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Rhopalosiphum rufiabdominale: first records from winter host plants in Europe

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Abstract

Aphid species *Rhopalosiphum rufiabdominale* (Sasaki) has been originally described from Japan where it is heteroecious holocyclic alternating between *Prunus* spp. and the underground parts of numerous species of herbaceous plants. Current knowledge is that it obligate alternation between winter and summer hosts in the East Asian region only, whilst populations reproducing by means of obligate parthenogenesis are distributed in warmer climates and in glasshouses worldwide. In 2013, two samples of *Rhopalosiphum* were collected in Bagnolo Mella of Brescia province in northern Italy from *Prunus armeniaca* (apricot) and *Prunus domestica* (common plum). The attribution of these two samples to *R. rufiabdominale* was confirmed both morphologically and by the application of two molecular markers, partial sequences of mitochondrial *COI* and nuclear *EF-1α* genes. This was the first record of *R. rufiabdominale* from its winter hosts *Prunus* spp. outside the East Asian region. Once holocyclic populations may exist in southern Europe, these aphids can inhabit the entire temperate region worldwide because they can thrive harsh winter conditions as overwintering egg.

Key words: Rice root aphid, mitochondrial *COI*, nuclear *EF-1α*, Europe, host plants.

Introduction

The aphid species *Rhopalosiphum rufiabdominale* (Sasaki) is thought to be an alien and/or invasive species worldwide (Kindler *et al*., 2004; Watts *et al*., 2008; Messing *et al*., 2012). In Europe, it is listed as an alien in the Register of the alien aphid species of Europe (Coeur d'acier *et al*., 2010). *R. rufiabdominale* has been originally described from Japan where (as in the whole East Asian region) it is heteroecious holocyclic alternating between *Prunus* spp. and the underground parts of numerous species of herbaceous plants (Torikura, 1991). Winter hosts include eighteen species of *Prunus* (mostly *P. glandulosa*, *P. mume*, *P. persica*, *P. yedoensis*, for other species see Doncaster, 1956; Holman, 2009). It was also recorded from *Malus*, *Chaenomeles*, *Pyrus*, *Rhodotypos* and *Sorbus* (Torikura, 1991; Blackman and Eastop, 2000; 2006; Holman, 2009). Reported summer hosts for *R. rufiabdominale* have included 52 genera of herbaceous plants belonging to 15 families, the most common species being those of the family Poaceae (Doncaster, 1956; Kindler *et al*., 2004; Holman, 2009). Current knowledge is that holocyclic (obligate alternation between winter and summer hosts) populations of this species inhabit East Asian region only, whilst anholocyclic ones (reproducing by means of obligate parthenogenesis) are distributed in warmer climates and in glasshouses worldwide (Blackman and Eastop, 2006). In Europe (Nieto Nafria *et al*., 2004), *R. rufiabdominale* has been mostly reported from southern countries (Spain, Italy, Greece, Portugal, France, Bulgaria), also occasionally from greenhouses in Poland and Finland (Labanowski, 2008). It is taken to be the thermophilous species with a more or less worldwide Pantropical distribution, colonising subtropical areas, like the Mediterranean, or even temperate territories, where it can live in warm biotopes or other conveniently sheltered habitats, such as greenhouses (Barbagallo *et al*., 2009). In European countries *R. rufiabdominale* was recorded only from herbaceous hosts belonging to families Araceae, Asteraceae, Poaceae, Ranunculaceae and Solanaceae (Holman, 2009).

In 2013, two samples of *Rhopalosiphum* were collected in Bagnolo Mella of Brescia province in northern Italy from *Prunus* spp. The aim of this paper is to present the evidence and discuss on the possible changes of the invasiveness of *R. rufiabdominale* in Europe due to holocycly.

Materials and methods

Sampling and morphology-based identification

In 2013, the first author have collected two samples of *Rhopalosiphum* in Bagnolo Mella of Brescia province in northern Italy from *Prunus armeniaca* (apricot) and *Prunus domestica* (common plum) (table 1). Microscope slides in Canada balsam were prepared according to Blackman and Eastop (2000). Morphology-based identification keys of *Prunus*-inhabiting aphid morphs of Torikura (1991) and Blackman and Eastop (2000; 2006) together with the morphological descriptions of apterous viviparous females (Doncaster, 1956; Torikura, 1991) were used for the morphological identification of our samples.

