INSECTA MUNDI A Journal of World Insect Systematics

0627

Revision of the taxonomic status of *Aphis floridanae* Tissot (Hemiptera: Aphididae) using morphological and molecular insight

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Date of issue: April 27, 2018

CENTER FOR SYSTEMATIC ENTOMOLOGY, INC., Gainesville, FL

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Insecta Mundi 0627: 1–10
ZooBank Registered: urn:lsid:zoobank.org:pub:9B87561C-3568-4820-88D3-824E0BDB8A3B

Published in 2018 by
Center for Systematic Entomology, Inc.
P.O. Box 141874
Gainesville, FL 32614-1874 USA
http://centerforsystematicentomology.org/

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Electronic copies (Online ISSN 1942-1354, CDROM ISSN 1942-1362) in PDF format
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Layout Editor for this article: Robert G. Forsyth

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Abstract. Morphological and cytochrome oxidase 1 (*Cox1*) data show that *Aphis floridanae* Tissot (Hemiptera: Aphididae) is not synonymous with *A. nasturtii* Kaltenbach. Instead, *A. floridanae* matches the morphological characters of *A. impatientis* Thomas. Additionally, the range of cytochrome oxidase 1 (*Cox1*) pair-wise distance of the multiple collections of *A. impatientis* on *Cornus* spp., *Impatiens* spp. and *Erechtites hieraciifolius* (L.) Raf. ex DC. is 0–0.39%. Therefore, we conclude that *A. floridanae* Tissot, 1933 is a junior synonym of *A. impatientis* Thomas, 1878, new synonymy. In addition, *A. impatientis* is re-described, including first descriptions of the ovipara and alate male of that species.

Key words. Morphology, *Aphis impatientis*, synonymy, genes, sequences, host plant.

Introduction

Aphis floridanae Tissot, 1933, is a North American species that feeds on endemic *Lactuca floridana* (L.) Gaertn and *Erechtites hieraciifolius* (L.) Raf. ex DC. Tissot (1933) provided morphological characters to distinguish *A. floridanae* from *A. rumicis* Linnaeus, 1758. Eastop and Hille Ris Lambers (1976) recognized it as a valid species, but Cook (1984) and Remaudière and Remaudière (1997) cited *A. floridanae* as a synonym of *A. nasturtii* Kaltenbach, 1843. Susan Halbert found specimens that appeared to be *A. floridanae* in suction traps in Florida. She noted that they resemble *A. salicariae* Koch, 1855, more than *A. nasturtii*, and found that the specimens keyed to *A. impatientis* Thomas, 1878 in Lagos-Kutz et al. (2016). After additional *A. floridanae*-like specimens were collected in Florida on *E. hieraciifolius*, we decided to gather morphological and molecular sequence data to elucidate the taxonomic status of *A. floridanae*.

Materials and Methods

Collection data of *A. impatientis* collected on *Lactuca floridana* and *Erechtites hieraciifolius* putative *A. floridanae* are provided in Table 1. Collection data of *A. impatientis* (aphids collected on *Impatiens* spp.) can be found in Lagos-Kutz et al. (2016). Species identification of slide-mounted materials was done

Table 1. Collection information of *Aphis impatientis* collected in Florida. INHS voucher and GenBank accession numbers are both included.

