New for Lithuania Aphid Species of the Tribe Eulachnini (Hemiptera: Aphididae, Lachninae): Is There Any Threat to Local Coniferous Forests?

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Abstract

Information about Eulachnini species in Lithuania concerns fragmentary faunistic data, therefore, their diversity, abundancy and ecological specificity is insufficiently studied. The aim of this work was to present available information on the new to Lithuania Eulachnini aphid species detected on coniferous plants in Lithuania in 2004 - 2017, with special regard to their potential impacts on forestry. Partial COI sequences (652 bp) were used to confirm morphology-based identification of the new to Lithuania Eulachnini species and to investigate their genetic diversity and relationships with samples collected in other countries. Twenty-seven aphid species of the tribe Eulachnini were detected in 2004 – 2017 in Lithuania. Eight of them are reported from Lithuania for the first time: *Cinara* (*Cinara*) *brauni*, *C.* (*C.*) *kochiana*, *C.* (*C.*) *neubergi*, *C.* (*C.*) *laricis*, *C.* (*C.*) *pectinatae*, *C.* (*C.*) *piceae*, *Eulachnus brevipilosus* and *E. nigricola*. Five species of Eulachnini (*C.* (*C.*) *piceae, C.* (*C.*) *nuda*, *C.* (*C.*) *piceicola*, *C.* (*C.*) *pinea*, and *C.* (*C.*) *pini*) appeared to be most promising honeydew producers because of their host plants, *Picea abies* and *Pinus sylvestris*, which are the most common conifers in Lithuania. Although five species of Eulachnini were listed in the identification key of forest pests in Lithuania, none of them were of particular importance for now. Out of the recently reported species, only *Cinara piceae* may be a potential forest pest as it appeared to be rather common in Lithuania and it has been reported as a pest of firs in nurseries of the Czech Republic and Northern Caucasus. Most of the samples from Lithuania represented COI haplotypes which were not previously detected in other countries.

Keywords: *Cinara*; coniferous trees; *Eulachnus*; Lithuania; mitochondrial COI

Introduction

Forests comprise approximately 30 % of Lithuania. Most of the forests are the pine and spruce stands (Valstybinių miškų urėdija 2019). For the present, information about Eulachnini species composition and ecological specificity in Lithuania is insufficient and concerns fragmentary faunistic data reporting 19 species of the tribe available in Lithuania (Rakauskas et al. 1992, Rakauskas et al. 2008). For comparison, 34 species have been recorded from Poland, 19 from Latvia and 21 from Belarus (Osiadacz and Halaj 2010, Halaj and Osiadacz 2015, Wojciechowski et al. 2015, Nieto Nafria et al. 2013).

All aphids of the tribe Eulachnini Baker, 1920 (Hemiptera: Aphididae, Lachninae) are phloem feeding insects inhabiting coniferous plants of families Pinaceae and Cupressaceae. Most of the species of the genus *Cinara* Curtis, 1835 feed on the bark of the woody parts of their hosts (roots, trunks, branches, twigs and shoots), whilst species of the subgenus *Schizolachnus* inhabit needles. Species of the genera *Eulachnus* del Guercio, 1909*, Esigella* del Guercio, 1909 and *Pseudessigella* Hille Ris Lambers, 1966 are known to feed only on the needles of the plant species of the family Pinaceae (Binazzi and Scheurer 2009, Chen et al. 2016, Blackman and Eastop 2018). Therefore, aphids of the tribe Eulachnini might cause serious damage in nursery gardens, parks, forests and cultivated areas (Murphy and Völkl 1996, Watson et al. 1999, Penteado et al. 2000, Hopmans and Elms 2013). On the other hand, honeydew of these aphid species supplies the raw material for the so called “forest honey” which is an important commercial product in Europe (Binazzi and Scheurer 2009).

European species of the aphid tribe Eulachnini belong to three genera: *Cinara*, *Eulachnus* and *Essigella*. Genus *Cinara* comprising of 49 species is the largest one. It is divided into four separate subgenera (*Cinara* Curtis, 1835, *Cedrobium* Remaudière, 1954, *Cupressobium* Börner, 1940 and *Schizolachnus* Mordvilko, 1909), and the nominal subgenus *Cinara* s. str. is the largest one (42 species) (Binazzi and Scheurer 2009, Blackman and Eastop 2018, Nieto Nafrìa et al. 2013, Chen et al. 2016, 2016a). Seventeen aphid species representing subgenera *Cinara* (12 species), *Cupressobium* (3) and *Schizolachnus* (2) of the genus *Cinara* are already registered in Lithuania (Rakauskas et al. 1992, Rakauskas et al. 2008). Another Eulachnini genus, a needle feeding *Eulachnus* is represented by 12 species in Europe (Kanturski et al. 2017), two of them being recorded in Lithuania (Rakauskas et al. 1992, Rakauskas et al. 2008). Species of the genus *Eulachnus* are reported as pests of pine stands (Murphy and Völkl 1996). Nearctic genus *Essigella* is represented in Europe by a single species *Essigella californica* (Essig 1909) introduced in Spain and France (for details see Seco Fernandez and Mier Durante 1992, Nieto Nafria et al. 2013). Unlike in Australia (Hopmans and Elms 2013), it does not cause any damage in Europe for now.