DNA-based identification of samples

To confirm the morphological identification, partial sequences of mitochondrial *COI* and nuclear *EF-1α* genes have been analysed and compared with other available sequences of common *Prunus*-inhabiting species of this genus, *Rhopalosiphum nymphaeae* (L.) and *Rhopalosiphum padi* (L.). In addition, available sequences of *Rhopalosiphum insertum* (Walker) were also included into analysis (for sample information, see table 1).

Table 1. Samples of *Rhopalosiphum* used in the present study. Sequences from GenBank (*in italics*) sample collection data were revealed by referring to publications. Sequences from BOLD Systems (**bold fonts**) sample information was taken from the sequence labels. Abbreviations: prov. - province; *P.* - *Prunus*; distr. - district; mun. - municipality.

Place, date, host plant, collection number	GenBank Accession No	
	COI	*EF-1α*
R. rufiabdominale		
Bagnolo Mella, Brescia prov., Italy, 2013.04.25, *P. armeniaca*, 13-6a	KJ776725	KJ776731
Bagnolo Mella, Brescia prov., Italy, 2013.04.30, *P. domestica*, 13-21	KJ776726	KJ776732
*Canada, 20 April 2005, Lycopersicon esculentum, CNC*HEM053450*	*EU701895*	-
Balliang, Australia, 9 October 2004, Triticum aestivum	*DQ499050*	-
GW, Hwengseong, Korea, 31-May-03, Prunus sp., 030531SH1	*GU457796*	*EU358937*
R. padi		
Bratoniškės, Vilnius distr., Lithuania, 2004.05.26, *P. padus*, 04-09	KJ722010	KJ722044
Narva, Estonia, 2008.06.27, Ida-Virumaa county, *P. padus*, B08-27	KJ722011	KJ722045
Skirgiškės, Vilnius distr., Lithuania, 2012.05.16, *P. tenella*, 12-09	KJ722012	KJ722046
Liubavas, Vilnius distr., Lithuania, 2012.06.05, *P. cerasifera*, 12-35	KJ722013	KJ722047
Akmeniai, Lazdijai distr., Lithuania, 2013.05.30, *P. padus*, 13-56	KJ722014	KJ722048
Merkinė, Varėna distr., Lithuania, 2013.05.31, *P. padus*, 13-61	KJ722015	KJ722049
Bratoniškės, Vilnius distr., Lithuania, 2013.06.12, *P. padus*, 13-77	KJ722016	KJ722050
Dobele, Latvia, 2013.07.03, *P. padus,* 13-115	KJ722017	KJ722051
Azarkrosti, Rēzekne mun., 2013.07.16, Latvia, *P. padus*,13-132	KJ722018	KJ722052
Balninkai, Molėtai distr., Lithuania, 2013.07.27, *P. padus*,13-151	KJ722019	KJ722053
Cesu distr., Latvia, 2010.07.09, *Hordeum vulgare*, B1	KJ722020	KJ722054
Tauragė distr., Lithuania, 2008.06.22, *Avena sativa*, LT, 9S3	KJ722023	KJ722057
Klaipėda distr., Lithuania, 2008.06.22, *Avena sativa*, 13S5	KJ722024	KJ722058
Kaunas distr., Lithuania, 2008.06.22, *Hordeum vulgare*, 2P8	KJ722022	KJ722056
Anykščiai distr., Lithuania, 2008.06.24, *Hordeum vulgare*, 30V5	KJ722026	KJ722060
Vilnius distr., Lithuania, 2008.06.26, *Hordeum vulgare*, 41V9	KJ722027	KJ722061
Pasvalys distr., Lithuania, 2008.06.24, *Hordeum vulgare*, 22T6	KJ722025	KJ722059
Limbazu distr., Latvia, 2010.07.11, *Avena sativa*, B6	KJ722021	KJ722055
China, P. dulcis, ZMIOZ27414	*KC286717*	-
New Zealand, D1	*KC008072*	-
New Zealand, A1	*KC008071*	-
China, Rosaceae, ZMIOZ 24386	*JX844414*	-
China, Rosaceae, ZMIOZ 24378	*JX844412*	-
China, Rosaceae, ZMIOZ 16577	*JX844386*	-
USA, TDWG-1117	*HQ979401*	-
India, KBRIIHR-191	*JX051427*	-
India, KBRIIHR-159	*JX051395*	-
India, KBRIIHR-158	*JX051394*	-
GW, Yangyang, Korea, 13 May 2003, Hordeum vulgare, 030513SH2	*GU457795*	*EU358936*
*Canada, 20 May 1993, P. virginiana, CNC*HEM007396*	*EU701894*	-
*Canada, 24 May 1998, P. nigra, CNC*HEM025924*	*EU701893*	-
*USA, Musa sp., CNC*HEM055880*	*EU701892*	-
Australia, 3	*EU179241*	-
Australia, 4	*FJ009050*	-
Fyansford, Australia, 19 October 2004, Poaceae, 2	*DQ499057*	-
Bundoora, Australia, 6 September 2004, Paspalum sp., 1	*DQ499056*	-
Finland	**AFNF033-12**	-
India	**HEMP003-12**	-
Canada	**MHAPH116-07**	-
Canada	**MHAPH117-07**	-
Canada	**MHAPH121-07**	-
Canada	**MHAPH132-07**	-
Canada	**MHAPH136-07**	-
New Zealand, T SB-2013	*KC008073*	-
New Zealand, Prunus spp.	-	*AY21979*

