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Identification of a new species of *Aphis* (Hemiptera: Aphididae) based on distinct morphology rather than DNA barcoding

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Abstract. *Aphis elena* Lagos-Kutz and Voegtlin, sp. nov. (Hemiptera: Aphididae), is described from specimens collected in Illinois, USA, on the North American native plant, *Pycnanthemum virginianum* (L.) T. Dur. & B.D. Jacks. ex B.L. Rob. & Fernald (Family: Lamiaceae). Both apterous and alate viviparae are described and illustrated, and a dichotomous key is provided to apterous and alate viviparae of the six species of *Aphis* that have been recorded on plants in the family Lamiaceae in North America. Neighbor-joining analysis of cytochrome oxidase 1 (*Cox1*) indicated a close relationship of the new species with *Aphis monardae* Oestlund, which also feeds on a member of Lamiaceae. The range of pair-wise distances for DNA barcoding of these species is 0.17–0.33%. The newly described *Aphis elena* is morphologically more similar to *Aphis gossypii* Glover than it is to *Aphis monardae*.

Key Words. Aphid**,** *Aphis elena,* genes, sequences, host plant, *Pycnanthemum virginianum*

Introduction

The family Lamiaceae has a cosmopolitan distribution, but species of the genus *Pycnanthemum,* commonly known as mountain-mint, are all native to North America. Most are very strongly scented and pungent, and are used in cooking and in making herbal tea (Harley et al. 2004; USDA-NRCS 2015). Many species of *Aphis* feed on the members of this plant family (Stroyan 1984; Blackman and Eastop 2006). In North America, five *Aphis* species are known to feed on mints: the native species *A. neomonardae* Rojanavongse and Robinson and *A. monardae* Oestlund; and the exotic species *A. fabae* Scopoli, *A. gossypii* Glover, and *A. nasturtii* Kaltenbach (Blackman and Eastop 2006; Lagos-Kutz et al. 2014). Molecular approaches have been used to resolve the taxonomic status of other *Aphis* species (Carletto et al. 2009; Komazaki et al. 2010; Favret and Miller 2011; Lagos et al. 2012; Coeur d'acier et al. 2014; Massimino Cocuzza and Cavalieri 2014; Lagos-Kutz et al. 2016). However, in some species of *Aphis*, DNA barcoding and even longer pieces of mitochondrial DNA are not sufficient for species delimitation and other factors such as host association and morphology need to be considered (Wang et al. 2011; Lagos-Kutz et al. 2014; Lee et al. 2014; Lagos-Kutz et al. 2016). In this study, we present a previously undescribed species of *Aphis* recently collected on *Pycnanthemum virginianum* (L.) T. Dur. & B.D. Jacks. ex B.L. Rob. & Fernald at Middlefork Savanna County Forest Preserve (Lake County, Illinois). *Aphis monardae* and *A. nasturtii* also have been found on different host plants in the same location.

Materials and Methods

Species identification of slide-mounted material was done by the first author using published keys (Oestlund 1887; Gillette 1927; Hottes and Frison 1931; Palmer 1952; Cook 1984; Voegtlin et al. 2004; Blackman and Eastop 2006) and authoritatively identified specimens in the insect collections of the Illinois Natural History Survey (INHS) and the University of Minnesota (UMSP). 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 for this study.

A total of 63 sequences for mitochondrial cytochrome oxidase 1 (*Cox1*) were retrieved from GenBank. *Aphis elena* (labeled as *Aphis* sp. 1 in Lagos-Kutz et al. (2014) KC897105, KC897106 - KC897107); *Aphis fabae* (JQ860273); *Aphis gossypii* (GU591547, KC897114, KC897118); *A. monardae* (KC897146, KC897149, KC897152, KC897155); *A. nasturtii* (KC897162), *A. oestlundi* Gillette (JQ860259), *Rhopalosiphum maidis* (Fitch) (JQ860263), and *Aphis* species that feed on plants of the family Lamiaceae published in Massimino Cocuzza and Cavalieri (2014).

Pair-wise distances for *Cox1* sequences were calculated using the Kimura 2-Parameter distance model (Kimura 1980) in PAUP 4.0b10 (Swofford 2001). PAUP was also used to generate Neighbor-joining tree to graphically represent the distance between sequences.