Species	Host	Date	Location	GPS	Collector	Collection voucher	GenBank accession #
A. impatientis Thomas, 1878	*Lactuca floridana*	07/21/1930	Tampa, Florida		F. S. Blanton	USNM: 44295	
	Lactuca floridana	07/21/1930	Tampa, Florida		F. S. Blanton	FSCA: F672-30	
	Erechtites hieraciifolius	09/17/1968	1 m. N of S271 on S270, Gadson County, Florida		R. Nielsson	FSCA: N188	
	Erechtites hieraciifolius	04/12/1972	Hendry County, Florida		W. Adlerz	FSCA: A72-73	
	Erechtites hieraciifolius	08/04/1998	Orlando, Orange County, Florida		T. Emery	FSCA: E98-2745	
	Erechtites hieraciifolius	06/22/2002	Fernandina Beach, Nassau County, Florida		J. Brambila	FSCA: E2002-2675	
	Erechtites hieraciifolius	06/06/2011	Pt. Pierce Teague Hammock, St. Lucie County, Florida	27.31, –80.54	K. Hibbard	INHS: 819336-819338	KY399471
	Erechtites hieraciifolius	08/21/2016	Arredondo, Alachua County, Florida	29.56, –82.42	S. Halbert	INHS: 819339-819340	KY399472–KY399473

based on using published keys (Voegtlin et al. 2004; Blackman and Eastop 2006; Lagos-Kutz et al. 2016) and authoritatively identified specimens in the insect collections of the Illinois Natural History Survey (INHS), U.S.D.A. Systematic Entomology Lab of Smithsonian National Museum of Natural History (USNM), and the Entomology Department of the Florida agricultural experimental station Florida State Collection of Arthropods (FSCA). Holotype of *A. floridanae* deposited in the USNM (44295) and paratypes in the FSCA (F672-30) were studied. Identifications of slide-mounted specimens were referenced to the aphid colony-mates used in the molecular analyses. Photographs of mounted specimens were taken using a Leica DM 2000 digital camera and SPOT Software 4.6 (Diagnostic Instruments, Inc., Michigan, USA). The SPOT software was used to take all measurements in millimeters for this study. A discriminant analysis of morphological characters and ratios was performed using JMP 13 (SAS Institute, Cary, NC).

DNA was extracted from one individual specimen from a field collection in Florida in 2011, and two individual specimens from a second field collection in Florida in 2016 (See Table 1 for more collection details). 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). PCR products were generated using 8 µl of PCR-grade water, 1.25 µl each of 20 µM F and R primers, 12.5 µl of Phusion Master Mix (Thermo Fisher Scientific Inc., Grand Island, NY), and 2 µl of genomic DNA solution. The thermocycle used to amplify *Cox1* was: 95º C for 2 min followed by 35 cycles of 98º C for 60s; 98° C for 15s; 54° C for 30s; 72° C for 30s. PCR products were submitted to W. M. Keck Center at the University of Illinois for sequencing. In addition, a total of 19 sequences for *Cox1* were retrieved from GenBank to estimate the pairwise distances for *Cox1* were calculated using the Kimura 2-parameter distance model (Kimura 1980) in PAUP 4.0b10 (Swofford 2001): *Aphis asclepiadis* Fitch (KC897221), *A. cornifoliae* Fitch (KC897553); *A. fabae* Scopoli (JQ860274); *A. gossypii* Glover (JQ860257); *A. impatientis* (KC905689, KC905690, KC905691, KC905692, KC905693, KC905694, KC905695, KC905696, KC897572, KC897573); *A. nasturtii* (KC897159, KC897162, KC897165, KC897168); *A. rumicis* (KC897183); and *A. salicariae* (KC897588).

After alignment and excluding the primer sites, 1290 bp were used in the analyses. A total of 22 sequences for COI from eight species were used in this study. The sequences of *A. impatientis* collected on *E. hieraciifolius* are available in GenBank under the following accession numbers: KY399471, KY399472, KY399473 (Table 1).

Results

The morphological characters and molecular evidence reveal that *A. floridanae* and *A. nasturtii* (Tables 2, 3) are distinct species that can be easily separated morphologically. The discriminant analysis of the morphological characters among *A. floridanae*, *A. impatientis* and *A. nasturtii* of both apterous and alate viviparae revealed that the ratios of Pt/B, URS/HT2, and SIPH/CA are the most useful characters to discriminate these species (Fig. 16), and that the other measured characters were rejected because of their high correlation (Table 2). Instead, the putative *A. floridanae* specimens conform the morphological characters described for the apterous and alate vivipara of *A. impatientis* (Fig. 1, 7) in dichotomous keys of the asclepiadis species group (Lagos-Kutz et al. 2016). This conclusion is also upheld with molecular evidence as the range of pair-wise distances among the collection on *E. hieraciifolius* in Florida and the collections on *Cornus* spp. and *Impatiens* spp. is 0–0.39% (Table 3).

