Systematics

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The Status of the Members of the *Aphis asclepiadis* Species Group (Hemiptera: Aphididae) in the United States of America

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Abstract

In North America, there is a morphologically defined group of *Aphis* species that use *Cornus* spp. as primary host plants and also are associated with plants in the family Apiaceae. We refer to them collectively as the *Aphis asclepiadis* species group and attempt to elucidate the taxonomic status of its members using sequences of mitochondrial cytochrome oxidase 1 (*Cox1*) and nuclear elongation factor 1-α (*EF1α1*) genes. The Bayesian phylogenetic analyses of the combined data of these two genes strongly supported a clade composed of the *A. asclepiadis* species group. This group includes the following North American native species: *A. asclepiadis, A. carduella, A. decepta, A. impatientis, A. neogillettei, A. nigratibialis, A. saniculae, A. thaspii,* and *A. viburniphila*, and the related exotic species, *A. salicariae*. Bayesian phylogenetic and Maximum Parsimony Network nested all the collections that match the diagnostic characters described for *A. asclepiadis* and *A. carduella*, and the ones described for *A. nigratibialis*. Moreover, the range of pair-wise distances between collections of *A. asclepiadis* and *A. carduella* are 0.00–0.73 and 0.00–0.87% for *Cox1* and *EF1α1*, respectively. Therefore, we conclude that *A. asclepiadis* Fitch 1851 is a senior synonym of *A. carduella* Walsh 1863, **syn. nov.** In addition, all the sequences of species morphologically identified as *A. impatientis* matched almost 100%. Biological studies showed that *Aphis impatientis* is a heteroecious species that alternates between *Cornus* and *Impatiens*. We also found that morphological characterization of the sexual morph is useful to differentiate species that feed on *Cornus* spp. as primary host plants.

Key words: aphid, host plant, morphology, phylogenetic relationship, sequence divergence

The genus *Aphis* (Hemiptera: Aphididae) is believed to have diversified rapidly as a consequence of the rapid radiation and diversification of herbaceous angiosperms during the Cretaceous period (Heie 1996). This recent and rapid diversification has led to the evolution of numerous extant species complexes as well as to the reliance on host identity as a principal character in species diagnoses. Within these *Aphis* spp. complexes, life cycles include the alternation of seasonally induced asexual and sexual reproduction, either with both reproductive modes occurring on the same host species (monoecy) or an alternation between two botanically distant hosts (heteroecy). This host alternation and close ecological host associations (Eastop 1971, Dixon 1973) have been one of the main sources of erroneous identification of *Aphis* spp., the most diverse aphid genus by a wide margin (Favret 2015).

There are six species of *Aphis* in North America known to feed on *Cornus* spp.: the monoecious native species *Aphis caliginosa* Hottes and Frison, *A. cornifoliae* Fitch, *A. neogillettei* Palmer, and *A. nigratibialis* Robinson, the heteroecious native *A. carduella* Walsh, and the heteroecious exotic, *A. salicariae* Koch (Palmer 1938, Fitch 1851, Koch 1855, Walsh 1863, Robinson and Chan 1969, Blackman and Eastop 2006). The secondary hosts of the heteroecious species are plants in the families Apiaceae and Asteraceae. In addition to the monoecious and heteroecious *Cornus*-feeding *Aphis* are the morphologically and genetically similar monoecious species found on apiaceous and asteraceous hosts: *A. asclepiadis* Fitch, *A. ceanothi* Clarke, *A. clydesmithi* Stroyan, *A. crassicauda* Smith & Eckel, *A. decepta* Hottes & Frison, *A. helianthi* Monell, *A. impatientis* Thomas, *A. saniculae* Williams, *A. spiraephila* Patch, *A. thaspii* Oestlund, and *A. viburniphila* Patch (Foottit et al. 2008, Lagos et al. 2014). Due to their host associations and the wide distribution of *Cornus* spp. in North America, some of these *Aphis* species are taxonomically problematic. For example, *A. carduella*

(=*helianthi* Monell) is highly polyphagous on its secondary hosts, preferring various Apiaceae species, but it is specialized on its primary host (Blackman and Eastop 2006). A symptom of the secondary host polyphagy of *A. carduella* is its several synonyms, each originally described as a host specialist: *A. oxybaphi* Oestlund and *A. yuccae* Cowen (Palmer 1952), *A. heraclella* Davis (Addicot 1981), *A. helianthi* Monell, and *A. pentstemonis* Williams (Cook 1984).

Compared with the polyphagous *A. carduella*, the other species of the *A. asclepiadis* group are relatively host-specific, whether on *Cornus* spp. or species of Apiaceae and Asteraceae. We hypothesize that the monoecious species on herbaceous hosts are derived from heteroecious species whose primary host was *Cornus*. However, given that aphid species identity historically has been confounded by host alternation, we further hypothesize that some of the apparently monoecious aphid species on *Cornus* may in fact be heteroecious, synonyms of apparently monoecious species on herbaceous hosts. We tested these hypotheses using molecular, morphological, and biological data and here present an elucidation of the taxonomic status of the members of the *A. asclepiadis* group in the United States.

Materials and Methods

Taxon Sampling

Aphids were collected on their respective host plants in Canada, France, Japan, and Madagascar, with the majority of the material originating from the American Midwest, United States (Supp. Table 1 [online only]). When possible, late instars were reared on a piece of the host until maturation. Adults were preserved in 95% ethanol and stored at –20° C for DNA extraction and slide preparation. A subset of adults from a colony was used to make archival microscope slides, following the technique described by Pike et al. (1991). Specimens were identified by Lagos-Kutz using published identification keys (Hottes and Frison 1931, Rojanavongse and Robinson 1977, Cook 1984, Blackman and Eastop 2006) and with reference to authoritatively identified specimens in the insect collections of the Illinois Natural History Survey (INHS), the University of Minnesota (UMSP), and the US National Museum of Natural History (USNM). Our specimens, as well as those located at UMSP and NMNH were examined under a compound microscope and photographed with a Leica DM 2000 digital camera. Photographs of mounted specimens were taken, morphological annotations were made, and standard anatomical measurements were made using SPOT Imaging Solutions 5.1 (A Division of Diagnostic Instruments, Inc., Michigan; Supp. Tables 2 and 3 [online only]). Newly acquired specimens are deposited at the INHS, Champaign, IL.

DNA Extraction, Amplification, and Sequencing

DNA was extracted from two or three individual specimens per collection. Individual specimens were crushed in a 1.5-ml microcentrifuge tube and DNA was purified using the QIAamp DNAmicrokit (QIAGEN Inc., Valencia, CA). The entire mitochondrial Cytochrome Oxidase I gene (*Cox1*) was amplified using two primer pairs: C1-J-1718 (Simon et al. 1994) and C1-J-2411 (Lagos et al. 2012), and C1-N-2509 (Lagos et al. 2012) and TL2-N-3014 (Simon et al. 1994). The nuclear gene Elongation Factor 1-α gene (*EF1α1*) was amplified with EF3F (Lagos et al. 2012) and EF2 (Palumbi 1996). Primers were synthesized by Invitrogen Corporation (Carlsbad, CA). PCR products were generated using PuReTaq Ready-To-Go PCR 0.2 ml beads (GE Healthcare, United Kingdom) to which