(Continued)

(Table 1 continued)

Place, date, host plant, collection number	GenBank Accession No *COI*	*EF-1α*
R. nymphaeae		
Kaniv State Nature Reserve, Cherkasy distr., Ukraine, 2006.06.17, *P. armeniaca*, 06-110	KJ721994	KJ722028
Göksun, Kahramanmaraş prov., Turkey, 2011.05.31, *P. cerasifera*, 11-29	KJ721995	KJ722029
Afşin, Kahramanmaraş prov., Turkey, 2011.05.31, *P. persica*, 11-36	KJ721996	KJ722030
Skirgiškės, Vilnius distr., Lithuania, 2012.05.16, *P. cerasifera*, 12-8a	KJ721997	KJ722031
Skirgiškės, Vilnius distr., Lithuania, 2012.05.16, *P. cerasifera*, 12-10a	KJ721998	KJ722032
Alytus, Lithuania, 2012.05.30, *P. domestica*, 12-29c	KJ721999	KJ722033
Daugai, Alytus distr., Lithuania, 2012.05.30, *P. domestica*, 12-31	KJ722000	KJ722034
Daugai, Alytus distr., Lithuania, 2012.05.30, *P. cerasifera*, 12-33a	KJ722001	KJ722035
Skirgiškės, Vilnius distr., Lithuania, 2012.06.05, *P. domestica*, 12-40	KJ722002	KJ722036
Daunorava, Joniškis distr., Lithuania, 2013.05.30, *P. armeniaca*, J13-110	KJ722009	KJ722043
Skirgiškės, Vilnius distr., Lithuania, 2013.05.22, *P. domestica*, 13-46a	KJ722003	KJ722037
Skirgiškės, Vilnius distr., Lithuania, 2013.05.22, *P. domestica*, 13-47a	KJ722004	KJ722038
Skirgiškės, Vilnius distr., Lithuania, 2013.05.22, *P. tenella*, 13-48	KJ722005	KJ722039
Akmeniai, Lazdijai distr., Lithuania, 2013.05.29, *P. cerasifera*, 13-53	KJ722006	KJ722040
Akmeniai, Lazdijai distr., Lithuania, 2013.05.30, *P. cerasifera*, 13-54a	KJ722007	KJ722041
Pawłowice, Lower Silesia, Poland, 2013.06.19, *P. cerasifera*, 13-93	KJ722008	KJ722042
Seoul, Gwanak, Korea, 15 August 2005, Nelumbo nucifera, 050815HJ1	*GU457794*	*EU35895*
China, P. dulcis, ZMIOZ26267, as R. rufiabdominale	*KC286718*	-
*USA, Hawaii, 15 March 2004, Nymphaea alba, CNC*HEM051877*	*EU701891*	-
Australia, isolate 1	*EU179243*	-
R. insertum		
Bagnolo Mella, Brescia prov., Italy, 2013.04.26, *Chaenomeles* sp., 13-10	KJ776722	KJ776728
Poncarale, Brescia prov., Italy, 2013.05.02, *Malus* sp., 13-31	KJ776723	KJ776729
Poncarale, Brescia prov., Italy, 2013.05.02, *Crataegus* sp., 13-32	KJ776721	KJ776730
Telšiai, Lithuania, 2013.05.15, *Malus* sp., 13-37	KJ776724	KJ776727
*Canada, 30 May 1993, Crataegus mollis, CNC*HEM007472*	*EU701889*	-
*Canada, 26 May 1993, Crataegus sp., CNC*HEM007427*	*EU701888*	-
Knoxfield, Australia, 19 October 2004, Poa annua	*DQ499047*	-