Results

Aphis elena Lagos-Kutz and Voegtlin sp. nov.

Diagnosis. Apterous viviparae similar to *A. gossypii*. Distinguished by the ratio of the length of siphunculus to the length of the cauda (SIPH/CA), 1–1.6 for *A. elena* and 1.3–2.5 for *A. gossypii*. Alate viviparae with few secondary sensoria in straight line restricted to antennal segments III (5–6) and IV (1–2). Distance from the base of antennal segment III to the first secondary sensorium (DBIII) 0.07–0.09. Forewing with median vein once-forked. Dark hind coxa. Cauda paler than siphunculus, with 6–7 setae. Ratio SIPH/CA=1–1.3. Marginal sclerites dusky.

Description. Apterous viviparae (n=22) (Table 1 and Figures 1–8). Color in life (Figure 18): Head, thorax and abdomen dark green, covered with white wax. Color of cleared specimens: Head: dark. Six antennal segments, rarely five (not included in morphometry): first, second, fifth and base of last antennal segment dusky; the remainder pale or slightly dusky. Ultimate rostral segment (URS) reaches the hind coxae. Thorax: Coxae dusky. Trochanters paler than coxae. Femora slightly dusky on distal half, basal tips pale. Tibiae pale, darkening near distal tip. Tarsi dusky. Abdomen: Cauda dusky and slightly spoon-shaped with inward curled setae. Siphunculi dark, imbricated with flange. Pre and post-siphuncular sclerite absent. Marginal sclerites pale. Marginal tubercles on abdominal II, III, and IV absent. Dorsum of abdomen without sclerites, cuticle with reticulation. Sub-genital plate dusky and complete.

Alate viviparae (n=6) (Table 1 and Figures 9–17). Color of cleared specimens: Head: Head and thorax dark. First and second antennal segments darker than other segments. Secondary sensoria restricted to antennal segments III and IV, arranged in a single row. URS reaches hind coxae, with 2 accessory setae. Thorax: Fore and middle femora dusky throughout. Hind femora dark except on pale base. Coxae dark. Trochanters paler than coxae. Tibiae pale, darkening near distal tip. Tarsi dusky or dark. Forewing with median vein once-forked. Abdomen: Cauda dusky, slightly spoon-shaped. Siphunculi dark, imbricated with flange. Marginal sclerites dusky. Pre-siphuncular sclerite absent. Post-siphuncular sclerite dusky. Marginal tubercles on abdominal II, III, and IV absent. Dorsum of abdomen with small transverse sclerites on VI, and VII and large transverse sclerite on VIII. Subgenital plate dusky and complete.

Type material. HOLOTYPE. Apterous vivipara. USA; Illinois; Lake County; Middlefork Savanna County Forest Preserve; on *Pycnanthemum virginianum* (L.) T. Dur. & B.D. Jacks. ex B.L. Rob. & Fernald 42.2620° N x 87.8962° W; 20.vi.2010; D. Lagos-Kutz. (INHS Insect Collection 511,252). Paratypes: 4 alate viviparae, 25 apterous viviparae, 511,243-510,259, Middlefork Savanna County Forest Preserve, 42.2620° N x 87.8962° W, Lake County, IL, 20.vi. 2010, on *Pycnanthemum virginianum,* D. Lagos-Kutz; 1 apterous vivipara, 511,363, Middlefork Savanna County Forest Preserve, 42.2620° N x 87.8962° W, Lake County, IL, 28.vi.2008, on *Pycnanthemum virginianum,* D. Voegtlin.

Biology. Two collections of alate and apterous viviparae females of *A. elena* were found in the summer (late June) of 2008 and 2010 on *Pycnanthemum virginianum* in Middlefork Savanna Forest Preserve, Lake Forest, Lake County, Illinois. The sexual morph was not found. It is likely that because the host plant is perennial, this aphid overwinters on the same host plant. Further observations need to be done to learn more about the biology of *A. elena.*

Etymology. This species is named after the first author's daughter's middle name, Katherine Elena.

