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Description of the oviparae and males of *Cinara* (*Cinara*) *piniphila* (Aphididae Lachninae)

Jurij DANILOV, Jekaterina HAVELKA, Rimantas RAKAUSKAS
Institute of Biosciences, Vilnius University, Lithuania

Abstract

Ovipara and winged male of *Cinara* (*Cinara*) *piniphila* (Ratzeburg) are described for the first time based on material from Lithuania. Sampling data and morphometric data are given for every morph together with information on partial sequences of mitochondrial COI. Phenological and bio-ecological data of monitored *C.* (*C.*) *piniphila* colonies are reported and discussed.

Key words: sexual morphs, life cycle, phenology, morphometric data.

Introduction

Cinara (*Cinara*) *piniphila* (Ratzeburg) (Aphididae Lachninae) was described as *Aphis piniphila* based on the relative lengths of antennae and rostrum in apterous and alate viviparous females collected on pine in Prussia (Ratzeburg, 1844). Later on, more detailed descriptions of these morphs appeared available, together with identification keys and information on its host specificity and distribution (Pašek, 1954; Heinze, 1962; Pintera, 1966; Szelegiewicz, 1977; Heie, 1995; Binazzi, 1990; Osiadacz and Halaj, 2009; Mamontova, 2012; Albrecht, 2017). Nowadays, *C.* (*C.*) *piniphila* is reported as a common species inhabiting *Pinus sylvestris* L. throughout Europe, also available in Turkey and China (Holman, 2009; Blackman and Eastop, 2019). Taxonomically, it is close to the *Cinara* (*Cinara*) *pinea* species group [*C.* (*C.*) *pinea* (Mordvilko), *C.* (*C.*) *pilosa* (Zetterstedt), *C.* (*C.*) *neubergi* (Arnhart)]. Ecologically, it shares shoots and young twigs of *P. sylvestris* with *C. pinea* and *Cinara* (*C.*) *hyperophila* (Koch), forming mutual colonies with those species in some cases (Szelegiewicz, 1962; 1968; Albrecht, 2017; Blackman and Eastop, 2019). Life cycle is supposed to be holocyclic, monoecious on *Pinus* (Albrecht, 2017), but it was not supported by the empiric evidence yet, sexual morphs were unknown (Blackman and Eastop, 2019).

The present paper is based on the recent observations of *C. piniphila* colonies in Lithuania that provided empirical data concerning life cycle and enabled description of earlier unknown sexual morphs (oviparae and winged males) of this aphid species.

Materials and methods

Observations of *C.* (*C.*) *piniphila* colonies inhabiting *P. sylvestris* were performed at two research sites in Lithuania (Figure 1A). Alksnynė (Neringa district, Curonian spit, 55°38'20"N 21°07'04"E) is on sandy coastal dunes, covered by naturally growing *P. sylvestris* (2-4 meters high) stand (Figure 1B). Puvočiai (Varėna district, Southeastern plain, 54°07'06"N 24°18'20"E) is on continental sandy dunes in the fluvioglacial plain covered by *P. sylvestris* multi-age stand. *C.* (*C.*) *piniphila* were recorded there on young pine plants (2 meters high) only. After having detected one male and two oviparous females of *C.* (*C.*) *piniphila* in Alksnynė on October 15 in 2017, *C.* (*C.*) *piniphila* colonies were visited in Alksnynė from 25 May till the 6 October in 2018 every 2-3 weeks. Puvočiai served as additional research site. In addition, samples of apterous viviparous females from Nida (Curonian spit) and Platakiai (Samogitian Upland) were also included. For sample information, see table 1. Microscope slides in Canada balsam were prepared according to Blackman and Eastop (2000). The material was explored using microscopes NICON ECLIPSE E200 and NICON SMZ800. Software INFINITY ANALYSE release 6.1 was used for measurements and taking pictures of microscope slides. The material is deposited at the Department of Zoology, Institute of Biosciences of the Vilnius University, Lithuania. For morphology-based identification, keys of Heie (1995) also Blackman and Eastop (2019) were used. In addition, partial sequences of mitochondrial COI (660 bp) of the same material were used to confirm morphology-based identification of aphids. DNA samples analysed in this paper together with previously published data used for comparison are given in table 2. Methods of DNA isolation, amplification, sequencing and data analysis were the same as described by Danilov *et al*. (2019).