To enable successful management of the local aphid species, regional species diversity and ecological specificity should be properly evaluated (Binazzi and Scheurer 2009, Durak 2014, Durak et al. 2016). Ecological and evolutionary relationships as well as taxonomy of *Cinara* species are still not clear and demand further studies in different parts of the distribution area of this aphid genus (Chen et al. 2016). The application of DNA-based methods proved to be a powerful tool for species delimitation and speciation process investigation of the genus *Cinara* (Jousselin et al. 2013, Chen et al. 2016, Meseguer et al. 2017). In this study, partial COI sequences were used to confirm morphology-based identification of new to Lithuania Eulachnini species as well as for the investigation of their genetic diversity and relationships with samples collected in other countries.

The aim of this work was to present available information on the new to Lithuania Eulachnini aphid species detected on coniferous plants in Lithuania between 2004 and 2017, with special regard to their potential impacts on forestry.

Table 1. Aphid samples used for the analysis of partial COI sequences (652 bp)

Locality, date, host plant, collection No (when available)	GenBank Accession No
Cinara (*Cinara*) *brauni* n=6	
Languedoc-Roussillon, France, 1998.07.01, *Pinus sylvestris*	KF639313, KF649558, ACEA053-14
Almaty, Kazakhstan, 2010.05.26, *Pinus nigra*	KF649394
Klaipėda, BG KU, 2017.06.24, *Pinus* sp., Da17-320	**MH396419**
Palanga, 2012.08.19, *Pinus nigra*, 12HA04602	**MH396418**
Cinara (*Cinara*) *kochiana* n=4	
PACA, France, 2009.08.17, *Larix decidua*	KF649382
PACA, France, 2010.09.15, *Larix decidua*	KF649462
Japan, 2012.08.22, *Larix leptolepis*	KP869241
Kapčiamiestis, 2017.08.07, *Larix decidua*, Da17-550	**MH396420**
Cinara (*Cinara*) *laricis* n=10	
China, 2002.07.05, *Larix* sp.	KP339517
China	KM501334
Languedoc-Roussillon, France, 1998.07.31, *Larix* sp.	KF639321
Ulan Bator city, Mongolia, 2009.06.22, *Larix sibirica*	JX035025
Valais, CH, 2011.08.22, *Larix decidua*	KF649554
Languedoc-Roussillon, France, 1998.07.31, *Larix decidua*	KF649556
PACA, France, 2010.09.15, *Larix decidua*	KF649464
PACA, France, 2009.07.11, *Larix decidua*	KF649369
Languedoc-Roussillon, France, 1998.07.31	ACEA039-14
Kapčiamiestis, 2017.09.28, *Larix decidua*, Da17-684	**MH396421**
Palanga, 2012.08.16, *Larix decidua*, 12HA04624	**MH396422**
Cinara (*Cinara*) *neubergi* n=5	
PACA, France, 2009.08.14, *Pinus mugo*	KF649380
Girionys, DA, 2017.06.23, *Pinus mugo*, Da17-311	**MH396423**
Smiltynė, 2017.06.27, *Pinus mugo*, Da17-356A	**MH396424**
Kairėnai, BG VU, 2017.07.07, *Pinus mugo*, Da17-436B	**MH396425**
Juodkrantė, 2017.07.22, *Pinus mugo*, Da17-515B	**MH396426**
Cinara (*Cinara*) *pectinatae* n=6	
Languedoc-Roussillon, France, 1998.07.31, *Abies alba*	KF639326, KF649557, ACEA040-14
Languedoc-Roussillon, France, 2009.07.29, *Abies alba*	KF649377
Languedoc-Roussillon, France, 2009.07.22, *Abies sp.*	KF649375
Kaunas, BG VMU, 2017.07.08, *Abies koreana*, Da17-446	**MH396427**
Cinara (*Cinara*) *piceae* n=14	
Japan, 2012.08.20, *Picea ghleni*	KP869234
Japan, 2012.08.30, *Picea ghleni*	KP869253
Alashan Left City, China, 2011.08.13, *Picea asperata*	JQ916814, JQ916815
Colorado, United States, *Picea*	KF649413, KF649414
New-Mexico, United States, *Picea*	KF649445
Skirgiškės, 2006.05.31, *Taxus baccata*, 06-10	**MH396433**
Žydaviškis, 2016.09.01, *Picea abies*, Da16-358	**MH396434**
Būtingė, 2017.06.12, *Picea abies*, Da17-227	**MH396431**
Šventoji, 2017.06.26, *Picea abies*, Da17-347	**MH396430**
Palanga, 2017.06.26, *Picea abies*, Da17-353	**MH396429**
Kaunas, BG VMU, 2017.07.08, *Picea abies*, Da17-445	**MH396432**
Klaipėda, BG KU, 2017.10.17, *Picea omorika*, Da17-715	**MH396428**
Eulachnus brevipilosus n=16	
Central Europe	KP637106- KP637118
Alksnynė, 2017.07.22, *Pinus sylvestris*, Da17-524	**MH396417**
Būtingė, 2017.07.26, *Pinus sylvestris*, Da17-528A	**MH396416**
Alksnynė, 2017.10.15, *Pinus sylvestris*, Da17-710	**MH396415**
Eulachnus nigricola n=3	
Central Europe	KP637143- KP637144
Klaipėda, BG KU, 2017.06.24, *Pinus heldreichii*, Da17-324	**MH396414**