20 µl of PCR-grade water, 1 µl each of 10 µM F and R primers, and 3 µl of genomic DNA solution were added. The thermocycle used to amplify *Cox1* and *EF1α1* was as follows: 95°C for 2 min followed by 40 cycles of 95°C for 30 s; 53°C for 30 s; 72°C for 2 min. PCR products were run for 40 min at 90 v on a 1% agarose gel and visualized with GelGreen (Biotium Inc, CA) nucleic acid stain. Most PCR products were purified using QIAquick (QIAGEN Inc., Valencia, CA) kits. PCR products that included the coamplification of nonspecific bands were gel purified using Zymoclean gel DNA recovery kit (Zymo Research, USA). The DNA concentration of purified PCR products was measured using a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Wilmington, DE) to determine the volume of DNA to use for the sequencing reaction (5 ng of DNA per 100 bp of sequenced product). PCR products were sequenced using 1.5 µl of BigDye Terminator v3.1, 0.75 µl dGTPBigDye Terminator v3.0, 0.75 µl of buffer, 1.6 µl of 2 µM primer, and 1 µl of dimethyl sulfoxide (DMSO; SIGMA-ALDRICH, St Louis, MO), and DNA volume. The sequencing reaction protocol was as follow: 96°C for 2 min followed by 25 cycles of 95°C for 20 s; 50°C for 5 s; 60°C for 4 min. Sequencing reactions were cleaned using Performa DTR Ultra 96-Well Plates (EdgeBioSystems, Gaithersburg, MD) and run on an ABI 3730 sequencer at the Keck Center (University of Illinois at Urbana-Champaign). Raw sequence data were assembled and trimmed using Sequencher 4.7 software (Gene Codes Corporation, Ann Arbor, MI). DNA sequences were aligned with Clustal X version 2.0 (Larkin et al. 2007). Reference sequences of *EF1α1* were retrieved from GenBank for comparison (von Dohlen and Teulon 2003, Kim and Lee 2008). Three intron regions were identified and used in this study. Nucleotide sequences were deposited in GenBank (Supp. Table 1 [online only]). Pairwise distances were obtained using PAUP 4.0b10 based on the Kimura two-parameter model (Swofford 2001).

Other DNA Sequences

Additional *Cox1* and *EF1α1* sequence data were obtained from GenBank: *Aphis asclepiadis* (KC897221–KC897223, KC905667, KC897540, KC897541–KC897542, KC897312–KC897313, KC905713, KC897466–KC897467, KC897468), *A. cornifoliae* (KC897553, AY219727), *A. fabae* (JQ860273–JQ860275, JQ860295–JQ860298), *A. glycines* (JQ860254–JQ860256, JQ860281–JQ860282), *A. gossypii* (KC897120–KC897122, KC897114–KC897117, KC897247, KC897240), *A. hederae* (KC897135–KC897137, KC897256–KC897257), *A. illinoisensis* (KC897138, KC897258), *A. impatientis* (KC897572–KC897573, KC897476), *A. nasturtii* (KC897165–KC897167, KC897282–KC897284), *A. neogillettei* (KC897580–KC897581, KC897480–KC897481), *A. nerii* (KC897169–KC897173, KC897289–KC897292), *A. nigratibialis* (KC897582–KC897583, KC897482–KC897483), *A. pastinaca* (KC897099–KC897101, KC897229–KC897230), *A. rumicis* (KC897183–KC897184, KC897299–KC897301), *A. salicariae* (KC897588–KC897590, KC897488), *A. saniculae* (KC897597–KC897599, KC897495–KC897496), *A. thaspii* (KC897602–KC897605, KC897499), *A. varians* (KC897609–KC897610, KC897501), *A. viburniphila* (KC897611–KC897612, KC897502–KC897503), *A.* (*Bursaphis*) *oenotherae* (KC897174–KC897178, KC897584–KC897585, KC951364–KC951365, KC897293–KC897295, KC897484, KC951361), *A.* (*Toxoptera*) *citricidus* (KC897617–KC897619, KC897505), *Hyadaphis tataricae* (KC897216–KC897217, KC897318), *Rhopalosiphum maidis* (JQ860263–JQ860265, JQ860287–JQ860288), and *Uroleucon helianthicola* (KC897218–KC897220, KC897319–KC897320).

Phylogenetic Analysis

Phylogenetic analysis was performed with a concatenated data set (2,368 bp) representing 92 full-length sequences and 24 taxa. To test for congruence, exhaustive pair-wise incongruence length difference tests (ILD, Farris et al. 1994) were performed in PAUP* v4.0b10 (Swofford 2001) using all taxa, 100 replicates, and parameters at default settings (uninformative discarded sites, gaps treated as missing data, state changes weighted equally). Modeltest 3.7 (Posada and Crandall 1998) was used to select the best-fit nucleotide substitution model for each gene. MrBayes 3.1.2 (Huelsenbeck and Ronquist 2003) was used to execute a Bayesian analysis partitioned into $Cox1$ and $EF1\alpha1$ data. For this analysis, four heated chains were run starting from a random tree. The number of generations was 5,000,000 with a tree sampling frequency 100 generations. The first 12,500 of the 50,000 sampled trees for each run were discarded, the remaining trees being summarized into a 50% majority rule consensus tree using MrBAYES's 'sumt' command. Support for nodes was assessed by posterior probability (PP). *Rhopalosiphum maidis* (Fitch) (Aphidinae: Aphidini) and *Hyadaphis tataricae* Aizenberg and *Uroleucon helianthicola* (Olive) (Aphidinae: Macrosiphini) were selected as outgroup. A parsimony network using 44 COI haplotypes of tentatively identified specimens of *A. asclepiadis* and *A. carduella* was constructed with TCS v. 1.21 (Clement et al. 2000) with a 95% connection limit.

Aphid Biology

Cornus racemosa Lamarck seedlings were grown in 12.7-cm-diameter pots and isolated in cages (34.5 by 34.5 by 57 cm^3). In order to induce the production of sexual morphs, aphids field-collected on *Cornus* spp. and matching the morphology and DNA sequences of *A. impatientis*, were reared on these plants for 6–8 wk in a growth chamber set at 12°C and a short photoperiod (8 L:16D).

Results

Phylogenetic Analysis

The ILD tests indicated no incongruence among loci ($P = 1.00$ on all). The likelihood settings for best-fit model were GTR+I+G for both genes. After alignment and excluding the primer sites, 1,290 and 1,078 bp for $Cox1$ and $EF1\alpha1$ (including gaps and introns) were used in the analysis, respectively, with a total of 2,368 bp. A restricted clade composed of the *A. asclepiadis* group was recovered with the exception of *A. caliginosa* (Fig. 1, Clade A, PP = 1.0). *Aphis asclepiadis* and the polyphagous *A. carduella*, collected from multiple host plants, grouped together (Fig. 1, Clade D, PP = 1.0), with *A. nigratibialis* as sister-group (Fig. 1, Clade C, PP = 1.0). The other *Aphis* species in this group form a large polytomy (Fig. 1, Clade B, PP:0.97).

Morphological Examination

We examined multiple collections of aphids from Asclepiadaceae, Apiaceae, Apocynaceae, Asteraceae, Cornaceae, Nyctaginaceae, and Malvaceae (Supp. Table 1 [online only]). Their morphological characters match the diagnostic characters of collections identified as *A. asclepiadis*, *A. carduella*, *A. helianthi*, and *A. nigratibialis* deposited at INHS, UMSP, and NMNH. However, we did find some morphological differences in body size (Supp. Table 2 [online only]) and body color pattern that varies from light green yellowish to dark green with a darker transversal band on the dorsum of abdominal segment V across populations collected on different host plants in the American West and Midwest. However, there are multiple

morphological characters that are useful to discriminate these species. The alate viviparae of *A. asclepiadis* and *A. carduella* have secondary sensoria restricted to antennal segment III (Fig. 3K) and pale hind tibia darkening distally (Fig. 4S); in contrast *A. nigratibialis* has secondary sensoria on antennal segments III and IV (Fig. 3P) and has hind tibia darkened throughout (Fig. 4T). In addition, they are morphologically differentiated by the presence or absence of dorsal abdominal and pre-siphuncular sclerites of alate viviparae; in *A. salicariae* these characters are present but in *A. neogillettei* they are absent (Supp. Table 2 [online only], Fig. 4D–F). In the alata, the hind tibia of *A. neogillettei* is paler than the hind tibia of *A. salicariae* (Fig. 4U–V).