In this study, the re-description is presented based on morphological data of both *A. floridanae* and *A. impatientis* (Table 2). Since *A. impatientis* was described based on a single alate specimen, a description of all the morphs including the ovipara and males collected from *Cornus racemosa* (Lagos-Kutz et al. 2016) is necessary.

Table 2. Morphological characters of *Aphis impatientis, A. floridanae and A. nasturtii*. For all measurements and counts the range is given and the mean is in parenthesis. All measurements are in millimeters.

	A. impatientis				*A. floridanae*		*A. nasturtii*	
Morphological characters/ Morphs	Apterae (n = 37)	Alatae (n = 43)	Oviparae (n = 8)	Male (n = 3)	Apterae (n = 23)	Alatae (n = 21)	Apterae (n = 22)	Alatae (n = 23)
Body	1.1–1.8 (1.5)	1.2–1.9 (1.5)	1.0–1.3 (1.1)	1.1–1.3 (1.2)	1.2–1.8 (1.5)	1.1–1.9 (1.6)	1.3–2.2 (1.9)	1.3–1.9 (1.6)
Ultimate Rostral Segment (URS)	0.07–0.09 (0.08)	0.07–0.09 (0.08)	0.06–0.07 (0.07)	0.07	0.08–0.10 (0.09)	0.07–0.1 (0.08)	0.10–0.12 (0.11)	0.09–0.11 (0.10)
Antennal segments:	6	6	5	6	6	6	6	6
III	0.15–0.33 (0.22)	0.20–0.35 (0.27)	0.17– 0.2 (0.20)	0.20–0.21 (0.21)	0.17–0.33 (0.24)	0.23–0.35 (0.28)	0.18–0.34 (0.28)	0.20–0.33 (0.27)
IV	0.09–0.21 (0.15)	0.12–0.23 (0.17)	0.07–0.11 (0.09)	0.13–0.14 (0.14)	0.10–0.21 (0.15)	0.13–0.23 (0.18)	0.12–0.23 (0.19)	0.13–0.24 (0.18)
V	0.10–0.17 (0.13)	0.10–0.18 (0.14)	—	0.13–0.14 (0.13)	0.10–0.17 (0.13)	0.11–0.16 (0.14)	0.12–0.19 (0.16)	0.11–0.19 (0.15)
Base of last ant. segm. (B)	0.08–0.12 (0.10)	0.08–0.13 (0.10)	0.07–0.09 (0.08)	0.08–0.09 (0.08)	0.07–0.12 (0.10)	0.08–0.13 (0.10)	0.09–0.12 (0.11)	0.09–0.12 (0.10)
Processus terminalis (Pt)	0.18–0.30 (0.25)	0.21–0.38 (0.26)	0.15–0.20 (0.18)	0.23	0.20–0.30 (0.25)	0.23–0.38 (0.27)	0.23–0.30 (0.27)	0.23–0.30 (0.26)
Secondary Sensoria on III	0	7–16 (12)	0	20–22 (21)	0	7–16 (12)	0	5–14 (10)
Secondary sensoria on IV	0	1–10 (4)	0	12–16 (14)	0	3–10 (6)	0	0–4 (3)
Secondary sensoria on V	0	0	0	1–9 (5)	0	0	0	0–3 (1)
Longest seta on ant.segm. III	0.004–0.013 (0.010)	0.004–0.012 (0.008)	0.007–0.011 (0.009)	0.007–0.008 (0.007)	0.007–0.0.13 (0.011)	0.006–0.012 (0.009)	0.009–0.016 (0.13)	0.008–0.016 (0.012)
Siphunculi (SIPH)	0.13–0.30 (0.20)	0.13–0.25 (0.17)	0.08–0.11 (0.09)	0.09–0.10 (0.09)	0.14–0.30 (0.22)	0.16–0.25 (0.19)	0.21–0.34 (0.28)	0.14–0.24 (0.19)
Cauda (CA)	0.11–0.20 (0.16)	0.10–0.17 (0.13)	0.10–0.14 (0.12)	0.09–0.10 (0.09)	0.13–0.20 (0.17)	0.11–0.17 (0.15)	0.15–0.21 (0.19)	0.11–0.16 (0.14)
Width of tubercle on abd. segm. I	0.005–0.020 (0.013)	0.005–0.022 (0.013)	0.005–0.009 (0.007)	0.008	0.010–0.018 (0.010)	0.010–0.022 (0.015)	0.013–0.026 (0.021)	0.010–0.019 (0.015)
Width of tubercle on abd. segm. VII	0.013–0.029 (0.021)	0.009–0.027 (0.018)	0.007–0.012 (0.009)	0.008–0.017 (0.012)	0.018–0.029 (0.022)	0.014–0.030 (0.022)	0.014–0.028 (0.021)	0.012–0.025 (0.017)
Hind Tibia	0.48–0.80 (0.65)	0.56–0.89 (0.70)	0.37–0.48 (0.44)	0.54–0.56 (0.55)	0.58–0.80 (0.67)	0.64–0.89 (0.75)	0.67–0.99 (0.85)	0.63–0.94 (0.78)
Second hind tarsus (HT2)	0.08–0.12 (0.10)	0.08–0.12 (0.10)	0.07–0.09 (0.08)	0.08–0.09 (0.09)	0.09–0.12 (0.10)	0.09–0.11 (0.10)	0.09–0.12 (0.11)	0.09–0.11 (0.10)
Pt/B	2.0–3.1 (2.5)	2.1–3.1 (2.6)	2.1–2.5 (2.3)	2.6–2.8 (2.7)	2.0–3.7 (2.7)	2.3–3.3 (2.7)	2.2–2.8 (2.5)	2.2–2.7 (2.5)