Samples from Lithuania are in bold. BG – Botanical Garden; DA – Dubrava Arboretum; KU – Klaipėda University; VMU – Vytautas Magnus University; VU – Vilnius University.

Materials and Methods

Sampling and identification of morphological species

Aphid material has been collected during 2004–2017 and included 328 samples of Eulachnini aphid species from various coniferous plant species in Lithuania. The entire list of samples is available from the authors on the request. Those used for molecular analysis are listed in Table 1. Microscope slides in Canada balsam were prepared according to Blackman and Eastop (2000). Ethanol preserved and mounted specimens are stored at the Life Sciences Centre, Vilnius University and/or Institute of Entomology of the Czech Academy of Sciences (České Budějovice). For morphology-based identification, keys of Blackman and Eastop (2018), as well as Heie (1995), Binazzi and Scheurer (2009) and Kanturski et al. (2017) were used.

DNA isolation, amplification, sequencing and data analysis

For molecular analysis, a single aphid individual from one sampled plant was considered as a unique sample. Total genomic DNA was extracted from a single aphid using DNeasy Blood & Tissue kit (Qiagen). For the amplification of COI fragments primers LCO-1490 and HCO-2198 (Folmer et al. 1994) were used. PCR amplification was carried out in a thermal cycler (Eppendorf) in 50 µl volumes containing 1 µl genomic DNA, 2.5 µl of each primer (0.5 µM), 25 µl of DreamTaq PCR master mix (Thermo Scientific) and 19 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 30 s, annealing at 47°C for 30 s, extension at 72 °C for 30 s (34 cycles in total), and final extension at 72 °C for 5 min (1 cycle). PCR products were purified with Gene Jet PCR purification kit (Thermo Scientific) and sequenced at Macrogen Europe (Amsterdam, the Netherlands) and Institute of Biotechnology (Life Sciences Centre, Vilnius University). The amplification primers were also used as sequencing primers. DNA sequences for each specimen were confirmed with both sense and anti-sense strands and aligned in the BioEdit Sequence Alignment Editor (Hall 1999). Partial COI sequences were tested for stop codons and none were found. GenBank Accession numbers for each sample are given in Table 1.

Additional sequences for comparison were downloaded from GenBank and BOLD systems (Table 1). Sequences were collapsed into haplotypes using FaBox 1.41 (Villesen 2007). To evaluate within-species sequence diversity uncorrected p-distances were calculated with MEGA 7 (Kumar et al. 2016) and statistical parsimony networks with 95 % implemented connection limit were built using TCS v 1.21 (Clement et al. 2000).

Results

Twenty-seven aphid species of the tribe Eulachnini were detected in 2004 – 2017 in Lithuania. Twenty-three of them belong to the genus *Cinara* (eighteen of subgenus *Cinara* s. str., three of *Cupressobium*, two of *Schizolachnus*) and four to the genus *Eulachnus*. Eight species are reported from Lithuania for the first time: *Cinara* (*C.*) *brauni, C.* (*C.*) *kochiana, C.* (*C.*) *neubergi, C.* (*C.*) *laricis, C.* (*C.*) *pectinatae, C.* (*C.*) *piceae, Eulachnus brevipilosus, E. nigricola.* Currently available information concerning distribution, biology and economic importance of new for Lithuania species is given below mostly after Nieto Nafria et al. (2013), Blackman and Eastop (2018), Binazzi and Scheurer (2009), Albrecht (2017), Kanturski et al. (2017).

