



Research Article

# Reexamination of *Rhopalosiphum* (Hemiptera: Aphididae) using linear discriminant analysis to determine the validity of synonymized species, with some new synonymies and distribution data

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## Abstract

Although 17 species of *Rhopalosiphum* (Hemiptera: Aphididae) are currently recognized, 85 taxonomic names have been proposed historically. Some species are morphologically similar, especially alate individuals and most synonymies were proposed in catalogues without evidence. This has led to both confusion and difficulty in making accurate species-level identifications. In an attempt to address these issues, we developed a new approach to resolve synonymies based on linear discriminant analysis (LDA) and suggest that this approach may be useful for other taxonomic groups to reassess previously proposed synonymies. We compared 34 valid and synonymized species using 49 measurements and 20 ratios from 1,030 individual aphids. LDA was repeatedly applied to subsets of the data after removing clearly separated groups found in a previous iteration. We found our

characters and technique worked well to distinguish among apterae. However, it separated well only those alatae with some distinctive traits, while those apterous which were morphologically similar were not well separated using LDA. Based on our morphological investigation, we transfer *R. arundinariae* (Tissot, 1933) to *Melanaphis* supported by details of the wing venation and other morphological traits and propose *Melanaphis takahashii* Skvarla and Miller as a replacement name for *M. arundinariae* (Takahashi, 1937); we also synonymize *R. momo* (Shinji, 1922) with *R. nymphaeae* (Linnaeus, 1761). Our analyses confirmed many of the proposed synonymies, which will help to stabilize the nomenclature and species concepts within *Rhopalosiphum*.

## Keywords

Aphidoidea, Aphididae, taxonomy, agriculture, species delimitation, linear discriminant analysis, *Melanaphis arundinariae*, *Rhopalosiphum nymphaeae*

## Introduction

The difficulty of aphid taxonomy and identification has been recognized as far back as the 18<sup>th</sup> Century by Carl Linnaeus (Walsh 1863) and is driven by multiple, often interconnected factors, including morphology and life history traits. Many aphids are heteroecious (Blackman and Eastop 2000) and host-specific morphology can vary enough that taxonomists have described the same species multiple times based on different host-associated life stages (Stern et al. 1997). Furthermore, aphid morphology can change substantially with abiotic factors such as temperature (e.g. Blackman and Spence 1994) and number of daylight hours (e.g. Hille Ris Lambers 1966), and biotic factors such as colony size (e.g. Watt and Dixon 1981), all of which cloud species delimitation. Lastly, most species diagnoses require measurements of body parts. Rarely are there one or more discrete characters that can separate species. Therefore, given the plasticity in aphid morphology and the paucity of discrete characters, aphid taxonomy desperately needs better ways to diagnose species.

Challenges in aphid taxonomy have resulted in different tools and methods to discern species. For example, Foottit et al. (2008) used mitochondrial DNA barcodes to distinguish among aphid species with high success. This high rate of success of DNA barcoding has led to the formation of regional barcoding databases to aid in the identification of aphid species (e.g. Coeur d'Acier et al. 2014). However, DNA barcoding is limited to freshly collected or alcohol-preserved specimens and cannot be used with cleared and slide-mounted specimens, which negates its use when comparing historic specimens.

Both statistical and non-statistical morphological tools have been developed for use with slide-mounted specimens. For example, online interactive keys have been developed to distinguish among large numbers of aphid species, but they lack statistical grounds for identification (e.g., Favret and Miller 2012). Multivariate statistics, specifically linear discriminant analysis (LDA), which requires taxonomic designation *a priori* and can use

multivariate analysis of variance (MANOVA) with discrete and continuous characters to test whether the group centroids differ (see Henderson 2006 for multivariate statistics introduction applied to taxonomy), have been applied to aphids to distinguish among aphid species and ecotypes, (e.g. Favret and Voegtlin (2004) for three species of *Cinara*, Lozier et al. (2008) for three species of *Hyalopterus*, Valenzuela et al. (2009) for three species of *Rhopalosiphum* and Bašilova (2010) for two species of *Cryptomyzus*). However, such studies have been limited to just a few species and have not been used to evaluate historic synonymies.

In this paper, we expand upon previous examples using discriminant analysis in aphid taxonomy to test whether multivariate statistics support currently recognized *Rhopalosiphum* species and their synonymies. Specifically, we use LDA to statistically compare 34 valid and synonymized *Rhopalosiphum* species to test the validity of historic synonymies. Valid species and synonymies were tested using iterative LDA analyses in which we removed the most distinct species clusters and reanalyzed the remaining species. We used LDA analyses with only valid species and applied the resulting discriminant functions to synonymized species to determine whether the synonymies are statistically correct using species specific LDA functions. The methods and analyses presented here are unique and repeatable with respect to previous aphid studies. No previous studies have tested taxonomic hypotheses in this manner. We use open source software and describe the analyses with sufficient detail to make them repeatable, which has been lacking from the literature. Lastly, our analyses are statistically robust with respect to LDA model assumptions as we thoroughly describe character transformations and missing data and their relationship to model assumptions.

Our example genus of aphids in need of comprehensive review and revision is *Rhopalosiphum* Koch, 1857 (Aphididae: Aphidinae: Aphidini: Rhopalosiphina) (Koch 1857). *Rhopalosiphum* currently comprises 17 recognized species, including some of the earliest named aphids (i.e. *R. padi* (Linnaeus, 1758) and *R. nymphaeae* (Linnaeus, 1761)) (Fig. 1, Table 1). Most *Rhopalosiphum* species are heteroecious and typically overwinter on rosaceous trees (Rosaceae: *Crataegus* Tourn. ex L., *Malus* Mill., *Prunus* L.) and feed on grasses, sedges, and related plants (Poales) throughout the summer (Table 2). A number are important grain (e.g. *R. maidis* (Fitch, 1856) and *R. padi*) and apple (*R. oxyacanthae*) pests that transmit more than 25 aphid-transmitted plant viruses (Chan et al. 1991) and are often intercepted at ports of entry and in aphid-monitoring suction traps (Strażyński 2010, Favret and Miller 2012, Skvarla et al. 2017). Those species that are not economically important have received comparatively little study. For example, *R. nigrum* Richards, 1960 and *R. padiformis* Richards, 1962 have not been discussed in scientific literature outside of their original descriptions and catalog entries. Examples like this complicate the identification of alates collected without host data (e.g., in suction traps) because the majority of taxonomic resources focus on apterae and not alates.

Table 1.

*Rhopalosiphum* species, associated original descriptions, type depositories, and number of specimens examined. Bold names are currently recognized as valid species with synonymized species listed immediately after. Citations marked with an asterisk (\*) are not included in the literature cited as the authors were unable to locate them.

Species	Original description	Primary type depository	Secondary type depositories	Number of apterae measured	Number of alatae measured	Notes
<b><i>R. albigubernum</i></b>	Zhang and Qiao (1997)			-	-	Unable to locate types.
<i>R. arundinariae</i>	Tissot (1933a)	USNM	NHMUK	3	5	Moved to <i>Melanaphis</i> .
<b><i>R. cerasifoliae</i></b>	Fitch (1855)	NHMUK	USNM	0	10	
= <i>Aphis furcata</i>	Patch (1914)	CNC	CNC	0	6	Lectotype and paralectotypes on one slide.
= <i>Aphis tahasa</i>	Hottes and Frison (1931)	USNM	NHMUK	27	10	
<b><i>R. chusqueae</i></b>	Pérez-Hidalgo et al. (2012)	CZULE		2	0	
<i>R. dryopterae</i>	Kan (1986)			-	-	Unable to locate types.
<b><i>R. enigmiae</i></b>	Hottes and Frison (1931)	INHS	NHMUK, USNM	120	32	
= <i>R. laconae</i>	Taber (1993)	USNM	NCSU	54	5	Unable to locate holotype, apparently lost or never deposited. Neotype designated from NCSU paratypes.
<b><i>R. esculentum</i></b>	Raychaudhuri and Roychoudhuri (1978)			-	-	Unable to locate types; syn. possible de <i>Aphis craccivora</i> (Remaudiere & Remaudiere 1997)
<b><i>R. maidis</i></b>	Fitch (1856)	USNM		93	51	
= <i>Aphis adusta</i>	Zehntner (1897a)			-	-	Unable to locate types.
= <i>Aphis africana</i>	Theobald (1914)	NHMUK	NHMUK	11	3	
= <i>Aphis cookii</i>	Essig (1911)	EMEC		0	13	

Species	Original description	Primary type depository	Secondary type depositories	Number of apterae measured	Number of alatae measured	Notes
= <i>Stenaphis monticelli</i>	del Guercio (1914a)			-	-	Unable to locate types.
= <i>Aphis obnoxia</i>	Mordvilko (1916)*			-	-	Unable to locate types.
= <i>Schizaphis setariae</i>	Rusanova (1962)*			-	-	Unable to locate types.
= <i>Aphis vulpiae</i>	del Guercio (1914b)			-	-	Unable to locate types.
= <i>R. zeae</i>	Rusanova (1960)*			-	-	Unable to locate types.
<i>R. musae</i>	Schouteden (1906)	NHMUK	NHMUK	27	7	Lecto- and paralectotypes.
= <i>R. sciripolii</i>	Gillette and Palmer (1932)	USNM	NHMUK, USNM	6	15	
<i>R. nigrum</i>	Richards (1960)	CNC	CNC, NHMUK, USNM	10	8	
<i>R. nymphaeae</i>	Linnaeus (1761)			58	14	
= <i>Aphis aquatica</i>	Jackson (1908)	OSUC (?)		-	-	Unable to locate types. Type depository not given in original description. Jackson worked at The Ohio State and likely deposited his types there, however a search for the specimens did not locate them.
= <i>Aphis butomi</i>	von Schrank (1801)			-	-	Unable to locate types.
= <i>Aphis infuscata</i>	Koch (1854a)			-	-	Unable to locate types.
= <i>R. najadum</i>	Koch (1854b)			-	-	Unable to locate types.
= <i>R. momo</i>	Shinji (1922)			-	-	Unable to locate types, apparently lost.
= <i>Aphis prunaria</i>	Walker (1848)	NHMUK		0	2	

Species	Original description	Primary type depository	Secondary type depositories	Number of apterae measured	Number of alatae measured	Notes
= <i>Aphis prunorum</i>	Dobrowljansky (1913)	Kiev Entomological Station (defunct)		-	-	Unable to locate types, apparently lost.
= <i>Hyadaphis spargani</i>	Theobald (1927)	NHMUK	NHMUK	6	0	
= <i>R. yoksumi</i>	Ghosh et al. (1971)	Entomology laboratory, Calcutta University		-	-	Types not examined.
<i>R. oxyacanthae</i>	von Schrank (1801)	NHMUK		0	9	
= <i>Aphis crataegella</i>	Theobald (1912)	NHMUK		-	-	
= <i>Aphis edentula</i>	Buckton (1879)	NHMUK	NHMUK	-	-	Holotypes and 2 paratypes on one slide, holotype indicated. Ovipara.
= <i>Aphis fitchii</i>	Sanderson (1902)	USNM	NHMUK	0	3	
= <i>Aphis insertum</i>	Walker (1849)	USNM		0	54	
= <i>Aphis macatata</i>	Walker (1849)	NHMUK		0	1	
= <i>R. viridis</i>	Richards (1960)	CNC	CNC, NHMUK	0	14	
= <i>R. mali bivincta</i>	Fitch (1855)	USNM		-	-	Unable to locate types, apparently lost. See also Favret et al. (2008)
= <i>R. mali fulviventris</i>	Fitch (1855)	USNM		-	-	
= <i>R. mali immaculata</i>	Fitch (1855)	USNM		-	-	Unable to locate types, apparently lost. See also Favret et al. (2008)
= <i>R. mali nigricollis</i>	Fitch (1855)	USNM		-	-	
= <i>R. mali nigriventris</i>	Fitch (1855)	USNM		-	-	
= <i>R. mali obsoleta</i>	Fitch (1855)	USNM		0	1	
= <i>R. mali pallidicornis</i>	Fitch (1855)	USNM		0	1	

Species	Original description	Primary type depository	Secondary type depositories	Number of apterae measured	Number of alatae measured	Notes
= <i>R. mali tergata</i>	Fitch (1855)	USNM		0	1	
= <i>R. mali thoracica</i>	Fitch (1855)	USNM		0	1	
= <i>R. mali triseriata</i>	Fitch (1855)	USNM		0	1	
<i>R. padi</i>	Linnaeus (1758)	NHMUK		49	44	Holotype not examined.
= <i>Siphocoryne acericola</i>	Matsumura (1917)			-	-	Unable to locate types.
= <i>Siphonaphis padi americana</i>	Mordvilko (1921)			-	-	Unable to locate types.
= <i>Aphis avenaestivae</i>	von Schrank (1801)			-	-	Unable to locate types.
= <i>Siphocoryne donarium</i>	Matsumura (1918)			-	-	Unable to locate types.
= <i>Siphocoryne fraxinicola</i>	Matsumura 1917			-	-	Unable to locate types.
= <i>Aphis holci</i>	Ferrari (1872)			-	-	Unable to locate types.
= <i>Aphis prunifoliae</i>	Fitch (1855)	USNM		24	24	
= <i>Aphis pseudoavenae</i>	Patch (1917)	CNC		2	11	
= <i>Aphis tritici</i>	Lawson (1866)*			-	-	Unable to locate types.
= <i>Aphis uwamizuskurae</i>	Monzen (1929)			-	-	Unable to locate types.
<i>R. padiformis</i>	Richards (1962)	CNC	CNC, NHMUK, USNM	4	0	
<i>R. parvae</i>	Hottes and Frison (1931)	USNM		3	0	
<i>R. rufiabdominale</i>	Sasaki (1899)	USNM	USNM	24	28	
= <i>Cerosipa californica</i>	Essig (1944)	EMEC	US Bureau of Entomology and Plant Quarantine (defunct)	0	1	Unable to locate paratypes, apparently lost.
= <i>R. fucanoi</i>	Moritsu (1947)*	Entomology Laboratory, Kyusyu Imperial University		-	-	Types not examined.