Dichotomous keys to apterous and alate viviparous females of the ***Aphis*** that feed on Lamiaceae in the North American Midwest. The dichotomous key presented below is based on specimens from collections made in the Midwest (may not be reliable in other geographic regions), and molecular data for specimens from these collections support our morphologically based identifications. Morphological data for these species is shown in Lagos et al. (2014), Lagos-Kutz et al. (2014), and Blackman and Eastop (2006). For some comparative morphometric data of European specimens of *A. fabae, A. gossypii* and *A. nasturtii* see Stroyan (1984), Heie (1986), Brown (1989) and García Prieto et al. (2005).

Key to apterous viviparae

1. URS with 4–7 accessory setae ..... ***A. neomonardae*** **Rojanavongse and Robinson**
— URS IV+V with 2 accessory setae **2**

2(1). Setae on antennal segment III longer than 0.020 mm ..... ***A. fabae*** **Scopoli**
— Setae on antennal segment III shorter than 0.020 mm **3**

3(2). Marginal tubercles on abdominal tergites II–V present ..... ***A. nasturtii*** **Kaltenbach**
— Marginal tubercles on abdominal tergites II–V absent **4**

4(3). Cauda pale, siphunculi distally dusky ..... ***A. gossypii*** **Glover** (in part)
— Cauda dusky or dark, siphunculi dark all throughout **5**

5(4). Siphunculi dark all throughout, cauda constricted **6**
— Siphunculi dusky or lighter at the base, cauda not constricted **7**

6(5). Cauda slightly constricted, with 5–9 setae. SIPH/CA 1–1.6. On *Pycnanthemum virginianum* (Figures 1–8) ..... ***A. elena*** **sp. nov.**
— Cauda distinctly constricted, with 5–7 setae. SIPH/CA 1.3–2.5. Polyphagous ..... ***A. gossypii*** **Glover** (in part)

7(5). Siphunculi lighter at the base, dusky distally. Cauda with 6–9 setae. Ratio of processus terminalis to base of antennal segment VI (PT/B) 1.7–2.9, SIPH/CA 1.3–1.7. On *Monarda* spp. ..... ***A. monardae*** **Oestlund**
— Siphunculi dusky or dark all throughout. Cauda with 4–7 setae. Ratio PT/B 2.6–4.1, SIPH/CA 1.3–2.5. Polyphagous ..... ***A. gossypii*** **Glover** (in part)

Key to alate viviparae

1. Forewing with median vein once-forked ..... ***A. elena* sp. nov.**
— Forewing with median vein two forks **2**

2(1). URS with 4–7 accessory setae ..... ***A. neomonardae* Rojanavongse and Robinson**
— URS with 2 accessory setae **3**

3(2). Setae on antennal segment III longer than 0.020 mm ..... ***A. fabae* Scopoli**
— Setae on antennal segment III shorter than 0.020 mm **4**

4(3). Marginal tubercles besides abdominal tergite 1 and 7 present ..... ***A. nasturtii* Kaltenbach**
— Marginal tubercles besides abdominal tergite 1 and 7 absent **5**

5(4). Cauda constricted with 4–6 setae, sclerites sometimes present on dorsum of abdominal segments I, II, and III. Ratio SIPH/CA 1.1–2.3. Secondary sensoria on antennal segment III (4–10), none on IV. DBIII 0.04–0.07. Ratio PT/B 2.1–3.6. Polyphagous ..... ***A. gossypii* Glover**
— Cauda tongue-shaped with 3–9 setae; sclerites never present on dorsum of abdominal segments I, II, and III. Ratio SIPH/CA 1.1–1.8. Secondary sensoria on antennal segment III (4–9), and sometimes on IV (0–3) DBIII 0.07–0.12. Ratio PT/B 1.9–3. On *Monarda* spp. ..... ***A. monardae* Oestlund**

Molecular data. The alignment of sequences within the 1290 bp amplified by Lagos-Kutz et al. (2014) show that the sequences amplified by Massimino Cucuzza and Cavalieri (2014) match in position 596 to 1204. Therefore, only 609 bp of *Cox1* were used to estimate the pair-wise distances between *A. elena* and the other *Aphis* species that feed on Lamiaceae. Interspecific pair-wise distances ranged from 2.52 to 4.80%, except between *A. monarda*e and *A. elena*, where the range of pair-wise distance was 0.17–0.33% (Table 2). The pair-wise distance between the two species is greater when estimated using 1290 bp. Graphical representation of the Kimura 2-parameter distances (Fig. 20) of *Cox1* for all taxa shows a cluster (A) with most of the species that feed on Lamiaceae except *Aphis oestlundi* Gillette. This aphid feeds on *Oenethera* (Onagraceae) and is a North American native along with *A. elena* and *A. monardae* (both feed on Lamiaceae). In Figure 20, the last three species form a distinct cluster labeled as B compared to the other mostly European species.