Aphis cornifoliae and *A. impatientis* can be distinguished easily with morphological characters (Supp. Table 3 [online only]). In *A. cornifoliae*, the mean lengths of the longest seta on antennal segment III for apterae and alatae are 0.012 and 0.010 mm, respectively (Fig. 3B), the mean widths of the marginal tubercle on abdominal segment I for apterae and alatae is 0.022 and 0.019 mm, respectively, and the mean width of the marginal tubercle on abdominal segment VII for both apterae and alatae is 0.024 mm. In both morphs, the siphunculi are strongly curved outwards, the cauda is parallel-sided and blunt, and pre-siphuncular sclerites are present (Fig. 4B–K). In *A. impatientis*, the mean lengths of the longest seta on antennal segment III of apterae and alatae are 0.010 and 0.008 mm, respectively (Fig. 3D), the mean width of the marginal tubercle on abdominal segment I for both apterae and alatae is 0.013 mm, and the mean width of the marginal tubercle on abdominal segment VII for apterae and alatae are 0.021 and 0.018 mm, respectively. In both morphs the tip of the siphunculi are curved outwards, the cauda is tapering, and pre-siphuncular sclerites are absent (Fig. 4C–L). *Aphis impatientis* has been collected on *Impatiens* spp. and *Cornus* spp. (see Supp. Table 1 [online only] for detailed collection information). It was not found in mixed colonies with other *Aphis* spp., and the specimens match morphological characters as well as $Cox1$ sequences (Fig. 1; Table 1). Moreover, under laboratory conditions oviparae and males were recovered for first time on *Cornus racemosa*. This finding allows us to infer that *Cornus* spp. is the primary host of *A. impatientis*.

Aphis saniculae and *A. thaspii* share the same host plant, *Zizia* spp. *Aphis saniculae* can be misidentified as *A. thaspii* because of the morphological similarities of the alata. The antennae of apterous *A. saniculae* have secondary sensoria (Fig. 3H), in contrast to the antennae of apterous *A. thaspii*, which lack secondary sensoria (Fig. 3I). The marginal tubercles on abdominal segments I and VII in *A. thaspii* are bigger than in *A. saniculae* (Supp. Table 3 [online only]). In addition, the color in life of the apterous and alate viviparae is distinct: *A. saniculae* individuals are golden yellow, and the apterous viviparae of *A. thaspii* have a brownish head and a waxy dark green abdomen. More images of these species can be seen in Lagos-Kutz et al. (2016).

The differentiation of sexual morphs can also be useful to discriminate species that share a primary host. The apterous oviparae of *A. impatientis* (Fig. 5A) have a hind tibia without pseudosensoria and is not swollen (Fig. 5F), and the male is winged with many secondary sensoria on antennal segments III, IV, and V (Fig. 5B). The apterous oviparae of *A. neogillettei* (Fig. 5C) have few pseudosensoria (1–9) on the hind tibia (Fig. 5G) and the apterous male has secondary sensoria apically on segment III (Fig. 5D). In *A. nigratibialis* the apterous oviparae (Fig. 5E) have many pseudosensoria (64–101) on the hind tibia (Fig. 5H).

Moreover, the comparison of the longest seta on antennal segment III shows that this morphological character is different among
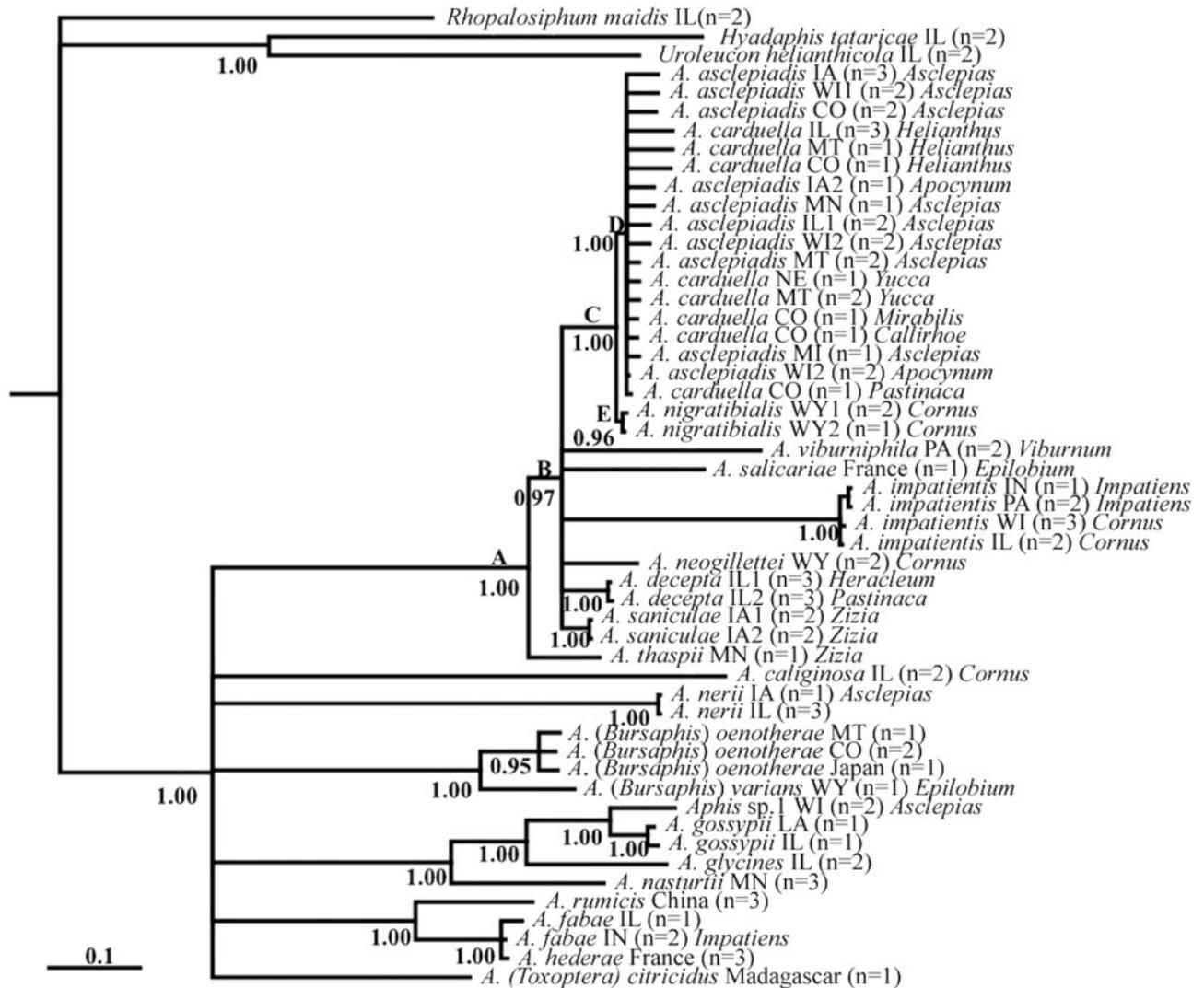
Fig. 1. Phylogenetic tree using a combined data set of *Cox1* and *EF1α1* sequences, inferred based on Bayesian analysis. Support values (posterior probabilities) are shown below branches. Values below 0.95 are not presented. Species names are followed by the collection locality [U.S.A.: CO (Colorado), IA (Iowa), IL (Illinois), IN (Indiana), LA (Louisiana), MO (Missouri), MN (Minnesota), MT (Montana), NE (Nebraska), SD (South Dakota), WI (Wisconsin)], the number of haplotypes, and the host plant genus.

the species of the *A. carduella* group that use *Cornus* spp. as primary host plants (Table 2, Fig. 6: F ratio = 241.87, df = 5, P < 0.0001).