Table 2. Continued.

	A. impatientis				*A. floridanae*		*A. nasturtii*	
Morphological characters/ Morphs	Apterae (n = 37)	Alatae (n = 43)	Oviparae (n = 8)	Male (n = 3)	Apterae (n = 23)	Alatae (n = 21)	Apterae (n = 22)	Alatae (n = 23)
URS/HT2	0.7–1.0 (0.8)	0.7–1.1 (0.8)	0.8–0.9 (0.9)	0.7–0.9 (0.8)	0.8–1.0 (0.9)	0.7–1.1 (0.9)	0.9–1.2 (1.0)	0.9–1.1 (1.0)
SIPH/CA	0.9–1.6 (1.3)	0.9–1.8 (1.3)	0.7–0.8 (0.8)	0.8–1.2 (1.0)	1.0–1.6 (1.3)	1.0–1.8 (1.3)	1.3–1.8 (1.5)	1.2–1.5 (1.4)
URS accesory setae	2	2	2	2	2–3 (2)	2–3 (2)	2–4 (2)	2
Caudal setae	4–10 (7)	4–10 (6)	5–6 (5)	6	6–10 (8)	6–10 (8)	6–10 (8)	6–9 (7)
Setae abd. tergite VIII	2–4 (3)	2–4 (2)	2	–	2–4 (3)	2–4 (2)	2	2
Anterior setae on subgenital plate	2–7 (4)	2–7 (4)	–	–	3–6 (4)	2–7 (4)	2	2–3 (2)
Marginal tubercles on II, III and IV	Absent	Absent	Absent	Absent	Absent	Absent	Present	Present

Table 3. Pairwise interspecific distances (%) of species morphologically associated to *Aphis impatientis* for COI calculated using Kimura 2-parameter.