Species	Original description	Primary type depository	Secondary type depositories	Number of apterae measured	Number of alatae measured	Notes
= <i>R. gnaphalii</i>	Tissot (1933b)	USNM		4	1	
= <i>Anuraphis mume</i>	Hori (1927)			-	-	Unable to locate types.
= <i>Yamataphis oryzae</i>	Matsumura (1917)			-	-	Unable to locate types.
= <i>Yamataphis papaveri</i>	Takahashi (1921)*	Taihoku agricultural experiment station (defunct)		-	-	Unable to locate types.
= <i>Pseudocerosipha pruni</i>	Shinji (1932)*			-	-	Unable to locate types.
= <i>Aresha setigera</i>	Blanchard (1939)			-	-	Unable to locate types.
= <i>Aresha shelkovnikovi</i>	Mordvilko (1921)			-	-	Unable to locate types.
= <i>Siphocoryne splendens</i>	Theobald (1915)	NHMUK		40	3	
= <i>R. subterraneum</i>	Mason (1937)	USNM		17	19	
<i>R. rufulum</i>	Richards (1960)	CNC	CNC, NHMUK	10	10	
<i>R. sanguinarium</i>	Baker (1934)		NHMUK (cotype)	0	1	
sp. nov. "ex. <i>Arisaema</i> "	undescribed	USNM		34	1	
<i>R. sp. T</i>	undescribed, proposed by Bulman et al. (2005)	Crop and Food Research, Lincoln, New Zealand		-	-	
<i>R. near insertum</i>	undescribed, proposed by Bulman et al. (2005)	Crop and Food Research, Lincoln, New Zealand		-	-	
<i>R. sp. x</i>	undescribed, proposed by Valenzuela et al. (2009)			-	-	

Table 2.

*Rhopalosiphum* hosts and distributions. Host author names have been omitted for space. Unless otherwise noted, information is compiled from the original description (see Table 1), Holman (2009), and Blackman and Eastop (2017).

Taxon	Primary host	Secondary host	Distribution	Notes	Additional references
<i>R. albigubernum</i>	<i>Citrus</i>		Baidicheng, Fengjie, Sichuan, China	Known only from the type series, which consists of two alate vivipara.	
<i>R. arundinariae</i>		<i>Arundinaria tecta</i>	Gainesville, FL	Known only from the type series, which were found as dense colonies on ventral side of younger <i>A. tecta</i> leaves that contained aptareae and alatae on 16 April 1930.	
<i>R. cerasifoliae</i>	<i>Prunus pennsylvanica</i> , <i>P. virginiana</i>	Cyperaceae, including <i>Schoenoplectus</i> , <i>Scirpus</i> , and <i>Eleocharis</i> . Also <i>Juncus</i> (Juncaceae)	North America, wherever host plants occur	Can persist throughout summer on primary host.	
<i>R. chusqueae</i>		<i>Chusquea tomentosa</i>	Costa Rica	Lives close to the nodes and well protected by leaves of <i>C. tomentosa</i> , so not easily detectable. Alatae and lifecycle unknown	
<i>R. dryopterae</i>	<i>Dryopteris filix-mas</i>		Kyrgyzstan		
<i>R. enigmæ</i>		<i>Typha</i> , especially <i>T. latifolia</i> ; also recorded from <i>Sparganium</i>	North America, wherever host plants occur, though apparently most common in the East (A. Jensen, pers. comm.). "R. laconæ" is morphology restricted to southeastern coastal plain.	Autoecious on cattails, no primary host known.	

Taxon	Primary host	Secondary host	Distribution	Notes	Additional references
<i>R. maidis</i>	<i>Prunus</i> , including <i>P. cornuta</i> (Pakistan), <i>P. mume</i> and <i>P. persica</i> (Korea), and <i>P. sargentii</i> (Japan).	<i>Zea</i> , <i>Sorghum</i> , <i>Hordeum</i> , other Poaceae; occasionally Cyperaceae and Typhaceae.	Cosmopolitan, but cannot survive outdoors in regions with severe winter climates	Most lineages are autoecious anholocyclic, with males and ovipara occurring only rarely. Heteroecious, androcyclic populations are known from Asia, where the species originated. In the Northern Hemisphere, lineages with different chromosome counts are associated with different hosts: $2n = 10$ colonize <i>Hordeum</i> and <i>Echinochloa crus-galli</i> , while $2n = 8$ colonize <i>Zea</i> and <i>Sorghum</i> ; lineages with $2n = 9$ , $2n = 11$ , and heterozygous $2n = 8$ are also known. In contrast, $2n = 8$ and $2n = 9$ lineages in Australia do not exhibit host preference. Morphological and early genetic investigations of North American lineages reported incomplete morphological separation and lack of genetic differences between the various parthenogenetic karyotype lineages of <i>R. maidis</i> , though future investigations may raise one or more lineages to species from within the complex.	Wilderthum and Walter (1932), Blackman (1954), Menon and Ghai (1969), Brown and Blackman (1988), Blackman et al. (1990), Blackman and Brown (1991), Barro (1992), Simon et al. (1995), Jauset et al. (2000)

Taxon	Primary host	Secondary host	Distribution	Notes	Additional references
<i>R. musae</i>	<i>Prunus beseyi</i> , <i>P. subcordata</i> , <i>P. fasciculata</i>	<i>Scirpus</i> ; also <i>Musa</i> , <i>Ensete</i> , <i>Sterlitzia</i>	WA to CO, MD (native); Europe, Middle East, Africa, and Australia (adventive)	Adventive populations outside of the native range are presumed to be anholocyclic. Specimen vouchers reportedly sent to the USNM are not present in the collection and were presumably never sent.	Taber (2003)
<i>R. nigrum</i>	<i>Crataegus</i>	<i>Avena sativa</i> , <i>Zizania aquatica</i> , <i>Alisma</i>	ON, MB, AK; unconfirmed reports from OR, UT		Pantoja et al. (2006)
<i>R. nymphaeae</i>	<i>Prunus</i>	Aquatic and semi-aquatic plants, including <i>Alisma</i> , <i>Juncus</i> , <i>Nelumbo</i> , <i>Nuphar</i> , <i>Sagittaria</i> , <i>Sparganium</i> and <i>Typha</i> . Occasionally other hosts, including <i>Canna</i> , <i>Glyceria</i> , <i>Lactuca</i> , <i>Triticum</i> , and <i>Tulipa</i>	Cosmopolitan		
<i>R. oxyacanthae</i>	Various Rosaceae including <i>Malus</i> , <i>Pyrus</i> , <i>Cotoneaster</i> , <i>Crataegus</i> , <i>Sorbus</i> , and <i>Prunus</i>	Various Poaceae, including <i>Agropyron</i> , <i>Agrostis</i> , <i>Festuca</i> , <i>Poa</i> ; occasionally cyperaceae and juncaceae.	North America (probable native range), Europe, North Africa, Japan	The name <i>R. insertum</i> Walker was used widely for the species in North America, but García Prieto et al. (2004) synonymized the species with <i>R. oxyacanthae</i> .	
<i>R. padi</i>	Primarily <i>Prunus virginiana</i> (North America) and <i>P. padus</i> (Europe), occasionally other <i>Prunus</i> . One record on <i>Syringa vulgaris</i> .	Primarily Poaceae, occasionally Asteraceae, Brassicaceae, Cyperaceae, Iridaceae, Juncaceae, Lilaceae, Typhaceae and other taxa.	Cosmopolitan	Can persist throughout summer on primary host.	
<i>R. padiformis</i>		<i>Poa</i> , <i>Triticum</i>	BC, MT	Primary host and associated morphs not known; alate males have been obtained in culture.	
<i>R. parvae</i>		<i>Carex</i> (US), <i>Scirpus lacustris</i> (Italy)	Native to North America (IL); Italy (adventive)	Primary host not known.	

Taxon	Primary host	Secondary host	Distribution	Notes	Additional references
<i>R. rufiabdominale</i>	Primarily <i>Prunus</i> ; also recorded from <i>Malus</i> , <i>Chaenomeles</i> , <i>Pyrus</i> , <i>Rhodotypos</i> , and <i>Sorbus</i> .	Underground parts of Poaceae (including cereals) and Cyperaceae. Can infest some dicots (e.g. Asteraceae, Solanaceae), especially in greenhouse and hydroponic situations.	Native to East Asia. Currently pan-tropical/subtropical and restricted to greenhouses in colder climates.	Heteroecious holocyclic in East Asia and Italy; autoecious anholocyclic in majority of introduced range. Rakauskas et al. (2015) noted that if the heteroecious holocyclic forms recently reported from Italy spreads the species may persist in cold climates in Europe.	Etzel and Pettit (1992), Ciampolini et al. (1993), Zilahi-Balogh et al. (2005)
<i>R. rufulum</i>	<i>Crataegus</i>	Primarily <i>Acorus</i> , also recorded from <i>Typha</i>	North America and Europe, wherever hosts are found.		
<i>R. sanguinarium</i>	<i>Crataegus mexicana</i>	Unknown, but reared on various Poaceae in lab conditions	Mexico		
sp. nov. "ex. <i>Arisaema</i> "	Unknown	<i>Arisaema</i>	Maryland		
<i>R. sp. T</i>	Unknown	Cereals	New Zealand		
<i>R. near insertum</i>	Unknown	Cereals	New Zealand; Victoria, Australia		
<i>R. sp. x</i>	Unknown	<i>Zea mays</i>	Victoria, Australia		

The taxonomic history of *Rhopalosiphum* is complicated. Many taxa with slightly-swollen siphunculi were historically included within *Rhopalosiphum*, but are now placed in a different tribe, Macrosiphini (e.g. *Hyadaphis* Börner, *Lipaphis* Mordvilko, *Rhopalomyzus* Mordvilko), or other aphidine genera (e.g. *Melanaphis* van der Goot, *Schizaphis* Börner) (Richards 1960, Blackman and Eastop 2017, Favret 2019). Börner (1952) was the first to restrict the definition of the genus as it is currently conceived.

Molecular studies (e.g. Kim and Lee 2008) suggest *Rhopalosiphum* is closely related to the rhopalosiphine genera *Melanaphis* and *Schizaphis*. However, no comprehensive molecular or morphological study has tested the monophyly of the genera and species contained therein (Valenzuela et al. 2009). Indeed, Halbert and Voegtlin (1998) suggested that *Rhopalosiphum arundinariae* (Tissot, 1933) is a North American representative of *Melanaphis* based on “wing venation [and] cuticular sculpturing”, though declined to officially move it to *Melanaphis*.

*Rhopalosiphum* species concepts are further complicated by the presence of cryptic species (Bulman et al. 2005, Valenzuela et al. 2009); holocyclic, heteroecious (alternating between sexual and asexual reproduction on primary and secondary hosts) and anholocyclic, autoecious (only asexual reproduction on secondary hosts) forms within the

same species (e.g. *R. padi*); and morphological plasticity within species that often makes morphological species determinations, especially of alate specimens, difficult (personal observation).

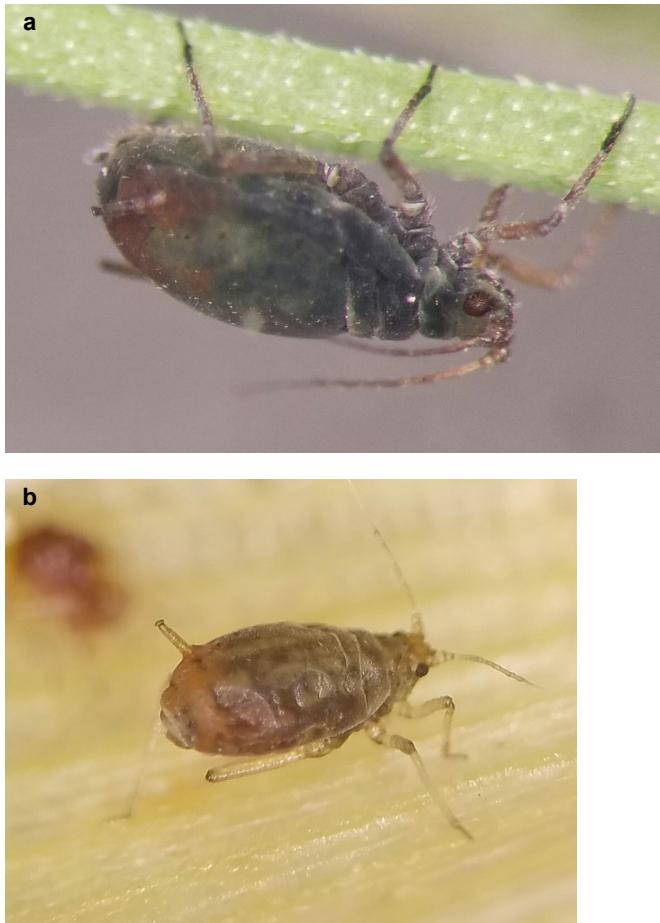


Figure 1.