Results

Oviparous female of *C. (C.) piniphila* (figures 2-3, table 3)

M a t e r i a l

34 oviparae, *P. sylvestris*, Alksnynė on the Curonian spit (55°38'N 21°7'E), Baltic coast of Lithuania, 15.X.2017, 2 - 24.IX.2018, leg. J. Danilov (slides Da17-700, Da18-424/433/436 - 441); 8 oviparae, *P. sylvestris*, Puvočiai in Varėna district (54°6'N 24°18'E), South Eastern plain of Lithuania, 27.IX.2018, leg. J. Danilov (slides Da18-453/454). Morphotypus: specimen in slide Da 18-437/ovip/1.

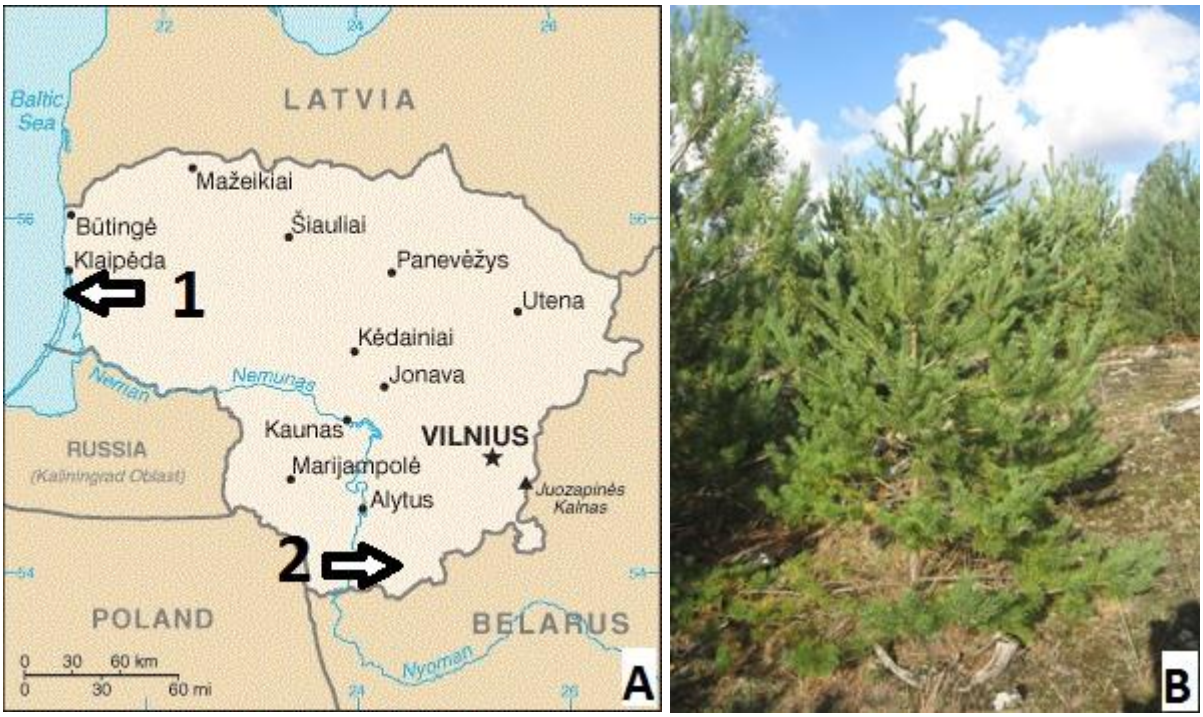

Figure 1. Research sites in Lithuania **(A)** showing *P. sylvestris* stand **(B)** on the first site (Alksnynė, 55°38'20"N 21°07'04"E).

Table 1. Aphid material from Lithuania used in this study. AVF - apterous viviparous females, ♀ - oviparous females, ♂ - males (winged).