Genetic Differentiation

The ranges of absolute pair-wise distances between the putative species *A. asclepiadis* and *A. carduella*, and *A. nigratibialis* for *Cox1* and *EF1α1* sequences, were 0.15–0.73% and 0.54–1.09%, respectively (Table 1). The Kimura 2-parameter pair-wise distance between sequences identified as *A. asclepiadis* and *A. carduella* (Supp. Table 1 [online only]) was 0–0.73 and 0–0.87 for *Cox1* and *EF1α1*, respectively (Table 1). The parsimony network indicated points of substitutions in COI sequences among the collections found in different locations and on similar host plants of putative *A. asclepiadis* and *A. carduella* in the United States (Fig. 2). The haplotype of *A. nigratibialis* is two substitutions away from the putative *A. asclepiadis* and *A. carduella* collected in Colorado on *Asclepias syriaca* L. and *Mirabilis multiflora* (Torr.) A. Gray, in Montana on *Helianthus* and *Yucca* spp., and in Nebraska on *Yucca* spp. The other species that feed on *Cornus* spp. (*A. impatientis*,

A. neogillettei, and *A. salicariae*) did not nest in the haplotype network. The exclusively *Cornus*-feeding *Aphis neogillettei* and *A. salicariae*, of Nearctic and Palaearctic origins, respectively, exhibit important differences. The interspecific pair-wise distances of *Cox1* and *EF1α1* were 3.33–3.41% and 1.31%, respectively (Table 1).

The pair-wise distances between *A. cornifoliae* and *A. impatientis* were 1.24–1.39% and 1.41–1.62% (Table 1), and between *A. saniculae* and *A. thaspii* were 0.77–0.78 and 1.52% for *Cox1* and *EF1α1*, respectively (Table 1).

Dichotomous Keys to Apterous and Alate Viviparae of the *Aphis asclepiadis* Species Group

For more comparative morphometric data and photographs of the species included in the following dichotomous key see the 3I online interactive key by Lagos-Kutz et al. (2016).

Key to Apterous Viviparae

1. Subgenital plate complete 2
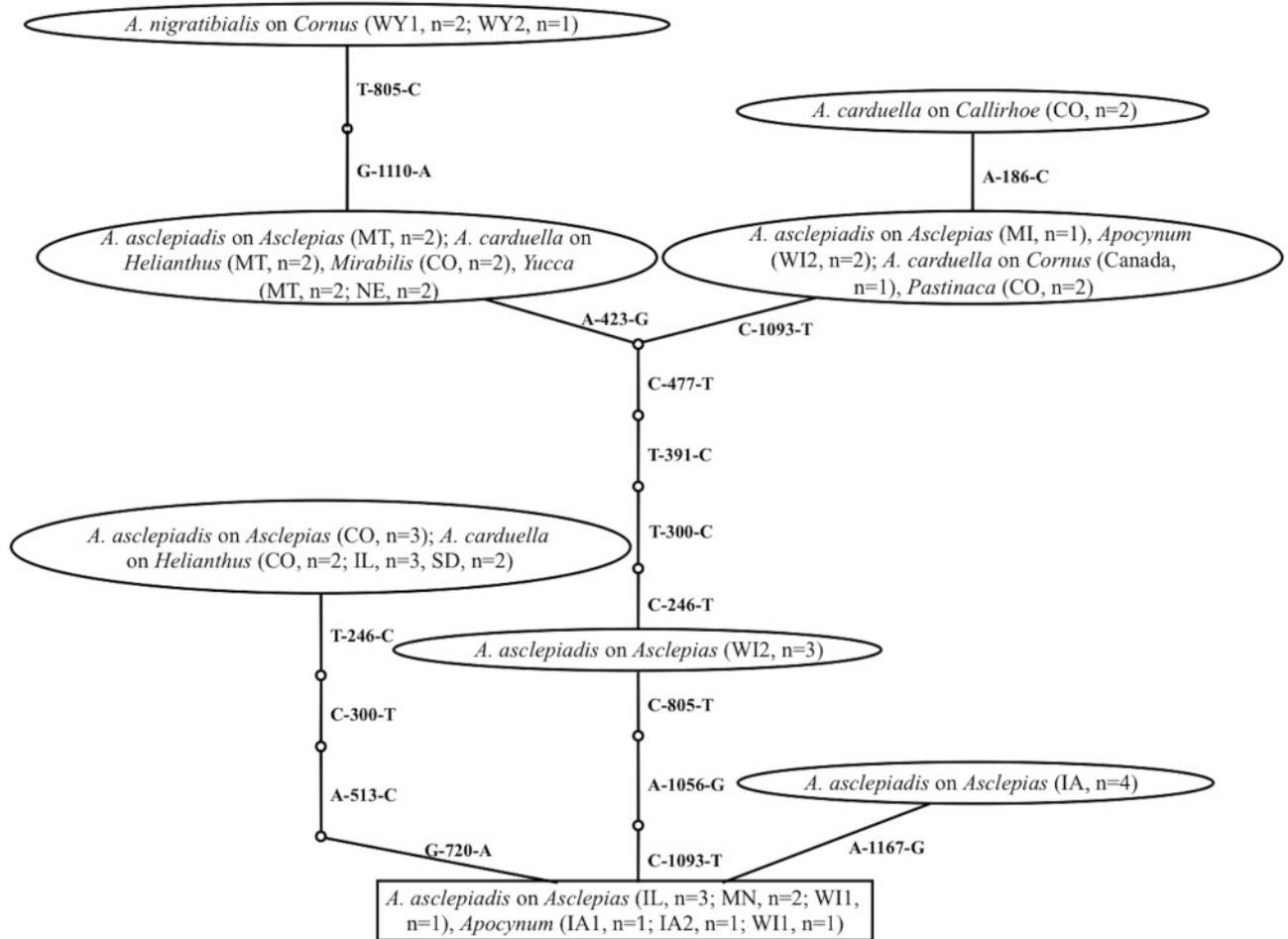
Fig. 2. Haplotype network of all the *Cox1* sequences of *Aphis* spp. that match the diagnostic morphological characters of *A. asclepiadis* and *A. carduella*, and the closely related *A. nigratibialis*. Nucleotides and numbers between nodes indicate the substituted nucleotide position. Ovals and rectangle contain the putative name species, location, host plant, and number of haplotypes whose pair-wise distances match 100%.