*Rhopalosiphum* live stereomicrographs.

a: *R. padi*. [doi](#)

b: *R. enigmae* [doi](#)

Finally, while 17 species are currently recognized, at least 85 specific names (which includes misspellings of valid names) have been applied to *Rhopalosiphum* (Favret 2019). Many of the names were synonymized in species catalogues (e.g. Eastop and Hille Ris Lambers 1976, Remaudière and Remaudière 1997), where the authors gave little or no comment on their reasoning for the moves, and the moves have not been confirmed since they were proposed. Thus, there are potentially valid species that have been improperly synonymized.

Herein, we confirm many historic synonymies using LDA, propose two new synonymies, and report new distribution data for *R. rufulum* Richards, 1960, based on material examined for the analyses.

## Materials and Methods

Most specimens examined are housed in the National Museum of Natural History Aphidomorpha Collection (USNM), which is currently housed at the USDA-ARS Beltsville Agricultural Research Center in Beltsville, Maryland, U.S.A. Additionally, types and other material were borrowed from the Essig Museum of Entomology (EMEC), Berkeley, California, U.S.A.; the Florida State Collection of Arthropods (FSCA), Gainesville, Florida, U.S.A.; the Illinois Natural History Survey Insect Collection (INHS), Champaign, Illinois, U.S.A.; the North Carolina State University Insect Museum (NCSU), Raleigh, North Carolina, U.S.A.; The Ohio State University Triplehorn Insect Collection (OSUC), Columbus, Ohio, U.S.A.; the personal collection of Andrew Jensen (AJ), Lakeview, OR, U.S.A.; The Canadian National Collection of Insects, Arachnids, and Nematodes (CNC), Ottawa, Ontario, Canada; the Natural History Museum (NHMUK), London, United Kingdom; and the Aphidological Collection of the University of León (CZULE). For a list of types, species, and number of specimens examined, see Table 1. Museum abbreviations follow Evenhuis (2017).

Slides were labelled with individual, sequential numbers (MS 0001–0980) and specimens assigned a number that was appended to the slide number (e.g. MS 0001-1 for the first specimen on the first slide). Specimens were examined using a Zeiss Axio Imager M1 stereomicroscope; micrographs were taken and measurements of various morphological features of adult female apterous and alate specimens made using AxioVision 4.9.1 software (Carl Zeiss AG, Oberkochen, Germany) (Fig. 2, Table 3). Measurements were hand-written in a notebook, then later manually entered into an Excel spreadsheet.

Table 3.  
Descriptions of measurements.

Measurement number	Measurement description or ratio
1	Antenna segment (AS) 1, length
2	Antenna segment (AS) 2, length
3	Antenna segment (AS) 3, length
4	Antenna segment (AS) 4, length
5	Antenna segment (AS) 5, length
6	Antenna segment (AS) 6 base, length
7	Antenna segment (AS) 6, process terminalis (pt), length
8	Head width across eyes

Measurement number	Measurement description or ratio
9	Ultimate rostral segment (RIV+V), length
10	Ultimate rostral segment (RIV+V), width
11	Hind femur, length
12	Hind tibia, length
13	Hind distitarsus, length
14	Siphunculus, length
15	Cauda, length
16	Abdominal segment 8 submedian seta, length
17	Body length (BL)
18	Siphunculus, width
19	RIV+V length: RIV+V width
20	RIV+V length: hind distitarsus length
21	Pt length: AS 6 base length
22	Siphunculus length: siphunculus width
23	Siphunculus length: cauda length
24	Siphunculus length: AS 3 length
25	RIV+V length: cauda length
26	BL: head width
27	AS 3 and 4 fused (0 = no, 1 = yes)
28–33	Angle, A1–A6 (see Fig. 2b)
34–36	Area, S1–S3 (see Fig. 2c)
37–57	Wing length, L1–L21 (see Fig. 2d)
58	L1:L2 (37:38)
59	L1:L3 (37:39)
60	L1:L4 (37:40)
61	L1:L5 (37:41)
62	L1:L6 (37:42)
63	L1:L7 (37:43)
64	L2:L3 (38:39)
65	L2:L4 (38:40)

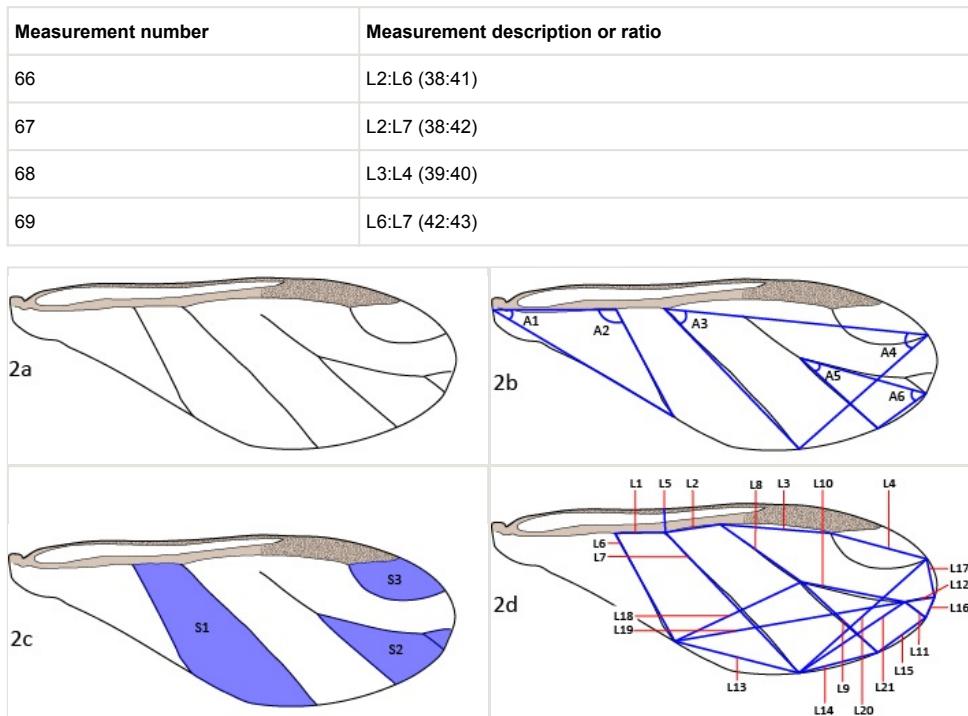


Figure 2.

Wing structures measured in *Rhopalosiphum alatae*.**a:** Wing schematic. [doi](#)**b:** Vein angles. [doi](#)**c:** Cell areas. [doi](#)**d:** Vein and point-to-point lengths. See Table 3 for explanation of alphanumeric codes. [doi](#)

Morphological terms and structures were adapted from Footit and Richards (1993). Throughout the text, the term aptera (pl. apterae) refers to wingless adult vivipara (pl. viviparae) and alata (pl. alatae) refers to winged adult vivipara (pl. viviparae). Body measurements were adapted from Footit et al. (2010). Wing measurements (Fig. 2) were adapted from Favret (2009). All measurements are in micrometers ( $\mu\text{m}$ ) and were taken from the right side of the body when possible. Species names and statuses follow Favret (2019). Measurement data are available as supplementary files (Suppl. materials 1, 2, 3, 4).

## Statistical analyses

We used linear discriminant analysis (LDA) in R (R Core Team 2017) using the MASS package (Venables and Ripley 2002) to test the synonymized and valid species names designations. The code we used to analyze the apterae datasets is available in Suppl. material 5

The analysis proceeded in several steps, which were similar for apterae and alatae, the first of which was data cleaning, described below. LDA is one of the most commonly employed discriminant function analyses, used both to identify useful characters distinguishing specimens of valid species and groups of species, and to form discriminant functions that could then be applied to specimens of uncertain taxonomic status to determine if they cluster with valid species or cluster separately, which would suggest a new species. This LDA approach was applied in a systematic iterative manner; the initial linear discriminant functions separated the most distinctive species, leaving a large amorphous cluster of the other known species. In the next step, the distinctive valid species were removed in that iteration, and the method applied to the remaining valid species. This could be followed by another iteration, until all valid species were separated to the extent possible using available morphological characters.

Prior to the analyses, the dataset was checked for incorrectly entered data. This was performed for both the valid and synonymized by constructing boxplots for each trait, broken down by species (e.g. Fig. 3). This allowed us to identify outlier measurements in the Excel database, which were compared against the hand-recorded measurements and in most cases were the result of inaccurately entered data (e.g. 10002 instead of 100.02 or 278 instead of 728). Incorrectly entered data that were discovered in this manner were corrected before additional analyses. The boxplots also gave a general picture of which species were more variable for which traits.

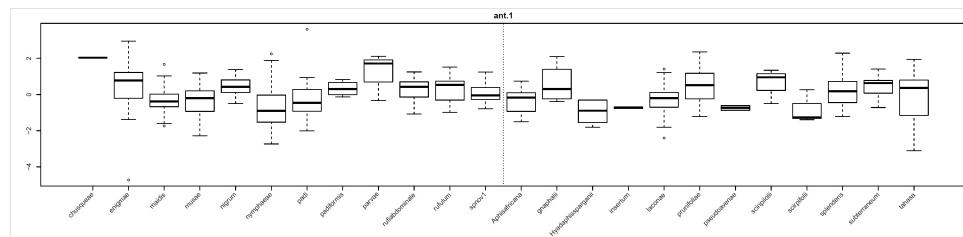


Figure 3. doi

Example of box plots created for a character (length of antennal segment I here) that were used to identify outlier measurements that should be doublechecked before further analyses. Note the outliers for *R. enigmæ* and *R. padi*, which were the result of miskeyed data.

## 'Size': as a trait and as an adjustment

We standardized the size measurements of each specimen, based on a 'size' variable (but also kept 'size' as a variable in the LDA, explained below). There are several reasons for this. One is that some species are more variable in size than others. If measures are not size adjusted, the LDA does not work as well; in fact, we implemented the size adjustments because size affected the usefulness of many of the measures, especially for taxa with a lot of size variability. Second, if one doesn't adjust for size, many of the measures are correlated through size, so less useful. Third, we wanted measures which made sense morphometrically, and these are often 'relative' measures, (e.g. antenna are relatively long for species A (in relation to its size) versus species B). If not adjusted for size, we would have to use ratios for more variables. Standardizing on size was very helpful for many traits and might be useful when developing criteria to separate species in other groups. Given that it is desirable to adjust for size, how does one best estimate 'size'? There is not a one-size-fits-all solution for this and, while we found one that seems to work well for these taxa, it may be improved upon or altered for other taxa. This variable was constructed by combining the body length, head width, and femur length using principal components (PC); head width and femur length were chosen as, among all measured characters, these were most highly correlated with body length. This is a dimension reduction technique, the idea being to create a single variable (the first PC) that best captures the variation in these three correlated measures. In cases where one or two of these measures were missing, the derived principal component measure (henceforth labelled 'relative size') for the specimen was imputed using linear regression based on the rest of the data set. Another method employed for developing a size measure is the use the geometric mean of the characters. We calculated the geometric means for data where we had all three characters and found the correlation between the geometric mean and first PC to be 0.9963; for this data set the two methods would yield essentially identical results.

Our relative size measure was retained as a character in the LDA analysis. It was also used to adjust all other size measures using linear regression, i.e. adjusted measures were residuals from regressing each of the size measures (dependent variable) on the relative size (independent variable). This resulted in smaller and larger individuals of the same species having similar adjusted measurements. Non-size measures (such as wing angles or ratios) were not adjusted using relative size, instead they were transformed by taking logs; this created measures that were closer to being normally distributed. All measures were then individually standardized (using all samples) to mean zero, standard deviation one (this helps one to interpret the coefficients of the linear discriminant analysis results). Missing values were then imputed by randomly sampling from the corresponding adjusted measure of other individuals of that same species. In the unusual case where this could not be done (e.g. all specimens were incomplete for this trait), the trait value was set to zero (which is the overall mean for each measure), removing its influence on the calculated discriminant functions. If all specimens of a species were naturally lacking a trait, a new character column was created for that trait, with either 0 (not missing) or 1 (missing). The end result was that a naturally missing trait could be used as a character when creating the

linear discriminant function and that incomplete or aberrant individuals were not dropped from the analysis because of missing data.

### Linear discriminant analyses

A first LDA was performed on the cleaned dataset for only the valid species (this included the three variables used to create relative size, as well as the adjusted size measure) using 49 measured values and 20 ratios calculated from those values. The first three latent axes were sufficient to explain 80% or more of the variability. We looked at which variables loaded most heavily on each axis and compared that to the boxplots created at an earlier step as a check that the methodology was working as expected. The linear discriminant functions derived from the valid species were then applied to all specimens (valid and synonymized) so the specimens could be mapped to the two dimensional space created from pairs of the latent axes. Species were suitably coded so they could be distinguished on the plots. Since there was only one individual of *R. sanguinarium* Baker, 1934 for both apterae and alatae, it was analyzed with the synonymized, rather than the valid, species.

We then identified clusters of specimens comprising a valid species. Sometimes a valid species was well separated from other valid species and sometimes not. For those well-separated valid species, we looked to see if any of the synonymized species occupied the same space and manually outlined the group. When this happened, we concluded that the synonymized species and valid species were likely the same and we removed them from further analyses. If a synonymized species formed a distinct cluster, we concluded that it was not synonymized with any of the valid (or other synonymized) species and considered it a separate species and also removed it from further analyses.

With the remaining species, we repeated the previously described procedure, producing another LDA using only the valid species that did not separate well in the previous analysis, and using the same independent variables. With fewer species and the same number of independent variables, the software sometimes had difficulty due to insufficient degrees of freedom. When this occurred, we reduced the number of independent variables by removing those that were highly correlated with others in a stepwise manner until there were no independent variables that were highly correlated with other independent variables, and then produced an LDA with the reduced set of independent variables. For alatae, this methodology needed to be repeated a third time to finish separating all the known species.

