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New records of invasive aphids (Hemiptera: Aphididae)  
on garlic mustard in the USA

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# New records of invasive aphids (Hemiptera: Aphididae) on garlic mustard in the USA

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**Abstract.** *Lipaphis alliariae* Müller (Hemiptera: Aphididae) was identified from specimens collected in Lake County, Ohio, on the invasive garlic mustard, *Alliaria petiolata* (Bieb.) Cavara & Grande (Brassicaceae). The identification was performed on apterous viviparae using morphological and molecular data. Body color and shape of the cauda discriminated *L. alliariae* and *L. pseudobrassicae* Davis. Measurements of morphological characters of both species were similar, but they have distinct characters distinguishing them from *L. erysimi* Kalténbach. Neighbor-joining analysis of cytochrome oxidase 1 (Cox1) barcoding indicated a close relationship of the aphids that feed on Brassicaceae, and the range of pair-wise distances for Cox1 barcoding of these species was 0.3–0.9%.

**Key words.** Aphid, *Lipaphis alliariae*, sequences, garlic mustard, *Alliaria petiolata*.

**ZooBank registration.** urn:lsid:zoobank.org:pub:2A838BC1-6652-4042-B326-8351FBB6C329

## Introduction

Garlic mustard, *Alliaria petiolata* (Bieb.) Cavara & Grande (Brassicaceae) is a highly invasive weed species in the United States. It was brought from Europe in the 1800s for herbal uses and erosion control (Munger 2001; USDA 2022). Records of aphids that feed on garlic mustard include *Aphis gossypii* Glover, *Brevicoryne brassicae* Linnaeus, *Lipaphis alliariae* Müller, *L. erysimi* Kalténbach, *Myzus ascalonicus* Doncaster, *M. ornatus* Laing, *M. persicae* (Sulzer), and *Rhopalosiphoninus latysiphon* Davidson (Blackman and Eastop 2022). Out of this list *L. alliariae* and *L. erysimi* were not included in the list of aphid species adventive to North America north of Mexico (Footitt et al. 2006; Skvarla et al. 2017). Our article presents new records of aphids on garlic mustard in the USA.

## Materials and Methods

Species identification of slide-mounted material was completed by using published morphological keys (Blackman and Eastop 2022; InfluentialPoints 2022). Photographs of the mounted specimens were taken using a Leica DM 2000 digital camera and SPOT Software 4.6 (Diagnostic Instruments, Inc., Michigan, USA). The latter was used to take all measurements in millimeters. Archival slides were deposited in the Illinois Natural History Survey Insect Collection Museum. Aphids collected on *A. petiolata* ( $n = 4$ ) identified as *L. alliariae* and aphids

collected on *Brassica oleracea* ( $n = 2$ ) and two individuals identified as *L. pseudobrassicae* Davis caught in suction traps located in Crookston and Lamberton, Minnesota on 16 July and 2 July 2021, respectively, were sequenced following a previously described DNA barcoding method (Coeur D'Acier et al. 2014). *Myzus persicae* and *R. padi* species were used as outgroup to root the neighbor-joining tree. Raw sequence data provided by Keck Center (University of Illinois at Urbana-Champaign) were assembled using Sequencher 4.7 (Gene Codes Corporation, Ann Arbor, MI). These sequences were blasted by using Nucleotide Blast: Basic Local Alignment Search Tool. A total of eight sequences for DNA barcoding was retrieved from GenBank: *L. erysimi* (KJ814966.1, KJ814967.1), *L. pseudobrassicae* (EU701713.1, MH183023.1, KR036803.1), *M. persicae* (KM115493.1 and JX844381.1) and *Rhopalosiphum padi* Linnaeus (KC286717.1). Pair-wise distances for DNA barcoding sequences were calculated using the Kimura 2-parameter distance model (Kimura 1980) in PAUP 4.0b10 (Swofford 2001). PAUP was also used to generate a neighbor-joining tree to graphically represent the distance between sequences.

## Results

**Morphological data.** There were two aphid species, *M. persicae* and *L. alliariae*, identified on *A. petiolata* collected in Lake Co., Ohio. The one species collected on *B. oleracea* was identified as *L. pseudobrassicae*. *Myzus persicae* is morphologically very distinct. To verify the identity of *L. alliariae* and *L. pseudobrassicae*, additional morphological and molecular work was completed. The measurements of antenna (III, base and processus terminalis), siphunculi, and cauda of *L. alliariae* overlapped with measurements from *L. pseudobrassicae* (Table 1). The mean ratio for both species of combined length of antennal segment III and processus terminalis to the length of siphunculi was 2.5–2.9 (Table 1), and the mean ratio of the length of processus terminalis to the siphunculi was 1.19–1.29. These are key characters to distinguish *L. alliariae* and *L. pseudobrassicae* from *L. erysimi*. The elongate triangular/tongue shape of the cauda (Fig. 1) and dark green body color (Fig. 3) of *L. alliariae* are distinct morphological characters compared to the spoony shape (Fig. 2) and yellowish green body color (Fig. 4) of *L. pseudobrassicae*. In addition, eggs of *L. alliariae* were found on dead garlic mustard leaves on 4 December 2021 (Fig. 5).