Cinara (*Cinara*) *brauni* Börner, 1940. This species has been already registered in Europe southwards from Lithuania, also from Sweden. Aphids form dense colonies on young shoots and one or two-year-old twigs of *Pinus nigra,* rarely *P. sibirica, P. sylvestris* and might cause damage to young host plants. These aphids are moderate honeydew producers, optionally dependent on ants. Lithuanian samples were from *Pinus nigra, P. heldreichii* and unidentified exotic *Pinus* sp. in coastal (Klaipėda) region of Lithuania.

Cinara (*Cinara*) *kochiana* (Börner, 1939). This species has been already registered in Europe southwards from Lithuania, also from Sweden. Aphids form dense colonies on older branches, trunks and open roots of *Larix* spp. and compulsory depend on ants as they produce a lot of honeydew. Lithuanian samples were from *Larix decidua* in southern Lithuania (Alytus). Noticeably, these were collected on third year twigs and third year stem of young trees.

Cinara (*Cinara*) *neubergi* (Arnhart, 1930). This species is common in mountainous regions of Europe, including Austria, Bulgaria, Germany, Italy, Switzerland, Poland and Ukraine. Aphids live on bark of twigs of *Pinus mugo* feeding among needle bases and produce a lot of honeydew. Lithuanian samples were from *Pinus mugo* in the coastal (Neringa, Klaipėda), central (Kaunas) and eastern (Vilnius) regions of Lithuania.

Cinara (*Cinara*) *laricis* (Hartig, 1839). This species is common throughout Europe. Aphids form small dense colonies on twigs of lower branches and trunks of young *Larix* spp. and are important honeydew producers in Austria, Germany, Switzerland and Italy. Lithuanian samples were from *Larix decidua* in the coastal (Klaipėda) and southern (Alytus) regions of Lithuania.

Cinara (*Cinara*) *pectinatae* (Nordlinger, 1880). This species has been already registered throughout Europe southwards from Lithuania, also in Sweden and Norway. Aphids feed on the needles, at the junction between petiole and stem on small branches of *Abies* spp. They are important honeydew producers in Central Europe. Lithuanian sample was from *Abies koreana* in the central (Kaunas) region of Lithuania.

Cinara (*Cinara*) *piceae* (Panzer, 1800). This species is common throughout Europe. These large-sized aphids (5–7 mm long) form large colonies on older branches and trunks of *Picea* spp., often move to roots in summer. These important honeydew producers can be harmful to host plants. Lithuanian samples were from *Picea abies*, *P. pungens* and *P. omorica* in the coastal (Klaipėda), central (Kaunas), northern (Panevėžys) and eastern (Vilnius) regions of Lithuania. In 2006, numerous colonies (apterae and larvae) of *C. piceae* were also found on the bark of trunk and branches of *Taxus baccata* 2 m height bush (Skirgiškės, Vilnius distr.). These appeared to be opportunistic invaders from the nearby growing overcrowded *P. pungens*, which failed to survive on atypical host (perished in two weeks).

Eulachnus brevipilosus Börner, 1940. This species has been already registered in Europe from mountain areas of Austria, the Czech Republic, Italy, Poland, Slovakia and Ukraine, also in Finland and Scandinavian Peninsula. Aphids feed on needles of *Pinus* spp. and are reported as pests causing serious damage to young and mature pine trees in nursery gardens, parks, forests and cultivated areas, especially in invasive distribution areas of aphid species (Kanturski et al. 2017). Lithuanian samples were from *P. sylvestris* in the coastal (Klaipėda, Neringa) and eastern (Utena) regions of Lithuania.

Eulachnus nigricola (Pašek 1953). This species has been already registered in Europe from mountain areas of Bulgaria, the Czech Republic, Denmark, France, Germany, Hungary, Italy, North Macedonia, Moldova, Poland, Romania, Slovakia, Spain and Switzerland. Until recently, it was regarded as montane element, but in Southern Poland, this species is very common also in anthropogenic areas (Kanturski et al. 2017). It was also found in Copenhagen (Kanturski 2017, personal communication). Aphids feed on needles of *Pinus nigra* (incl. var. *pallasiana*, var. *salzmanii*), also *P. heldreichii*, *P. massoniana P. leucodermis*. Lithuanian samples were from *P. nigra* and *P. heldreichii* in coastal (Klaipėda) region.

Partial COI sequences (652 bp) were used to confirm morphology-based identification of the new to Lithuania Eulachnini species by comparing with available sequences from public databases (see Table 1). The number of downloaded sequences was from 1 to 13 per species. Average proportion of differences (p-distances) within species varied from 0.05% in *C. pectinatae* to 2.36% in *C. piceae* (Table 2). Two COI haplotypes were detected in *E. nigricola*, *C. neubergi* and *C. pectinatae*, 3 in *C. brauni* and *C. kochiana*, 5 in *C. laricis*, 7 in C. *piceae* and 9 in *E. brevipilosus* (Table 3).