Location	Host plant	Date	Sample No	Morphs
Alksnynė, Curonian Spit	*Pinus sylvestris*	27.VI.2017	Da17-361	AVF
		15.X.2017	Da17-698	♀, ♂
		15.X.2017	Da17-700	
		25.V.2018	Da18-112	AVF
		9.VII.2018	Da18-325	
		11.VIII.2018	Da18-414	AVF, ♂
			Da18-415	AVF, ♀
		2.IX.2018	Da18-423	AVF, ♂
			Da18-424	AVF, ♀, ♂
			Da18-427	AVF, ♂
		14.IX.2018	Da18-433	♀, ♂
		24.IX.2018	Da18-436	AVF, ♀, ♂
			Da18-437	♀, ♂
			Da18-438	
			Da18-439	
			Da18-440	
			Da18-441	
Puvočiai, Southeastern Plain		22.VI.2017	Da17-307	AVF
		8.VI.2017	Da17-193	
		27.IX.2018	Da18-453	♀, ♂
			Da18-454	
Nida, Curonian Spit	*Pinus mugo*	28.VI.2017	Da17-376	AVF
Platakiai, Samogitian Upland	*Pinus sylvestris*	27.V.2018	Da18-134	

Table 2. Partial COI sequences (660 bp) of *C.* (*C.*) *piniphila* used in this study.

GenBank Accession No	Sample information	COI haplotype code
Lithuania		
MK829821	Puvočiai, 22.VI.2017, *Pinus sylvestris*, Da17-307	Hap4
MK829822	Alksnynė, 27.VI.2017, *Pinus sylvestris*, Da17-361	Hap1
MK829823	Nida, 28.VI.2017, *Pinus mugo*, Da17-376	Hap1
MK829820	Alksnynė, 15.X.2017, *Pinus sylvestris*, Da17-698	Hap1
MK829824	Alksnynė, 24.IX.2018, *Pinus sylvestris*, Da18-436	Hap1
MK829825	Platakiai, 27.V.2018, *Pinus sylvestris*, Da18-134	Hap1
China (Chen *et al.*, 2012)		
JX034937	Inner Mongolia, 1.VIII.2004, *Pinus sylvestris*, 15766	Hap3
JX034939	Inner Mongolia, 13.VIII.2004, *Pinus sylvestris*, 15921	Hap2