	A. asclepiadis	*A. cornifoliae*	*A. fabae*	*A. gossypii*	*A. impatientis*	*A. nasturtii*	*A. rumicis*
A. cornifoliae	6.88						
A. fabae	7.75	7.82					
A. gossypii	8.77	9.75	7.39				
A. impatientis	6.96–7.05	1.25–1.41	7.99–8.26	9.84–10.01	0–0.39		
A. nasturtii	7.98–8.24	8.16	6.44	6.69	7.98–8.24		
A. rumicis	8.6–8.77	8.42	4.34	7.82	8.60–8.77	6.52	
A. salicariae	3.52	6.96	7.84	8.77	6.96–7.14	7.73	7.91

Aphis impatientis Thomas, 1878

Aphis floridanae Tissot, 1933, **new synonymy**

Diagnosis. Despite of the overlapping of the ratios: Pt/B, SIPH/CA and URS/HT2, they seem to be useful for discrimination and there are other descriptive characters that can be used for diagnosis. The apterous viviparae of *Aphis impatientis* can be distinguished from *A. nasturtii* by the color of siphunculi that are dark throughout and curved outwards. The antennal segments III of *A. impatientis* and *A. nasturii* have secondaria sensoria arranged in a row and scattered respectively. Also, both morphs of *A. impatientis* lack marginal tubercles on abdominal segments II–V while *A. nasturtii* has marginal tubercles on all abdominal segments. Lastly, the body color of both morphs of *A. impatientis* and *A. nasturtii* is brownish and greenish respectively. For more comparative morphometric data and photographs of these species, *A. salicariae* and *A. rumicis* see Lagos-Kutz et al. (2016) and Lagos-Kutz et al. (2018).

Description. Apterous viviparae (n = 37) (Table 2 and Fig. 1–6). *Color in life* (Fig. 14–15). Head**,** siphunculi and cauda black. Abdomen brown with white wax. Antennal segments one and six dusky, the other segments light-orange. Legs light-orange and dusky. Thorax and abdomen brown dusted with white wax. *Color on slide and morphological characters. Head:* dusky with 2 frontal setae. Antennal tubercles undeveloped. Antennae six- segmented, shorter than body. Antennal segments: one and six dusky; the other segments pale. Secondary sensoria absent on all antennal segments. Rostrum extending to mesocoxae. *Thorax:* Coxae dusky. Trochanters paler than coxae. Fore femora dusky. Middle and hind femora dark, pale near the base. Tibiae pale, darkening near distal tip. Tarsi dusky. *Abdomen:* Cauda dark, parallel sided and blunt. Siphunculi dark, curved outwards with flange. Marginal sclerites pale. Pre-siphuncular and post-siphuncular sclerites absent. Marginal tubercles present on abdominal segments I and VII, absent from II, III, and IV. Dorsum of abdomen without sclerites. Subgenital plate dusky, complete. Dorsal cuticle with reticulation.

Alate viviparae (**n = 42**) **(Table 2 and Fig. 7–11).** *Color in life* (Fig. 15). Head and thorax black. Abdomen brown, shiny, without wax. Wings dusky, transparent. Femora black, and tibiae light-yellow. *Color on slide and morphological characters. Head:* Dark with 2 frontal setae. Antennal tubercles undeveloped. Antennae six-segmented, shorter than body. All antennal segments dark. Antennal segments III and IV with secondary sensoria arranged in a single row. Rostrum does not reach the metacoxae. *Thorax:* Coxae dark. Trochanters paler than coxae. Fore femora dusky. Middle and hind femora dark except at the base. Tibiae pale, darkening near distal tip. Tarsi dark. *Abdomen:* Cauda dark, finger shaped. Siphunculi dark, weakly curved outwards with flange. Marginal sclerites dark. Pre-siphuncular sclerite absent. Post-siphuncular sclerite dark. Marginal tubercles present on abdominal segments I and VII. Dorsal abdomen with transverse sclerites on VII, and VIII. Subgenital plate dusky, complete.