**Specimens examined.** Apterous viviparae of *L. alliariae*, USA; Ohio; Lake County; Holden Forests & Gardens; on *A. petiolata*, 41.6299° N × 81.306° W; 19.vii.2021, R. Troutman. (INHS Insect Collection 1011143–1011156). Apterous viviparae of *Myzus persicae*, USA; Ohio; Lake County; Holden Forests & Gardens; on *A. petiolata*, 41.6299° N × 81.306° W; 9.viii.2021, R. Troutman (INHS Insect Collection 1011171–1011172; GenBank accessions OM964623–OM964624). Apterous viviparae of *L. alliariae*, USA; Ohio; Lake County; Holden Forests & Gardens; on *A. petiolata*, 41.6299° N × 81.306° W; 19.viii.2021, R. Troutman (INHS Insect Collection 1011133–1011142; GenBank accessions OM964625–OM964626). Apterous viviparae of *L. pseudobrassicae*, USA; Illinois; Champaign County; Idea Garden; on *Brassica oleracea*, 40.0962° N × 88.2183° W; 8.viii.2021, D. Lagos-Kutz. (INHS Insect Collection 1011157–1011170; GenBank accession numbers OM964627–OM964628). Alate vivipara of *L. pseudobrassicae*, USA; Minnesota; Redwood County; suction trap; 44.2397° N × 95.3153° W; 2.vii.2021,

**Table 1.** Measurements of morphological characters of *Lipaphis alliariae* and *L. pseudobrassicae*. Length of antennal segment III (ANT III), length of the base of the last antennal segment (ANT B), length of the processus terminalis (ANT PT), length of siphunculi (SIPH), length of cauda (CAU) and corresponding ratios. All measurements are in millimeters. For all measurements and counts the range is given and the mean is in parentheses.

Aphids	ANT III	ANT B	ANT PT	SIPH	CAU	ANT PT/SIPH	ANT PT/ANT B	ANT III/SIPH	SIPH/CAU	ANT III+ANT PT/SIPH
<i>L. alliariae</i> ( $n = 16$ )	0.20–0.36 (0.27)	0.09–0.11 (0.10)	0.21–0.27 (0.24)	0.17–0.23 (0.20)	0.12–0.16 (0.14)	1–1.4 (1.19)	2.1–2.6 (2.34)	1.1–1.8 (1.31)	1.2–1.7 (1.42)	2.2–2.8 (2.5)
<i>L. pseudo-brassicae</i> ( $n = 23$ )	0.21–0.35 (0.27)	0.08–0.12 (0.10)	0.17–0.29 (0.23)	0.14–0.23 (0.18)	0.11–0.15 (0.12)	1.1–1.5 (1.29)	1.9–2.7 (2.27)	1.2–1.7 (1.53)	1.3–1.8 (1.41)	2.4–3.4 (2.9)



**Figures 1–2.** Body of apterous vivipara with distinctly different cauda pointed out. Figures 1 and 2 were magnified to 76.4 $\times$ . Scale bar = 1 mm. 1) *Lipaphis alliariae*. 2) *Lipaphis pseudobrassicae*. Photograph: D. Lagos-Kutz.