Most of the samples from Lithuania represented COI haplotypes, which were not previously detected in other countries (Table 3, Figure 1). Samples of *C. brauni* collected in Lithuania belonged to two COI haplotypes, one of them (H2) was unique, while another one (H1) was the same with sample from Kazakhstan. Unique COI haplotype (H1) was also detected in Lithuanian sample of *C. kochiana*. It differed from samples collected in France (H2) by single nucleotide substitution. In case of *C. laricis*, one sample from Lithuania represented unique haplotype (H2), differing by four nucleotides from the most abundant COI haplotype (H1), which included samples from Lithuania, France and China. COI sequences of *C. neubergi* from Lithuania were identical and were different from the single sample from France by three nucleotide substitutions. Partial COI sequences of *C. pectinatae* samples from France (H2) differed by one nucleotide from the sample collected in Lithuania (H1). All COI haplotypes of Lithuanian *C. piceae* samples (H1, H2 and H4) were not detected in samples collected in other countries. The closest haplotype was H3, representing samples from Japan. Partial COI sequences of *E. nigricola* from Lithuania (H2) differed from other available European samples (H1) by two nucleotides. Unique

Table 3. COI haplotypes of seven Eulachnini species new to Lithuania. Sample information is given in Table 1

Haplotype code	Number of sequences	Sequences belonging to haplotype
		Cinara (*Cinara*) *brauni*
H1	2	Da17-320, KF649394
H2	1	12HA04602
H3	3	KF639313, KF649558, ACEA053-14
		Cinara (*Cinara*) *kochiana*
H1	1	Da17-550
H2	2	KF649382, KF649462
H3	1	KP869241
		Cinara (*Cinara*) *laricis*
H1	7	Da17-684, KF639321, KF649554, KF649556, KF649464, KF649369, ACEA039-14
H2	1	12HA04624
H3	1	KP339517
H4	1	KM501334
H5	1	JX035025
		Cinara (*Cinara*) *neubergi*
H1	4	Da17-311, Da17-356A, Da17-436B, Da17-515B
H2	1	KF649380
		Cinara (*Cinara*) *pectinatae*
H1	1	Da17-446
H2	5	KF639326, KF649557, KF649375, KF649377, ACEA040-14
		Cinara (*Cinara*) *piceae*
H1	1	Da17-715
H2	4	06-10, Da17-227, Da17-347, Da17-353, Da17-445
H3	2	KP869234, KP869253
H4	1	Da16-358
H5	2	JQ916814, JQ916815
H6	2	KF649413, KF649414
H7	1	KF649445
		Eulachnus brevipilosus
H1	1	KP637118
H2	5	KP637108-KP637111, KP637117
H3	1	KP637116
H4	1	KP637115
H5	1	KP637114
H6	2	KP637106, KP637113
H7	1	KP637112
H8	1	KP637107
H9	3	Da17-524, Da17-528A, Da17-710
		Eulachnus nigricola
H1	2	KP637144, KP637143
H2	1	Da17-324

Table 2. Average and range of pairwise within-species sample divergences (*p*-distances) of partial COI sequences (652 bp) for seven species of the tribe Eulachnini

Species	Number of samples	Average; range of divergence, %	Number of haplotypes
Cinara (*Cinara*) *brauni*	n=6	0.14; 0.00 – 0.31	3
Cinara (*Cinara*) *kochiana*	n=4	1.94; 0.00 – 3.83	3
Cinara (*Cinara*) *laricis*	n=10	0.47; 0.00 – 1.23	5
Cinara (*Cinara*) *neubergi*	n=5	0.18; 0.00 – 0.46	2
Cinara (*Cinara*) *pectinatae*	n=6	0.05; 0.00 – 0.15	2
Cinara (*Cinara*) *piceae*	n=14	2.36; 0.00 – 5.37	7
Eulachnus brevipilosus	n=16	0.29; 0.00 – 0.67	9
Eulachnus nigricola	n=3	0.22; 0.00 – 0.33	2