Table 2. Primers and PCR parameters used in the present study.

	COI fragment	*EF-1α* fragment
Primers	LCO-1490 5'-GGTCAACAAATCATAAAGATATTGG-3' HCO-2198 5'-TAAACTTCAGGGTGACCAAAAAATCA-3' (Folmer *et al*., 1994)	Eloaphis-F 5'-TCACCTTGGGTGTAAAACAATTGA-3' Eloaphis-R 5'-CAATAGACCAGTTTCAACACGACCT-3' (Turčinavičienė *et al*., 2006)
Initial denaturation	94 °C for 2 min	95 °C for 10 min
Denaturation	94 °C for 30"	95 °C for 30"
Annealing	49 °C for 30"	57 °C for 30"
Extention	72 °C for 2 min	72 °C for 30"
Number of cycles	35	32
Final extention	72 °C for 10 min	72 °C for 5 min

A single aphid individual from one sampled plant was considered as a unique sample. Total genomic DNA was extracted from a single aphid using the DNeasy Blood & Tissue kit (Qiagen), which involved at least a 2 h digestion of tissue with proteinase K. PCR amplification was carried out in a thermal cycler (Eppendorf) in 50 µl volumes containing 2 µl genomic DNA, 5 µl of each primer (1 µM), 5 µl of PCR-reaction buffer, 5 µl of dNTP mix (2 mM each), 4-8 µl of 25 mM $MgCl_2$ and 1.25 U of AmpliTaq Gold 360 polymerase (5U/µl) and ddH_2O to 50 µl. Primer sequences and amplification parameters are given in table 2. PCR products were subjected to electrophoresis on 2% TopVision agarose (Fermentas, Lithuania), stained with GelRed and sized against a MassRuler Low Range DNA ladder (Fermentas, Lithuania) under UV light. PCR products were purified and sequenced at Institute of Biotechnology of the Vilnius University (Vilnius, Lithuania). The amplification primers were also used as sequencing primers.

DNA sequences for each specimen were confirmed

Table 3. *COI* haplotypes of four *Rhopalosiphum* species revealed by construction of haplotype network using TCS 1.21 software (Clement *et al*., 2000). Sample information is given in table 1.

Haplotype number	Number of sequences	Sample numbers
	R. padi (n = 44)	
1	1	KC286717
2	4	KC008072; EU179241; 04-09; 13-61
3	1	KC008071
4	3	JX844414; JX844412; JX844386
5	25	HQ979401; EU701894; EU701893; EU701892; FJ009050; AFNF033-12; MHAPH116-07; MHAPH117-07; MHAPH121-07; MHAPH132-07; MHAPH136-07; 12-09; 12-35; 13-56; 13-77; 13-115; 13-132; 13-151; B1; 9S3; 13S5; 2P8; 30V5; 41V9; 22T6
6	6	JX051427; JX051395; JX051394; GU457795; DQ499057; KC008073
7	1	DQ499056
8	1	HEMP003-12
9	1	B6
10	1	B08-27
	R. rufiabdominale (n = 5)	
1	5	GU457796; EU701895; DQ499050; 13-6a; 13-21
	R. nymphaeae (n = 20)	
1	3	KC286718 (as *R. rufiabdominale*); GU457794; EU701891
2	1	EU179243
3	13	12-8a; 12-10a; 12-31; 12-33a; 12-40; 06-110; 11-29; 13-48; 13-46a; 13-47a; J13-110; 13-53; 13-54a
4	1	12-29c
5	1	11-36
6	1	13-93
	R. insertum (n = 7)	
1	1	EU701889
2	1	EU701888
3	1	DQ499047
4	4	13-10; 13-32; 13-31; 13-37

