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Research

Taxonomy

Taxonomy and Natural History of Cattail Aphids, *Rhopalosiphum enigmae* (Hemiptera: Aphidomorpha: Aphididae), Including a New Synonymy and Notes on Ant and Parasitoid Associates of *Rhopalosiphum*

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Abstract

We designate a neotype for *Rhopalosiphum laconae* Taber 1993 and synonymize it with *Rhopalosiphum enigmae* Hottes and Frison 1931 (Hemiptera: Aphididae) based on geographic, morphological, and molecular evidence. We list 16 new state/province records and provide notes on morphology and natural history for *R. enigmae*. Additionally, we review and include new ant and parasitoid associates of *Rhopalosiphum* Koch, 1854 (Hemiptera: Aphididae).

Key words: Aphidomorpha, Aphidoidea, Aphididae, *Rhopalosiphum enigmae*

Cattails (Poales: Typhaceae: *Typha* L.) are one of the most recognizable wetland plants due to their generally large size, distinctive flower and seedheads, and tendency to form large single-species stands (Fig. 1). Four species are present in Eastern North America: the native *Typha latifolia* L. (broadleaf cattail) and *Typha domingensis* Pers. (southern cattail), introduced *Typha angustifolia* L. (narrowleaf cattail), and hybrid *Typha* ×*glauca* Godr. (*T. angustifolia* × *T. latifolia*) (hybrid or white cattail) (USDA 2017). Cattails provide many benefits to wildlife but can be considered a nuisance due to their rapid growth and tendency to form single-species stands. Insects can cause considerable damage and mortality to cattails, which may be viewed positively or negatively, depending on the situation (e.g., pestiferous invasive cattails or crop grown for biofuel), although in general their ecological importance is not well studied (Penko 1985). Most of the relatively few studies of cattail-feeding insects have focused on Lepidoptera (e.g., Cole 1931, Judd 1952, Beule 1979, Andrews et al. 1981, Penko et al. 1983, Cassani 1985, Penko and Pratt 1986a,b), although a couple have examined other taxa or the entire community (e.g., Claassen 1921, McDonald 1951, Penko 1985, Penko and Pratt 1987).

Twenty-four species of aphids (Hemiptera: Aphididae) have been recorded from *Typha* worldwide, to which Blackman and Eastop (2017) provided a key. The majority of aphid species recorded from *Typha* are polyphagous or feed mainly on Poaceae and Cyperaceae and use cattails only occasionally (Table 1). Only four aphid species feed primarily on *Typha*: the Palearctic *Aphis typhae* Mamontova, 1959 and *Schizaphis rosazevedoi* Ilharco, 1961 and Nearctic *Rhopalosiphum enigmae* (Hemiptera: Aphididae) Hottes and Frison 1931 and *Rhopalosiphum laconae* Taber 1993, which are the focus of this article and hereafter collectively referred to as cattail aphids.

Species of *Rhopalosiphum* Koch, 1854 are easily distinguished from other *Typha*-feeding aphids by having abdominal marginal tubercles I and VII that occur dorsal to adjacent spiracles and the apterae exhibit a polygonal reticulate pattern comprised of small spicules on the dorsum of the abdomen (Fig. 2). *R. enigmae* and *R. laconae* can be distinguished from their polyphagous congeners by the relatively longer, parallel-sided, and heavily imbricated siphunculi and longer processus terminalis (Table 2).

R. enigmae (Fig. 3) is widespread in North America wherever *Typha* occurs (Fig. 4), but little is known about its ecology. It is reportedly monoecious holocyclic on *Typha*, but has also been recorded from *Sparganium* L. (Blackman and Eastop 2017). Specimens are not commonly encountered in collections and some authors (e.g., Richards 1960) consider it a rare species in the environment. Individuals are typically found under *Typha* leaf sheaths, although Penko and Pratt (1987) reported that it was occasionally found in galleries of lepidopteran stem borers. A single hymenopteran parasitoid, *Lysiphlebus testaceipes* (Cresson, 1880),

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Fig. 1. Cattails (*Typha*).

Table 1. Aphids associated with *Typha*

Species	Range	Typical secondary host(s)
Aphis fabae (Scopoli, 1763)	Cosmopolitan	Polyphagous
Aphis gossypii (Glover, 1877)	Cosmopolitan	Polyphagous
Aphis typhae (Mamontova, 1959)	Palearctic (Ukraine)	*Typha*
Ceruraphis eriophori (Buckton, 1879)	Palearctic, Nearctic	Cyperaceae
Hyalopterus amygdali (Blanchard, 1840)	Palearctic, possibly Nearctic	*Phragmites*
Hyalopterus pruni (Geoffroy, 1762)	Nearctic, Palearctic	*Phragmites*, occasionally *Arundo donax*
Hysteroneura setariae (Thomas, 1878)	Nearctic, pantropical	Poaceae, occasionally Cyperaceae and seedling Arecaceae
Metopolophium dirhodum (Walker, 1849)	Pantemperate	Cyperaceae, Poaceae
Mordvilkoiella skorkini	Palearctic (Russia, Ukraine)	*Phragmites australis*
Myzus persicae (Sulzer, 1776)	Cosmopolitan	Polyphagous
Rhopalosiphum enigmae (Hottes and Frison 1931)	Nearctic	*Typha*
Rhopalosiphum laconae (Taber 1993)	Nearctic (North Carolina)	*Typha*
Rhopalosiphum maidis (Fitch, 1856)	Cosmopolitan, but cannot survive outdoors in regions with severe winter climates	Poaceae, occasionally Cyperaceae
Rhopalosiphum nymphaeae (Linnaeus, 1761)	Cosmopolitan	Polyphagous on aquatic and semi-aquatic plants
Rhoaplosiphum padi (Linnaeus, 1758)	Cosmopolitan	Polyphagous
Schizaphis rosazevedoi (Ilharco, 1961)	Ethiopian, Palearctic	*Strelitzia reginae, Typha*
Schizaphis rotundiventris (Signoret, 1860)	Nearly cosmopolitan, including Nearctic	*Cyperus*, occasionally Poaceae and other monocots (*Acorus*, young Arecaceae)
Schizaphis scirpi (Passerini, 1874)	Palearctic	Typhaceae, Cyperaceae, occasionally other wetland monocots (Araceae, Juncaceae, Iridaceae)
Sipha glyceriae (Kaltenbach, 1843)	Nearctic, Palearctic	Poaceae, especially wetland species; occasionally other monocots, including Alismataceae, Cyperaceae, Juncaceae, and Typhaceae, and Ceratophyllaceae
Sitobion avenae (Fabricius, 1775)	Nearly cosmopolitan, including Nearctic	Poaceae and other monocots, occastionally certain dicots
Sibobion fragariae (Walker, 1848)	Nearly cosmopolitan, including Nearctic	Poaceae

Modified from Blackman and Eastop (2017).

has been reported to attack the species (Supplementary Appendix 1), and although ants are known to attend other *Rhopalosiphum* species, no such interactions have been previously reported for *R. enigmae* (Supplementary Appendix 2). Hottes and Frison (1931) and Richards (1960) provided descriptions of the apterous and alate parthenogenic females, alate males, and apterous oviparae.

R. laconae is known only from the type series, which was collected from *Typha* at three localities in coastal North Carolina (Taber 1993). Nothing is known about its ecology, including associated parasitoids or ant associates. It is distinguished from *R. enigmae* by having larger lateral abdominal tubercles on segments 1 and 7 (those on 7 35–50 μm vs. 20–30 μm in basal diameter), having lateral abdominal tubercles on segments 2–6 always present rather than sporadically present (Fig. 5), and shorter processus terminalis (pt:base of antenna VI 4.0–5.0 vs. 4.6–6.3).