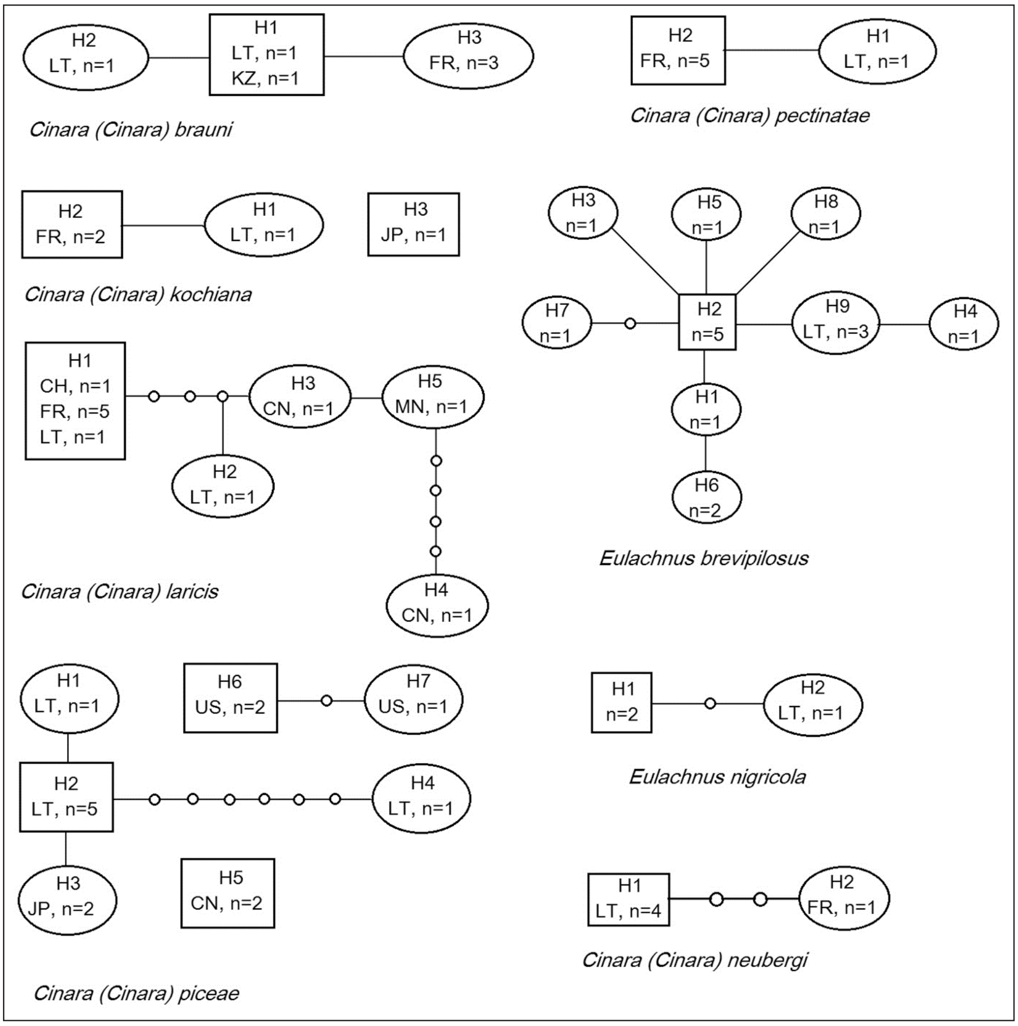

Figure 1. Haplotype networks for partial COI sequences (652 bp, 95% connection limit, gaps treated as missing data) of new to Lithuania species of Eulachnini. The haplotype with the highest outgroup probability is displayed as a square, while others are displayed as ovals. For sample information, see Tables 1 and 3. CH – Switzerland, CN – China, FR – France, JP – Japan, KZ – Kazakhstan, LT – Lithuania, MN – Mongolia, US – United States of America

COI haplotype was detected in all samples of *E. brevipilosus* from Lithuania (H9) and it differed from the most abundant haplotype (H2) by single nucleotide.

Discussion and Conclusions

Current research added eight species of Eulachnini to the earlier reported 19 species in the faunal list of Lithuania. With 27 species on the list, Lithuanian Eulachnini appear to be rather well studied when compared with adjacent countries – 19 from Latvia, 21 from Belarus (Nieto Nafria et al. 2013). Thirty-four species have been recorded from Poland, due to the presence of mountainous regions in Southern Poland, and establishment of alien species *Cinara curvipes* (Patch 1912, Osiadacz and Hałaj 2009, Hałaj and Osiadacz 2015).

For the present, economical value of Eulachnini species in Lithuania mostly concern their honeydew production and 13 species out of 27 registered are known as important producers of honeydew (Binazzi and Scheurer 2009). Out of them, *C. (C.) piceae, C. (C.) nuda*, *C. (C.) piceicola*, *C. (C.) pinea*, and *C. (C.) pini* appear most promising because their host plants, *Picea abies* and *Pinus sylvestris*, are the most common conifers in Lithuania, as have already been noted by the Lithuanian beekeepers (Straigis and Amšiejus 2002). To increase the effective management of honeydew resource, a proper knowledge of the local specificity of honeydew producing conifer aphid species is inevitable including the precise identification of aphid species.