Discussion

Aphis elena was included in Lagos-Kutz et al. (2014) as *Aphis* sp.1. The phylogenetic analysis of its sequences of *Cox1* and nuclear elongation factor 1-α (EF1-α) placed it close to *A. monardae*. They both feed on plants (*Monarda* and *Pycnanthemum*) of the endemic family of North America, Lamiaceae (USDA-NRCS 2015); hosts on which *A. gossypii* has also been recorded (Blackman and Eastop 2006). However, diagnosis of morphological characters and body color in life are especially useful to discriminate between apterous viviparae of *A. gossypii*, *A. monardae* and *A. elena* (Fig. 18–19; also see Lagos-Kutz et al. 2014). The low sequence divergence (less than 1%) of mitochondrial DNA between *Aphis* species that are morphologically distinct is not a surprise, this has been found in multiple previous studies. For example, *A. grossulariae* Kaltenbach vs. *A. schneideri* (Börner) (Wang et al. 2011), *A. gossypii* vs. *A. sedi* Kaltenbach (Lagos-Kutz et al. 2014), and *A. asclepiadis* Fitch vs. *A. nigratibialis* Robinson in Robinson & Chen (Lagos-Kutz et al. 2016). Moreover, we used DNA barcoding to compare our data with closely-related species (Foottit et al. 2008; Coeur d'acier et al. 2014; Massimino Coccuza and Cavalieri 2014). However, the pair-wise distances estimated from DNA barcoding region are lower than those found using the 1290 bp (Lagos-Kutz et al. 2014). This indicates that there are more substitutions in the DNA barcoding region, and it may be useful to evaluate different *Cox1* regions or genes for more taxonomic and phylogenetic inferences (Kim and Lee 2008; Carletto et al. 2009; Favret and Miller 2011; Lee et al. 2014). Thus, DNA barcoding cannot be used alone for species identification, and the taxonomic

knowledge of the taxa along with other traits of the organism should be considered to prevent species misidentification (DeSalle 2006; Meier 2008).