– Subgenital plate divided on anterior half 5

2. Secondary sensoria on antennal segments III (15–23), IV (1–10), and V (0–1). Pt/B 1–1.5. URS/HT2 1.1–1.2. Length of longest seta on antennal segment III 0.056–0.081 (Fig. 3J). Shape of cauda parallel side and blunt (Fig. 4R). SIPH/CA 1.4–1.5. On *Viburnum* spp..*viburniphila*

– Antennal segment III without secondary sensoria 3

3. Length of longest seta on antennal segment III shorter than 0.030. Pt/B 2.3–2.8. URS/HT2 0.9–1.1 (Fig. 3I). Cauda with parallel sides with constriction near base, blunt and relatively short. SIPH/CA 1.2–1.8 (Fig. 4Q). On *Cicuta maculata*, *Thaspium* spp., *Sanicula* spp., and *Zizia* spp. ....*thaspii*

– Length of longest seta on antennal segment III longer than 0.0304

4. Length of longest seta on antennal segment III 00.036–0.077 (Fig. 3E). Pt/B 1.1–2.1. Abdominal tergite VIII with 4–6 setae (Fig. 4M). On *Cornus* spp. ....*neogillettei*

– Length of longest seta on antennal segment III 0.041–0.066 (Fig. 3G). Pt/B 1.8–2.3. Abdominal tergite VIII with 6–12 setae (Fig. 4O). On *Cornus* spp. and *Epilobium* spp ....*salicariae*

5. Hind tibia dark throughout (Fig. 4T). Pt/B 2.5. URS/HT2 0.9. SIPH/CA 1.9 (Fig. 4N). On *Cornus* spp ....*nigratibialis*

– Hind tibia pale or dusky, darkening near distal tip 6

6. Siphunculi cylindrical and straight. Secondary sensoria on antennal segments III (3–24), IV (3–12), and V (1–6). On III, secondary sensoria restricted to the distal half distal, scattered on IV and V.

Length of longest seta on antennal segment III 0.012–0.021 (Fig. 3H). Pt/B 1.9–2.4. URS/HT2 0.8–1.1. Shape of cauda tapering. SIPH/CA 1.4–1.9 (Fig. 4P). On *Zizia* spp. and *Cicuta maculata* ....*saniculae*

– Siphunculi cylindrical and curved outwards. Antennal segment III without secondary sensoria 7

7. Ultimate rostral segment with 3–5 accessory setae. Pt/B 2.5–3.6. URS/HT2 1–1.2. Shape of cauda is nearly parallel side with slight constriction near the base, blunt and relatively short. SIPH/CA 1.7–2.8. On *Pastinaca sativa* and *Heracleum maximum*... *decepta*

– Ultimate rostral segment with 2 accessory setae 8

8. Length of ultimate rostral segment longer than 0.09. Length of longest seta on antennal segment III shorter than 0.040 (Fig. 3A). Pt/B 1.6–2.2. URS/HT2 0.8 1.0. Shape of cauda tapering. SIPH/CA 1.2–1.9 (Fig. 4J). Polyphagous and primary host, *Cornus* spp. *asclepiadis*

– Length of ultimate rostral segment less than 0.09 9

9. Length of longest seta on antennal segment III 0.007–0.016 (Fig. 4B). Width of marginal tubercle on abdominal segment I 0.013–0.029, width of marginal tubercle on abdominal segment VII 0.020–0.029. SIPH/CA 1.2–2.0 (Fig. 4K). On *Cornus* spp. . ...*cornifoliae*

– Length of longest seta on antennal segment III 0.004–0.013 (Fig. 3D). Width of marginal tubercle on abdominal segment I 0.005–0.020, width of marginal tubercle on abdominal segment
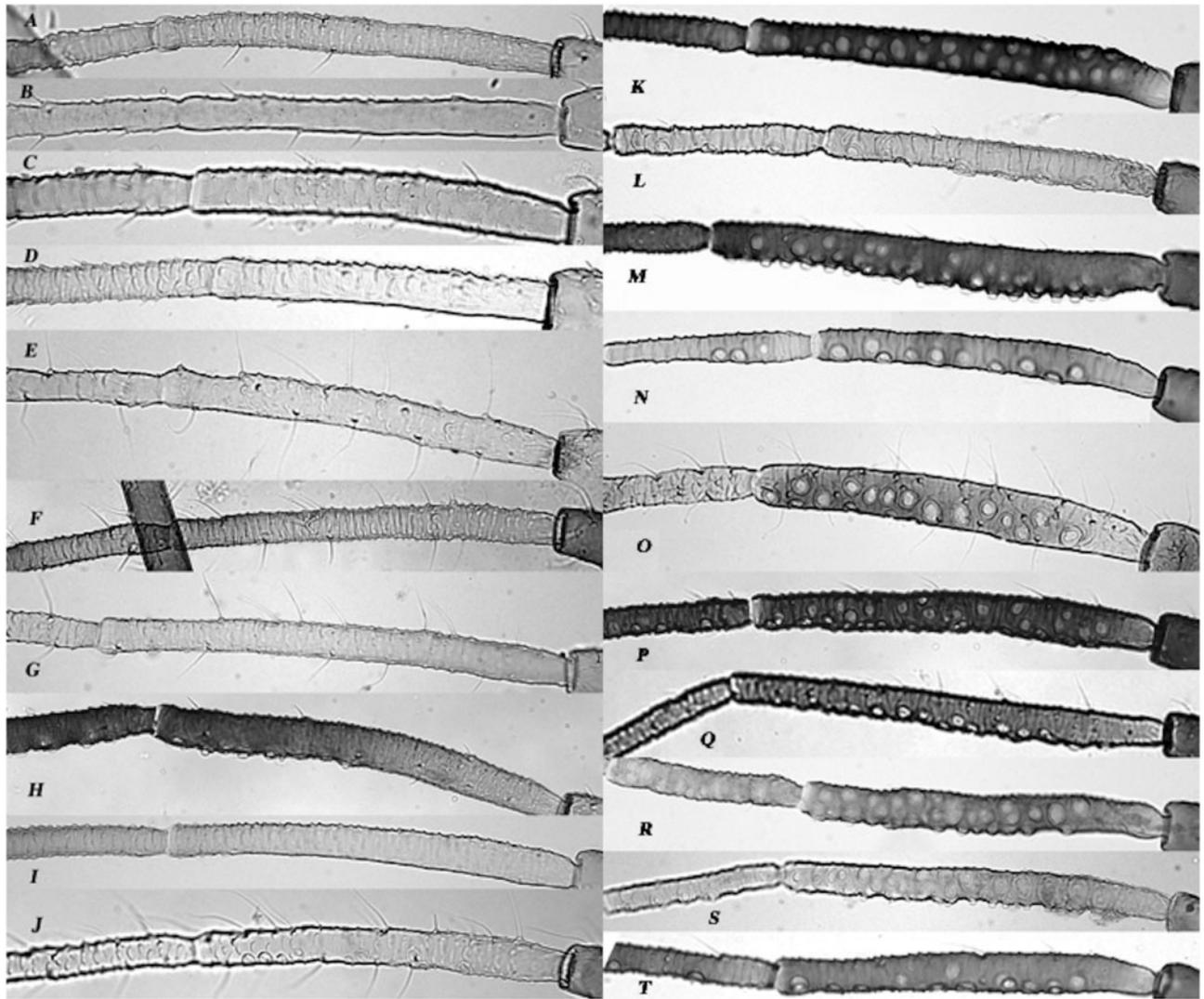
Fig. 3. Antennal segments II, III, and IV of apterous viviparae in the *A. asclepiadis* group. (**A**) *A. asclepiadis*, (**B**) *A. cornifoliae*, (**C**) *A. decepta*, (**D**) *A. impatientis*, (**E**) *A. neogillettei*, (**F**) *A. nigratibialis*, (**G**) *A. salicariae*, (**H**) *A. saniculae*, (**I**) *A. thaspii*, (**J**) *A. viburniphila*. Antennal segments II, III, and IV of alate viviparae. (**K**) *A. asclepiadis*, (**L**) A. cornifoliae, (**M**) *A. decepta*, (**N**) *A. impatientis*, (**O**) *A. neogillettei*, (**P**) *A. nigratibialis*, (**Q**) *A. salicariae*, (**R**) *A. saniculae*, (**S**) *A. thaspii*, (**T**) *A. viburniphila*.