During collection efforts for a revision of *Rhopalosiphum*, cattail aphid specimens were collected from Maryland that exhibited characteristics intermediate between *R. enigmae* and *R. laconae*. Additional collections from West Virginia, Pennsylvania, and Delaware revealed a grade of morphology from that typical of

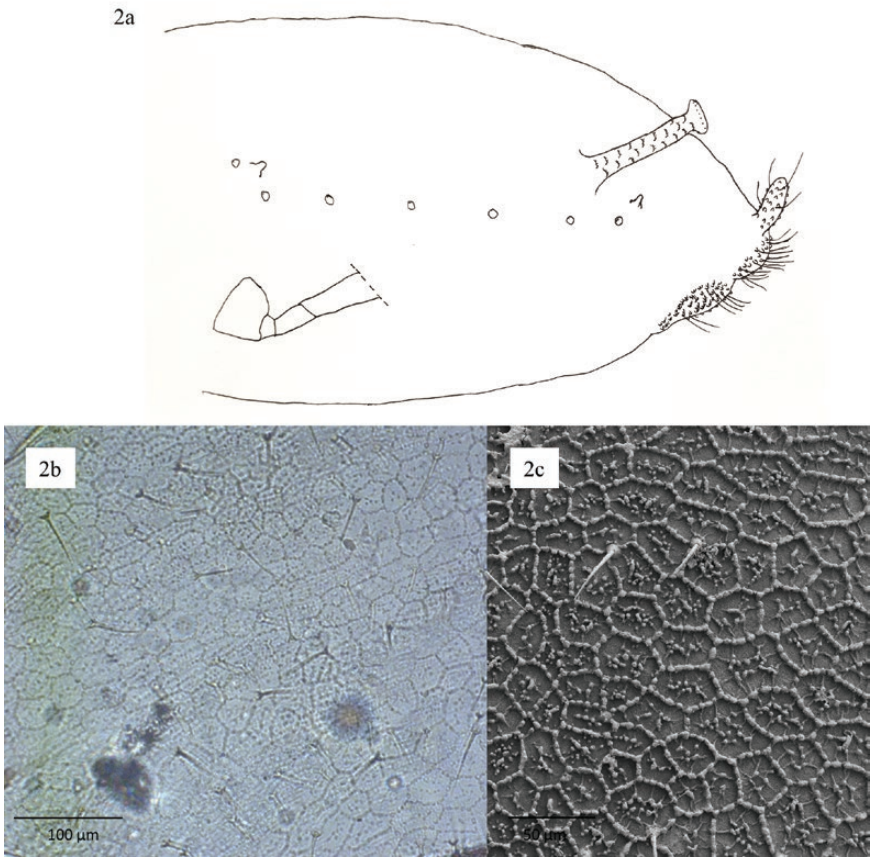

Fig. 2. Diagnostic characteristics of *Rhopalosiphum*. (a) Abdominal tubercles 1 and 7 dorsal of adjacent spiracles. (b) Compound micrograph of dorsal reticulate pattern of apterae. (c) LT-SEM image of dorsal reticulate pattern of apterae.

Table 2. Distinguishing characteristics of *Typha*-feeding *Rhopalosiphum*

Species	Siphunculi shape	Imbrications	Siphunculi:cauda (apterae)	Antennae pt:base of VI (apterae)	Siphunculi:cauda (alatae)	Antennae pt:base of VI (alatae)	*n* = apterae/ alatae
Rhopalosiphum enigmae	Parallel-sided	Heavy	2.3–3.0 (2.0–4.0)	4.5–6.0 (4.0–6.9)	2.2–2.5 (1.9–2.7)	4.8–5.6 (4.5–6.3)	120/32
Rhopalosiphum laconae	Parallel-sided	Heavy	2.2–2.7 (2.0–2.9)	4.8–5.8 (4.1–6.3)	1.9–2.3 (1.9–2.3)	5.7–6.3 (5.7–6.3)	54/5
Rhopalosiphum maidis	Parallel-sided	Heavy	1.2–1.5 (0.8–1.7)	1.8–2.3 (1.7–3.2)	1.2–1.4 (1.0–1.6)	2.0–2.5 (1.9–2.6)	91/46
Rhopalosiphum nymphaeae	Inflated apically	Light	2.0–2.5 (1.8–2.8)	3.3–4.0 (3.0–4.2)	1.9–2.4 (1.7–2.5)	3.4–4.0 (2.8–4.2)	58/13
Rhopalosiphum padi	Parallel-sided	Light	1.6–2.1 (1.3–2.3)	4.3–5.3 (3.3–5.5)	1.6–1.9 (1.4–2.1)	4.0–5.3 (3.6–5.7)	52/44

The first range noted encompasses at least 90% of the variability observed, while the range noted parenthetically encompasses the entire range observed.

R. enigmae through intermediates to that typical of *R. laconae*. Because the XXV International Congress of Entomology was fortuitously held in Orlando, FL, the authors decided to collect a transect of cattail-associated *Rhopalosiphum* from Maryland to Florida while en route, including the type localities of *R. laconae*. This resulted in fresh material for morphological and molecular investigations of the relationship between the two species, which is one topic of this article. During these collections, associated parasitoid wasps, ants, and coccinellids were found; this spurred an extensive literature search for records of parasitoids, ants, and coccinellids associated with *R. enigmae* and *Rhopalosiphum* more generally, which is also discussed.

Materials and Methods

Terminology

The following museum abbreviations follow Evenhuis (2017): National Museum of Natural History Aphidomorpha Collection (USNM) in Beltsville, MD; Florida State Collection of Arthropods (FSCA), Gainesville, FL.; Illinois Natural History Survey Insect Collection (INHS), Champaign, IL; North Carolina State University Insect Museum (NCSU), Raleigh, NC; and Canadian National Collection of Insects, Arachinds, and Nematodes (CNC), Ottawa, ON, Canada. Additional collection abbreviations include the personal collection of Andrew Jensen (AJ), Lakeview, OR; and Symbiota Collections of Arthropods Network (SCAN). Species names follow Favret (2017). Morphological terms were adapted from Foottit and Richards (1993). State and province abbreviations follow those of the USPS (2015) and Canada Post (2011).

Specimen Collection, Curation, and Identification

Cattail aphids were located by pulling back the outermost leaf sheaths of cattails and visually searching for aphids. Early in the season this was done in a random fashion; later in the season, aphid

Fig. 3. *R. enigmae* Hottes and Frison 1931. (b) Photo: Claude Pilon. (d) Photo: Tom Murray. Photos (b) and (d) used with permission.

Fig. 4. Range of *R. enigmae*. Closed circles represent individual collections, open circles represent state record without locality information. Locality information from Smith and Parron (1978), Penko and Pratt (1987), Murray (2009), and slide label data including SCAN specimens. A record from Newfoundland was not included for clarity. A specimen reported from Cuernavaca, Morelos, Mexico, was not examined and is not included for space and clarity.

colonies could often be more precisely located by scanning cattail stands for ant activity. Once found, aphids were collected into 95% ethanol using a camel hair brush, piece of grass, or other reasonably soft tool conveniently at hand. As aphids reproduce asexually during the summer, one individual or colony was typically collected per locality. If parasitized aphids were found, the cattail leaf was cut and stored in a 1 gallon self-sealing bag until the parasitoids emerged, whereupon they were stored in 70% ethanol. Ants and coccinellids associated with aphid colonies were also collected when encountered; they were initially stored in 70% ethanol and later point mounted for identification.

GPS coordinates of collection localities were measured using the GPS Status & Toolbox (MobiWIA Ltd. 2017) app on a Galaxy S7 mobile phone (Samsung, Seoul, South Korea). Ethanol-preserved specimens from three localities were obtained from collaborators.

Slide-mounted material for morphological investigation and biogeographic range construction were borrowed from the FSCA, INHS, NCSU, CNC, and AJ. Additional specimens were found by searching SCAN, though such material was not borrowed and used only for locality information.

The paratypes of *R. laconae* housed in the NCSU collection were not labeled as *R. laconae* or as paratypes. It was determined that the material examined consisted of the paratypes by matching the slide label data to the collection information provided in the original description by Taber (1993).

Aphids, ants, and coccinellids were identified by MJS and parasitoid and hyperparasitoid wasps were identified by Mike Gates, Matt Buffington, and Bob Kula (USDA-ARS Systematic Entomology Laboratory). Aphid species determinations were based on characters listed in the description of *R. laconae* (Taber 1993) and used to separate the species in keys by Blackman and Eastop (2017) (i.e., the

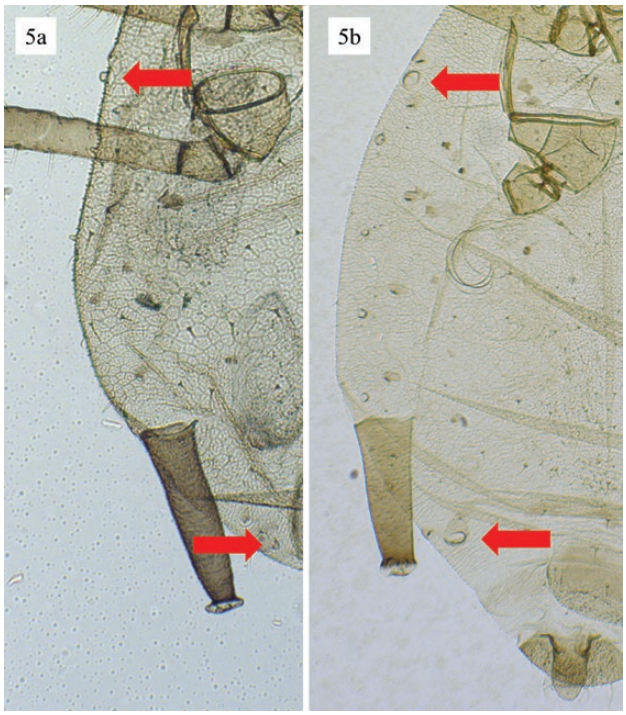

Fig. 5. Morphological comparison of *R. enigmae* (a) and *R. laconae* (b). Arrows indicate abdominal tubercles 1 and 7. Note that the apparent differences in the constriction at the apex of the siphunculi are artifacts of slide mounting.

presence/absence of abdominal tubercles 2–6 and size of abdominal tubercles 1 and 7 as observed in slide-mounted individuals) and by comparison to the type series of both species and material available in USNM. Ants were identified by eye and using the keys by Fisher and Cover (2007) and Coovert (2005) and information and images available on AntWeb (2017); *Crematogaster pilosa* identifications were confirmed by James Trager. Coccinellids were identified by eye.