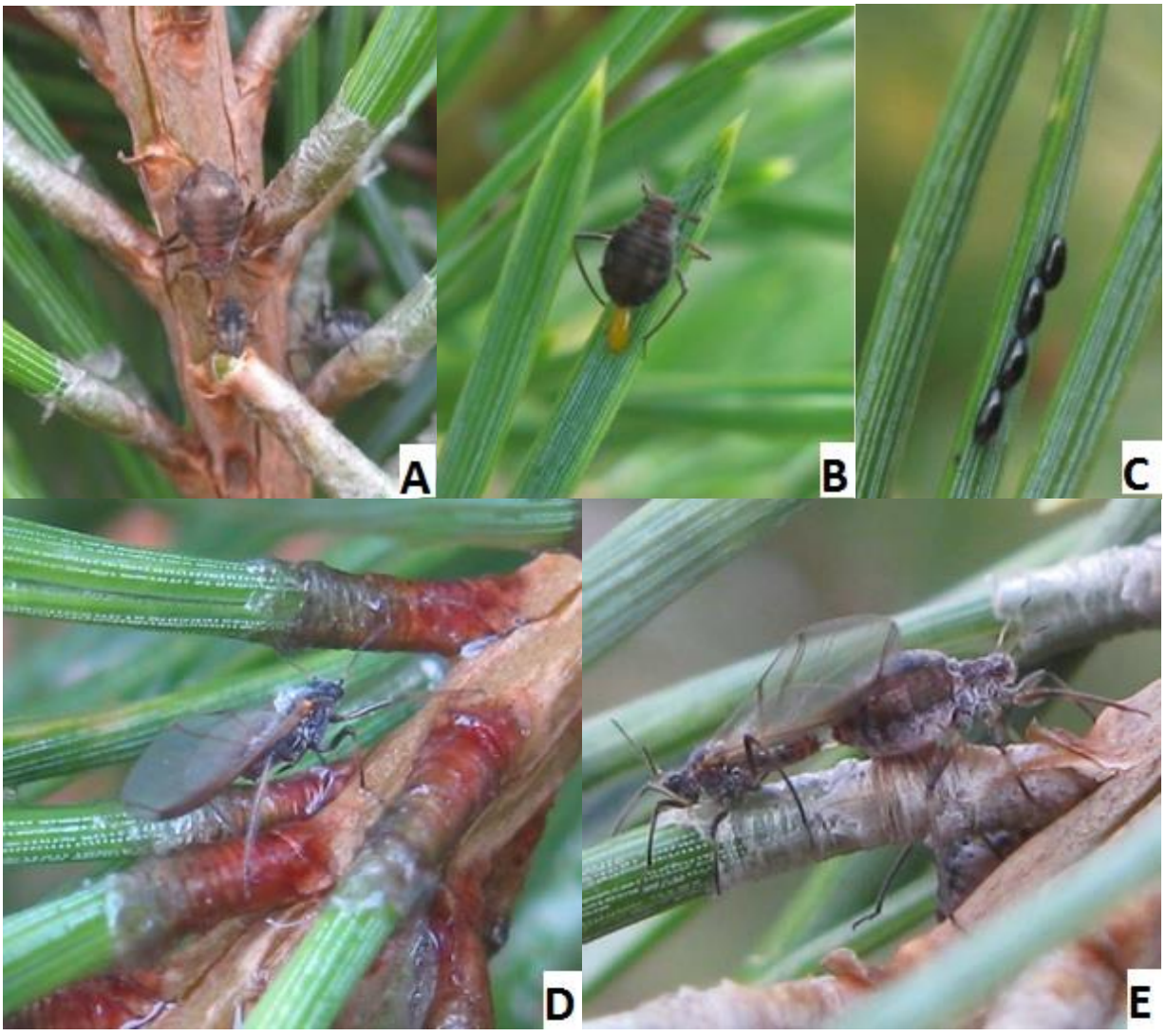

Figure 2. *C.* (*C.*) *piniphila* on *P. sylvestris* (Alksnynė, 55°38'20"N 21°07'04"E): apterous viviparous female **(A)**, ovipara with fresh egg **(B)**, mature eggs on needles **(C)**, winged male **(D)**, mating pair **(E)**.

D e s c r i p t i o n

Elongated oval, slightly egg-shaped, body length 2.82-3.74 mm. Lifetime body colour brown with darker markings, without any conspicuous wax powdering. Siphunculi dark. Legs dark, proximal parts (up to 50%) yellowish. Tarsi dark. Cleared specimen with brown head, light brown thorax, translucent abdomen with small (0.02-0.06 mm) setae bearing scleroites on tergites I-VII. Siphunculi and siphuncular sclerites dark. Cauda and genital plate dark brown. Siphuncular cones blackish, with evident apical flange. Tergite VIII with a well sclerified solid bar interrupted in the middle.