VII 0.013–0.029. SIPH/CA 0.9–1.6 (Fig. 4L). On *Impatiens* spp. and *Cornus* spp ....*impatientis*

Key to Alate Viviparae

1. Subgenital plate divided on anterior half and hind tibia dark throughout. Secondary sensoria on antennal segments III (28–37) and IV (5–12). Pt/B 1.8–3.1. Length of longest seta on antennal segment III 0.023–0.034 (Fig. 3P). URS/HT2 0.8–1.3. Width of the marginal tubercle on abdominal segment I 0.026–0.035. Width of the marginal tubercle on abdominal segment VII 0.029–0.035. Shape of siphunculi cylindrical and straight. Shape of cauda oblong, pointed and constricted in the middle. Cauda 8–9 setae. SIPH/CA 1.3–1.7 (Fig. 4E) . ...*nigratibialis*
– Subgenital plate divided on anterior half and hind tibia pale or dusky, darkening near distal tip 2
2. Scattered secondary sensoria on antennal segments 3
– Secondary sensoria restricted to internal margin or in a row 7

3 Secondary sensoria restricted to antennal segment III 4
– Secondary sensoria on III and IV, or III, IV, and V antennal segments...5
4. Antennal segment III with 14–31 secondary sensoria. Length of longest seta on third segment antennal 0.010–0.038 (Fig. 3K). Pt/B 1.8–3.1. URS/HT2 0.9–1.3. Width of the marginal tubercle on abdominal segment I 0.014–0.030. Width of the marginal tubercle on abdominal segment VII 0.014–0.028. Shape of cauda oblong, pointed and constricted in the middle. Cauda 6–13 setae. SIPH/CA 1.1–2.4 (Fig. 4A). .... *asclepiadis*
– Antennal segment III with 36–53 secondary sensoria. Length of longest seta on third segment antennal 0.018–0.025 (Fig. 3M). Pt/B 2.8–4.3. Ultimate rostral segment 3–5 accessory setae. URS/HT2 1–1.3. Width of the marginal tubercle on abdominal segment I 0.030–0.040. Width of the marginal tubercle on abdominal segment VII 0.030–0.050. Shape of cauda parallel sid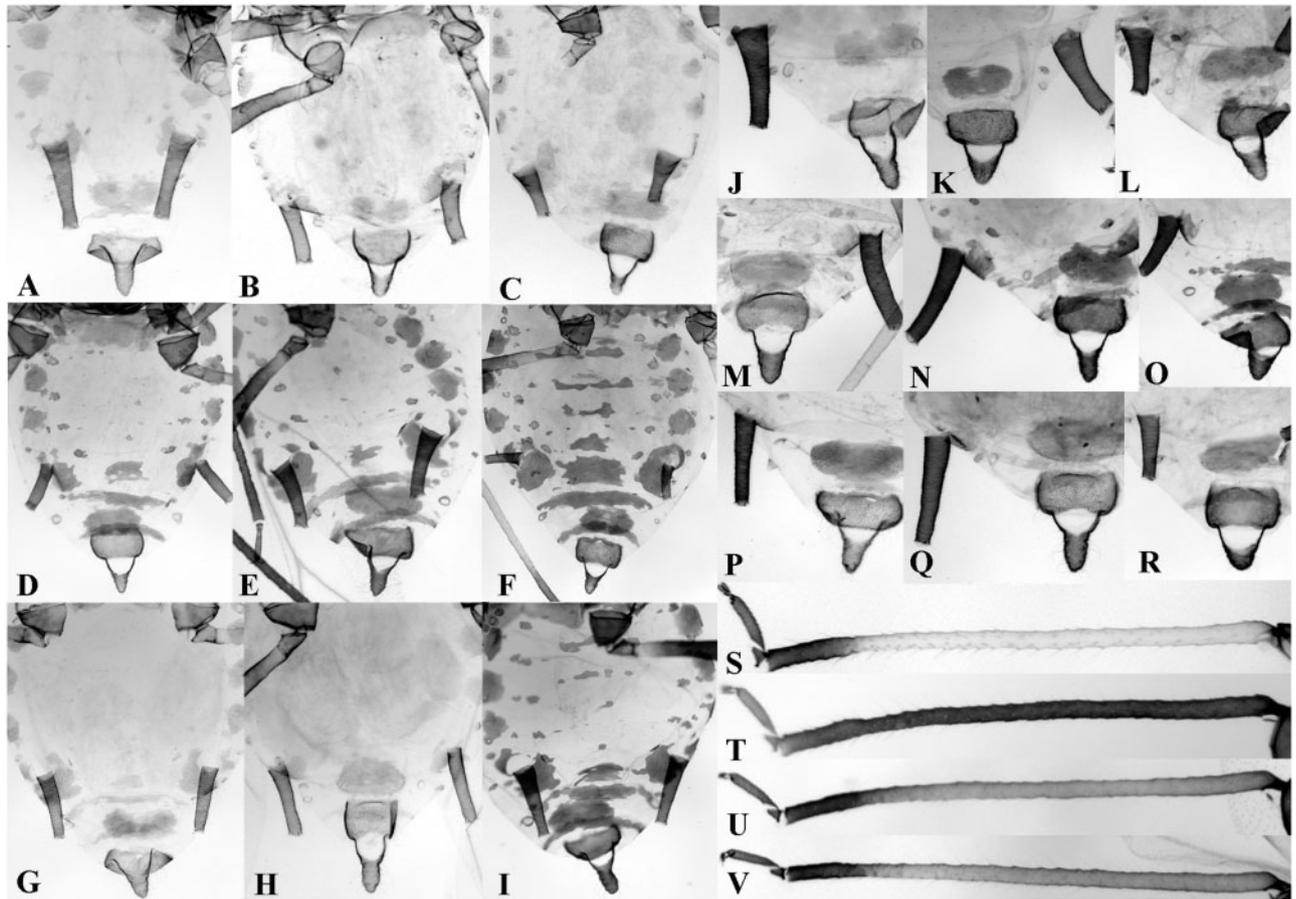e and blunt reticulation. Cauda 13–17 setae. SIPH/CA 1.9–2.3...*decepta*

Fig. 4. Abdomens of alate viviparae in the *A. asclepiadis* group. (**A**) *A. asclepiadis*, (**B**) *A. cornifoliae*, (**C**) *A. impatientis*, (**D**) *A. neogillettei*, (**E**) *A. nigratibialis*, (**F**) *A. salicariae*, (**G**) *A. saniculae*, (**H**) *A. thaspii*, (**I**) *A. viburniphila*. Last abdominal segments of apterous viviparae. (**J**) *A. asclepiadis*, (**K**) *A. cornifoliae*, (**L**) *A. impatientis*, (**M**) *A. neogillettei*, (**N**) *A. nigratibialis*, (**O**) *A. salicariae*, (**P**) *A. saniculae*, (**Q**) *A. thaspii*, (**R**) *A. viburniphila*. Hind tibia of alate viviparae. (**S**) *A. asclepiadis*, (**T**) *A. nigratibialis*, (**U**) *A. neogillettei*, (**V**) *A. salicariae*.