Photographs of aphid colonies were taken in the field with the same Galaxy S7 mobile phone. Stereomicrographs of individual aphids were taken through the eye piece of a Wild M8 stereomicroscope (Wild, now a subsidiary of Leica, Wetzlar, Germany) using the mobile phone. Specimens were cleared using KOH, processed through a dehydration series, and mounted in Canada balsam following standard procedures (Miller et al. 2013). Slide-mounted specimens were examined using a Leica DMN compound microscope. Compound micrographs and measurements were made using AxioVision (Zeiss 2013) implemented through a Zeiss Axio Imager M1 microscope (Carl Zeiss Microscopy, Oberkochen, Germany). Focus stacked compound micrographs were created using Helicon Focus (Helicon 2016). Measurements are in micrometers (μm).

Low-Temperature SEM

Specimens were observed in the low-temperature scanning electron micrographs (LT-SEM) as described in Bolton et al. (2014). Briefly, the specimens preserved in 70% ethanol or were obtained from fresh tissue; secured to 15 cm × 30 cm copper plates using ultra smooth, round (12 mm diameter), carbon adhesive tabs (Electron Microscopy Sciences, Inc., Hatfield, PA). The specimens were frozen conductively, in a Styrofoam box, by placing the plates on the surface of a pre-cooled (–196°C) brass bar whose lower half was submerged in liquid nitrogen (LN_2). After 20–30 s, the holders containing the frozen samples were transferred to a Quorum PP2000 cryo-prep chamber (Quorum Technologies, East Sussex, UK) attached to an S-4700 field emission scanning electron microscope (Hitachi High Technologies America, Inc., Dallas, TX). The specimens were etched inside the cryo-transfer system to remove any surface contamination (condensed water vapor) by raising the temperature of the stage to –90°C for 10–15 min. Following etching, the temperature inside the chamber was lowered below –130°C, and the specimens were coated with a 10 nm layer of platinum using a magnetron sputter head equipped with a platinum target. The specimens were transferred to a pre-cooled (–130°C) cryostage in the SEM for observation. An accelerating voltage of 5kV was used to view the specimens. Images were captured using a 4pi Analysis System (Durham, NC). Individual images were re-sized and placed together to produce a single figure using Adobe Photoshop CS 5.0.

Molecular Methods

R. enigmae, *R. laconae*, *Rhopalosiphum musae* (Schouteden, 1906), and *Rhopalosiphum nymphaeae* (Linnaeus, 1761) specimens were sent to the Foottit laboratory at the CNC and Matthew Lewis at the USDA-ARS Systematic Entomology Laboratory for DNA extraction and sequencing. The two labs employed the following protocols:

USDA-ARS-SEL: DNA was extracted from whole bodies using the DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA). PCR amplification of the DNA barcode region of cytochrome c oxidase subunit I (COI) was performed using primers PcoF1 (Park et al. 2010) and LepR1 (Hebert et al. 2004). PCRs were performed on a Tetrad 2 thermocycler (Bio-Rad, Hercules, CA) with the following 'touchdown' program: initial denaturation for 2 min at 92°C, 12 touchdown cycles from 58 to 46°C (10 s at 92°C, 10 s at 58–46°C, 1 min at 72°C), 27 cycles at 10 s at 92°C, 10 s at 45°C, 1 min at 72°C, and a final extension for 7 min at 72°C. PCR products were enzymatically purified for sequencing using ExoSAP-IT (Affymetrix, Santa Clara, CA). Sequences were generated with the amplifying primers using the BigDye Terminator v3.1 Sequencing kit (Applied Biosystems, Foster City, CA) and fractionated on an ABI 3730XL Genetic Analyzer. Raw sequences were edited and aligned in Geneious R10 (Biomatters, New Zealand).

CNC: DNA was extracted non-destructively from whole bodies using modified CTAB chloroform/phenol/ extraction and PCR was performed using primers LCO1490 and HCO2198 (Folmer et al. 1994) on a Eppendorf Mastercycler with program as follows: initial denaturation for 2 min at 95°C, 5 cycles of 15 s at 95°C, 20 s at 45°C, 1 min at 72°C, 30 cycles of 15 s at 95°C, 20 s at 51°C, 1 min at 72°C, and a final extension for 10 min at 72°C. Subsequent processing as above.

Resulting sequences from both labs were checked for contamination with BLASTn searches of NCBI's nr database. Sequences and specimen records have been deposited in the Barcode of Life Data System (BOLD) and GenBank (Table 3).

When multiple specimens were collected from a single plant or multiple collections were taken at a single locality, DNA extracted and amplified from 2 to 3 specimens in order to ensure that at least one specimen from every locality was successfully sequenced; in a few instances, individuals from the same locality were sequenced by both labs. Identical genetic sequences were recovered in every case in which multiple specimens were sequenced from the same locality. Such duplicate sequences were excluded from further phylogenetic analyses. After DNA extraction, aphid cuticles were slide mounted and assigned to species as described above.

Phylogenetic Analyses

Outgroups for the following analyses included *Melanaphis donacis* (Theobald, 1917), *R. musae*, and *R. nymphaeae*. *M. donacis*, a

Table 3. Collection information and GenBank accession numbers

Species	State/ province	County	Locality	Coordinates	Plant association/ collection method	Date	Collected by	Collection number	GenBank Accession number
M. donacis									KF639526.1
R. enigmae	AB	Vermilion River	Vermilion Provincial Park	53°21.690′N, 110°52.200′W	*Typha latifolia*	12-June-2009	E. Maw, R.G. Foottit	CNC#HEM063600	GU668768
R. enigmae	AB	Bonnyville	Truman, Hwy 55 at Sand River	54°28.074′N, 111°11.160′W	*Typha latifolia*	14-July-2009	E. Maw, R.G. Foottit	CNC#HEM063643	GU668766
R. enigmae	ON	Ottawa	Mer Bleu Cons. Area	45°24.252′N, 75°33.882′W	*Typha latifolia*	1-August-2008	E. Maw et al.	CNC#HEM061388	KR038471.1
R. enigmae	ON	Ottawa	Ottawa	45°23.652′N, 75°42.198′W	*Typha latifolia*	25-September-2009	G. Miller, E. Maw	CNC#HEM064177	GU668786
R. enigmae	DE	Kent	Felton	39°00.730′N, 75°35.746′W	*Typha*	28-July-2016	M. J. Skvarla & G. Miller	MS 16-0728-006	MF123452
R. enigmae	DE	Sussex	Georgetown	38°44.570′N, 75°25.602′W	*Typha*	28-July-2016	M. J. Skvarla & G. Miller	MS 16-0728-001	MF123417
R. enigmae	DE	Sussex	Selbyville	38°28.778′N, 75°14.093′W	*Typha*	28-July-2016	M. J. Skvarla & G. Miller	MS 16-0728-004	
R. enigmae	DE	Sussex	Selbyville	38°28.778′N, 75°14.093′W	*Typha*	28-July-2016	M. J. Skvarla & G. Miller	MS 16-0728-005	MF123422
R. enigmae	FL	Collier	Immokalee		Suction trap	10–17-February-2016	S. Halbert	E2016-536-2	MF123420
R. enigmae	FL	Collier	Immokalee		Suction trap	24-February-2016–2-March-2016	S. Halbert	E2016-731-1	MF123449
R. enigmae	FL	Polk	Lake Alfred	28°08.869′N, 81°44.301′W	*Typha*	25-September-2016	M. J. Skvarla	MS 16-0925-001	MF123434
R. enigmae	FL	Polk	Winter Haven	28°03.350′N, 81°44.050′W	suction trap	11–18-February-2016	S. Halbert	E2016-535-2	MF123437
R. enigmae	FL	St. Johns	St. Augustine	29°54.959′N, 81°24.846′W	*Typha*	22-September-2016	M. J. Skvarla	MS 16-0922-001	MF123448
R. enigmae	FL	Walton	Point Washington	30°22.185′N, 86°06.391′W	*Typha*	25-April-2016	K. E. Schnepp	KS 16-0425-001	MF123433
R. enigmae	GA	McIntosh	Darien	31°22.536′N, 81°25.862′W	*Typha*	22-September-2016	M. J. Skvarla	MS 16-0922-003	MF123435
R. enigmae	MD	Anne Arundel	Russett	39° 06.267′N, 76°48.004′W	*Typha*	10-May-2016	M. J. Skvarla	MS 16-0510-001	MF123421
R. enigmae	MD	Anne Arundel	Russett	39° 06.267′N, 76°48.004′W	*Typha*	21-April-2016	M. J. Skvarla	MS 16-0421-001	MF123425
R. enigmae	MD	Anne Arundel	Russett	39° 06.267′N, 76°48.004′W	*Typha*	19-April-2017	M. J. Skvarla	MS 16-0419-001	
R. enigmae	MD	Dorchester	Linkwood	38°33.105′N, 75°57.521′W	*Typha*	28-July-2016	M. J. Skvarla & G. Miller	MS 16-0728-009	MF123453, MF123430
R. enigmae	MD	Frederick	Frederick	39°22.860′N, 77°24.410′W	*Typha*	15-June-2016	M. J. Skvarla	MS 16-0615-001	MF123450, MF123447
R. enigmae	MD	Queen Anne's	Chester	38°57.294′N, 76°17.981′W	*Typha*	28-July-2016	M. J. Skvarla & G. Miller	MS 16-0728-002	MF123432
R. enigmae	MD	Queen Anne's	Grasonville	38°57.850′N, 76°13.253′W	*Typha*	28-July-2016	M. J. Skvarla & G. Miller	MS 16-0728-008	MF123424, MF123439
R. enigmae	MD	Queen Anne's	Grasonville	38°57.850′N, 76°13.253′W	*Typha*	28-July-2016	M. J. Skvarla & G. Miller	MS 16-0728-010	
R. enigmae	MD	Talbot	Easton	38°49.014′N, 76°03.686′W	*Typha*	28-July-2016	M. J. Skvarla & G. Miller	MS 16-0728-003	MF123418, MF123445
R. enigmae	MD	Washington	Hancock	39°42.142′N, 78°11.429′W	*Typha*	15-July-2016	M. J. Skvarla	MS 16-0715-002	
R. enigmae	MD	Washington	Hancock	39°42.142′N, 78°11.429′W	*Typha*	15-July-2016	M. J. Skvarla	MS 16-0715-003	
R. enigmae	MD	Wicomico	Salisbury	38°22.178′N, 75°32.160′W	*Typha*	28-July-2016	M. J. Skvarla & G. Miller	MS 16-0728-007	MF123431