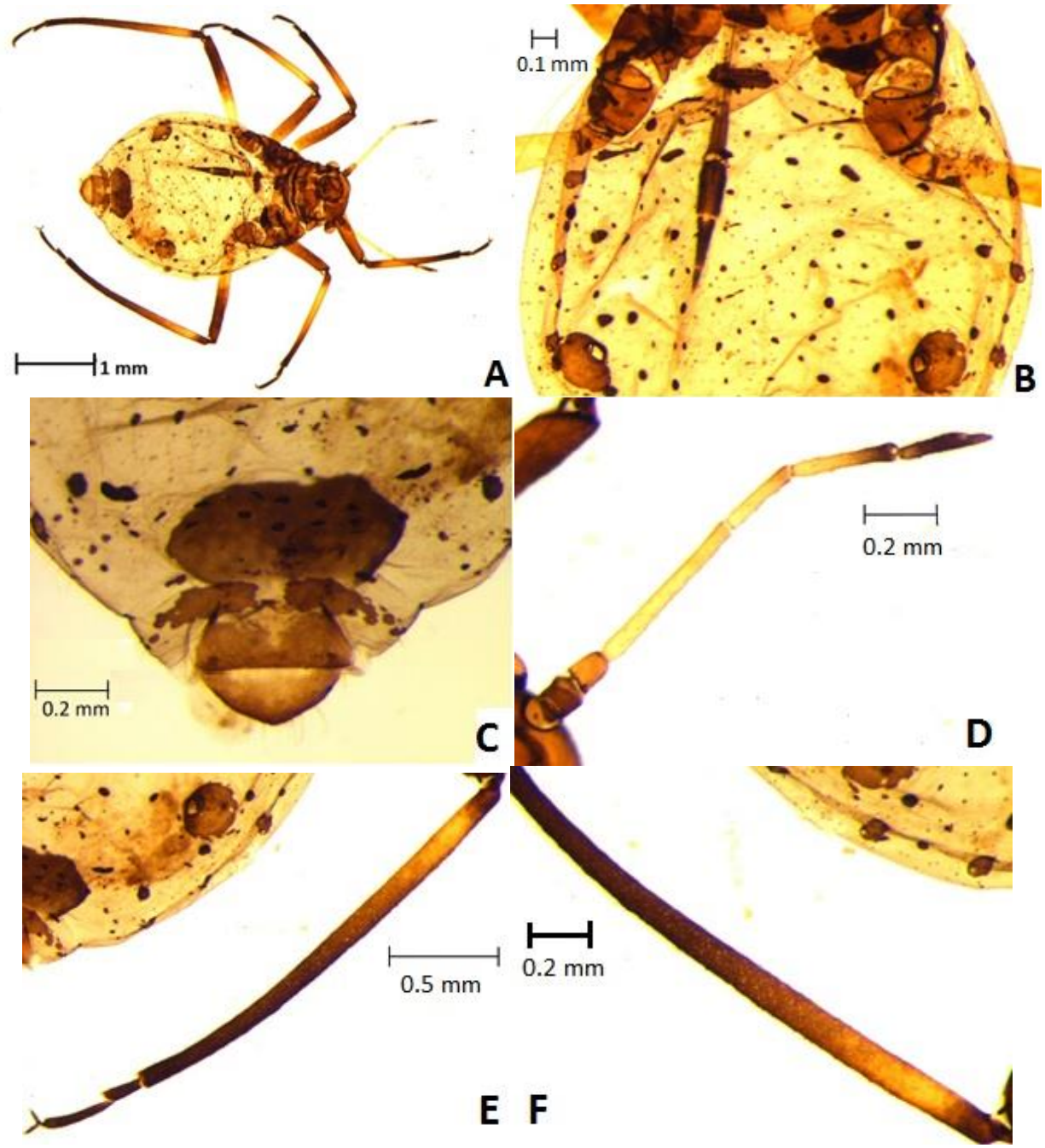

Figure 3. *C.* (*C.*) *piniphila* - ovipara morphotype (*P. sylvestris*, Alksnynė, 55°38'20"N 21°07'04"E): general view **(A)**, abdominal tergites I-V **(B)**, distal tergites **(C)**, antenna **(D)**, hind tibia and tarsus **(E)**, hind tibia **(F)**.

Antennal segment I, distal part of antennal segment V and most of the antennal segment VI brown. Antennal segment II brownish. The remaining parts of antennal segments white. Femora rather uniformly brown, except the very basal parts of first and second femora and up to 25% of proximal parts of hind femora whitish. First and second tibiae whitish with the distal parts (up to 30 %) and tarsi dark. Hind tibiae somewhat swollen, bearing plenty scent plaques, those being more numerous at the proximal half. Apical rostral segments uniformly dark. Antennal segments lack any secondary rhinaria, except that sometimes one is available on segment V. Mesosternal tubercle absent. Processus terminalis of VI antennal segment with 4 subapical setae. Setae on antennal segment III subequal to basal width of respective ant. segment. For more morphometric data see table 3.

Winged male of *C (C.) piniphila* (figures 2 and 4, table 3)

M a t e r i a l

27 males, *P. sylvestris*, Alksnynė on the Curonian spit, Baltic coast of Lithuania, 15.X.2017, 11.VII - 24.IX.2018, leg. J. Danilov (slides Da17-700, Da18-414/423/424/427/433/436 - 441); 3 males, *P. sylvestris*, Puvočiai in Varėna district, South Eastern plain of Lithuania, 27.IX.2018, leg. J. Danilov (slides Da18-453/454). Morphotypus: specimen in slide Da18-437/al♂/1.

Table 3. Morphometric data, [min-max (mean ± SD), lengths in mm] of the respective amphigonic morphs of *C.* (*C.*) *piniphila*.