Table 1. Ranges of pairwise interspecific and intraspecific distances (%) using COI and EF1-∝ calculated using Kimura 2 Parameter of species within the *A. asclepiadis* group that feed on *Cornus*

Species/ Genes	A. asclepiadis		A. cornifoliae		A. impatientis		A. neogillettei		A. nigratibialis		A. salicariae	
	COI	EF1-α	COI	EF1-α	COI	EF1-α	COI	EF1-α	COI	EF1-α	COI	EF1-α
A. asclepiadis	0.00–0.73	0.00–0.87										
A. cornifoliae	6.27–6.34	1.52–2.29	0.00	0.00								
A. impatientis	6.35–6.70	1.42–2.19	1.24–1.39	1.41–1.62	0.00–0.15							
A. neogillettei	2.17–2.71	0.66–0.99	6.35–6.58	1.86	6.12–6.51	1.42–1.54	0.00–0.85	0.00				
A. nigratibialis	0.15–0.73	0.54–1.09	6.27	1.73	6.51	1.30–1.42	3.02	0.55	0.00	0.22		
A. salicariae	3.02–3.41	1.41–2.17	6.58	2.38	6.74	2.06–2.17	3.33–3.41	1.31	3.02	1.51	0.00	0.00

5. Length of longest seta on third segment antennal 0.038–0.051. Secondary sensoria on antennal segment III (15–23), IV (1–10), V (0–1) (Fig. 3T). Pt/B 2.6–3.3. URS/HT2 1.1–1.2. Width of marginal tubercle on abdominal segment I 0.023–0.031. Width of marginal tubercle on abdominal segment VII 0.029–0.036. Shape of siphunculi cylindrical and straight. Shape of cauda slightly spoon-shaped. Cauda 12–22 setae. SIPH/CA 1.1–1.5 (Fig. 4I) ....*viburniphila*

– Length of longest seta on third segment antennal less than 0.020 6

6. Length of longest seta on third segment antennal 0.010–0.019. Secondary sensoria on antennal segments III (25–39), IV (6–18), V (3–8) (Fig. 3P). Pt/B 2.1–2.6. URS/HT2 0.6–1. Width of marginal tubercle on abdominal segment I 0.029–0.047. Width of marginal tubercle on adominal segment VII 0.027–0.040. Shape of cauda tapering (Fig. 4G)..*saniculae*

– Length of longest seta on third segment antennal 0.007–0.012. Secondary sensoria on antennal segments III (30–49), IV (4–14), V (0–4) (Fig. 3S). Pt/B 2.2–3. URS/HT2 0.9–1.1. Width of marginal tubercle on abdominal segment I 0.017–0.029. Width of marginal tubercle on abdominal segment VII 0.018–0.027. Shape of cauda slightly spoon-shaped (Fig. 4H)...........................*thaspii*

7. Length of longest setae on segment antennal III 0.030 or longer 8
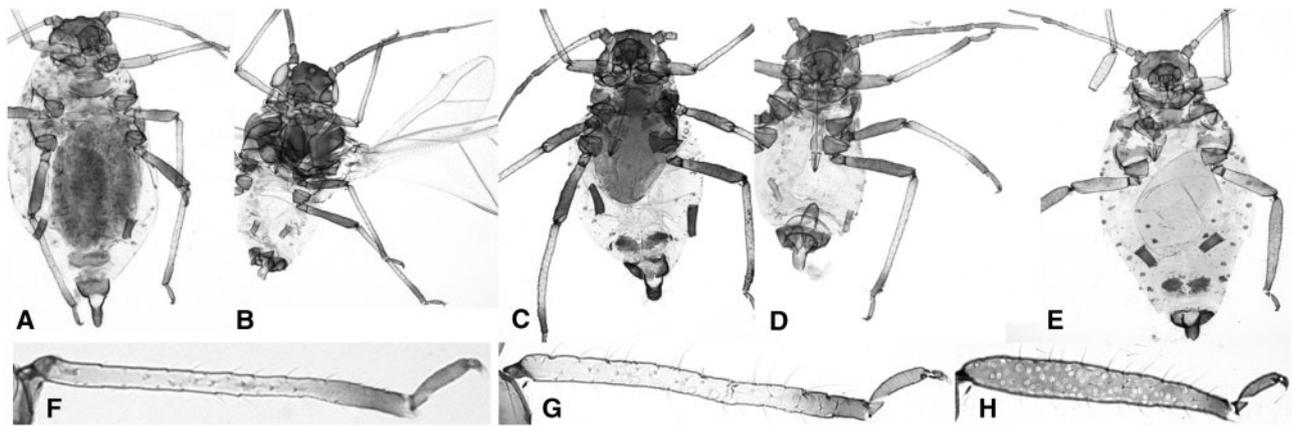
Fig. 5. Sexual morphs of selected species of the *A. asclepiadis* group. (**A**) Apterous oviparae of *A. impatientis*. (**B**) Alate male of *A. impatientis*. (**C**) Apterous ovipara of *A. neogillettei*. (**D**) Apterous male of *A. neogillettei*. (**E**) Apterous ovipara of *A. nigratibialis*. Hind tibiae of apterous oviparae. (**F**) *A. impatientis*, (**G**) *A. neogillettei*, (**H**) *A. nigratibialis*.

Table 2. Mean (±SE) length of the longest seta on antennal segment III of apterous and alate viviparae of species in the *Aphis asclepiadis* group, in millimeters

Species	Longest seta on antennal segment III	
	n	Mean (±SE)
A. asclepiadis	281	0.019 ± 0.0005c
A. cornifoliae	35	0.011 ± 0.0014d
A. impatientis	37	0.008 ± 0.0014d
A. neogillettei	50	0.051 ± 0.0011a
A. nigratibialis	5	0.032 ± 0.0034b
A. salicariae	32	0.052 ± 0.0018a

Means followed by different letters within each morphological character compared by species using Tukey–Kramer HSD confidence quantile are significantly different (q or studentized range statistic of morphological character is 2.863; $P < 0.05$).

– Length of longest setae on segment antennal III less than 0.030....... 9

8. Siphunculi cylindrical and straight ((Fig. 4D). Length of longest seta on segment antennal III 0.030–0.062 (Fig. 3O). Pt/B 1.5–2.2. URS/HT2 0.8–1.0. Width of marginal tubercle on abdominal segment I 0.023–0.047. Width of marginal tubercle on abdominal segment VII 0.020–0.042. Pre-siphuncular sclerite absent. Tergite abdominal VIII 4–5 setae. ....*neogillettei*

– Siphunculi cylindrical and curved outwards (Fig. 4F). Antennal segment III with 17–23 secondary sensoria mostly restricted to the internal margin. Length of longest seta on segment antennal III 0.038–0.051 (Fig. 3Q). Pt/B 2.0–2.5. Width of marginal tubercle on abdominal segment I 0.018–0.035. Width of marginal tubercle on abdominal segment VII 0.021–0.034. Pre-siphuncular sclerite present. Dorsal abdomen with small transversal sclerites on abdominal segments II, III, IV, and V; and large transversal sclerites on VI, VII, and VIII. Tergite abdominal VIII 4–9 setae..*salicariae*

9. Pre-siphuncular sclerite present. Secondary sensoria on antennal segments III (5–12) and IV (0–3). Length of longest seta on third segment antennal 0.007–0.015 (Fig. 3L). Width of marginal tubercle on abdominal segment I 0.012–0.027. Width of marginal tubercle on abdominal segment VII 0.016–0.028. Shape of siphunculi cylindrical and strongly curved outwards. Shape of cauda parallel side and blunt (Fig. 5B). .... *cornifoliae*

– Pre-siphuncular sclerite absent. Secondary sensoria on antennal segments III (7–16) and IV (1–10). Length of longest seta on third segment antennal 0.004–0.012 (Fig. 3N). Width of marginal tubercle on abdominal segment I 0.005–0.022. Width of marginal tubercle on abdominal segment VII 0.009–0.027. Shape of siphunculi cylindrical and weakly curved outwards. Shape of cauda tapering. SIPH/CA 0.9–1.8 (Fig. 4C)..................*impatientis*