### Notes on *Rhopalosiphum* species not included in linear discriminant analyses

*Rhopalosiphum dryopterae* Kan, 1986. Halbert and Voegtlind (1998) and Jenson and Holman (2000) suggested *R. dryopterae* is a species of *Dysaphis*. The authors have been unable to locate the type specimens or a copy of the original description by Kan (1986), though Holman, now deceased, based his suggestion on it (Jensen, pers. comm.). We hesitate to move *R. dryopterae* without having seen specimens or the description, although concede that it is likely not a species of *Rhopalosiphum* given the weight of expert opinion and do not treat it further herein.

*Rhopalosiphum esculentum* Raychaudhuri and Roychoudhuri, 1978. Remaudière and Remaudière (1997) suggested *R. esculentum* may be a synonym of *Aphis craccivora* Koch, 1854. Unfortunately, the description of *R. esculentum* does not include characters that would definitively place the species in either Aphidina or Rhopalosiphina (i.e. lateral tubercles I and VII dorsal to adjacent spiracles). Raychaudhuri and Roychoudhuri (1978) report the presence of “rhopalosiphine reticulations” on the thorax and abdomen. However, *A. craccivora* also has reticulations and the lack of accompanying illustrations in the description of *R. esculentum* leaves it ambiguous as to whether the reticulations are composed of solid lines as in *A. craccivora* (similar to Fig. 4a, b) or spicules as in *Rhopalosiphum* (Fig. 4c). Certain characters in the description agree with *A. craccivora* (e.g. segments I, II, apex of V, and VI dark or dusky, the shape of and imbrications on the siphunculi). Finally, *A. craccivora* is the only aphid species reported to feed on *Manihot esculenta* Crantz, the reported host plant (Blackman and Eastop 2017), which is native to South America and grown as a food crop in India, where *R. esculentum* was described. We, therefore, agree that *R. esculentum* is likely to be a synonym of *A. craccivora* and do not include it in subsequent analyses. However, as we have not examined the holotype of *R. esculentum* to confirm our suspicions, we decline to formally synonymize the two species herein.

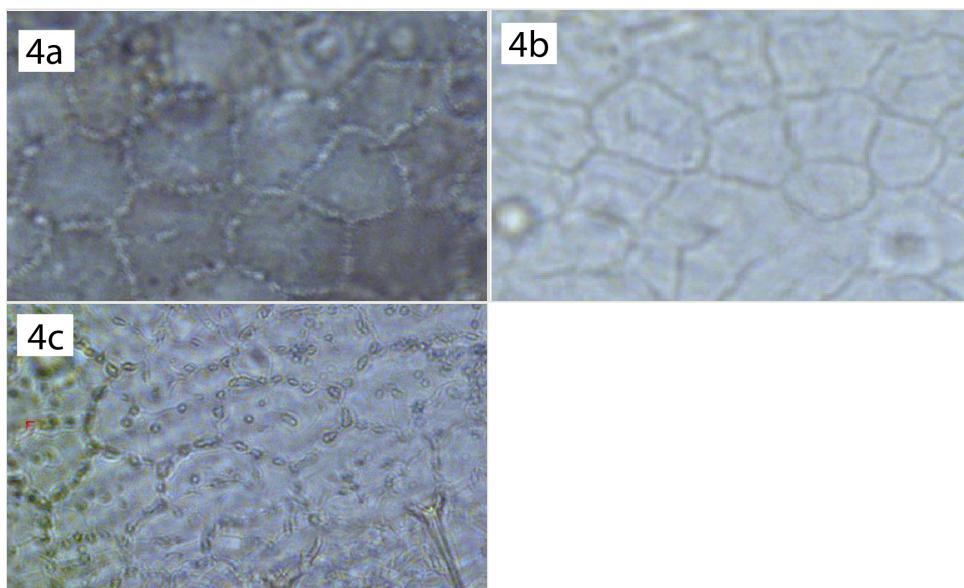


Figure 4.

Dorsal cuticle.

- a: *M. pyraria* (Passerini, 1861). [doi](#)
- b: *R. arundinariae* (Tissott, 1933). [doi](#)
- c: *R. enigmae* Hottes and Frison, 1931. [doi](#)

*Rhopalosiphum momo* Shinji, 1922. Halbert and Voegtlin (1998) reported that the description of *Rhopaloiphum momo* “is suggestive of” *R. nymphaeae*, but did not formally

synonymize the two species. An undated, unattributed translation of the original description is available in the USDA-ARS Systematic Entomology Laboratory library and is reproduced here in full:

(40) *Rhopalosiphum momo* SHINJI, n. sp. / pp. 791

Characteristics: Body green or pale. Antennae somewhat longer than body, III shorter than IV and V taken together, with 16–18 subcircular sensorial, IV and V subequal in length, the former with one sensorium in the middle and the latter with a subapical one; flagellum of VI about 3 times as long as the base. Antennae as a whole infuscated and each segment has a few hairs. Cornicles with a basal 2/3 part green or pale, the remaining 1/3 part swollen and infuscated.

Host plant: *Prunus* [sic] *persica* L.

Date of collection: 2 June 1920

Locality: Miyakonojo, Nakajo (Nagano Pref.)

*Rhopalosiphum arundinariae* (Tissot, 1933). Halbert and Voegtlin (1998) suggested that *R. arundinariae* belongs within *Melanaphis* based on “wing venation [and] cuticular sculpturing” (they also suggested bamboo as a host plant is a *Melanaphis* but not a *Rhopalosiphum* characteristic; however, *R. chusqueae* Pérez Hidalgo and Villalobos Muller, 2012, which feeds on bamboo, was subsequently described and placed unambiguously within *Rhopalosiphum* based on morphological and molecular characters, thus establishing bamboo-feeding as a *Rhopalosiphum* characteristic). We agree with Halbert and Voegtlin (1998) and did not include it in the linear discriminant analyses (see Results for additional details).

We were unable to locate apterae of *R. cerasifoliae* (Fitch, 1855) to include in the analyses. However, we included specimens of the junior synonym *R. tahasa* Hottes, 1950 in order to understand what might happen when synonymized species do not have a presumed conspecific valid species for comparison.

## Results

In total, 1,030 *Rhopalosiphum* specimens (625 apterae, 405 alatae), representing 34 valid and synonymized species, were measured (Table 1). This resulted in 50,470 independent measurements and 20,600 ratios calculated from those measurements (Suppl. materials 1, 2, 3, 4). Additionally, 6 wing vein angles were measured for 62 *Melanaphis* (6 species) and 99 *Schizaphis* (7 species) specimens (Table 4).

Table 4.

Wing angle measurements for species of *Melanaphis*, *Rhopalosiphum*, and *Schizaphis*. Ranges are followed parenthetically by the average for each measurement. Generic measurements, which are listed in bold for easy comparisons, were calculated by adding together species in each genus. *Rhopalosiphum arundinariae* Tissot 1933a was not included in any generic summary and is presented by itself to allow easy comparisons to *Melanaphis* and *Rhopalosiphum*.

Genus species	Original description	Specimens measured	A1	A2	A3	A4	A5	A6
<i>Melanaphis</i>	van der Goot (1917)	62	25.2– 42.6 (30.6)	92.9– 118.7 (106.9)	44.9– 60.6 (52.6)	37.9– 53.1 (43.5)	27–48.1 (36.8) (42.7)	36–53.1
<i>M. bambusae</i>	Fullaway (1910)	9	27–34.7 (31.6)	92.9–104 (101)	55.6– 60.6 (57.9)	41.1– 53.1 (45.4)	34.1– 47.1 (41.3)	41–53.1 (45.7)
<i>M. donacis</i>	Passerini (1861)	4	27–30.8 (29.6)	108.7– 113.9 (111.1)	44.9–49 (46.9)	42.6–45 (43.4)	27–30.2 (28.7)	36–44.7 (41.5)
<i>M. japonica</i>	Takahashi (1919)	2	32–32 (32)	111.9– 111.9 (111.9)	54.6– 56.7 (55.7)	42.6– 45.2 (43.9)	30.1– 31.7 (30.9)	40.6– 43.3 (42)
<i>M. pyraria</i>	Passerini (1861)	10	27.8– 35.1 (32.1)	106.9– 118.7 (112.6)	46–53.8 (49.7)	42–47 (44.3)	27.9– 38.3 (33.4)	38.9– 48.6 (44.9)
<i>M. sacchari</i>	Zehntner (1897b)	36	25.2– 42.6 (29.7)	94.8– 112.1 (105.7)	45.6– 56.3 (52.4)	37.9– 48.2 (42.8)	29.9– 48.1 (37.9)	37.4– 49.7 (41.6)
<i>M. sorini</i>	Halbert et al. (2000)	1	29.5– 29.5 (29.5)	113.7– 113.7 (113.7)	48.2– 48.2 (48.4)	42.8– 42.8 (42.8)	33.4– 33.4 (33.4)	42.2– 42.2 (42.2)
<i>R. arundinariae</i>	Tissot (1933a)	5	<b>29.5–34</b> (31.5)	<b>102.2–</b> <b>114.1</b> (108.5)	<b>45.3–</b> <b>55.7</b> (48.8)	<b>43.8–</b> <b>45.6</b> (44.5)	<b>35.1–</b> <b>37.5</b> (36.5)	<b>44.7–</b> <b>49.1</b> (47.1)
<i>Rhopalosiphum</i>	Koch (1854a)	342	26.6– 44.6 (34.6)	96.7– 123.5 (113.1)	28.8– 44.6 (36.1)	38.7– 55.1 (47.1)	15.2– 47.4 (25.9)	37.3– 62.8 (50.3)
<i>R. cerasifoliae</i>	Fitch (1855)	23	29.7– 40.2 (33.6)	106.3– 122 (114.2)	33.4– 41.2 (37.8)	41.8– 48.8 (45.3)	17.7– 42.1 (26.7)	43.7– 52.6 (47.8)

Genus species	Original description	Specimens measured	A1	A2	A3	A4	A5	A6
<i>R. enigmæ</i>	Hottes and Frison (1931)	38	26.6– 38.3 (32.3)	110.3– 123.5 (115.8)	33–43 (37.6)	39.9– 51.8 (45.3)	19.9– 47.4 (30.4)	37.3– 53.7 (48.6)
<i>R. maidis</i>	Fitch (1856)	53	28.1– 44.6 (34.1)	101.7– 123.2 (112.7)	28.9– 40.5 (35.2)	41.9– 54.3 (46.3)	15.6– 36.9 (24.9)	40.8– 60.1 (49.7)
<i>R. musae</i>	Schouteden (1906)	15	30.6– 39.2 (34.5)	111.2– 119.6 (116)	31.1– 36.3 (34.1)	42.6– 50.8 (46)	15.2– 25.3 (19.9)	43.4– 53.4 (49.2)
<i>R. niger</i>	Richards (1960)	8	29.2– 35.2 (31.7)	112.8– 122.1 (116.4)	36.6–42 (38.5)	44.5– 49.2 (46.9)	21.5– 32.6 (26.8)	48.4– 55.5 (51.2)
<i>R. nymphææ</i>	Linnaeus (1761)	14	29.6– 40.8 (34.8)	105.3– 118 (112.4)	35.3– 44.4 (40.3)	44.3–52 (47.6)	19.6– 46.7 (25.7)	46.1–58 (49.1)
<i>R. oxyacanthæ</i>	von Schrank (1801)	73	30.7– 42.6 (35.9)	105.5– 120.9 (112.6)	32.6– 44.6 (36)	42.4– 51.5 (47.5)	17–41.3 (25.6)	42.3– 58.6 (50.6)
<i>R. padi</i>	Linnaeus (1758)	72	27–43.3 (35.7)	96.7– 123.1 (110.9)	28.8– 41.1 (35.1)	38.7– 52.6 (48)	17.5– 35.7 (23.6)	42.9– 62.8 (51.7)
<i>R. rufiabdominale</i>	Sasaki (1899)	43	28.5– 39.7 (34.4)	105.5– 120.3 (113.8)	29.6– 40.1 (35)	43.7– 55.1 (48.9)	18.6– 36.1 (27.6)	45.6– 58.5 (51.6)
<i>R. sanguinarium</i>	Baker (1934)	1	40.9– 40.9 (40.9)	101.3– 101.3 (101.3)	39.8– 39.8 (39.8)	44.6– 44.6 (44.6)	29.6– 29.6 (29.6)	47.8– 47.8 (47.8)
<i>Schizaphis</i>	Börner (1931)	99	26.3– 41.5 (31.7)	100– 123.4 (112.4)	29.5– 44.9 (38.8)	38.1– 52.2 (44.2)	18.5– 53.8 (37.9)	33.6–58 (46.3)
<i>S. caricis</i>	Schouteden (1906)	2	28.6– 33.8 (31.2)	111.1– 117.9 (114.5)	37–40.2 (38.6)	46.3– 46.3 (46.3)	29.4– 29.4 (29.4)	51.4– 51.4 (51.4)
<i>S. graminum</i>	Rondani (1852)	41	28.4– 35.5 (31.1)	103.7– 118.5 (111.9)	36.3– 44.9 (40.6)	38.1– 44.6 (41.4)	32.7– 53.8 (39)	40.8– 51.1 (44)