Apterous oviparae (n = 8) (Table 2). *Color in life.* Same color pattern as apterous vivipara. *Color on slide and morphological characters* (Fig. 12). *Head:* Dusky without frontal setae. Antennal tubercles undeveloped. Antennae five-segmented, shorter than body. Antennal segments dusky. Rostrum reaches mesocoxae. Thorax: Coxae dusky. Trochanters pale. Fore femora pale throughout, mid and fore femora dusky except at base. Tibia pale throughout. Tarsi dusky. Hind tibia not swollen and without pseudosensoria. *Abdomen:* Siphunculi dusky, strongly curved outwards with reduced flange. Cauda dusky, oblong and pointed. Pre-siphuncular and post-siphuncular sclerites absent. Marginal tubercles present only on abdominal segments I and VII. Marginal tubercles on abdominal segments II, III, and IV absent. Dorsum of abdomen without sclerites. Subgenital plate dark, and divided.

Alate male (n = 3) (Table 2). *Color in life.* Same color pattern as alate vivipara. *Color on slide and morphological characters* (Fig. 13). *Head:* Dark without frontal setae. Antennal tubercles undeveloped. Antennae six-segmented, shorter than body. Antennae with secondary sensoria scattered on segments III, IV, and V. Antennal segments dusky. Rostrum reaches mesocoxae. *Thorax:* Coxae dusky. Trochanters pale. Fore femora pale throughout, mid and fore femora dusky except at base. Tibia pale, darkening near distal tip. Tarsi dusky. *Abdomen:* Siphunculi short and slightly curved outwards. Cauda dusky, oblong and pointed. Marginal tubercles present on abdominal segments I and VII. Male genitalia dark with 2 short claspers anteriorly and aedeagus centrally.

Discussion

Aphis floridanae is currently treated as a synonym of *A. nasturtii*. Morphological and molecular comparisons of multiple collections showed that these species are not synonymous (Fig. 1–11, Tables 2, 3). Instead, we conclude that *A. floridanae* Tissot, 1933 is a junior synonym of *A. impatientis* Thomas, 1878 and, in accordance with the Principle of Priority of the International Code on Zoological Nomenclature (Article 23, ICZN 1999), refer to *A. floridanae* as *A. impatientis*. The wide distribution of *A. impatientis* and its secondary host plants, such as *L. floridana*, *E. hieraciifolius* and native North American *Impatiens*, and primary host *Cornus* in North America (USDA-NRCS. 2018) have led to such an issue within the species, and there still more to explore about the distribution and host plant association of this species. Host alternation provides an opportunity for aphids to acquire new hosts and may be a key to the rapid diversification of some groups of aphids (Eastop 1971; Dixon 1973; von Dohlen and Moran 2000), but can result in problematic taxonomy when polyphagous species are described from different hosts. While traditional morphological techniques demonstrated that *A. floridanae* is distinct from *A. nasturtii* and similar to *A. impatientis*, we reaffirmed the usefulness of mitochondrial DNA sequences for matching aphid life stages as has been demonstrated within polyphagous aphid species on different host plants (Zhang et al. 2008; Coeur d'acier et al. 2014). Lastly, the genetic variation found in this study (0.39%) is not different from other polyphagous *Aphis* species, such as *A. asclepiadis* (Foottit et al. 2008; Lagos-Kutz et al. 2016), and *A. gossypii* (Foottit et al. 2008; Favret and Miller 2011; Lagos-Kutz et al. 2014).