Table 3. Continued

Species	State/ province	County	Locality	Coordinates	Plant association/ collection method	Date	Collected by	Collection number	GenBank Accession number
R. enigmae	NC	Brunswick	Bolivia	34°02.502′N, 78°14.617′W	*Typha*	21-September-2016	M. J. Skvarla	MS 16-0921-011	
R. enigmae	NC	Carteret	Bouge	34°41.834′N, 77°03.287′W	*Typha*	21-September-2016	M. J. Skvarla	MS 16-0921-010	
R. enigmae	NC	Craven	Ernul	35°14.611′N, 77°03.616′W	*Typha*	21-September-2016	M. J. Skvarla	MS 16-0921-001	
R. enigmae	NC	Craven	New Bern	35°02.983′N, 77°00.086′W	*Typha*	21-September-2016	M. J. Skvarla	MS 16-0921-002	
R. enigmae	NC	Halifax	Tillery	36°13.909′N, 77°27.336′W	*Typha*	20-September-2016	M. J. Skvarla	MS 16-0920-004	
R. enigmae	NC	Onslow	Jacksonville	34°44.522′N, 77°29.848′W	*Typha*	21-September-2016	M. J. Skvarla	MS 16-0921-005	
R. enigmae	NC	Onslow	Swansboro	34°41.544′N, 77°07.147′W	*Typha*	21-September-2016	M. J. Skvarla	MS 16-0921-003	
R. enigmae	NC	Onslow	Swansboro	34°41.544′N, 77°07.147′W	*Typha*	21-September-2016	M. J. Skvarla	MS 16-0921-004	
R. enigmae	NC	Pender	Montague	34°27.275′N, 78°03.059′W	*Typha*	21-September-2016	M. J. Skvarla	MS 16-0921-009	
R. enigmae	NC	Pender	Watha	34°38.618′N, 77°54.196′W	*Typha*	21-September-2016	M. J. Skvarla	MS 16-0921-006	
R. enigmae	NC	Pitt	Greenville	35°30.425′N, 77°19.405′W	*Typha*	21-September-2016	M. J. Skvarla	MS 16-0921-007	
R. enigmae	NC	Pitt	Greenville	35°30.425′N, 77°19.405′W	*Typha*	21-September-2016	M. J. Skvarla	MS 16-0921-008	
R. enigmae	PA	Adams	Gettysburg NMP	39°48.106′N, 77°14.078′W	*Typha*	09-June-2016	M. J. Skvarla	MS 16-0609-001	
R. enigmae	PA	Bedford	Bedford	40°02.120′N, 78°31.200′W	*Typha*	15-July-2016	M. J. Skvarla	MS 16-0715-004	MF123440
R. enigmae	PA	Lancaster	Lancaster	40°02.602′N, 76°14.706′W	*Typha*	4-June-2016	M. J. Skvarla	MS 16-0603-001	
R. enigmae	PA	Lancaster	New Danville	39°59.175′N, 76°19.523′W	*Typha*	4-June-2016	M. J. Skvarla	MS 16-0603-002	
R. enigmae	PA	Westmoreland	Ligonier	40°15.874′N, 72°16.021′W	*Typha*	15-July-2016	M. J. Skvarla	MS 16-0715-005	MF123416, MF123443
R. enigmae	PA	Westmoreland	Paintertown	40°22.115′N, 79°42.011′W	*Typha*	6-May-2016	M. J. Skvarla	MS 16-0506-001	
R. enigmae	PA	Westmoreland	Paintertown	40°22.115′N, 79°42.011′W	*Typha*	6-May-2016	M. J. Skvarla	MS 16-0506-002	
R. enigmae	SC	Colleton	Waterboro	32°52.612′N, 80°42.765′W	*Typha*	22-September-2016	M. J. Skvarla	MS 16-0922-002	MF123427, MF123426
R. enigmae	SC	Jasper	Hardeeville	32°16.336′N, 81°04.678′W	*Typha*	22-September-2016	M. J. Skvarla	MS 16-0922-004	MF123451, MF123444
R. enigmae	SC	Sumter	Pinewood	33°44.301′N, 80°27.855′W	*Typha*	22-September-2016	M. J. Skvarla	MS 16-0922-005	MF123413
R. enigmae	VA	Chesterfield	Chester	37°21.139′N, 77°24.199′W	*Typha*	20-September-2016	M. J. Skvarla	MS 16-0920-002	
R. enigmae	VA	Chesterfield	Chester	37°21.139′N, 77°24.199′W	*Typha*	20-September-2016	M. J. Skvarla	MS 16-0920-003	
R. enigmae	WV	Raleigh	Bradley	37°52.831′N, 81°13.521′W	*Iris*?	7-May-2016	M. J. Skvarla	MS 16-0507-001	MF123414, MF123436
R. enigmae	WV	Raleigh	Bradley	37°52.831′N, 81°13.521′W	*Iris*?	7-May-2016	M. J. Skvarla	MS 16-0507-002	MF123415
R. musae	OR	Lake	8 mi NW of Lakeview		*Prunus subcordata*	3-May-2016	A. Jensen	MS 16-1206-001	MF123446, MF123419
R. musae									EU179242.1
R. nymphaeae	BC	Vancouver	Vancouver, Queen Elizabeth Park	54°41.160′N, 124°56.220′W	*Callitriche stagnali*	12-June-2005	C.-K. Chan	CNC#HEM054279	KR045003.1
R. nymphaeae	MD	Frederick	Adamstown	39°17.696′N, 77°25.887′W	*Nymphaea*	14-September-2016	M. J. Skvarla	MS 16-0914-001	MF123438, MF123441, MF123442
R. nymphaeae	ON	Ottawa	Ottawa	45°23.580′N, 75°42.240′W	*Butomus umbellatus*	2-October-2009	E. Maw	CNC#HEM064179	
R. nymphaeae	OR	Lake	3 mi N of Lakeview		*Alisma*	1-September-2016	A. Jensen	MS 16-1206-002	MF123411

member of the subtribe Rhopalosiphina, is thought to be closely related to *Rhopalosiphum* and was used to root the phylogenetic tree. The COI sequence for *M. donacis* was obtained from GenBank (Table 3). *R. musae* and *R. nymphaeae* were included in order to help root the phylogenetic tree, and to determine the percent difference in COI between species and thus provide a baseline comparison for the percent difference in COI between *R. enigmae* and *R. laconae*. In addition to COI sequences produced de novo and available through GenBank, seven *R. enigmae*, one *R. nymphaeae*, and one *R. musae* were available through the Barcode of Life Database and included in analyses (Table 3).

Bayesian analyses were performed with MrBayes (3.2.6) using the Extreme Science and Engineering Discovery Environment (XSEDE) infrastructure on the Cipres Portal (Miller et al. 2010). Each analysis consisted of four simultaneous runs, each with four chains sampling every 1,000 generations for 1.11 million generations, under a GTR+I+Γ model of molecular evolution. The analysis was automatically ended when the split frequencies fell below 0.01; 25% of the trees were discarded as burn-in. The resulting majority-rule consensus trees were viewed with Dendroscope 3 (v. 3.5.7) (Huson and Scornavacca 2012); tree image files were then exported in PDF format and edited for final figures in Adobe Illustrator CS6 (Adobe Systems, San Jose, CA).