with both sense and anti-sense strands and aligned in the BioEdit Sequence Alignment Editor (Hall, 1999). Partial sequences of mitochondrial *COI* were tested for stop codons and none were found. The sequence data have been submitted to the GenBank, accession numbers are given in table 1. Additional partial sequences of mitochondrial *COI* and nuclear *EF-1α* of *Rhopalosiphum* spp. were downloaded from GenBank and BOLD Systems (table 1). Sample information was gathered by referring to publications or information provided in these databases.

Sequences of both fragments were collapsed into haplotypes and statistical parsimony networks (95 % implemented connection limit) were constructed using TCS v 1.21 (Clement *et al*., 2000). For analysis of partial *COI* sequences gaps were treated as missing data, while for *EF-1α* fragment gaps were treated as 5th state. The sequences representing each haplotype were used for phylogenetic reconstructions with sequences of *Aphis pomi* de Geer and *Aphis spiraecola* Patch as outgroup species. Analyses included Neighbor joining (NJ), Maximum parsimony (MP), Maximum likelihood (ML) and Bayesian inference in phylogeny (BI). NJ, MP and ML analyses were performed using MEGA 5 (Tamura *et al*., 2011). For NJ and distance analyses Kimura 2-parameter (K2P) model of base substitution was used. ML analysis was performed using Tamura-Nei model with Invariable sites (TN93+I model) for *COI* and Tamura 3-parameter model with Gamma distribution (T92+G) for *EF-1α*, which were selected by MEGA 5 model selection option (Tamura *et al*., 2011). Bootstrap values for NJ, MP and ML trees were generated from 1000 replicates. Bayesian analysis was conducted in MrBayes 3.2.1 (Ronquist and Huelsenbeck, 2003) using Hasegawa-Kishino-Yano model with Gamma distribution (HKY+G) for *COI* and General Time Reversible model with Gamma distribution (GTR+G) for *EF-1α*, which were selected by jModeltest (Posada, 2008). One run for 2,000,000 generations with tree sampling every 1,000 generations was performed using the coalescence model of molecular clock.

Results

COI fragment

In this study 76 partial *COI* sequences of four species of the genus *Rhopalosiphum* were analyzed. These sequences were collapsed into 21 haplotype: 10 of *R. padi*, 6 of *R. nymphaeae*, 4 of *R. insertum* and 1 of *R. rufiabdominale* (table 3). The maximum parsimony (MP) analysis of partial *COI* sequences representing 21 haplotype resulted in 176 equally parsimonious trees (length = 168, CI = 0.69, RI = 0.89). ML tree (TN93+I model)