Discussion

Based on our morphological examination and analysis of molecular data, we conclude that *A. asclepiadis* Fitch, 1851 is synonymous with *A. carduella* Walsh, 1863 and *A. helianthi* Monell, 1879. The oldest name that applies in accordance with the Principle of Priority of the International Code on Zoological Nomenclature (Article 23, ICZN 1999) is *A. asclepiadis* Fitch, 1851. Therefore, *A. asclepiadis* is the senior synonym of *A. carduella* **syn. nov.** The morphological assessment of the samples included in this study (Supp. Table 1 [online only]) overlap greatly (Supp. Table 2 [online only]). Also, the range of the longest seta on antennal segment III presented in Blackman and Eastop (2006) to discriminate *A. asclepiadis* and *A. helianthi* were not useful to discriminate the North American populations presented in this study and identified as *A. asclepiadis*. Instead, we found that this morphological character is different across the species included in the *A. asclepiadis* group that uses *Cornus* as primary host (Table 2 and Fig. 6). Wide morphological variations have been found on other highly polyphagous aphids such as *A. gossypii* (Margaritopoulos et al. 2006, Lagos 2007), and *A. fabae* (Müller 1986, Gorur et al. 2005). Moreover, the high value of intraspecific sequence divergence (Table 1) suggests that there is potential high gene flow among the *Aphis* species included in the *A. asclepiadis* group that use *Cornus* as primary host. Such gene flow is possible when many species share the same primary overwintering host plant (Petit and Excoffier 2009). For example, cases of hybridization have been observed under laboratory and field conditions between *A. schneideri* and *A. grassulariae* (Rakauskas 2003).

The original description of *A. impatientis* did not include sexual morph (Thomas 1878). Hottes and Frison (1931) stated that *A. impatientis* and *A. cephalanthi* were synonyms. They suggested that *Impatiens* spp. was the secondary host and *Cephalanthus* spp. the primary, but Lagos et al. (2014) provided morphological and molecular evidence that both species were valid. Moreover, in this study
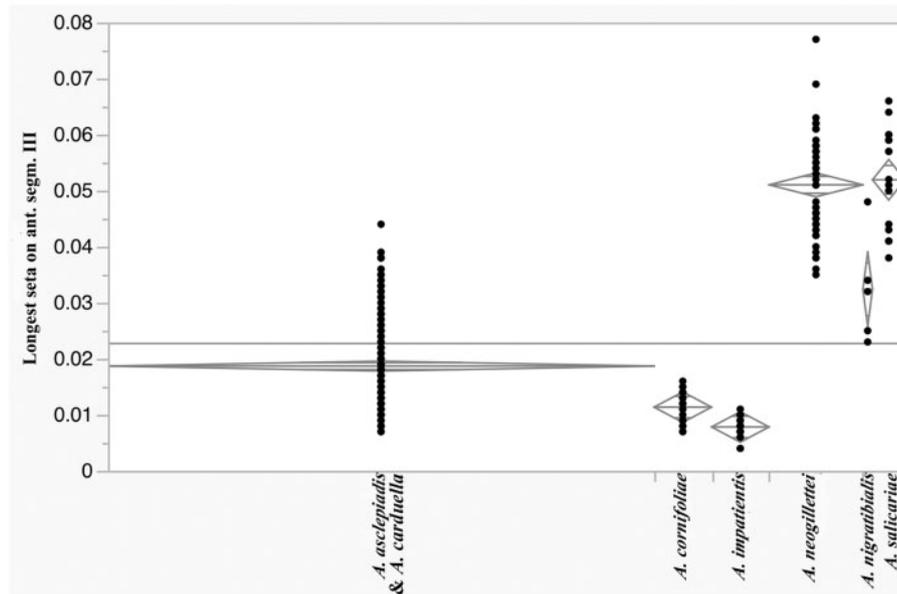
Fig. 6. Analysis of variance of the longest seta of the third antennal segment of apterous and alate vivipara of *A. asclepiadis*, *A. cornifoliae*, *A. impatientis*, *A. neogillettei*, *A. nigratibialis*, and *A. salicariae*. The gray line represents the median. The diamond represents the means and standard error.

we clarified the life cycle of *A. impatientis*, and that *Cornus* spp. is its primary host plant.

The pair-wise sequence divergences of *Cox1* obtained in this study (Table 1) fall within the ranges presented in Foottit et al. (2008), Coeur d'acier et al. (2014), and Lagos et al. (2014). They found that the range of interspecies pair-wise sequence divergence for *Aphis* species was 0.15–11.05%. Foottit et al. (2008) estimated the maximum intraspecific value for *A. helianthi* (0.31%) from samples collected in Canada and United States (Utah and Washington) and it is lower than that obtained in this study (0.73%). However, Foottit et al. (2008) and Lagos-Kutz et al. (2014) found for other polyphagous species such as *A. gossypii* (0.62 and 0.54%, respectively) values almost as high as those found in this study.

In 1931, Hottes and Frison claimed that *A. impatientis* Thomas, 1878 is synonymous with *A. cephalanthi* Thomas, 1878, but later authors have maintained these species as different taxa (e.g., Remaudière and Remaudière 1997, Blackman and Eastop 2006). In Lagos et al. (2014), the phylogenetic analyses of COI sequences indicated that *A. cephalanthi* does not belong to the *A. asclepiadis* group, and therefore cannot be a synonym of *A. impatientis*. Morphological examination of the specimens collected by Hottes and Frison in 1929 on *Cephalanthus occidentalis* (INHS: Sl.6375) revealed that all have a longer rostrum (0.12 mm), longer setae on antennal segment III (0.013–0.018), longer width of the marginal tubercle on abdominal segment I (0.015–0.018), and longer siphunculi (0.18–0.20) than *A. impatientis* (Supp. Table 3 [online only]). In addition, *A. cephalanthi* has marginal tubercles on abdominal segments II, III, and IV (Lagos 2007). *Aphis impatientis* is the sister species of *A. cornifoliae* (Lagos et al. 2014). *Aphis impatientis* feeds on the annual *Impatiens* spp., and through morphological and molecular correlation, we found that its primary host plant is *Cornus* spp. (Supp. Tables 1 and 3 [online only], and Table 1). Therefore, this species is heteroecious holocyclic. We found that the presence of wings on the males, the number of pseudosensoria on the hind tibia of oviparae, and the shape of the hind tibia of oviparae (swollen or flat) are useful to differentiate *A. cornifoliae*, *A. impatientis*, *A. neogillettei* and *A. nigratibialis* (Fig. 5). All four species use *Cornus* spp. as primary host. In *A. asclepiadis* the male is alate and the apterous

ovipara has many pseudosensoria on its swollen hind tibia; in *A. cornifoliae* the male is apterous and the apterous ovipara has slightly swollen hind tibiae with few, scattered, large, and flat pseudosensoria (Palmer 1952); in *A. salicariae* the male is alate and the apterous ovipara has strongly swollen hind tibiae with 150–200 pseudosensoria (Stroyan 1984, Heie 1986). Moreover, in this group there is a correlation between the formation of wingless or winged males and the type of life cycle. Species with a monoecious life cycle produce only apterous males (*A. cornifoliae* and *A. neogillettei*) while those with a heteroecious life cycle produce alate males (*A. asclepiadis*, *A. impatientis*, and *A. salicariae*). Under laboratory conditions, however, *A. impatientis* produced both apterous and alate sexual morphs on *Cornus racemosa*. The production of apterous and alate sexual morphs is not surprising: the first author has observed under laboratory conditions (mostly on senescing plants of *Glycine max*) that *A. glycines* produce alate males and apterous oviparae. Under natural conditions, winged gynoparae and males are produced on *Glycine max*, and the gynoparae produce apterous oviparae on *Rhamnus* spp.

Acknowledgments

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