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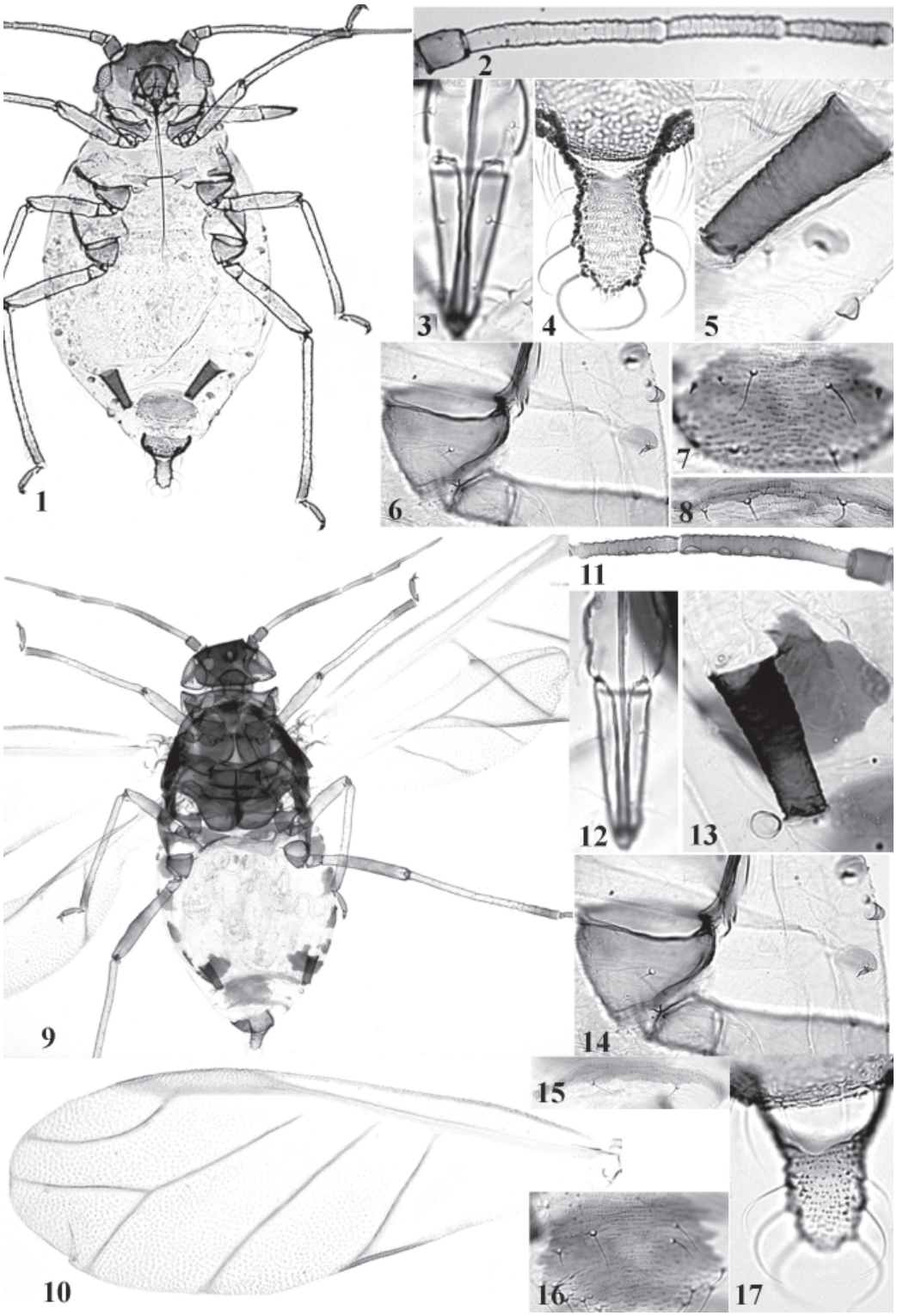
Table 1. Morphological characters of *A. elena* sp. nov. For all measurements and counts the range is given and the mean is in parenthesis. All measurements are in millimeters.

