were less abundant. Aphidiid *Pauesia* sp. (Braconidae, Aphidiinae) was also reported by Smith [23] as a parasitoid of *Cinara* species, closely related to *C. splendens* in the USA.

4. Discussion

The nearctic aphid *C. splendens* was found in South Bohemia (the Czech Republic) in 2009. A thick layer of wax on the body of virginogenous parthenogentic females of the *C. splendens*, its presence in dense colonies of *G. coweni*, as well as the absence of ants, is probably the main reason why this rather large aphid escapes the attention of forest entomologists in Europe. Aphids of this species are very similar to those of *C. pseudotaxifoliae* and *C. pseudotsugae*, differing in the color of the hind tibia, which is pale in *C. splendens* and dark over the distal third or more in the other two species.

This exotic aphid species survived successfully under new ecological conditions, but its population density remained quite low. It belongs to the second group of alien aphid species (Havelka, Starý, in prep). These exotic species survive in a new place, but in normal abiotic conditions they do not cause damage to cultural plants.

Higher population density of *C. splendens* occurred in 2009 and 2019, only due to a synergistic effect of the dry weather and the presence of dense *G. coweni* colonies, which is a key pest of Douglas fir in the Czech Republic. Under those conditions, slight damage of *P. menziesii*, including needle yellowing and falling, has been evident. High population density was usually reduced by polyphagous predators. Coccinellid beetles were the principal predator species, while the aphidophagous hover flies were less abundant. *Cinara splendens* is a typical representative of the K-strategy species, which are characterized by a continuous presence in its locality at low population density and low reproduction rate. Together with a weak ability to migrate due to a low number of alate viviparous females in the population, this exotic aphid species cannot be a potential pest of *P. menziesii* in Central Europe. Honeydew of *C. splendens* is not collected by Hymenoptera and has therefore no importance as a source of honey for beekeeping. For comparison, another Nearctic species of the genus *Cinara*, *C. curvipes*, may be an example of an R-strategy exotic aphid species in Europe, as it has a wide range of host plants, disperses as aerial plankton, forms vast colonies with a large proportion of winged morphs, has a small number of natural enemies and may reproduce anholocyclically during a "warm" winter season [5].

In general, it appears that *C. splendens* will have not great economic importance in Central Europe. Theoretically, however, the geographical isolation of an exotic species in a new environment can cause unexpected qualitative changes [21].

5. Conclusions

Nearctic aphid *C. splendens* was discovered on ornamental Douglas fir in South Bohemia in 2009. It was the first record of this species in the Palaearctic region. After a decade of observation, it was proved that *C. splendens* was able to overwinter as egg and reproduce successfully on its host tree throughout the growing season in the new environment. The study of its bionomy showed that this monophagous aphid species had low ability to migrate and low population density, which was also reduced by local predators, three species of Coccinellidae (Coleoptera) and two species of Syrphidae (Diptera). During the decade of observations, there were only two population explosions of *C. splendens* caused

by a synergistic effect of dry weather and high population density of adelgid *G. coweni*, a key pest of Douglas fir in Czech Republic. The honeydew produced by *C. splendens* is not collected by ants, bees and other Hymenoptera, therefore, this aphid species has no influence on beekeeping as a potential source of honey. In general, it appears that *C. splendens* will have not great economic importance in Central Europe.

Supplementary Materials: The following are available online at http://www.mdpi.com/1999-4907/11/9/911/s1, Table S1: General morphometric data of *Cinara splendens* samples from Czech Republic.

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