Percent difference in COI was determined by comparing sequence data for pairs of individuals in BioEdit (Hall 1999) using the 'calculate identity/similarity for two sequences' function. At least one such pair comparison was made within and between each clade and additional within-clade comparisons were made when multiple morphologies existed within a single clade, such that 19 pair comparisons were made within the larger *R. enigmae* + *R. laconae* clade, 1 pair comparison was made within the *R. musae* clade, 2 pair comparisons were made within the *R. nymphaeae* clade, and 1 pair comparison was made between *R. enigmae* + *R. laconae* and each of the outgroup clades.

Deposition

Freshly collected aphid specimens, aphids processed for molecular investigations by the USDA-ARS Systematic Entomology Laboratory, and the neotype of *R. laconae* were deposited in the USNM Aphidoidea Collection. Specimens sent to the Foottit laboratory for molecular investigations were deposited in the CNC. Ant, coccinellid, and parasitoid wasp specimens are deposited in the appropriate USNM collections.

Nomenclature

This article has been registered in Zoobank (www.zoobank.org). The LSID number is: urn:lsid:zoobank.org:pub:DE305539-03BD-473E-AA5B-87B079E61E0E

Results and Discussion

R. laconae Types

Holotype and paratypes of *R. laconae* were reported to be deposited in the USNM (Taber 1993); however, an extensive search, including correspondence with the author, did not find any such specimens. It is unclear whether the specimens were ever deposited or perhaps lost after deposition, but they apparently no longer exist. In order to avoid future confusion about the identity of *R. laconae* due to the lack of a name-bearing specimen, an apterous female paratype collected from the type locality previously housed at NCSU was designated as the neotype (Fig. 6) and deposited in the USNM collection.

Collections and Phylogenetic Analysis

Forty collections of cattails aphids were made across nine U.S. states, including within a few miles of the type locality of *R. laconae*; specimens with morphology corresponding to *R. enigmae*, *R. laconae*, and forms with intermediate morphology were found (Fig. 7, Table 3). *R. laconae* is present along the east coast of the United States from Delaware south through Georgia, a much larger range than originally reported. However, forms with morphology intermediate between *R. enigmae* and *R. laconae* exist throughout much of the range and especially near areas where *R. enigmae* occurs. These intermediate forms include specimens with small abdominal marginal tubercles 1 and 7 but abdominal marginal tubercles 2–6 always present, specimens with large abdominal marginal tubercles 1 and 7 but abdominal marginal tubercles 2–6 sporadically present or absent, *R. laconae* specimens with a long processus terminalis (up to a ratio of pt: base of antenna VI of 6.3), and specimens with long and short dorsal abdominal setae. Examining morphology alone, it

Fig. 6. Neotype slide of *R. laconae*.

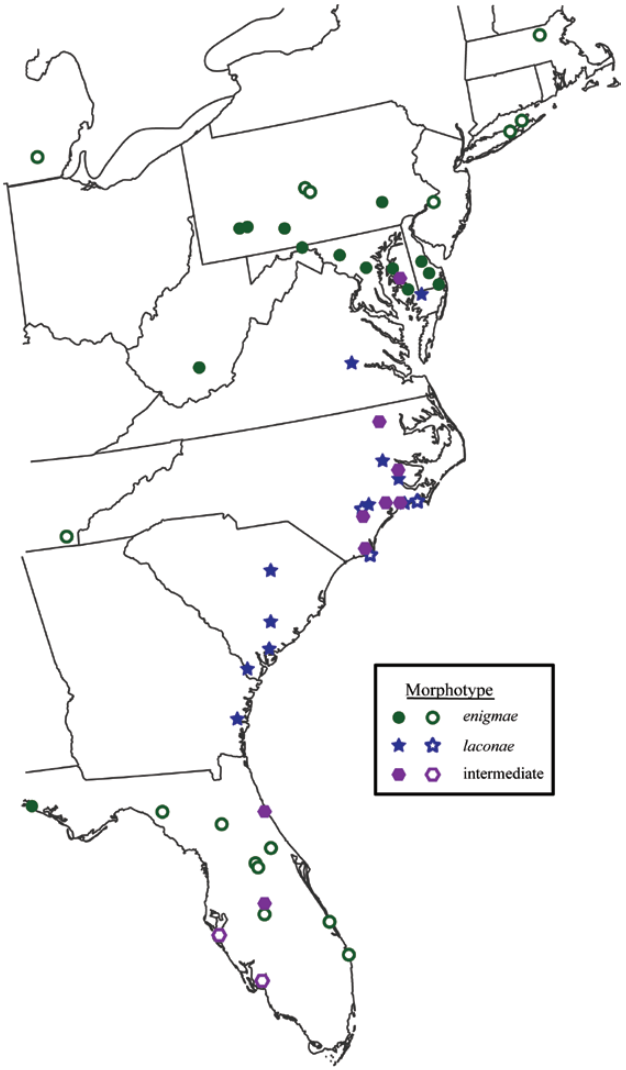

Fig. 7. Map of cattail aphid collections. Closed symbols represent collections included in the phylogenetic analysis, open symbols represent locality records without corresponding DNA.

was unclear whether *R. enigmae* and *R. laconae* are separate species with large hybrid zones or if they are a single species that exhibits a continuum of morphology across a large geographic area.

COI sequence data were obtained for 69 *R. enigmae* or *R. laconae* individuals from 49 localities (Table 3), 49 of which were included in the analyses. Additionally, COI sequence data were obtained for six *R. musae* and four *R. nymphaeae* individuals from two and three localities, respectively. The resultant phylogenetic hypothesis had well-supported (posterior probably >95%) clades that corresponded to *R. nymphaeae*, *R. musae*, and *R. enigmae* + *R. laconae* (Fig. 8). Within the *R. enigmae* + *R. laconae* clade there was some structure, including three clades that were well supported, two of which contain either *R. enigmae* or *R. laconae* exclusively. However, *R. enigmae*, *R. laconae*, and intermediate forms were interspersed throughout the larger *R. enigmae* + *R. laconae* clade, so the two well-supported subclades are better explained by their geographic closeness than by morphological similarity.

R. nymphaeae, *R. musae*, and *R. enigmae* + *R. laconae* exhibited less than 1% difference in COI within each clade and 4.6–7% difference between clades (Table 4). This level of variation is typical of within- and between-species difference in COI reported in *Rhopalosiphum* (Valenzuela et al. 2009) and other aphids (Foottit et al. 2008, Foottit et al. 2009, Wang et al. 2011, Rebijith et al. 2013).

The lack of genetic differentiation within COI, lack of phylogenetic structure within the *R. enigmae* + *R. laconae* clade, and morphological gradation from *R. enigmae* through intermediate forms into *R. laconae* along a geographic gradient strongly suggest that *R. enigmae* and *R. laconae* are not separate species. We therefore declare that *R. laconae* is a junior synonym of *R. enigmae*.

New State Records

R. enigmae has been previously reported from CA, CO, FL, ID, IL, LA, MN, NC, NJ, NY, OK, PA, UT, BC, AB, MB, NB, ON, QC, and SK (Hottes and Frison 1931, Smith and Parron 1978, Taber 1993, Maw et al 2000). The species is newly recorded from DE, GA, MA, MD, MI, NE, SC, TN, OR, VA, WA, WV, NL, NS, PE, and Morelos, Mexico.

Notes on Morphology

After the synonymization of *R. laconae* with *R. enigmae*, the following characters should be expanded to include the diversity found in *R. laconae*. Abdominal marginal tubercles 1 and 7 can be small to large (those on segment 7 20–50 µm in basal diameter), rather than small (those on segment 7 20–30 µm in basal diameter); abdominal tubercles 2–6 present or absent; and the ratio of the processus terminalis to the base of antennal segment VI 4.0–6.3.

Hottes and Frison (1931) and Richards (1960) provided descriptions of *R. enigmae* alate and apterous viviparae, ovipare, males, and nymphs. We expand upon those works and note morphological variation not included in earlier descriptions. Unless otherwise indicated, these notes pertain to apterous vivipara.

The body color of living specimens has been described as 'dark reddish brown to greenish brown' (Hottes and Frison 1931). While most specimens are reddish brown (Fig. 3a,f), a minority of specimens are light to dark green (Fig. 3b,c) and may exhibit a faint red patch between the siphunculi similar to that found in *R. padi* (Linnaeus, 1758), or dark brown (Fig. 3d). The color of living nymphs, which has not previously been noted, is light yellow to umber (Fig. 3f).

While Hottes and Frison (1931) noted that nymphs 'usually [have] five-segmented antennae', adults have been described as having antennae with six segments; the character was considered stable enough that Richards (1960) used it in his key to *Rhopalosiphum* species. However, 14.7% (25/170) of specimens examined had antennal segments III and IV fused, which would be considered five-segmented. Additionally, we found that when the character is present, many, if not all, of the individuals in a colony had fused antennal segments, so examining a series of individuals collected from one locality may not be helpful.