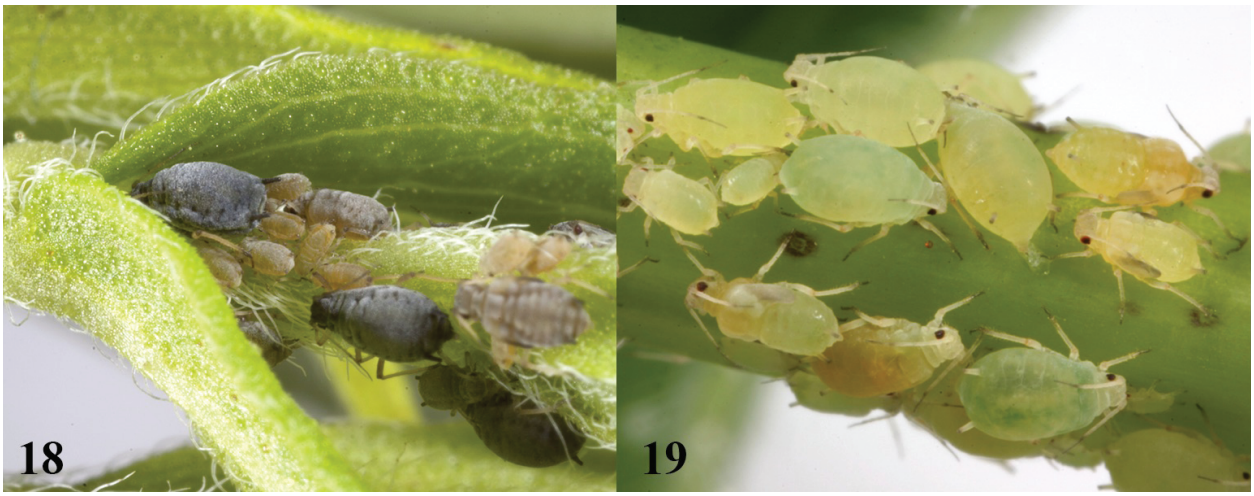
Characters/ Morphs	Apterae (n=22)	Alatae (n=6)
Length of body	1.3-1.6 (1.5)	1.3-1.6 (1.45)
Length of ultimate rostral segment(URS)	0.10-0.13 (0.11)	0.10-0.12 (0.11)
Number of antennal segments:	5-6	6
Length of antennal segment III (ASIII)	0.15-0.23 (0.20)	0.20-0.22 (0.21)
Length of antennal segment IV (ASIV)	0.07-0.15 (0.12)	0.10-0.15 (0.12)
Length of antennal segment V (ASV)	0.09-0.15 (0.11)	0.12-0.15 (0.13)
Base of antennal segment VI (B)	0.08-0.10 (0.09)	0.09-0.10 (0.10)
Processus Terminalis (PT)	0.16-0.20 (0.18)	0.2
Number of secondary sensoria on ASIII	0	5-6 (6)
Number of secondary sensoria on ASIV	0	1-2 (1)
Number of secondary sensoria on ASV	0	0
Distance from the base of ASIII to the first sensorium (DBIII)		0.07-0.09 (0.07)
Length of longest seta on ASIII	0.007-0.010 (0.008)	0.008-0.014 (0.010)
Length of siphunculus (SIPH)	0.11-0.20 (0.16)	0.10-0.15 (0.12)
Length of cauda (CA)	0.12-0.14 (.13)	0.10-0.11 (0.10)
Width of the marginal tubercle on abdominal segment I	0.015-0.030 (0.021)	0.015-0.021 (0.019)
Width of the marginal tubercle on abdominal segment VII	0.017-0.031 (0.024)	0.017-0.031 (0.025)
Length of hind tibiae	0.38-0.51 (0.45)	0.45-0.66 (0.57)
Length of hind tarsus 2 (HT2)	0.08-0.10 (0.09)	0.08-0.09 (0.08)
PT/B	1.8-2.4 (2)	2-2.1 (2.1)
URS/HT2	1.2-1.4 (1.3)	1.3-1.4 (1.3)
SIPH/CA	1-1.6 (1.2)	1-1.3 (1.1)
Number of accesory setae on URS	2	2
Number of setae on cauda	5-9 (7)	6-7 (7)
Number of setae on tergite of abdominal segment VIII	2-4 (2)	2-4 (3)
Number of setae on subgenital plate anterior part	2-6 (4)	3-6 (4)

Table 2. Ranges of interspecific pair-wise distances (%) for DNA barcoding. Calculated using the Kimura 2-Parameter.

	Aphis elena
Aphis affinis	2.69-2.86
Aphis alienus	3.03-3.20
Aphis balloticola	2.86-3.03
Aphis brunellae	3.55-3.72
Aphis clinopodii	3.22-3.39
Aphis gossypii	3.04-3.21
Aphis lamiorum	2.87-3.04
Aphis monardae	0.17-0.33
Aphis nepetae	4.63-4.80
Aphis origani	2.86-3.03
Aphis passeriniana	3.73-4.25
Aphis pulegii	2.86-3.03
Aphis serpylii	2.87-3.21
Aphis stachydis	2.69-3.22
Aphis teucrii	2.86-3.03
Aphis verticillatae	3.21-3.73



Figures 1–17. Holotype (INHS: 511,252 collection number) of *Aphis elena* **1–8)** Apterous vivipara. **1)** Body. **2)** Antennal segments: II–V. **3)** Ultimate rostral segment. **4)** Cauda. **5)** Siphunculus and marginal tubercle on abdominal segment VII. **6)** Marginal tubercle on abdominal segment I, and hind coxa. **7)** Setae on subgenital plate. **8)** Setae on abdominal tergite VIII. **9–17)** Alate vivipara. **9)** Body. **10)** Fore wing. **11)** Antennal segments: II–IV. **12)** Ultimate rostral segment. **13)** Siphunculus and marginal tubercle on abdominal segment VII. **14)** Marginal tubercle on abdominal segment I, and hind coxa. **15)** Setae on abdominal tergite VIII. **16)** Cauda. **17)** Setae on sub-genital plate.



Figures 18–19. Colonies of aphids on their respective perennial host plants. 18) *Aphis elena* sp. nov. on *Pycnanthemum virginianum* (L.) T. Dur. & B.D. Jacks. ex B.L. Rob. & Fernald. Photograph: David Voegtlin, emeritus University of Illinois at Urbana-Champaign, Illinois. 19) *Aphis monardae* Oestlund on *Monarda fistulosa* L. Photograph: David Voegtlin, emeritus INHS of University of Illinois at Urbana-Champaign, Illinois.