	Ovipara	Winged male
N of specimens	34	27
Lengths of:		
body	2.82-3.74 (3.28 ± 0.26)	2.26-2.93 (2.58 ± 0.17)
hind tibia	1.73-2.43 (2.13 ± 0.14)	1.55-2.10 (1.81 ± 0.12)
antennal segment III	0.44-0.57 (0.50 ± 0.03)	0.42-0.57 (0.50 ± 0.04)
antennal segment IV	0.15-0.25 (0.21 ± 0.02)	0.20-0.27 (0.22 ± 0.02)
antennal segment V	0.24-0.33 (0.29 ± 0.02)	0.27-0.33 (0.29 ± 0.02)
antennal segment VI b	0.15-0.20 (0.18 ± 0.01)	0.15-0.19 (0.17 ± 0.01)
antennal segment VI p.t.	0.05-0.08 (0.06 ± 0.01)	0.05-0.08 (0.06 ± 0.01)
rostrum segment IV (RIV)	0.20-0.25 (0.23 ± 0.01)	0.17-0.21 (0.19 ± 0.01)
rostrum segment V	0.11-0.14 (0.12 ± 0.01)	0.09-0.11 (0.10 ± 0.01)
2nd segment of hind tarsus (HTII)	0.31-0.37 (0.34 ± 0.02)	0.26-0.32 (0.29 ± 0.01)
Numbers of secondary rhinaria on:		
antennal segment III	0	36-55 (47.26 ± 4.77)
antennal segment IV	0	11-21 (15.41 ± 3.18)
antennal segment V	0-1 (0.88 ± 0.03)	7-14 (10.00 ± 1.76)
Length of the first segment of hind tarsus (HTI):		
basal	0.04-0.06 (0.05 ± 0.00)	0.03-0.05 (0.04 ± 0.00)
dorsal	0.11-0.15 (0.13 ± 0.01)	0.08-0.12 (0.10 ± 0.01)
ventral	0.18-0.22 (0.20 ± 0.01)	0.14-0.18 (0.16 ± 0.01)
distal	0.06-0.09 (0.08 ± 0.01)	0.05-0.07 (0.06 ± 0.01)
Lengths of the longest setae on:		
abdomen tergite V (LHATV)	0.03-0.05 (0.04 ± 0.01)	0.05-0.11 (0.08 ± 0.02)
abdomen tergite VII	0.03-0.07 (0.04 ± 0.01)	0.06-0.11 (0.08 ± 0.01)
antennal segment III (LHASIII)	0.03-0.06 (0.04 ± 0.01)	0.06-0.08 (0.07 ± 0.01)
hind tibia dorsal side	0.04-0.07 (0.06 ± 0.01)	0.06-0.16 (0.13 ± 0.02)
hind tibia ventral side	0.06-0.08 (0.07 ± 0.01)	0.08-0.13 (0.10 ± 0.01)
Numbers of:		
setae on antennal segment II	9-15 (11.06 ± 1.57)	9-15 (12.04 ± 1.84)
setae on antennal segment VI b.p.	5-10 (7.65 ± 1.19)	6-10 (8.04 ± 1.23)
setae on antennal segment VIII	13-22 (17.26 ± 2.12)	13-19 (15.19 ± 1.54)
accessory setae rostral segment IV	5-7 (6.15 ± 0.60)	6-8 (7.07 ± 0.94)
Maximum diameters of:		
setae producing sclerite on abdomen tergite V (HSATV)	0.02-0.06 (0.04 ± 0.01)	0.02-0.06 (0.03 ± 0.01)
siphuncular cones	0.23-0.44 (0.31 ± 0.05)	0.21-0.33 (0.26 ± 0.03)
siphuncular pores	0.08-0.10 (0.09 ± 0.00)	0.05-0.07 (0.06 ± 0.01)
scleroites on abdomen tergites II-IV	0.08-0.22 (0.12 ± 0.03)	0.04-0.08 (0.06 ± 0.01)
base of antennal segment III (BANTIII)	0.04-0.06 (0.05 ± 0.00)	0.04-0.06 (0.05 ± 0.00)
Ratios:		
HT I (ventral side)/HT II	0.53-0.64 (0.58 ± 0.02)	0.51-0.59 (0.54 ± 0.02)
R IV/HT I	0.99-1.38 (1.14 ± 0.07)	1.05-1.29 (1.18 ± 0.06)
R IV/HT II	0.58-0.78 (0.66 ± 0.04)	0.57-0.70 (0.64 ± 0.04)
LHATV/HSATV	0.52-3.07 (1.11 ± 0.56)	1.33-4.14 (2.45 ± 0.64)
LHASIII/BANTIII	0.62-1.17 (0.80 ± 0.12)	1.14-1.69 (1.45 ± 0.13)