Genus species	Original description	Specimens measured	A1	A2	A3	A4	A5	A6
<i>S. minuta</i>	van der Goot (1917)	1	31.5– 31.5 (31.5)	115–115 (115) (31.5)	41.7– 41.7 (41.7)	42.3– 42.3 (42.3)	38.9– 38.9 (28.9)	45.8– 45.8 (45.8)
<i>S. muhlenbergiae</i>	Phillips and Davis (1912)	2	27.3– 27.3 (27.3)	109.6– 109.6 (109.6)	35.7– 43.4 (39.6)	38.25– 40.9 (39.6)	41.1– 44.6 (42.9)	43.7– 49.3 (46.5)
<i>S. nigra</i>	Baker (1918)	44	26.3– 41.5 (31.7)	100– 123.4 (113.5)	29.5– 42.1 (37.5)	43–52.2 (46.5)	18.5– 46.8 (37.5)	33.6–58 (47.9)
<i>S. palustris</i>	Theobald (1929)	3	30–38.5 (34.1)	105.3– 112.1 (107.7)	33.7– 39.5 (37.1)	46.5– 49.2 (48.3)	27.8– 43.8 (35)	46.8–53 (49.5)
<i>S. rotundiventris</i>	Signoret (1860)	6	30.6– 40.4 (35)	107– 115.8 (110.8)	33.6– 37.7 (36.3)	44.5– 50.6 (47.2)	31.9– 38.9 (35.5)	46.2– 53.6 (48.7)

## New synonymies and distribution data

The type material of *R. momo* is apparently lost, so, left with only the description, we concur with Halbert and Voegtlind (1998) that *R. momo* is synonymous with *R. nymphaeae* and formally synonymize them for the following reasons:

- 1) *Rhopalosiphum nymphaeae* is the only species in the genus with siphunculi that are green basally and dark and expanded apically, as was described in *R. momo*.
- 2) Seven species of *Rhopalosiphum* utilize *Prunus* as a primary host (Table 2), five of which occur in Japan: *R. maidis*, *R. nymphaeae*, *R. oxyacanthae*, *R. padi*, and *R. rufiabdominale*. Of these candidate species, only *R. nymphaeae* is not eliminated based on differences in color.
  - a) The body of *R. momo* is described as “green or pale”. *Rhopalosiphum padi* and *R. rufiabdominale* have a distinctive red patch between the siphunculi and *R. oxyacanthae* have dark green stripes that would probably have been noted in the description if present.
  - b) The antennae of *R. momo* are “whol[ly] infuscated”. Antenna segment III of *R. maidis* is pale, rather than concolorous with the other dark segments, and can be pale or dark in *R. padi*.
- 3) One potential complication is that Richards (1960), who published the last review of *Rhopalosiphum*, reported that alate *R. nymphaeae* have zero secondary rhinaria on antennal segment IV. However, Heie (1986) reports a range of 0–8 secondary rhinaria on antennal segment IV and multiple examples in the USNM collection have 1–4 (although

most have zero). The single secondary rhinaria on antennal segment IV reported for *R. momo*, therefore, does not exclude the possibility they are synonymous with *R. nymphaeae*.

The morphological characters suggested by Halbert and Voegtlin (1998) support the placement of *R. arundinariae* (Tissot, 1933) within *Melanaphis*. The reticulate pattern on the dorsal abdomen of *R. arundinariae* (Tissot, 1933) is formed by smooth lines, as in *M. pyraria* (Passerini, 1861), rather than small spicules, as in *Rhopalosiphum* (Fig. 4). The gestalt of the wing veins is more difficult to classify as there can be high levels of plasticity in the veins. However, the angle A3 is consistently different and non-overlapping between *Rhopalosiphum/Schizaphis* and *Melanaphis*: *Rhopalosiphum* (28.8–44.9°), *Schizaphis* (29.5–44.6°) and *Melanaphis* (44.9–60°) (Table 4). The angle A3 of *R. arundinariae* is 45.3–55.7°, so supports its placement within *Melanaphis*. We, therefore, transferred *Rhopalosiphum arundinariae* (Tissot, 1933) to *Melanaphis* and did not include it in additional analyses.

Pursuant to Article 57 of the International Code of Zoological Nomenclature (International Commission on Zoological Nomenclature 2000), *Melanaphis arundinariae* (Takahashi 1937) is considered a junior homonym of *Melanaphis arundinariae* (Tissot 1933a). We therefore suggest *Melanaphis takahashii* Skvarla, Kramer, Owen, and Miller, in honor of Ryoichi Takahashi, who originally described the species, as a replacement name for *Melanaphis arundinariae* (Takahashi, 1937).

While sorting undetermined *Rhopalosiphum* specimens in the USNM collection, three slides of specimens collected from *Acorus* in North America were discovered. The specimens match the measurements and brief descriptions of European *R. rufulum* apterous viviparae (Stroyan 1972, Heie 1986). These specimens represent the first collections of *R. rufulum* from secondary hosts in North America and extend the known range southeast of previous collections. In Europe, colonies on *A. calamus* are reported to grow so large that “the plants look bespattered with black mud” (Heie 1986), so, while the species is rarely collected in North America, it is presumably common where the appropriate host plants are present. The collection data are as follows:

CANADA: 3 female apterae, locality unknown (label states “at HO-17214”), ex *Acorus calamus*, 29-IX-1952, J. Adams, USNM; UNITED STATES: Massachusetts: 2 female apterae, Hampshire Co., Amherst, ex. *Acorus*, 31-V-1964, M. Smith, USNM; New York: 3 nymphs, Suffolk Co., Greenport, ex. *Acorus calamus*, 25-VI-1963, R. Latham, USNM.

## Morphometric analyses

In the first LDA of apterae, *R. maidis* and its synonym *R. africana* (Theobald, 1914) formed a distinct cluster in the plots of LD1 x LD2 and LD1 x LD3 (Fig. 5). *Rhopalosiphum nymphaeae* L., 1761) and its synonym *R. sparganii* (Theobald, 1925) formed a distinct cluster in the LD1 x LD2 plot (Fig. 4a) and *R. sp. nov. “ex. Arisaema”* formed a distinct cluster in the plot of LD1 x LD3 (Fig. 4b). These five species were removed prior to performing the second LDA.

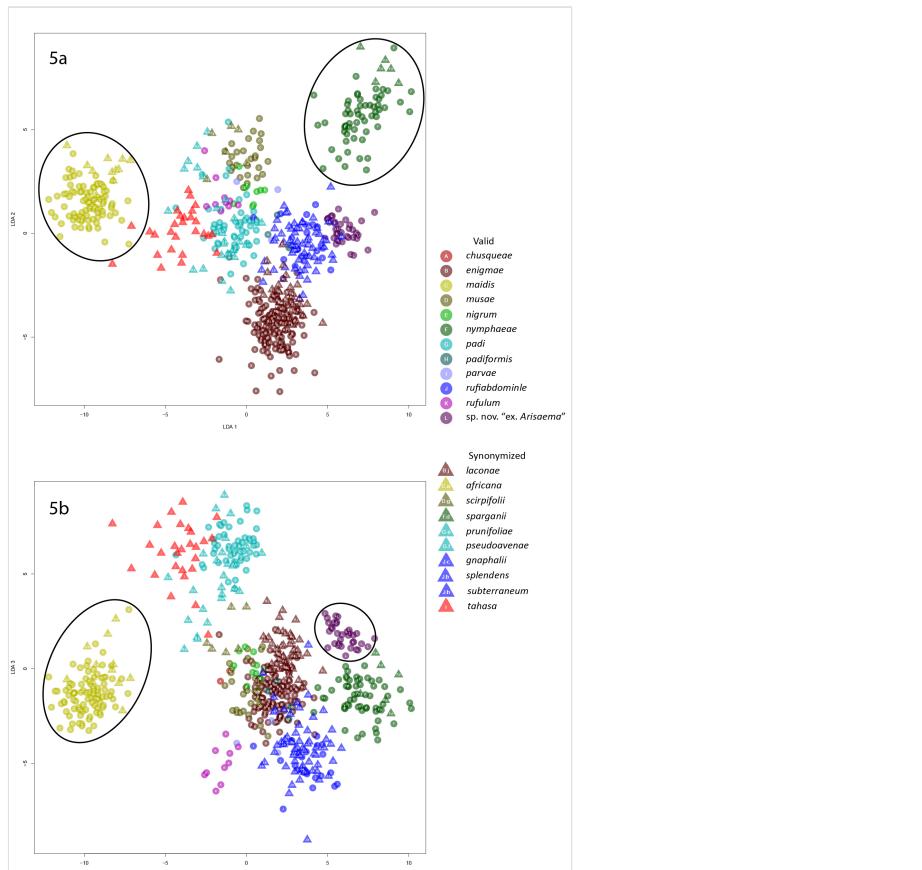
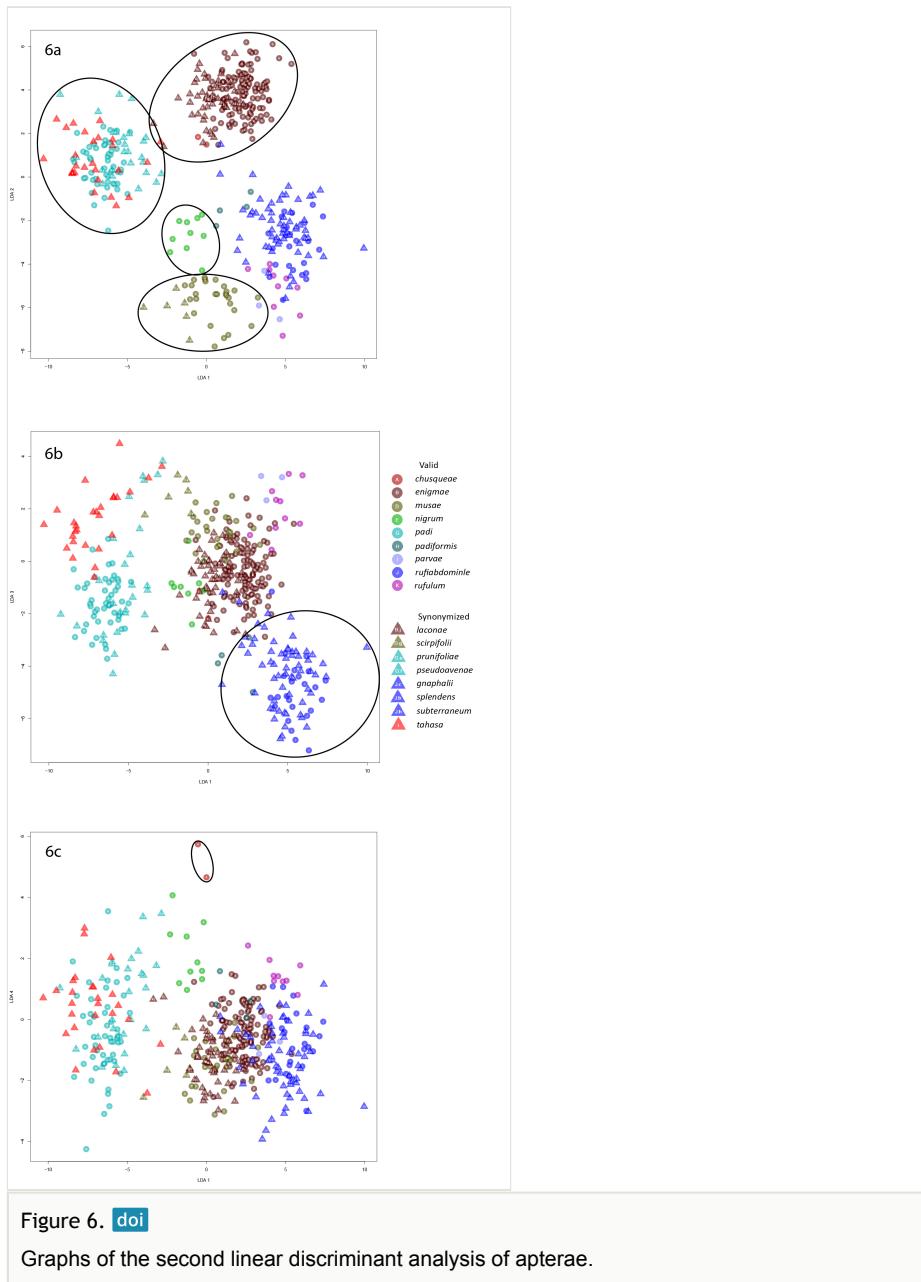


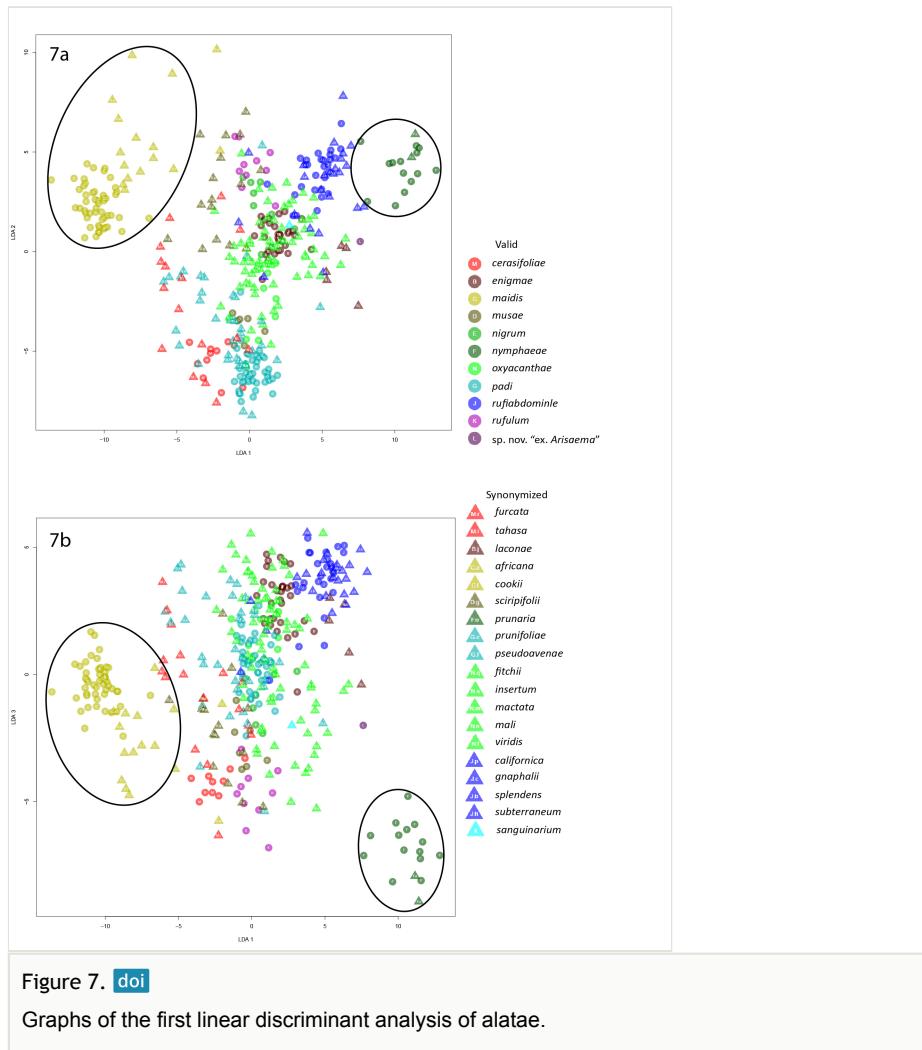
Figure 5. [doi](#)

Graphs of the first linear discriminant analysis of apterae.