The length and shape of dorsal abdominal setae (long and pointed or short and capitate), which is measured in relation to the width of the siphunculi, is used to separate some species of *Rhopalosiphum*. *R. enigmae* has been described as having setae 'equal to or much longer than diameter of the [siphunculi] just proximal to the flange' (Richards 1960). However, we collected multiple colonies in which individual aphids had long or short setae and a single individual that had long and short setae on opposite sides of the body! The ratio of abdominal setae VIII to the width of the base of the siphunculi ranged from 0.16 to 1.21 (mean = 0.52, median = 0.51, n = 126).

Some *Rhopalosiphum* species have distinctive patterns of wax; *R. nymphaeae*, for instance, has wax on the legs, cauda, lateral thorax, and a strip of wax medially on the head that is obvious without

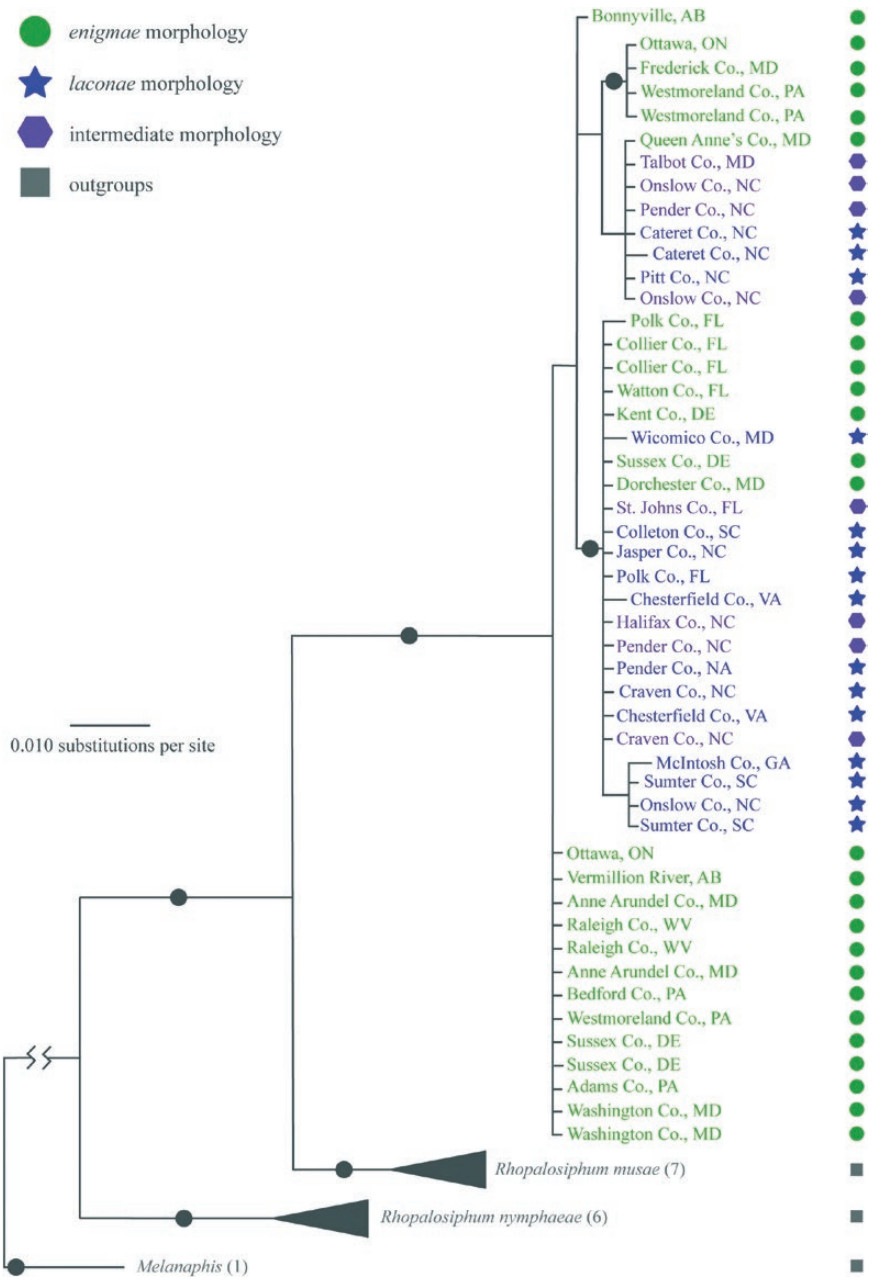

Fig. 8. Phylogenetic hypothesis inferred using Baysean analysis based on COI sequence data. Posterior probabilities greater than 95% are represented by black circles.

Table 4. Percent difference in COI within each clade

	R. enigmae and *R. laconae*	*R. musae*	*R. nymphaeae*	*Melanaphis pyraria*
R. enigmae and *R. laconae*	0–0.7			
R. musae	4.6	0.0		
R. nymphaeae	7.0	6.4	0.4	
M. pyraria	9.0	8.9	8.1	-

magnification. However, *Rhopalosiphum* wax patterns have been investigated little as the wax is destroyed when aphids are cleared in KOH and slide mounted. In *R. enigmae*, Hottes and Frison (1931) noted that alate viviparae have a 'pair of small wax glands on the anterior ventro-lateral region' of the mesothorax, but did not mention wax further and wax is generally not apparent in live or unmounted specimens in ethanol. When wax is apparent, it is confined to the legs, antennae, and dorsum of the head (Fig. 3b). When examined using LT-SEM, every apterous adult and nymph exhibited this wax pattern (Fig. 9). In addition to large wax extrusions visible using a stereomicroscope, LT-SEM images revealed a wax pruinescense on *R. enigmae* covering everywhere examined except the apex of the tibia, tarsi, and apex of

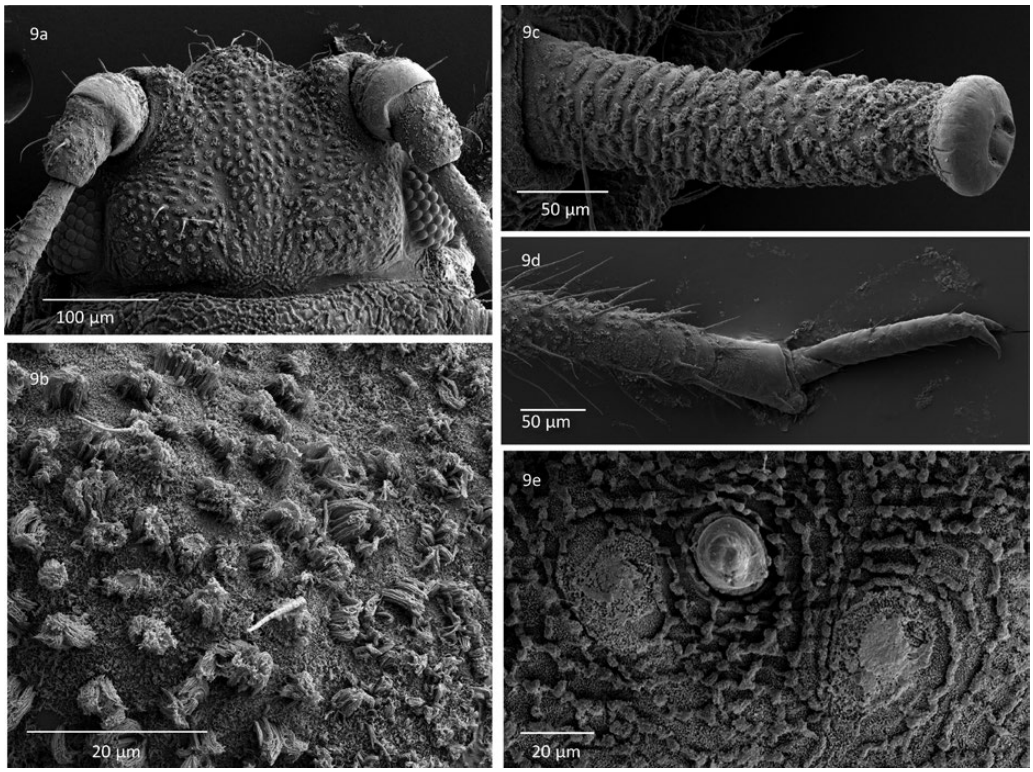

Fig. 9. LT-SEM micrographs of *R. enigmae*. Images taken from multiple specimens. (a) Head. (b) Close up of head showing wax blooms and waxy powder between blooms. (c) Siphunculus. (d) Hind leg. (e) Abdominal spiracles 1 and 2 and marginal abdominal tubercle 1. Note the waxy plates over the spiracles.

the siphunculus. Additionally, the spiracles are apparently covered with a waxy plate. The function of this wax is unknown, but should be investigated further as it may prove useful in species recognition.