The application of molecular taxonomy tools can be important both for aphid species life stages association (Zhang et al. 2008) and the detection of cryptic species (Rakauskas et al. 2011). DNA fragments can also be used for tracing invasion history (Lozier et al. 2009). In case of new to Lithuania Eulachnini aphids, revealing the area of their origin is almost impossible for now, because of the scarcity of available sequences for comparative analyses (see Table 1 for details). Species of the genus *Cinara* belonging to the "*pinea* group" (for details see Blackman and Eastop 2018), are an example when molecular taxonomy tools are inevitable.

Eulachnini species are not included in the list of important forest pests in Lithuania for now (Valstybinė Miškų Tarnyba 2019). Out of the recently reported species, only *C. piceae* appeared to be rather common, already registered in the coastal (Klaipėda), central (Kaunas), northern (Panevėžys) and eastern (Vilnius) regions of Lithuania. The species has been reported as a pest of firs in nurseries of the Czech Republic and Northern Caucasus (Stary 1976). Situation in Lithuania requires constant monitoring because alien species of Eulachnini may establish novel insect-plant interactions as was documented for *C. curvipes* in Poland (Hałaj and Osiadacz 2015).

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References

Albrecht, A. Ch. 2017. Illustrated identification guide to the Nordic aphids feeding on conifers (Pinophyta) (Insecta, Hemiptera, Sternorhyncha, Aphidomorpha). *European Journal of Taxonomy* 338: 1–160.

Binazzi, A. and Scheurer, S. 2009. Atlas of the honeydew producing conifer aphids of Europe. ARACNE, Roma, 127 pp.

Blackman, R. L. and Eastop, V. F. 2000. Aphids on the World's Crops. An Identification and Information Guide. John Wiley and Sons Ltd., Chichester, 466 pp.

Blackman, R. L. and Eastop, V. F. 2018. Aphids on the World's Plants: An Identification and Information Guide. Available online at: http://www.aphidsonworldsplants.info/ [accessed February 2019]

Chen, R., Jiang, L., Chen, J. and Qiao, G. 2016. DNA barcoding reveals a mysterious high species diversity of conifer-feeding aphids in the mountains of southwest China. *Scientific Reports* 6: 20123.

Chen, R., Favret, C., Jiang, L., Wang, Z. and Qiao, G. 2016 a. An aphid lineage maintains a bark-feeding niche while switching to and diversifying on conifers. *Cladistics* 32: 555-572. DOI: 10.1111/cla.12141

Clement, M., Posada, D. and Crandall, K. A. 2000. TCS: a computer program to estimate gene genealogies. *Molecular Ecology* 9: 1657-1659.

Durak, R. 2014. Life cycle, seasonal and interannual polymorphism in a monoecious aphid *Cinara mordvilkoi* (Hemiptera: Aphidoidea: Lachnidae). *European Journal of Entomology* 111(3): 357–362.

Durak, R., Węgrzyn, E. and Leniowski, K. 2016. Do all aphids benefit from climate warming? An effect of temperature increase on a native species of temperate climatic zone *Cinara juniperi*. *Ethology Ecology and Evolution* 28(2): 188-201.

Folmer, O., Black, M., Hoeh, W., Lutz, R.and Vrijehoek R. 1994. DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3: 294-299.

Hałaj, R. and Osiadacz, B. 2015. On foreign land: the conquest of Europe by *Cinara curvipes* (Patch, 1912). *Deutsche Entomologische Zeitschrift* 62(2): 261-265.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium* 41: 95-98.

Heie, O. E. 1995. The Aphidoidea of Fennoscandia and Denmark VI. Aphidinae. Part 3 of Macrosiphini and Lachnidae. *Fauna Entomologica Scandinavica* 31: 222 pp.

Hopmans, P. and Elms, S. R. 2013. Impact of defoliation by *Essigella californica* on the growth of mature *Pinus radiata* and response to N, P and S fertilizer. *Forest Ecology and Management* 289: 190–200.

Jousselin, E., Cruaud, A., Genson, G., Chevenet, F. and Foottit, R. G. 2013. Is ecological speciation a major trend in aphids? Insights from a molecular phylogeny of the conifer-feeding genus Cinara. *Frontiers in Zoology* 10: 56.

Kanturski, M., Kajtoch, L. and Wieczorek, K. 2017. European species of the aphid genus *Eulachnus* Del Guercio, 1909 (Hemiptera: Aphididae: Lachninae): revision and molecular phylogeny. *Zootaxa* 4356: 1 – 81.

Kumar, S., Stecher, G., and Tamura, K. 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* 33:1870–1874.

Lozier, J. D., Roderick, G. K. and Mills, N. J. 2009. Tracing the invasion history of mealy plum aphid, *Hyalopterus pruni* (Hemiptera: Aphididae), in North America: a population genetics approach. *Biological Invasions* 11 (2): 299–314.