In the second linear discriminant analysis of apterae, *R. padi* and its synonyms *R. prunifoliae* (Fitch, 1855) and *R. pseudoavenae* (Patch, 1917) formed distinct clusters in both the LD1 x LD2 and LD1 x LD3 plots (Fig. 6); *Rhopalosiphum tahasa* (Hottes, 1950) also clustered with *R. padi*, although this may be an artifact of the fact that the species with which it is currently synonymized, *R. cerasifoliae*, was not included in the analyses. In the LD1 x LD2 plot, *Rhopalosiphum enigmae* and its synonym *R. laconae* (+ *R. chusqueae*), *R. musae* (Schouteden, 1906) and its synonym *R. scirpifolii* Gillette & Palmer, 1932 and *R. nigrum* Richards, 1960 all formed distinct clusters. In the LD1 x LD3 plot, *Rhopalosiphum rufabdominale* (Sasaki, 1899) and its three synonyms *R. gnaphalii* Tissot, 1933, *R. subterraneum* Mason, 1937 and *R. splendens* (Theobald, 1915) clustered together although there was some overlap of *R. splendens* with *R. enigmae* + *R. laconae*. *Rhopalosiphum chusqueae* formed a distinct cluster in the LD1 x LD4 plot. *Rhopalosiphum parvae* Hottes and Frison, 1931 and *R. rufulum* clustered together in all plots, not forming separate clusters in any plot.

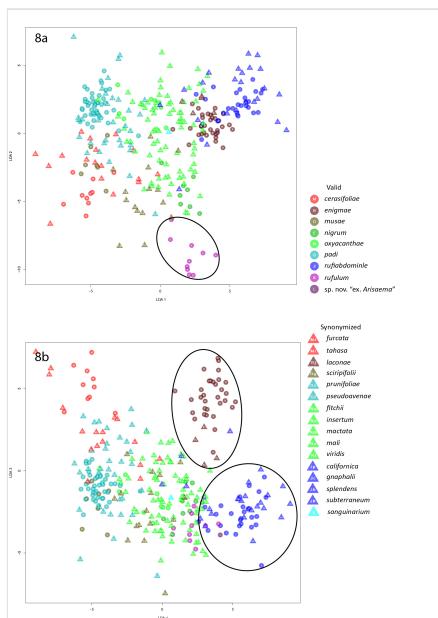


In the first linear discriminant analysis of alatae, *R. maidis* formed a cluster with its synonyms *R. cookii* (Essig, 1911) and *R. africana* and *R. nymphaeae* clustered with its synonym *R. prunaria* (Walker, 1848) in the plots of LD1 x LD2 and LD1 x LD3 (Fig. 7). These five species were removed prior to performing the second LDA except for the three most outlying *R. africana*, which were paratypes.

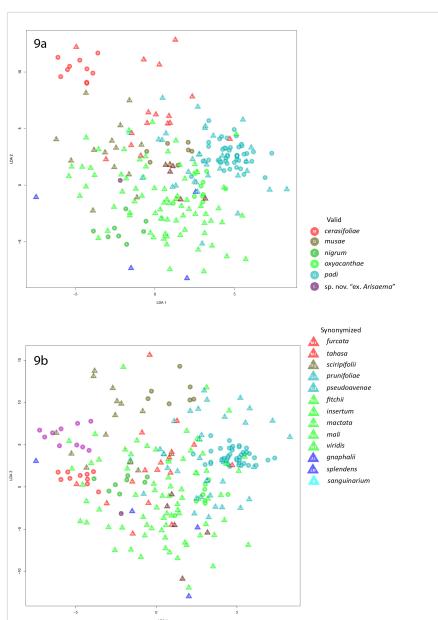


In the second linear discriminant analysis of alatae, *R. rufulum* (along with one of the outlier *R. africana* not removed from the analyses) formed a distinct cluster in the LD1 x LD2 plot (Fig. 8). *Rhopalosiphum enigmae* and its synonym *R. laconae* formed a distinct cluster in the LD1 x LD3 plot and were removed prior to performing the third LDA. *Rhopalosiphum rufiabdominale* and its synonyms *R. californica* (Essig, 1944), *R. splendens* (Theobald, 1915), and *R. subterraneum* Mason, 1937 formed a distinct cluster in the LD1 x LD3 plot with minimal overlap with *R. oxyacanthae* synonyms and so were also removed prior to the third LDA.

None of the remaining species formed distinct clusters in the third linear discriminant analysis (Fig. 9).

**Figure 8.** doi

Graphs of the second linear discriminant analysis of alatae.

**Figure 9.** doi

Graphs of the third linear discriminant analysis of alatae.

## Discussion

### Current species and natural history

The synonymization of *R. momo* with *R. nymphaeae* and movement of *M. arundinariae* (Tissot, 1933) to *Melanaphis* brings the total number of described *Rhopalosiphum* species to 17, two of which (*R. dryopterae* and *R. esculentum*) are questionably assigned to the genus, pending examination of the type material, with an additional four undescribed species known.

The discovery of *R. rufulum* on *Acorus* in North America and the presence of an undescribed *Rhopalosiphum* species on *Arisaema* in eastern North America echoes the sentiment of Skvarla et al. (2018) – who found that *R. enigmae*, which was once considered a rare species, was present in every cattail stand examined – that many rarely collected *Rhopalosiphum* species are likely abundant in the correct habitats/secondary hosts and that apparent rarity may be due to inadequate collecting efforts on non-crop plants. Indeed, we expect that additional surveys of semi-aquatic plants, especially monocots, will continue to produce new species of *Rhopalosiphum* in North America and probably East and Southeast Asia.

### Morphological analyses in the molecular era

Determining species boundaries and synonymies using molecular techniques has become the standard by which most modern taxonomy and systematics are measured. Indeed, with the continually lower costs and greater ease with which highly degraded DNA can be extracted and sequenced from historic museum specimens (Gilbert et al. 2007, Bi et al. 2013, Tin et al. 2014, McCormack et al. 2015, Blaimer et al. 2016, Sproul and Maddison 2017), determining species validity and synonomies using old specimens, including type material, has never been easier (e.g. Kirchman et al. 2009, Mutanen et al. 2015, McGuire et al. 2018). However, most aphids and other slide-mounted arthropods present a relatively unique challenge in that all of the DNA is destroyed when specimens are cleared for mounting (although the authors have seen a few decades-old aphids that were not cleared before being slide-mounted in balsam and have wondered if it would be possible to free them from the mounting medium and extract DNA). Thus, even in this era when molecular techniques dominate, there is still a need for robust morphological comparisons for certain groups, especially aphids, as has been demonstrated here.

### LDA methodology applied to apterae confirms most species and their synonymies

In general, the linear discriminant analyses were successful when applied to apterae. The analyses successfully grouped all synonymized species with their associated valid species over two iterations and we concluded that most of the synonymizations we tested are sound. This demonstrates that linear discriminant analyses can be used to test synonymizations when DNA is unavailable and provides a new method to examine and use

historic, slide-mounted specimens. Additionally, *R. sp. nov. "ex. Arisaema"* formed a distinct group when it was included as a valid species. This supports its status as a valid, but undescribed, species and demonstrates that linear discriminant analysis can be used to distinguish potentially undescribed species from valid described species based on morphological similarities.

However, a few notable problems exist. First, *R. parvae* and *R. rufulum* consistently clustered together, but never as a distinct group away from other species. This may indicate that they are synonymous, although several factors indicate they are distinct species: they feed on different secondary hosts (*Carex* and *Acorus*, respectively), have non-overlapping ranges (Illinois versus New England and adjacent Canada), and have several morphological differences that separate them (Table 5). However, this is based on very few individuals (3 and 10 specimens, respectively). Without additional specimens from a wider geographic range and considering the above evidence, we elected to leave them as separate species, although acknowledge the LDA indicated these species concepts should be revisited in the future.

Table 5.

Morphological differences between *R. parvae* and *R. rufulum*. Measurement and ratio ranges are followed parenthetically by the mean and number of specimens measured.

Species	ant-6	caud-l	bl	rost-l:rost-w	ant-pt:ant-6	bl:head	bl:ant-pt
<i>R. parvae</i>	61.7–66.8 (64.7, 3)	91.8–103.1 (98.4, 3)	1410.2–1437.0 (1427.9, 3)	2.13–2.16 (2.15, 2)	3.37–3.77 (3.56, 3)	3.48–3.84 (3.60, 3)	5.80–6.90 (6.22, 3)
<i>R. rufulum</i>	77.1–91.0 (83.6, 9)	94.9–131.6 (119.7, 9)	1829.0–2186.0 (1969.3, 10)	1.37–2.06 (1.78, 9)	2.42–3.45 (3.14, 9)	4.15–5.04 (4.61, 10)	6.65–9.71 (7.50, 9)

Unfortunately, we were unable to include verified apterous *R. cerasifoliae* in the analyses due to lack of specimens. We included specimens of *R. tahasa*, which is synonymized with *R. cerasifoliae*, in the analyses to see if they would still form a distinct cluster without valid *R. cerasifoliae* to act as a guide in the discriminant function. Instead, the *R. tahasa* specimens clustered well with *R. padi*, which it is not synonymized with, rather than forming a distinct cluster. This association has not previously been suggested in the literature, but without the inclusion of *R. cerasifoliae*, it is impossible to determine if *R. tahasa* should instead be synonymized with *R. padi*. This issue suggests that it is extremely important to include all valid species when creating the discriminant functions, so that synonymized species can be properly plotted. Additionally, assuming that *R. tahasa* is synonymous with *R. cerasifoliae*, it suggests that valid species analyzed with the synonymized species (either because they are incorrectly synonymized with a different species or because, as in this case, a synonymized species is included without specimens of its associated valid species) may not form distinct clusters.

## LDA methodology worked less well for classifying alatae using morphological traits

The analyses of alatae were less decisive. Intuitively, species with the most distinctive apterae – *R. maidis*, *R. nymphaeae*, *R. enigmae*, and *R. rufiabdominale* – also had the most distinctive alatae and formed distinct clusters with their associated synonymized species in the first two LDA. However, the remaining species failed to form distinct clusters, even after a third LDA. Additionally, a number of synonymized species – *R. furcata*, *R. fitchii*, *R. insertum*, *R. mactata*, *R. mali*, and *R. viridis* – were only represented by alate specimens and did not cluster with the species with which they are synonymized. Based on our analyses, we cannot confirm that these synonymies are correct, although we also do not propose any be raised as valid species pending additional investigation.

Aphidologists have found that *Rhopalosiphum* alatae captured without host plant data (e.g., in suction traps) are difficult to identify to species due to similar, conserved morphologies. While published keys to alatae are available (i.e. Richards 1960), they can be difficult to use because no key includes all described species and specimens often do not neatly fit within one couplet or another. Rather than revealing new, perhaps subtle, morphological characters that can be used to distinguish alatae, the suboptimal results from the alatae analyses reinforces the perception that they are very similar morphologically and reticent to identification without additional information (e.g. associated apterae, host plant data).

## LDA loadings to create taxonomic keys

The loadings (coefficients) for each character on the discriminant functions could conceivably be used to create taxonomic keys. However, we do not suggest following that path for this group for a number of reasons: measures we used were adjusted by size, based on our data, so this same adjustment would need to be made for all measures (except wing venation angles); variables were then standardized to mean zero, variance one, so each variable would need to be standardized in the same way we did (again, the standardization we used was based on our data); the functions are built largely on continuous characters, rather than naturally dichotomous ones and all characters contribute to each discriminant function. However, since the variables were standardized, their importance (i.e. weighting) to the discriminant function can be evaluated, based on the absolute value of the coefficient. One could determine a cut-off for which variables were important. We looked at this for all the discriminant functions and found that, for any reasonable cut-off, some discriminant functions were largely determined by just a few characters (which would be useful for creating a key), but many were largely determined by at least 12 (which would be less useful). Since placement in the plane for any specimen is determined by two discriminant functions, many with large contributions from many characters, creating a usable key from these results would not be an easy exercise. One possible statistical approach to creating keys from continuous characters that should be explored is the use of regression trees.