D e s c r i p t i o n

Body length 2.26-2.93 mm. Lifetime body with blackish head and pterothorax and light bronzy abdomen. Siphuncular cones blackish, small to medium sized, with evident apical flange. Legs dark, proximal parts (up to 50%) yellowish. Tarsi dark. Cleared specimen with brown head, light brown pterothorax, translucent abdomen with small (0.02-0.06 mm) setae bearing scleroites on tergites I-VII. Siphunculi and siphuncular sclerites dark. Cauda and genital plate dark brown. Tergite VIII with a well sclerified solid bar interrupted in the middle. Antennal segments I, II-VI and distal half of antennal segment III brown. Proximal half of antennal segment III white. Femora rather uniformly brown, except that the very basal parts of first and second femora and up to 25% of proximal parts of hind femora whitish. Front and median tibiae whitish with the very proximal and distal ends and tarsi dark. Hind tibiae dark with the light areas closer to proximal end, which is dark. Apical rostral segments uniformly dark. Antennal segments III, IV, and V bearing secondary rhinaria. Processus terminalis of VI antennal segment with 4 subapical setae. Setae on antennal segment III up to 1.6x basal width of respective antennal segment. For more morphometric data see table 3.

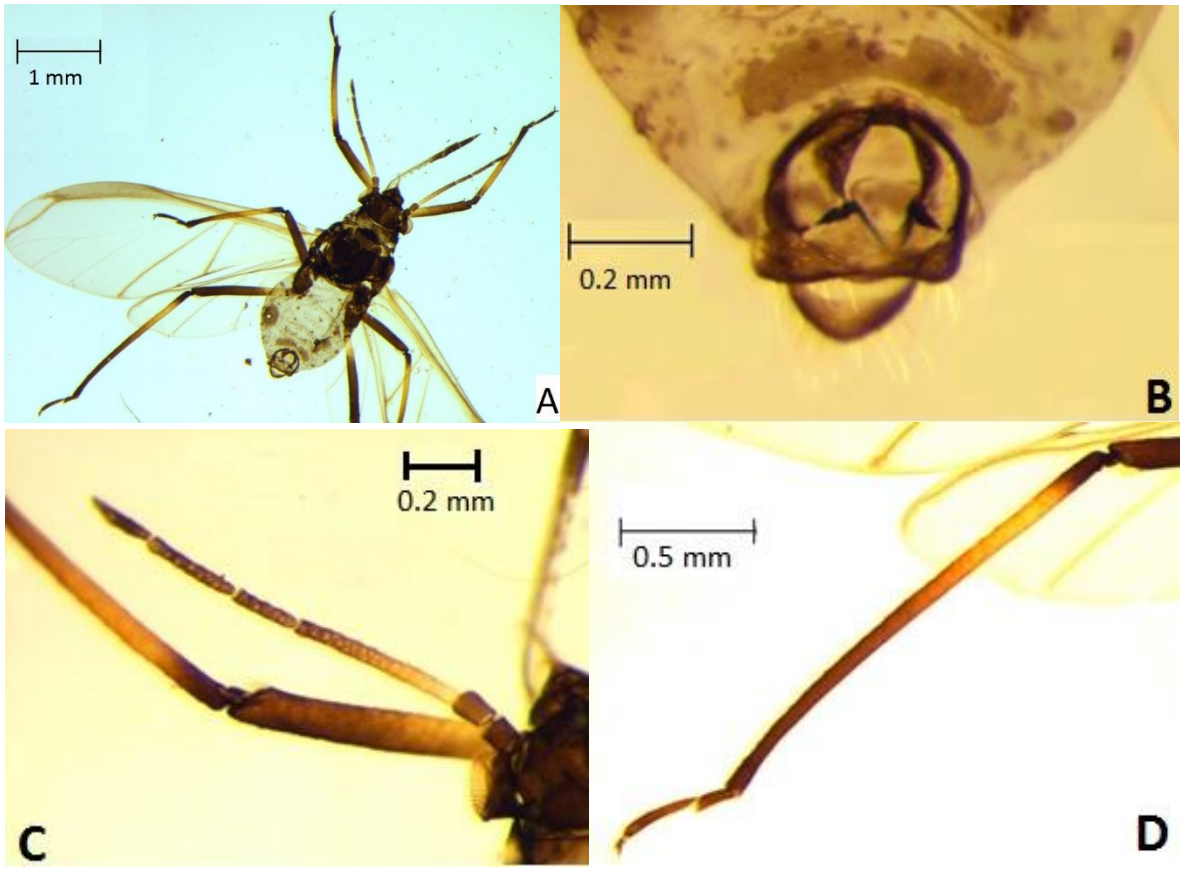

Figure 4. *C.* (*C.*) *piniphila* - winged male morphotype (*P. sylvestris*, Alksnynė, 55°38'20"N 21°07'04"E): general view **(A)**, distal tergites **(B)**, antenna **(C)**, hind tibia and tarsus **(D)**.