Natural History

While exact population numbers were not recorded, the authors estimate that cattail aphids were found in 70–80% of early season (April–June) and 90–100% of late season (July–September) cattail stands in the Eastern U.S. based on their experience collecting specimens during 2016; the abundance of aphids within individual cattail stands varied dramatically from a single nymph per 100 plants to a colony of at least 10 individuals per five plants. Cattail aphids were always found under or in cattail leaves and stalks, such that the leaves had to be peeled back to expose the aphids. As cattails grow, new leaves emerge from the middle of the plant and the outermost leaves die back, become dry and papery, and are often tightly appressed to the stalk; when this happens, the aphids move deeper into the stalk and onto new growth if the leaves are not too tightly appressed or onto younger shoots nearby. The aphids may also move into lepidopteran borer galleries (Penko and Pratt 1987, Fig. 10).

An extensive literature review of ants attending all species of *Rhopalosiphum* found no previous records of ants associated with *R. enigmae* (Supplementary Appendix 2). However, when ants had access to cattail aphids (e.g., cattails were in direct contact with soil or, if in standing water, connect to dry soil via bent plants), they were often found to attend the aphids. The presence of ants or ant activity, such as dirt and detritus around a cattail stem, proved an excellent indicator for the presence of an aphid colony. Eleven ant species are now known to attend *R. enigmae*—based on historic slide label data (three species) and freshly collected material (eight species)—(Supplementary Appendix 2). While such mutualisms have been reported for other *Rhopalosiphum*, all of the interactions

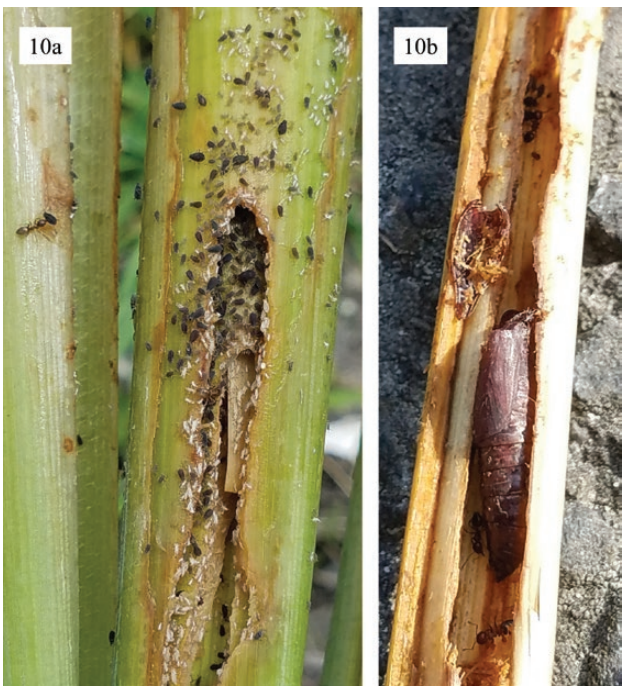

Fig. 10. Photographs of cattail aphids in lepidopteran borer galleries. Note the attendant ants, *Nylanderia faisonensis* (a) and *Crematogaster pilosa* (b).

with *R.enigmae* reported herein are new. This is also apparently the first report of the uncommonly collected wetland specialist *Crematogaster pilosa* Emery, 1895 tending aphids (AntWeb 2017).

Ten ant species were found to tend *R. enigmae* north of central North Carolina. However, the red imported fire ant (RIFA), *Solenopsis*

invicta Buren, 1972, was the only species found tending *R. enigmae* in areas where it has become established the Southeast. Where adventive, RIFA are reported to reduce diversity of native ant species by dominating access to limited resources (e.g., aphid honeydew) and competitive exclusion, and have been shown to alter native ant and arthropod community assemblages (Porter and Savignano 1990, Gotelli and Arnett 2000, Kaplan and Eubanks 2005, Tschinkel 2006).

Fig. 11. Cattail aphid mummies.

Such competitive exclusion of native ants is apparent within the cattail community, although additional studies are needed to quantify if and how the broader cattail arthropod community is affected by RIFA attendance of cattail aphids.

R. enigmae is reported to be autoecious holocyclic on *Typha* because sexuales and ovipare have been collected from cattails but not woody primary hosts typical of other *Rhopalosiphum* (e.g., *Prunus*, *Malus*, and *Pyrus*) (Hottes and Frison 1931, Richards 1960). While fundatrices are undescribed, early-instar nymphs have been collected on *Typha* as early as late April in Maryland (e.g., MS 16-0421-001) when other *Rhopalosiphum* are confined to primary hosts. If cattail aphids are indeed autoecious on *Typha*, an important question to answer is where eggs are laid in the fall as 1) cattail habitat is often flooded by spring rain, which could inundate eggs and 2) cattails produce new shoots every season, so young aphids do not have the benefit of hatching onto suitable host plants. One possibility is that ants move aphid eggs into their own nests during the winter, as has been documented in other ant-aphid mutualisms (Way 1963).

On three occasions, the coccinellid *Diomus terminatus* (Say, 1852) was collected under cattail leaf sheaths in association with cattail aphids (KS 16-0425-001, MS 16-0506-002, MS 16-0921-002). *Diomus terminatus* is a generalist aphid predator known to feed on a wide variety of aphids, including other *Rhopalosiphum* (Gordon 1976, Tifft et al. 2006), but has not been associated with *R. enigmae*. The beetles were not observed to feed on *R. enigmae*, but considering their proximity and propensity to feed on other aphids, such a scenario is likely.

An extensive literature search found that many hymenopteran parasitoids and hyperparasitoids have been recorded from four economically important and/or commonly encountered *Rhopalosiphum* species: *R. maidis* (Fitch, 1856) (63 spp.), *R. nymphaeae* (25 spp), *R. oxyacanthae* (Schrank 1801) (27 spp.), and *R. padi* (86 spp.) (Supplementary Appendix 1). However, we were only able to locate

Fig. 12. Cattail aphid parasitoids and hyperparasitoids. (a) *Aphelinus* (Aphelinidae) (b,c) Aphidiinae (Braconidae). (d) *Alloxysta* (Figitidae). (e) *Dendrocerus* (Megaspilidae). (f) *Asaphes* (Pteromalidae). (g) *Pachyneuron* (Pteromalidae).

a few parasitoid records for four *Rhopalosiphum* species, including cattail aphid and no records for the seven remaining species. While it is understandable that species of economic importance have been investigated more thoroughly, this disparity highlights how little attention non-pest species have received, which is interesting given aphids can be found in extremely high abundance in select habitats. Heie (1986), e.g., described colonies of *R. rufulum* Richards 1960 on *Acorus* L. as so dense that 'the plants look bespattered with black mud'.

On 3 June 2016 MJS located a large colony of *R. enigmae* (collection code MS 16-0603-001) in which hundreds of aphid mummies were observed (Fig. 11). The aphids were located on cattails in standing water and not attended by ants. The hymenopteran parasitoid *Aphelinus* (Aphelinidae), and hyperparasitoids *Alloxysta* (Figitidae), *Dendrocerus* (Megaspilidae), *Asaphes* and *Pachyneuron* (Pteromalidae) were reared from the parasitized cattail aphids, all of which are new parasitoid/host records for *R. enigmae* (Fig. 12, Supplementary Appendix 1). Two aphidiine braconids were also reared, but not identified beyond subfamily. Besides this single event, no parasitized aphid mummies were found among the hundreds of cattails aphids observed in the field. However, the COI sequence from one aphid specimen sequenced for the molecular species investigation from Chester, VA (MS 16-0920-003) matched aphidiine braconid sequences (91% similar to *Lipolexis gracilis*, 88% similar to *Praon* sp.) when BLASTn searches of NCBI's nr database were conducted. The aphid was not obviously parasitized when it was selected for sequencing but must have contained a wasp larva. This sequence was not uploaded to GenBank as the species identity of the parasitoid is unknown, but is available upon request.

The new parasitoid and hyperparasitoid records presented here and in Supplementary Appendix 1 suggest the parasitoid community associated with *R. enigmae* and non-pest *Rhopalosiphum* more generally is diverse. The lack of previous parasitoid records associated with *R. enigmae* is due in part to lack of interest and investigation, although the difficulties the authors had in finding parasitoids outside of the single incident mentioned suggests that parasitoids may be more abundant during certain seasons. Indeed, we speculate that the generally cryptic nature of cattail aphids and ant attendance deter parasitism and that when aphids are relatively exposed in the spring and early summer (i.e., when leaves are less tightly spaced and lepidopteran galleries do not yet exist) and/or ant mutualists are absent, cattail aphids can be heavily exploited by parasitoids.

Finally, cattail aphid is an often abundant and easily located species that has received little study in large part due to its status as a non-pest, which is exemplified by the fact that nearly every natural history observation reported herein is new. We hope that the new observations and extensive reference section will spur future research into this interesting but understudied species.

Supplementary Data

Supplementary data are available at *Insect Systematics and Diversity* online.

Acknowledgments

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References Cited

Andrews, N. J., M. Penko, M. D. Mattson, and D. C. Pratt. 1981. The establishment of cattails on a Minnesota peatland. Minnesota Department of Natural Resources, St. Paul, MN.

AntWeb. 2017. Antweb, version 6.47.3. https://www.antweb.org

Beule, J. D. 1979. Control and management of cattails in southeastern Wisconsin. Tech. Bull. No. 112. Wisconsin Department of Natural Resources, Madison, WI.