## Summary

Two species of *Rhopalosiphum* were moved or synonymized, bringing the total number of species in the genus to 17. While LDA has previously been used to distinguish between a limited number of species or ecotypes, the use of it to confirm previously proposed synonymies using historic slide-mounted specimens that lack DNA is novel and yielded promising results. In particular, the analyses confirmed most synonymizations when apterae were analyzed. However, while the most distinct alate *Rhopalosiphum* and associated synonymies were recovered in the LDA, many species and associated synonymies were not recovered as distinct. The failure of the analyses with some of the alatae using phenotypic traits mirrors problems previously documented for this morphologically similar group.

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## References

- Baker AC (1918) Another *Toxoptera* feeding on sedge (Homoptera; Aphididae). *Psyche* 25 (4): 88-93.
- Baker JM (1934) Algunos áfidos mexicanos. *Anales del Instituto de Biología de la Universidad Nacional de México* 5 (3): 209-222.
- Barro PJD (1992) Karyotypes of cereal aphids in South Australia with special reference to *Rhopalosiphum maidis* (Fitch) (Hemiptera: Aphididae). *Australian Journal of Entomology* 31 (4): 333-334. <https://doi.org/10.1111/j.1440-6055.1992.tb00518.x>
- Bašilova J (2010) The application of discriminant analysis to identify *Cryptomyzus* aphids. *Žemdirbystė* 97 (4): 99-106.
- Bi K, Linderöth T, Vanderpool D, Good J, Nielsen R, Moritz C (2013) Unlocking the vault: next-generation museum population genomics. *Molecular Ecology* 22 (24): 6018-6032. <https://doi.org/10.1111/mec.12516>
- Blackman RL (1954) The males of *Rhopalosiphum maidis* (Fitch) and a discussion on the use of males in aphid taxonomy. *Proceedings of the Entomological Society of London* 20: 84-86.
- Blackman RL, Halbert SE, Carroll TW (1990) Association between karyotype and host plant in corn leaf aphid (Homoptera: Aphididae) in the Northwestern United States. *Environmental Entomology* 19: 609-611.

- Blackman RL, Brown PA (1991) Morphometric variation within and between populations of *Rhopalosiphum maidis* with a discussion of the taxonomic treatment of permanently parthenogenetic aphids. *Entomologia Generalis* 16 (2): 97-113.
- Blackman RL, Spence JM (1994) The effects of temperature on aphid morphology, using a multivariate approach. *European Journal of Entomology* 91: 7-22.
- Blackman RL, Eastop VF (2000) Aphids on the world's crops: an identification and information guide. 2. John Wiley & Sons Ltd, 476 pp.
- Blackman RL, Eastop VF (2017) Aphids on the world's plants. <http://www.aphidsonworldplants.info/>. Accessed on: 2017-3-13.
- Blaimer B, Lloyd M, Guillory W, Brady S (2016) Sequence Capture and Phylogenetic Utility of Genomic Ultraconserved Elements Obtained from Pinned Insect Specimens. *PLOS ONE* 11 (8): 1-20. <https://doi.org/10.1371/journal.pone.0161531>
- Blanchard EE (1939) Estudio sistemático de los afidoideos argentinos. *Physis* 17: 857-1003.
- Börner C (1931) Mitteilungen über Blattläuse. *Anzeiger für Schädlingskunde* 7 (1): 8-11.
- Börner C (1952) Europe centralis Aphides. Die Blattläuse mittleeuropas: Namen, Synonyme, Wirtspflanzen, Generationzyklen. *Mitteilungen der Thüringischen Botanischen Gesellschaft Supplement* 3: 1-259.
- Brown PA, Blackman RL (1988) Karyotype variation in the corn leaf aphid, *Rhopalosiphum maidis* (Fitch), species complex (Hemiptera: Aphididae) in relation to host-plant and morphology. *Bulletin of Entomological Research* 78: 351-363.
- Buckton GB (1879) *Monograph of British Aphides*. 2. The Ray Society, London, 176 pp.
- Bulman SR, Stukens MA, Eastop VF, Teulon DA (2005) *Rhopalosiphum* aphids in New Zealand. II. DNA sequences reveal two incompletely described species. *New Zealand Journal of Zoology* 32: 37-45.
- Chan CK, Forbes AR, Raworth DA (1991) Aphid-transmitted viruses and their vectors of the world. 1991-3E. *Agriculture Canada Technical Bulletin*, 216 pp.
- Ciampolini M, di Perna V, Maiolini C (1993) Damage by root aphids to vegetable crops in greenhouses in Lazio. *Informatore-Agrario* 49: 59-63.
- Coeur d'Acier A, Cruaud A, Artige E, Genson G, Clamens A, Pierre E, Hudaverdian S, Simon J, Jousselin E, Rasplus J (2014) DNA barcoding and the associated PhylAphidB@se website for the identification of European aphids (Insecta: Hemiptera: Aphididae). *PLOS One* 9 (6): e97620. <https://doi.org/10.1371/journal.pone.0097620>
- del Guercio G (1914a) Generi e specie nouve di afididi o nuovi per la fauna Italiana. *Redia* 9: 169-19.
- del Guercio G (1914b) Specie nouve di afidini le Graminacee in Italia a confront con quelle conosciute. *Redia* 9: 197-212.
- Dobrowljansky WW (1913) Contribution to the biology of aphids of fruit trees and berry bushes. *Entomologische Station de Landwirtschaftlichen Syndikate in Kiew* 3-49.
- Eastop VF, Hille Ris Lambers D (1976) Survey of the world's aphids. Dr. W. Junk b.v. Publishers, The Hague, 573 pp.
- Essig EO (1911) Aphididae of Southern California V. *Pomona College Journal of Entomology* 3 (4): 404-411.
- Essig EO (1944) A new aphid of guayule and notes on other species of *Cerosiphaphila*. *Hilgardia* 16 (4): 177-184.

- Etzel RW, Pettitt FL (1992) Association of *Verticillium lecanii* with population reduction of red rice root aphid (*Rhopalosiphum rufiabdominalis*) on aeroponically grown squash. *Florida Entomologist* 75: 605-606.
- Evenhuis NL (2017) The insect and spider collections of the world website. <http://hbs.bishopmuseum.org/codens/>. Accessed on: 2017-2-15.
- Favret C, Voegtlin DJ (2004) Host-based morphometric differentiation in three *Cinara* species (Insecta: Hemiptera: Aphididae) feeding on *Pinus edulis* and *P. monophyla*. *Western North American Naturalist* 64 (3): 364-375.
- Favret C, Miller G, Jensen A (2008) Fitch's *Aphis mali* varieties are *Rhopalosiphum oxyacanthae* (Hemiptera: Aphididae). *Entomological News* 119 (5): 521-523. <https://doi.org/10.3157/0013-872X-119.5.521>
- Favret C (2009) Wing morphometry helps diagnose cryptic species and resurrect *Mindarus pinicolus* (Hemiptera: Aphididae). *Annals of the Entomological Society of America* 102 (6): 970-981.
- Favret C, Miller GL (2012) AphID. <http://AphID.AphidNet.org/>. Accessed on: 2016-11-14.
- Favret C (2019) Aphid species file. <http://Aphid.SpeciesFile.org>. Accessed on: 2019-5-14.
- Ferrari PM (1872) Aphididae Liguria. *Annali del Museo Civico di Storia Naturale di Genova* 2: 49-85.
- Fitch A (1855) Report on the noxious, beneficial and other insects of the state of New York. *Transactions of the New York State Agricultural Society* 14: 705-880.
- Fitch A (1856) Report on the noxious, beneficial and other insects of the state of New York. *Transactions of the New York State Agricultural Society* 15: 550.
- Foottit RG, Richards WR (1993) The insects and arachnids of Canada. Part 22. The genera of aphids of Canada (Homoptera: Aphidoidea and Phylloxeroidea). Centre for Land and Biological Resources, Research Branch, Ottawa, 766 pp.
- Foottit RG, Maw HE, Von Dohle CD, Herbert PD (2008) Species identification of aphids (Insecta: Hemiptera: Aphididae) through DNA barcodes. *Molecular Ecology Resources* 8 (6): 1189-1201.
- Foottit RG, Maw HEL, Pike KS, Miller RH (2010) The identity of *Pentalonia nigronervosa* Coquerel and *P. caladii* van der Goot (Hemiptera: Aphididae) based on molecular and morphometric analysis. *Zootaxa* 2358 (1): 25-38. <https://doi.org/10.11646/zootaxa.2358.1.2>
- Fullaway DT (1910) Report of the entomologist. Annual Report of the Hawaiian Agricultural Experiment Station 1909 17-46.
- García Prieto F, Pérez Hidalgo N, Mier Durante MP, Nieto Nafría JM (2004) Updated check-list of Iberian-Balearic Aphidini (Hemiptera, Aphididae). *Graellsia* 60: 197-214.
- Ghosh AK, Banerjee H, Raychaudhuri DN (1971) Studies on the aphids (Homoptera: Aphididae) from Eastern India. *Proceedings of the Zoological Society, Calcutta* 24: 99-111.
- Gilbert MTP, Moore W, Melchior L, Worobey M (2007) DNA Extraction from Dry Museum Beetles without Conferring External Morphological Damage. *PLoS ONE* 2 (3). <https://doi.org/10.1371/journal.pone.0000272>
- Gillette CP, Palmer MA (1932) Six new aphids from Colorado. *Annals of the Entomological Society of America* 25 (1): 136-151.

- Halbert S, Remaudière G, Webb S, Remaudiere G (2000) Newly established and rarely collected aphids (Homoptera: Aphididae) in Florida and the southeastern United States. *The Florida Entomologist* 83 (1): 79-91. <https://doi.org/10.2307/3496232>
- Halbert SE, Voegtlin DJ (1998) Evidence for the North American origin of *Rhopalosiphum* and Barley Yellow Dwarf Virus. In: Nieto Nafría JM, Dixon AF (Eds) *Aphids in natural and managed ecosystems*. Universidad de León, León, 351–356 pp.
- Heie OE (1986) The Aphidoidea (Hemiptera) of Fennoscandia and Denmark, Part 3. Vinderup Bogtrykkeri A/S, Vinderup, 317 pp.
- Henderson A (2006) Traditional morphometrics in plant systematics and its role in palm systematics. *Botanical Journal of the Linnean Society* 151 (1): 103-111. <https://doi.org/10.1111/j.1095-8339.2006.00526.x>
- Hille Ris Lambers D (1966) Polymorphism in Aphididae . *Annual Review of Entomology* 11 (1): 47-78. <https://doi.org/10.1146/annurev.en.11.010166.000403>
- Holman J (2009) Host Plant Catalog of Aphids. Palaearctic Region. Springer, Berlin, 1216 pp.
- Hori M (1927) Some new aphids from Hokkaido. *Insecta Matsumurana* 1 (4): 196-198.
- Hottes FC, Frison TH (1931) The plant lice, or Aphididae, of Illinois. *Bulletin of the Illinois Natural History Survey* 19: 121-447.
- International Commission on Zoological Nomenclature (2000) International code of zoological nomenclature. <http://www.iczn.org/code>. Accessed on: 2018-1-15.
- Jackson CF (1908) Notes on the Aphididae. I. Observations on a semi-aquatic aphid, *Aphis aquaticus* n. sp. *Ohio Naturalist* 8 (3): 243-249.
- Jauset AM, Muñoz MP, Pons X (2000) Karyotype occurrence and host plants of the corn leaf aphid (Homoptera: Aphididae) in a Mediteranean Region. *Annals of the Entomological Society of America* 93 (5): 1116-1122.
- Jenson AS, Holman J (2000) *Macrosiphum* on ferns: taxonomy, biology and evolution, including the description of three new species (Hemiptera: Aphididae). *Systematic Entomology* 25: 339-372.
- Kan AA (1986) Fauna and ecology of the root aphids of the Middle Asian and Kazakhstan. Tashkent, Mechnat 1-214.
- Kim H, Lee S (2008) A molecular phylogeny of the tribe Aphidini (Insecta: Hemiptera: Aphididae) based on the mitochondrial tRNA/ COII, 12S/16S and the nuclear EF1 $\alpha$  genes. *Systematic Entomology* 33: 711-721.
- Kirchman J, Witt C, McGuire J, Graves G (2009) DNA from a 100-year-old holotype confirms the validity of a potentially extinct hummingbird species. *Biology Letters* 6 (1): 112-115. <https://doi.org/10.1098/rsbl.2009.0545>
- Koch CL (1854a) Die Pflanzenläuse Aphiden getreu nach dem Leben abgebildet und beschrieben. Verlag von J.L. Lotzbeck, Nürnberg, 73-100 pp.
- Koch CL (1854b) Die Pflanzenläuse Aphiden getreu nach dem Leben abgebildet und beschrieben. Verlag von J.L. Lotzbeck, Nürnberg, 37-72 pp.
- Koch CL (1857) Die Pflanzenläuse Aphiden getreu nach dem Leben abgebildet und beschrieben. Verlag von J.L. Lotzbeck, Nürnberg, 335 pp.
- Linnaeus C (1758) *Systema naturæ per regna tria naturæ, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonymis, locis. Tomus I. Editio decima, reformata*. Holmiae: Impensis Direct. Laurentii Salvii, 824 pp. URL: <https://www.biodiversitylibrary.org/bibliography/542#/summary>