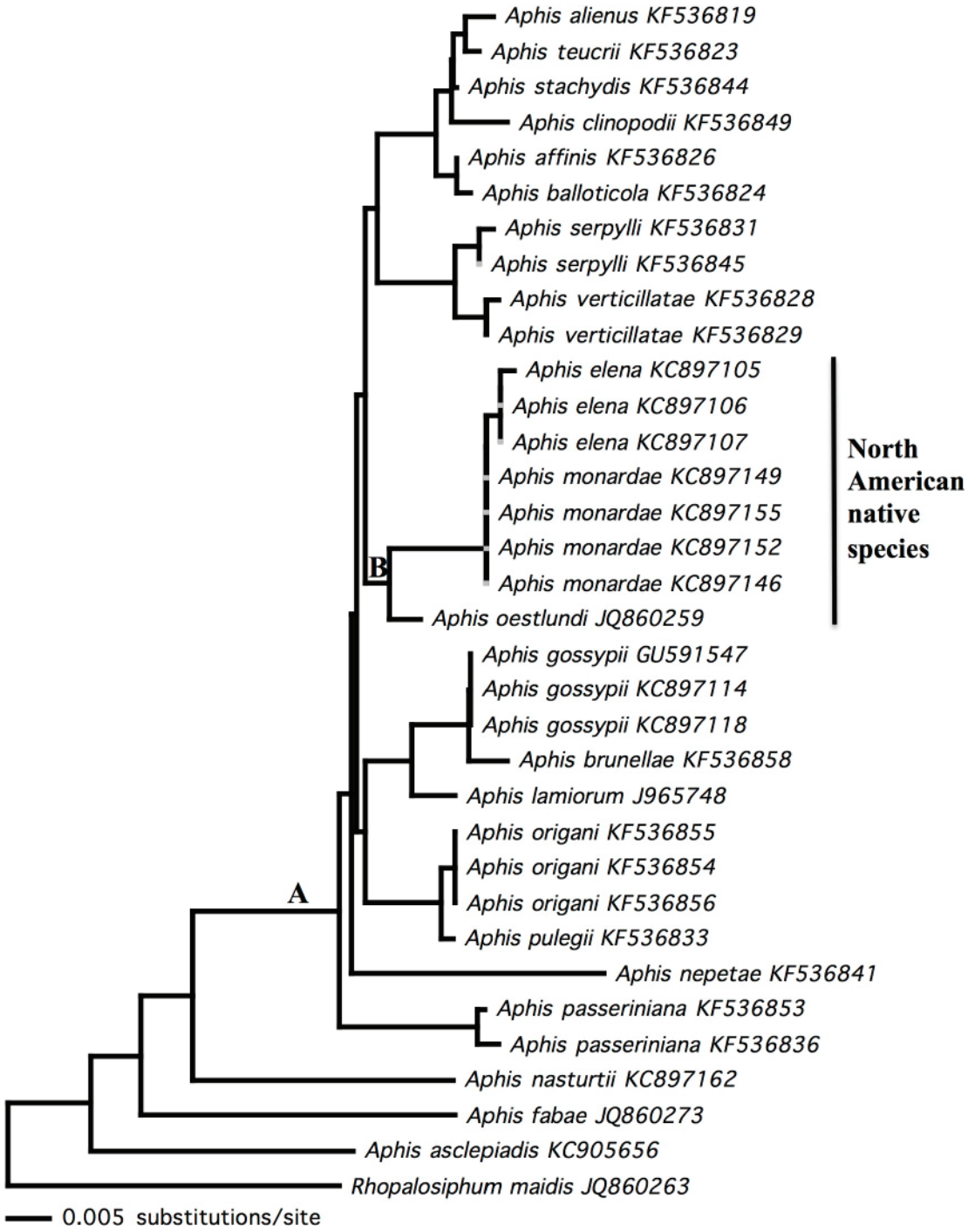


Figure 20. Neighbor-joining tree of K2P distances of DNA barcodes of targeted species. Species names are followed by the GenBank accession numbers.