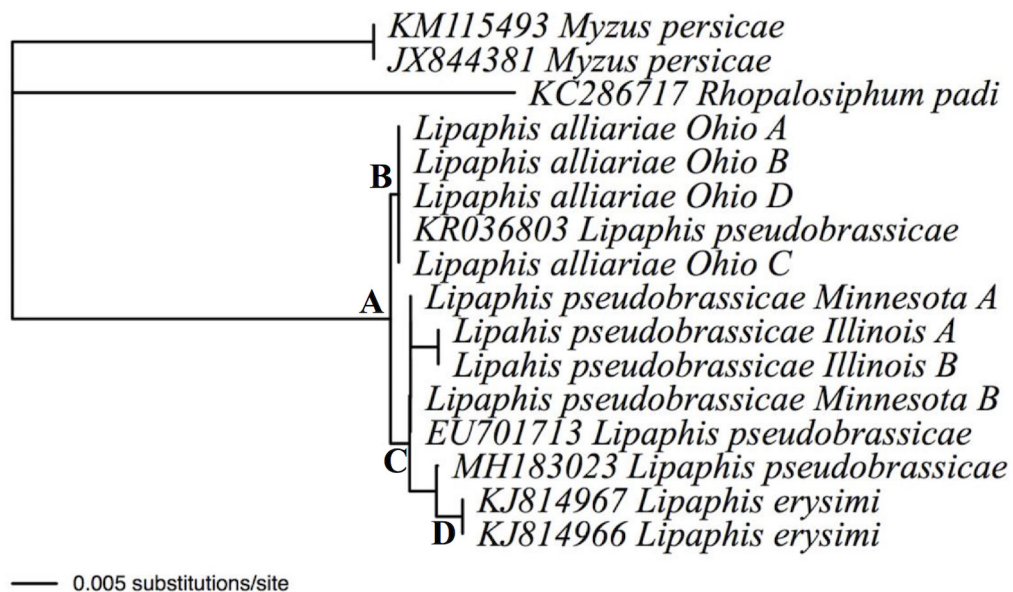
**Figures 3–5.** Colonies of *Lipaphis* species on Brassicaceae. 3) Apterous vivipara of *L. alliariae* on garlic mustard, *Alliaria petiolata* (Bieb.) Cavara & Grande. 4) Apterous vivipara of *L. pseudobrassicae* on kale, *Brassica oleracea* L. 5) Black shiny egg of *L. alliariae* on *A. petiolata*. Photographs 3 and 4 were taken by Doris Lagos-Kutz, and 5 was taken by Rebecah Troutman.

B. Potter (INHS Insect Collection 1011174; GenBank accession number OM964629). Alate vivipara of *L. pseudobrassicae*, USA; Minnesota; Polk County; suction trap; 47.7978° N  $\times$  96.6209° W; 16.vii.2021, I. MacRae (INHS Insect Collection 1011173; GenBank accession number OM964630).

**Molecular data.** The pair-wise distances between *M. persicae* and *Lipaphis* species ranged from 8.6% to 9.8%. The pair-wise distance between *L. alliariae* and *L. erysimi* was 0.9, and between *L. alliariae* and *L. pseudobrassicae* ranged from 0.3% to 0.6% when estimated using DNA barcoding sequences (623 bp) (Table 2). Graphical representation of the Kimura 2-parameter distances (Fig. 6) for all taxa shows a cluster (A) with most of the species

**Table 2.** Ranges of interspecific pair-wise distances (%) for DNA barcoding of *Lipaphis alliariae*, *L. erysimi*, and *L. pseudobrassicae*. Calculated using the Kimura 2-parameter model.

	<i>L. alliariae</i>		<i>L. erysimi</i>		<i>L. pseudobrassicae</i>		
	Ohio	KJ814966.1	KJ814967.1	EU701713.1	Illinois	Minnesota	MH183023.1
<i>L. alliariae</i> Ohio A–D (n = 4)	0						
<i>L. erysimi</i> KJ814966.1 (n = 1)	0.9	0					
<i>L. erysimi</i> KJ814967.1 (n = 1)	0.9	0	0				
<i>L. pseudobrassicae</i> EU701713.1 (n = 1)	0.3	0.6	0.6	0			
<i>L. pseudobrassicae</i> Illinois A–B (n = 2)	0.6	0.9	0.9	0.3	0		
<i>L. pseudobrassicae</i> Minnesota (n = 2)	0.3	0.6	0.6	0	0.3	0	
<i>L. pseudobrassicae</i> MH183023.1 (n = 1)	0.6	0.3	0.3	0.3	0.6	0.3	0
<i>L. pseudobrassicae</i> KR036803.1 (n = 1)	0	0.9	0.9	0.3	0.6	0.3	0.6

**NJ tree****Figure 6.** Neighbor-joining tree (K2P) distances using DNA barcoding sequences to compare species of aphids in the genus *Lipaphis*.

that feed on Brassicaceae. In Figure 6, *L. alliariae* form a distinct cluster (B) compared to the other European species.

## Discussion

*Lipaphis alliariae* is morphologically like *L. erysimi* and *L. pseudobrassicae*. Also, these three species have low sequence divergence (less than 1%) based on mitochondrial DNA barcoding (Table 2). Although not helpful for identification when sequences of *L. alliariae* were blasted by using GenBank, the caudal shape of *L. alliariae* and *L. pseudobrassicae* (triangular/tongue and spoony respectively) and body color of live specimens (dark green and yellowish green respectively) help distinguish the two species. Also, for both species the mean ratio (2.5–2.9) of the combined length of antennal segment III and processus terminalis to the siphunculi, and mean ratio of the length of processus terminalis to the siphunculi (1.19–1.29) confirm that these two species are distinct from *L. erysimi*, with ratios lower than 2.4 and 0.8–1.15 respectively (InfluentialPoints 2022). Further studies need to be done to learn more about the distribution and seasonal phenology of *L. alliariae* in the USA, as well as to evaluate if this aphid species has an impact on the invasive garlic mustard plant with potential as a management tool or biocontrol in the future.

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