- Linnaeus C (1761) Fauna Svecica sistens animalia Sveciae regni: Mammalia, Aves, Amphibia, Pisces, Insecta, Vermes. Distributa per classes et ordines, genera et species, cum differentiis specierum, synonymis auctorum, nominibus incolarum, locis natalium, descriptionibus insectorum. Editio altera. Sumtu & Literis Direct, Stockholm, 578 pp.
- Lozier JD, Foottit RG, Miller GL, Mills NJ, Roderick GK (2008) Molecular and morphological evaluation of the aphid genus *Hyalopterus* Koch (Insecta: Hemiptera: Aphididae), with a description of a new species. Zootaxa 1688 (1): 1-19. <https://doi.org/10.11646/zootaxa.1688.1.1>
- Mason PW (1937) A new root aphid (Homoptera: Aphididae). Proceedings of the Entomological Society of Washington 39 (6): 166-167.
- Matsumura S (1917) A list of the Aphididae of Japan, with description of new species and genera. Journal of the College of Agriculture, Tohoku Imperial University 7: 351-414.
- Matsumura S (1918) New Aphidinae of Japan. Transactions of the Sapporo Natural History Society 7 (1): 1-22.
- McCormack J, Tsai WE, Faircloth B (2015) Sequence capture of ultraconserved elements from bird museum specimens. Molecular Ecology Resources 16 (5): 1189-1203. <https://doi.org/10.1111/1755-0998.12466>
- McGuire JA, Cotoras DD, O'Connell B, Lawalata SZS, Wang-Claypool CY, Stubbs A, Huang X, Wogan GOU, Hykin SM, Reilly SB, Bi K, Riyanto A, Arida E, Smith LL, Milne H, Streicher JW, Iskandar DT (2018) Squeezing water from a stone: high-throughput sequencing from a 145-year old holotype resolves (barely) a cryptic species problem in flying lizards. PeerJ 6: e4470. <https://doi.org/10.7717/peerj.4470>
- Menon MG, Ghai S (1969) The oviparous form of *Rhopalosiphum maidis* (Fitch) (Homoptera). Oriental Insects 3: 383-388.
- Monzen K (1929) Studies on some gall producing aphides and their galls. Monograph Saito Ho-on Kai 1: 1-80.
- Mordvilko AK (1921) Les pucerons des graminées (Aphidodea), Pars I. Izvestija Severnoi oblastnoi stancii Zashchity rastenii ot vreditelei 3 (3): 1-72.
- Mutanen M, Kekkonen M, Prosser SJ, Hebert PN, Kaila L (2015) One species in eight: DNA barcodes from type specimens resolve a taxonomic quagmire. Molecular Ecology Resources 15 (4): 967-984. <https://doi.org/10.1111/1755-0998.12361>
- Pantoja A, Alvarez JM, Munyaniza J, Hagerty A, Adams T (2006) Aphids and leafhoppers associated with potatoes in Alaska. In: Potato Association of America Proceedings, Potato Association of America 2006 Meeting. 193-194 pp.
- Passerini DJ (1861) Additamenta ad indicem aphidinarum quas hucusque in Italia legit. Atti della Società Italiana di Scienze Naturali in Milano 3 (398): 401.
- Patch EM (1914) Maine aphids of the rose family. Bulletin of the Maine Agricultural Experiment Station 233: 253-280.
- Patch EM (1917) The aphid of chokecherry and grain. Bulletin of the Maine Agricultural Experiment Station 267: 211-224.
- Pérez-Hidalgo N, Martínez-Torres D, Collantes-Alegre JM, Villalobos Muller W, Nieto Nafría J (2012) A new species of *Rhopalosiphum* (Hemiptera, Aphididae) on *Chusquea tomentosa* (Poaceae, Bambusoideae) from Costa Rica. ZooKeys 166: 59-73. <https://doi.org/10.3897/zookeys.166.2387>

- Phillips WJ, Davis JJ (1912) Papers on Aphididae. Studies on a new species of *Toxoptera*, with an analytical key to the genus and notes on rearing methods. United States Department of Agriculture Bureau of Entomology, Technical Series 25 (1): 1-1.
- Rakauskas R, Havelka J, Bernotiene R (2015) *Rhopalosiphum rufiabdominale*: first records from winter host plants in Europe. *Bulletin of Insectology* 68 (1): 73-81.
- Raychaudhuri DN, Roychoudhuri D (1978) A new species of *Rhopalosiphum* (Homoptera: Aphididae) from South India. *Science and Culture* 44 (5): 239-40.
- R Core Team (2017) A language and environment for statistical computing. R Foundation for Statistical Computing. URL: <https://www.R-project.org/>
- Remaudière G, Remaudière M (1997) Catalogue des Aphididae du monde. Institut National de la Recherche Agronomique, Paris, 473 pp.
- Richards WR (1960) A synopsis of the genus *Rhopalosiphum* in Canada. *Canadian Entomologist* 13: 1-51.
- Richards WR (1962) A new species of *Rhopalosiphum*. *Canadian Entomologist* 94 (9): 969-972.
- Rondani C (1852) *Aphis graminum* n. sp. *Nuove Ann. Sci. Nat. Bologna* 6: 9-11.
- Sanderson ED (1902) Report of the Entomologist. Thirteenth Annual Report of the Deleware College Agricultural Experiment Station 1-213.
- Sasaki C (1899) *Toxoptera rufiabdominalis* n. sp. Report of the Hokkaido Agricultural Experiment Station 17: 202.
- Schouteden H (1906) Catalogue des aphides de Belgique. Mémoires de la Société Entomologique de Belgique 12: 189-246.
- Shinji O (1922) New genera and species of Japanese Aphididae. *Dobutsugaku Zasshi* (Zoological Magazine) 24 (407): 787-800. [In Japanese].
- Signoret VA (1860) Faune des Hémiptères de Madagascar I. *Annales de la Société Entomologique de France* 8 (1): 177-206.
- Simon JC, Herbert PD, Carillo C, de Melo R (1995) Lack of clonal variation among Canadian populations of the corn leaf aphid, *Rhopalosiphum maidis* Fitch (Homoptera: Aphididae). *Canadian Entomologist* 127: 623-629.
- Skvarla M, Halbert S, Foottit R, Jensen A, Maw E, Miller G (2017) An update to the adventive aphids (Hemiptera: Aphidoidea) of America North of Mexico, with notes on intercepted species. *Proceedings of the Entomological Society of Washington* 119 (1): 90-111. <https://doi.org/10.4289/0013-8797.119.1.90>
- Skvarla M, Miller G, Bauchan G, Lewis M, Foottit R, Maw E (2018) Taxonomy and natural history of cattail aphids, *Rhopalosiphum enigmae* Hottes & Frison, 1931 (Hemiptera: Aphidomorpha: Aphididae), including a new synonymy and notes on ant and parasitoid associates of *Rhopalosiphum*. *Insect Systematics and Diversity* 2 (2): 1-14. URL: <http://zoobank.org/DE305539-03BD-473E-AA5B-87B079E61E0E>
- Sproul J, Maddison D (2017) Sequencing historical specimens: successful preparation of small specimens with low amounts of degraded DNA. *Molecular Ecology Resources* 17 (6): 1183-1201. <https://doi.org/10.1111/1755-0998.12660>
- Stern D, Aoki S, Kuroso S (1997) Determining aphid taxonomic affinities and life cycles with molecular data: a case study of the tribe Cerataphidini (Hormaphididae: Aphidoidea: Hemiptera). *Systematic Entomology* 22 (1): 81-96.
- Strażyński P (2010) Population structure of *Rhopalosiphum padi* (Linnaeus, 1758) (Hemiptera, Aphidoidea) in Wielkopolska region in 2003-2008 in the context of winter cereals threat of BYDV expansion. *Aphids and Other Hemipterous Insects* 16: 91-106.

- Stroyan HL (1972) Additions and amendments to the check list of British aphids (Homoptera: Aphidoidea). *Transactions of the Royal Entomological Society of London* 124 (1): 37-79.
- Taber SW (1993) A new *Rhopalosiphum* on cattail (Homoptera: Aphididae). *Pan-Pacific Entomologist* 69 (4): 323-328.
- Taber SW (2003) Range extension, new hosts, new morphs, and a synonym of the sedge aphid, *Rhopalosiphum musae* (Schouteden). *Southwest Entomologist* 28 (1): 81-83.
- Takahashi R (1919) Seven species of Aphididae . *Sapporo Journal of Natural History* 7: 194-201.
- Takahashi R (1921) Aphidæ of Formosa. Part I. Report of the Department of Agriculture, Government Research Institute, Formosa 20: 1-97.
- Takahashi R (1937) Additions to the aphids fauna of Formosa (Hemiptera), IV. *Philippine Journal of Science* 63 (1): 1-18.
- Theobald FV (1912) List of Aphididae of Hastings District. *Hastings and E. Sussex Naturalist* 2: 9-20.
- Theobald FV (1914) African Aphididae . *Bulletin of Entomological Research* 4: 313-337.
- Theobald FV (1915) African Aphididae. Part II. *Bulletin of Entomological Research* 6: 103-153.
- Theobald FV (1927) The Plant Lice or Aphididae of Great Britain. Vol II. Headley Bros., London, 411 pp.
- Theobald FV (1929) The Plant Lice or Aphididae of Great Britain. Vol III. Headley Bros., London, 364 pp.
- Tin MM, Economo EP, Mikheyev AS (2014) Sequencing Degraded DNA from Non-Destructively Sampled Museum Specimens for RAD-Tagging and Low-Coverage Shotgun Phylogenetics. *PLoS ONE* 9 (5): 1-9. <https://doi.org/10.1371/journal.pone.0096793>
- Tissot AN (1933a) Five new species of *Anuraphis* and *Aphis* . *Florida Entomologist* 16 (4): 49-60.
- Tissot AN (1933b) Additions to the aphid fauna of Florida. *Florida Entomologist* 17 (3): 37-45.
- Valenzuela I, Eastop VF, Ridland PM, Weeks AR (2009) Molecular and morphometric data indicate a new species of the aphid genus *Rhopalosiphum* (Hemiptera: Aphididae). *Annals of the Entomological Society of America* 102 (6): 914-924.
- van der Goot P (1917) Zur Kenntnis der Blattläuse Java's. Contributions à la Fauna des Indes Néerlandaises 3: 1-301.
- Venables WN, Ripley BD (2002) Modern Applied Statistics with S. 4th. Springer, New York, 498 pp.
- von Schrank FP (1801) Blattlaus, Fauna Boica. Durchgedachte Geschichten der in Baiern einheimischen und zahmen Thiere. *Fauna Boica* 2 (1): 102-4140.
- Walker F (1848) Descriptions of Aphides. *Zoologist* 6: 2217-2221-2246-2252.
- Walker F (1849) Descriptions of new British aphides. *Zoologist* 7: 43-57.
- Walsh BD (1863) On the genera of Aphidae found in the United States. *Proceedings of the Entomological Society of Philadelphia* 1: 294-311.
- Watt AD, Dixon AF (1981) The role of cereal growth stages and crowding in the induction of alatae in *Sitobion avenae* and its consequences for population growth. *Ecological Entomology* 6 (4): 441-447.

- Wildermuth VL, Walter EV (1932) Biology and control of the corn leaf aphid with special reference to the southwestern States. U.S. Department of Agriculture Technical Bulletin 306: 1-21.
- Zehntner L (1897a) De plantenluizen van het suikerriet. Arch Suikerind Ned-Ind 5.
- Zehntner L (1897b) ? Archief voor de Java-Suikerindustrie 5 (1): 552.
- Zhang G, Qiao G (1997) Homoptera: Pemphigidae, Anoeciidae, Lachnidae and Aphididae . In: Xang X (Ed.) Insects of the Three Gorge Reservoir Area of Yangtze River. 1. Chongqing Press, Chongqing, 374-384 pp.
- Zilahi-Balogh GM, Footit RG, Ferguson G, Shipp JL (2005) New records for *Rhopalosiphum rufiabdominale* (Saski) (Hemiptera: Aphididae) on greenhouse tomatoes and peppers. Journal of the Entomological Society of Ontario 136: 85-87.

## Supplementary materials

### Suppl. material 1: *Rhopalosiphum* measurements, alatae, valid species [doi](#)

**Authors:** Michael Skvarla, Gary Miller

**Data type:** Morphological, measurement

**Brief description:** Length measurements (given in µm) and wing angles for alatae of valid *Rhopalosiphum* species.

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### Suppl. material 2: *Rhopalosiphum* measurements, apterae, synonymized species [doi](#)

**Authors:** Michael Skvarla, Gary Miller

**Data type:** Morphological, measurements

**Brief description:** Length measurements (given in µm) and wing angles for apterae of valid *Rhopalosiphum* species.

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### Suppl. material 3: *Rhopalosiphum* measurements, alatae, synonymized species [doi](#)

**Authors:** Michael Skvarla, Gary Miller

**Data type:** Morphological, measurements

**Brief description:** Length measurements (given in µm) and wing angles for alatae of synonymized *Rhopalosiphum* species.

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### Suppl. material 4: *Rhopalosiphum* measurements, apterae, synonymized species [doi](#)

**Authors:** Michael Skvarla, Gary Miller

**Data type:** Morphological, measurements

**Brief description:** Length measurements (given in µm) and wing angles for apterae of synonymized *Rhopalosiphum* species.

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**Suppl. material 5: Code to analyze apterae datasets in R** [doi](#)

**Authors:** Kramer, M.

**Data type:** R Code

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