Discussion and conclusions

In this study, six partial COI sequences of *C.* (*C.*) *piniphila* from West Palaearctic were analysed together with previously published two sequences from the East Palaearctic (table 2). All sequences were collapsed into four COI haplotypes. Two of them represented samples from Lithuania, other two - samples from China (table 2). In haplotype network (figure 5) samples from China were closer to each other and differed by one nucleotide substitution. Similarly, samples from Lithuania were closer to each other and differed by three nucleotide substitutions. The difference between samples from Eastern and Western Palaearctic were from 3 to 5 nucleotides. Sequence data were especially helpful when justifying that collected amphigonic morphs are conspecific with other samples of *C.* (*C.*) *piniphila*. Aphids both from Lithuania (this study) and China (Chen *et al.*, 2012) were identified by the morphological characters of viviparous females already approved in morphology-based identification keys. Sequences from Lithuania represent the most numerous haplotype H1, which consisted of DNA samples extracted both from viviparous (collected from *P. sylvestris* and *Pinus mugo* Turra) and amphigonic morphs (tables 1-2). The finding of amphigonic morphs of *C.* (*C.*) *piniphila* confirms earlier presuppositions (Albrecht, 2017; Blackman and Eastop, 2019) on the holocycle of this aphid species.

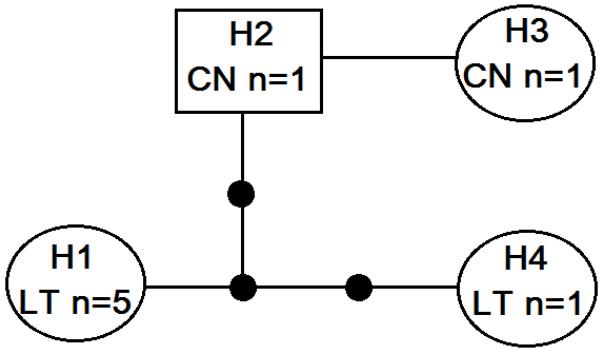

Figure 5. Network showing relationships between COI fragment haplotypes (660 bp) for *C.* (*C.*) *piniphila* samples. Black circles represent hypothetical haplotypes that separate observed haplotypes. The haplotype with the highest outgroup probability is displayed as a rectangle. CN - China, LT - Lithuania.

Our data support earlier reports (Szelegiewicz, 1968; Blackman and Eastop, 2019; Albrecht, 2017) on the close microhabitat similarity of *C.* (*C.*) *piniphila*, *C.* (*C.*) *pinea* and *C.* (*C.*) *hyperophila* on the same host plant: we found mutual colonies of these three species on shoots and young twigs of *P. sylvestris*. In addition, information on the life cycle peculiarities of *C.* (*C.*) *pini-*

phila in the northern part of the of the Central European floristic province appear available. Namely, emergence of wingless oviparous females and winged males in the research areas of Lithuania coincided, starting from the mid-August. Shoots of the principal aphid host, *P. sylvestris* become entirely wooden by that time. Amphigonic morphs dominated against parthenogenetic ones from the mid-September, when the oldest needles of their host plant had turned yellow. Oviparous females were available till the October 6, whilst last males - September 27. The first eggs (both yellow and shining black, deposited along the needles), were noted on the September 14. Fresh (yellow) eggs were available till the September 24. We have failed to find sexual morphs on *P. mugo*.

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Authors' addresses: Rimantas RAKAUSKAS (corresponding author, e-mail: rimantas.rakauskas@gf.vu.lt), Jurij DANILOV, Jekaterina HAVELKA, Institute of Biosciences, Vilnius University, Saulėtekio al. 7, LT-10257 Vilnius, Lithuania.

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