Acknowledgments

Support was provided by funds from the North Central Soybean Research Program. Many thanks to Dr. Gary Miller, Research Leader of the USDA Beltsville ARS Systematic Entomology Laboratory, for sending Tissot's slides and for his quick correspondence, and Dr. James Haudenshield, Research Plant Pathologist, USDA-ARS, for input on improving sequences quality. We gratefully acknowledge the collaboration of Dr. Michael Skvarla, insect identification laboratory directory and extension educator, Pennsylvania State University, USA, and Dr. Nicholás Pérez-Hidalgo, researcher of the Department of genetics, University of Valencia, Spain, for their valuable comments that help to improve the quality of this manuscript.

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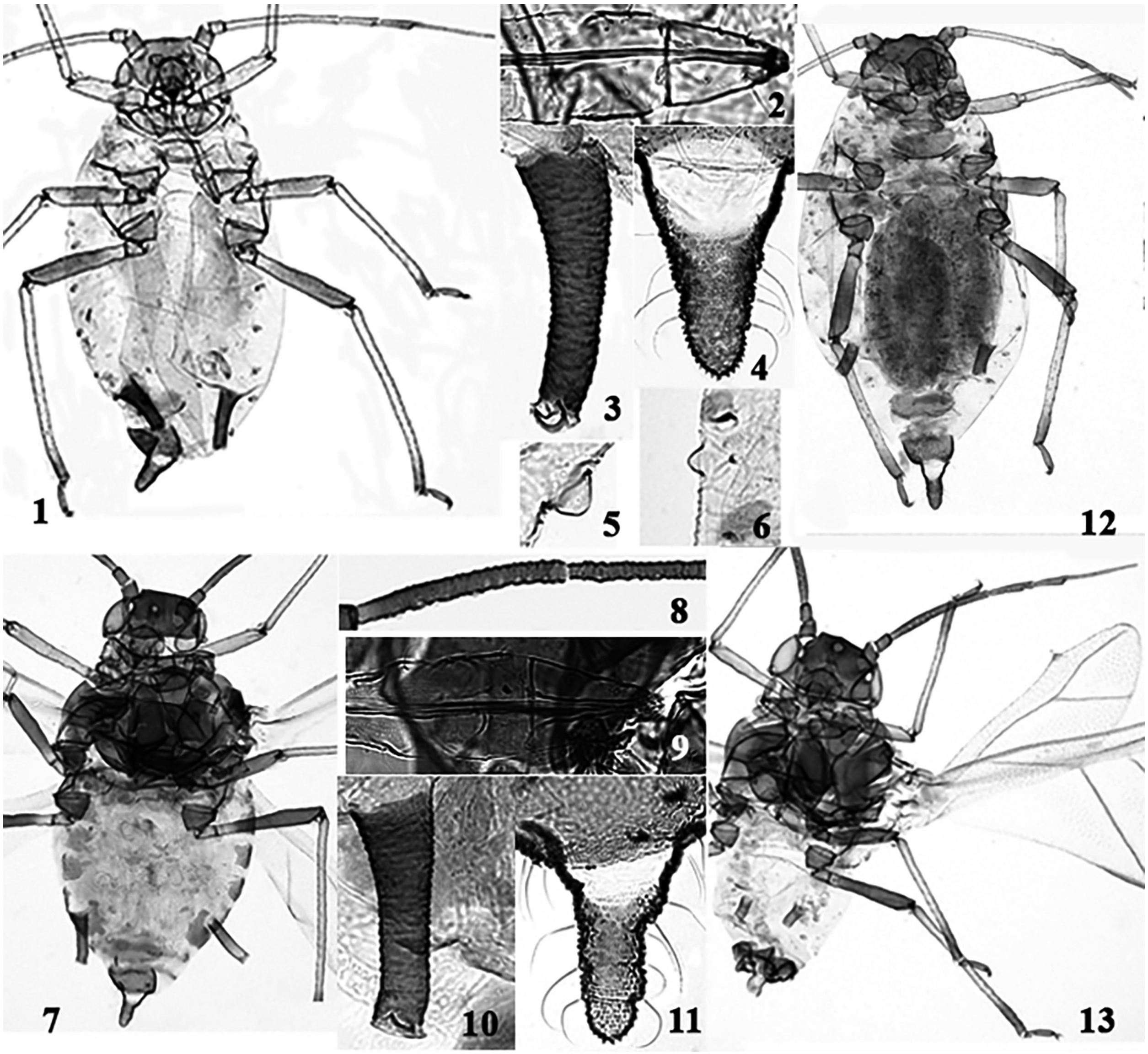
Received February 21, 2018; accepted March 30, 2018.
Review editor Joe Eger.