Meseguer, A., Manzano-Marin, A., Coeur d'acier, A., Clamens, A.-L., Godefroid, M. and Jousselin, E. 2017. *Buchnera* has changed flatmate but the repeated replacement of co-obligate symbionts is not associated with the ecological expansions of their aphid hosts. *Molecular Ecology* 26: 2363–2378.

Murphy, S. T. and Völkl, W. 1996. Population dynamics and foraging behaviour of *Diaeretus leucopterus* (Hymenoptera: Braconidae), and its potential for the biological control of pine damaging *Eulachnus* spp. (Homoptera: Aphididae). *Bulletin of Entomological Research* 86(04): 397 – 405.

Nieto Nafría, J. M., Andreev, A. V, Binazzi, A., Mier Durante M. P., Pérez Hidalgo, N., Rakauskas, R. and

Stekolshchikov, A.V. 2013. Fauna Europaea: Aphidoidea. Fauna Europaea, version 2018. Available online at: https://fauna-eu.org. Last accessed in: February 2019.

Osiadacz, B. and Halaj, R. 2009. The Aphids (Hemiptera: Sternorrhyncha: Aphidinea) of Poland. A Distributional Checklist. *Polish Entomological Monographs* 6: 1-96.

Osiadacz, B. and Halaj, R. 2010. Systematic review of aphids (Hemiptera: Sternorrhyncha: Aphidomorpha) of Poland with host plant index. *Silesian Natural History Monographs* I: 1-191.

Penteado, S.R.C., Trentini, R.F., Iede, E.T. and Filho, W.R. 2000. Pulgao do *Pinus*: nova praga lorestal. *Serie Tecnica Instituto de Pesquisas e Estudos Florestais* 13: 97–102.

Rakauskas, R., Rupais, A. and Juronis, V. 1992. The check-list of Lithuanian Aphidodea. *New and rare for Lithuania insect species. Records and Descriptions of 1992*: 83–100.

Rakauskas, R., Havelka, J. and Bašilova, J. 2008. Contribution to the knowledge of the aphid (Hemiptera, Sternorrhyncha: Phylloxeroidea, Aphidoidea) fauna of the Curonian Spit, Lithuania. *Acta Zoologica Lituanica* 18(2): 90-107.

Rakauskas, R., Turčinavičienė, J. and Bašilova, J. 2011. How many species are there in the subgenus *Bursaphis* (Hemiptera: Sternorrhyncha: Aphididae)? COI evidence. *European Journal of Entomology* 108 (3): 469–479.

Seco Fernandez, M. V., and Mier Durante, M. P. 1992. Presencia en Espańa del pulgón verde de los pinos americanos: *Essigella* (Hom., Aphididae: Cinarinae). *Boletín De La Asociación Española De Entomología* 16: 255–256. (in Spanish, with English abstract)

Stary, P. 1976. *Cinara piceae* (Panz.) (Hom. Lachnidae), as pest of young European fir trees (*Abies alba* Mill.), and its natural enemy complex in Czechoslovakia. *Studia Entomologica Forestalia* II (10): 171-180.

Straigis, J., and Amšiejus, A. 2002. Lipčiaus tiekėjai ir skruzdėlės [Honeydew providers and ants]. LŽUŪ, Kaunas, 27 pp. (*in Lithuanian*).

Valstybinė Miškų Tarnyba, 2019. Vabzdžiai miškų kenkėjai. [Insect Forest Pests]. Available online at: http://www.amvmt.lt/index.php/vabzdziai-misku-kenkejai (*in Lithuanian*)

Valstybinių miškų urėdija, 2019. Available online at: http://www.gmu.lt/forest_resources/ (*in Lithuanian*)

Villesen, P. 2007. FaBox: an online toolbox for fasta sequences. *Molecular Ecology Resources* 7 (6): 965–968.

Watson G.W., Voegtlin, D.J., Murphy, S.T. and Foottit, R.G. 1999. Biogeography of the *Cinara cupressi* complex (Hemiptera: Aphididae) on Cupressaceae, with description of a pest species introduced into Africa. *Bulletin of Entomological Research* 89: 271–283.

Wojciechowski, W., Depa, L., Kanturski, M., Wegierek, P. and Wieczorek, K. 2015. An annotated checklist of the Aphids (Hemiptera: Aphidomorpha) of Poland. *Polish Journal of Entomology* 84: 383–420.

Zhang, H.-C., Zhang, D. and Qiao, G.-X. 2008. Association of aphid life stages using DNA sequences: A case study of tribe Eriosomatini (Hemiptera: Aphididae: Pemphiginae). *Insect Science* 15(6): 545-551.