Blackman, R. L., and V. F. Eastop. 2017. Aphids on the world's plants. http://www.aphidsonworldsplants.info/

Bolton, S. J., H. Klompen, G. R. Bauchan, and R. Ochoa. 2014. A new genus and species of Nematalycidae (Acari: Endeostigmata). J. Natural History 48:1359–1373.

Canada Post. 2011. Addressing guidelines. https://www.canadapost.ca/tools/pg/manual/PGaddress-e.asp#1442131

Cassani, J. R. 1985. Biology of *Simyra henrici* (Lepidoptera: Noctuidae) in Southwest Florida. Fla. Entomol. 68: 645–652.

Claassen, P. W. 1921. *Typha* insects: their ecological relationships. Cornell University Agricultural Experiment Station, Memoir 47. Cornell University, Ithica, NY.

Cole, A. C. 1931. *Typha* insects and their parasites. Entomol. News. 42: 6–11.

Coovert, G. A. 2005. The ants of Ohio. Ohio Biological Survey, Inc., Columbus, OH.

Evenhuis, N. L. 2017. The insect and spider collections of the world website. http://hbs.bishopmuseum.org/codens/ (accessed 15 February 2017).

Favret, C. 2017. Aphid species file. Version 5.0/5.0. http://Aphid.SpeciesFile.org.

Fisher, B. L., and S. P. Cover. 2007. Ants of North America. A guide of genera. University of California Press, Berleley, CA.

Folmer, O., M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3: 294–299.

Foottit, R. G., and W. R. Richards. 1993. The insects and arachinds of Canada. Part 22. The genera of aphids of Canada (Homoptera: Aphidoidea and Phylloxeroidea). Centre for Land and Biol. Resources Res., Ottawa, ON, Canada. 766 pp.

Foottit, R. G., H. E. L. Maw, C. D. von Dohlen, and P. D. N. Hebert. 2008. Species identification of aphids (Insecta: Hemiptera: Aphididae) through DNA barcodes. Mol. Ecol. Resour. 8: 1189–1201.

Foottit, R. G., H. E. L. Maw, and K. S. Pike. 2009. DNA barcodes to explore diversity in aphids (Hemiptera Aphididae and Adelidgidae). Redia 92: 87–91.

Gordon, R. D. 1976. The Scymnini (ColeopteraL Cocinellidae) of the United States and Canada: key to genera and revision of *Scymnus*, *Nephus*, and *Diomus*. Bul. Buffalo Soc. Nat. Sci. 28: 341–346.

Gotelli, N. J., and A. E. Arnett. 2000. Biogeogrpahic effects of red fire ant invasion. Ecology Letters 3: 257–261.

Hall, T. A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium 41: 95–98.

(Helicon) Helicon Soft Ltd. 2016. Helicon Focus, version 6.7.1. Helicon Soft Ltd, Kharkiv, Ukraine.

Hebert, P. D. N., E. H. Penton, J. M. Burns, D. H. Janzen, and W. Hallwachs. 2004. Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. Proc. Nat. Acad. Sci. U.S.A. 101: 14812–14817.

Heie, O. E. 1986. The Aphidoidea of Fennoscandia and Denmark III. Pterocommatinae and Aphidinae, Aphidini. Fauna ent. scand. 17: 1–314.

Hottes, F. C., and T. H. Frison 1931. The plant lice, or Aphididae, of Illinois. Bull. Ill. Nat. Hist. Surv. 19: 121–447.

Huson, D. H., and C. Scornavacca. 2012. Dendroscope 3: an interactive tool for rooted phylogenetic trees and networks. Syst. Biol. 61: 1061–1067.

Judd, W. W. 1952. The white veined dagger, *Simyra henrici* Grt. (Phalaenidae), and its parasites reared from cat-tail, *Typha* spp. Nova Scotian Inst. Sci. Proc. Trans. 23: 115–119.

Kaplan, I., and M. D. Eubanks. 2005. Aphids alter the community-wide impact of fire ants. Ecology 86: 1640–1649.

Maw, H. E. L., R. G. Foottit, K. G. A. Hamilton, and G. G. E. Scudder. 2000. Checklist of the Hemiptera of Canada and Alaska. NRC Research Press, Ottawa, ON, Canada.

McDonald, M. E. 1951. The ecology of the Pointe Mouillee marsh, Michigan, with special reference to the biology of cat-tail (*Typha*). Dissertation. University of Michigan, Ann Arbor, Michigan.

Miller, M. A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees, pp. 1–8. In Proceedings of the Gateway Computing Environments Workshop, 14 Nov. 2010, New Orleans, LA.

Miller G. L., G. Bauchan, C. E. Mitter, and A. Tracy. 2013. Preparing soft-bodied arthropods for microscope examination: Aphids (Insecta: Hemiptera: Aphididae). https://www.ars.usda.gov/northeast-area/beltsville-md/beltsville-agricultural-research-center/systematic-entomology-laboratory/docs/sels-slide-mounting-tutorial-videos/.

MobiWIA Ltd. 2017. GPS Status & Toolbox. https://mobiwia.com/gpsstatus

Murray, T. 2009. Aphid on cattail reeds. http://bugguide.net/node/view/355368/bgpage (accessed 19 April 2017).

Park, D. S., S. J. Suh, H. W. Oh, and P. D. Hebert. 2010. Recovery of the mitochondrial COI barcode region in diverse Hexapoda through tRNA-based primers. BMC Genomics 11: 423.

Penko, J. M. 1985. Ecological studies of *Typha* in Minnesota: *Typha* insect interactions, and the productivity of floating stands. M.S. thesis, University of Minnesota, St. Paul, MN.

Penko, J. M., and D. C. Pratt. 1986a. Effects of *Bellura obliqua* on *Typha latifolia* productivity. J. Aquatic. Plant Manage. 24: 24–28.

Penko, J. M., and D. C. Pratt. 1986b. The growth and survival of early instars of *Bellura oblique* (Lepidoptera: Noctuidae) on *Typha latifolia* and *Typha angustifolia*. Great Lakes Entomol. 19: 35–42.

Penko, J. M., and D. C. Pratt. 1987. Insect herbivory in Minnesota *Typha* stands. J. Freshwater Ecol. 4: 235–244.

Penko, J. M., E. Gorham, and D. C. Pratt. 1983. The relative suitability of two species of cattail (*Typha*) as host plants for *Bellura obliqua*. Minn. Acad. Sci., 51st Annu. Spring Meet., Univ. Minnesota-Duluth.

Porter, S. D., and D. A. Savignano. 1990. Invasion of polygyne fire ants decimates native ants and disrupts arthropod community. Ecology 71: 2095–2106.

Rebijith, K. B., R. Asokan, N. K. Krishna Kumar, V. Krishna, B. N. Chairanya, and V. V. Ramamurthy. 2013. DNA barcoding and elucidation of cryptic aphid species (Hemiptera: Aphididae) in India. Bull. Entomol. Res. 103: 601–610.

Richards, W. R. 1960. A synopsis of the genus *Rhopalosiphum* in Canada. Can. Entomol. 92 (Supp. 13): 1–51.

Smith, C. F., and C. S. Parron. 1978. An annotated list of Aphididae (Homoptera) of North America. Tech. Bull. 255. North Carolina Agriculture Experiment Station, North Carolina State University, Raleigh, NC.

Taber, S. W. 1993. A new *Rhopalosiphum* on cattail (Homoptera: Aphididae). Pan-Pacif. Entomol. 69: 323–328.

Tifft, K. H., N. C. Leppla, L. S. Osborne, and J. P. Cuda. 2006. Reading *Diomus terminates* (Coleoptera: Coccinellidae) on the corn leaf aphid, *Rhopalosiphum maidis* (Homoptera: Aphididae). Fla. Entomol. 89: 263–265.

Tschinkel, W. R. 2006. The fire ants. Harvard University Press, Cambridge, MA.

(USDA) United Stated Department of Agriculture. 2017. "Typha". Natural Resources Conservation Service PLANTS database. https://plants.usda.gov/core/profile?symbol=TYPHA

(USPS) United States Postal Service. 2015. Publication 28 – Postal addressing standards. Appendix B. Two-letter state and possession abbreviations. http://pe.usps.gov/text/pub28/28apb.htm.

Valenzuela, I., V. F. Eastop, P. M. Ridland, and A. R. Weeks. 2009. Molecular and morphometric data indicate a new species of the aphid genus *Rhopalosiphum* (Hemiptera: Aphididae). Ann. Entomol. Soc. Am. 102: 914–924.

Wang, J.-F., L.-Y. Jiang, and G.-X. Qiao. 2011. Use of mitochondrial COI sequence to identify species of the subtribe Aphidina (Hemiptera, Aphididae). ZooKeys 122: 1–17.

Way, M. J. 1963. Mutualism between ants and honeydew-producing Homoptera. Ann. Rev. Entomol. 8: 307–344.

(Zeiss) Carl Zeiss Microscopy. 2013. AxioVision SE64, version 4.9.1. Carl Zeiss Microscopy, Oberkochen, Germany.