Figures 1–13. *Aphis impatientis*. **1–6)** Apterous vivipara. **1)** Body. **2)** Ultimate rostral segment. **3)** Siphunculus. **4)** Cauda. **5)** Marginal tubercle on abdominal segment VII. **6)** Marginal tubercle on abdominal segment I. **7-11)** Alate vivipara. **7)** Body. **8)** Antennal segments: III–IV. **9)** Ultimate rostral segment. **10)** Siphunculus. **11)** Cauda. **12)** Apterous ovipara, body. **13)** Alate male, body.

Figures 14–15. Colony of *Aphis impatientis*. **14)** On *Erechtites hieraciifolius*. Photograph: Lyle Buss, senior biological scientist. University of Florida, Institute of Food and Agricultural Sciences, Florida. **15)** On *Impatiens capensis*. Photograph: David Voegtlin, emeritus INHS of University of Illinois at Urbana-Champaign, Illinois.

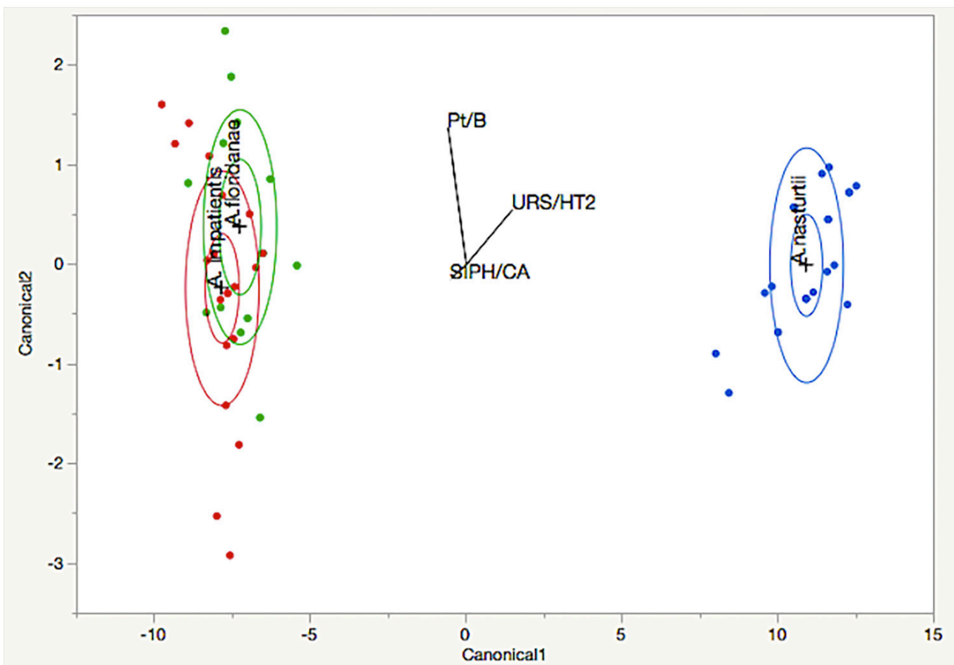


Figure 16. Discriminant analysis of 57 specimens of *Aphis impatientis*, *A. floridanae* and *A. nasturtii* based on the analysis of the ratios: Pt/B, USR/HT2 and SIPH/CA.