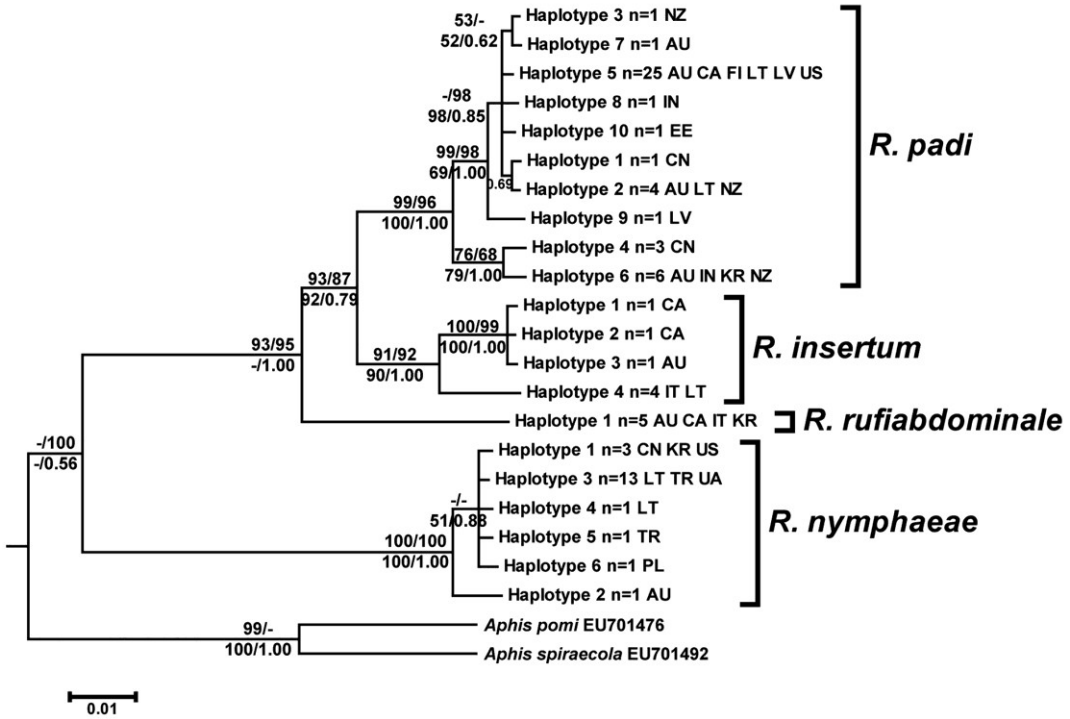

Figure 1. Bayesian Inference (BI) tree showing phylogenetic relationships among four *Rhopalosiphum* species based on haplotypes of partial sequences of mitochondrial *COI* (619 positions in final set). Numbers above branches indicate support of NJ (left, > 50%) and MP (right, > 50%) bootstrap test with 1,000 replicates, and numbers below branches indicate support of ML (left, > 50%) bootstrap test with 1,000 replicates and posterior probabilities of BI analysis (right, > 0.50). The number of sequences representing particular haplotype is given next to its label. Sample numbers / sequence accession numbers are presented in table 3. AU - Australia, CA - Canada, CN - China, EE - Estonia, FI - Finland, IT - Italy, IN - India, KR - Korea, LV - Latvia, LT - Lithuania, NZ - New Zealand, PL - Poland, TR - Turkey, UA - Ukraine, US - United States of America.

Table 4. Range of pairwise interspecific sample divergences of mitochondrial *COI* gene and *EF-1α* gene fragments (K2P model) for four species of the genus *Rhopalosiphum*.

Species 1	Species 2	Average; range of divergence, %	
		COI	*EF-1α*
R. rufiabdominale	*R. nymphaeae*	7.72; 7.33-8.59	7.53; 7.14-7.82
R. rufiabdominale	*R. padi*	5.47; 4.54-6.31	2.17; 2.01-2.42
R. rufiabdominale	*R. insertum*	4.58; 4.53-4.71	0.40; 0.20-0.60
R. nymphaeae	*R. padi*	8.18; 7.33-9.71	7.39; 6.91-7.81
R. padi	*R. insertum*	4.16; 3.50-5.05	2.01; 1.81-2.22
R. insertum	*R. nymphaeae*	8.11; 6.97-9.34	7.37; 6.92-7.82

showed similar topology, the same as MP, NJ (K2P model) and BI (HKY+G model) analyses. NJ, MP and ML bootstrap values over 50 % together with BI posterior probabilities over 0.50 are given at respective nodes of the same tree in figure 1. Five sequences of *R. rufiabdominale* were identical and represented one *COI* haplotype, which appeared as a separate node in all phylogenetic trees (figure 1). Noticeably, one *COI* sequence referred as *R. rufiabdominale* (GenBank accession No KC286718, tables 1 and 3) clustered together with those of *R. nymphaeae* haplotype No 1 in haplotype networks. This could be explained by erroneous morphology-based identification of the sample. Remaining sequences representing samples of three other species of *Rhopalosiphum*, also formed well-defined clusters in all phylogenetic trees (figure 1). Interspecific pairwise sample *COI* sequence divergences between these four species ranged from 3.50 to 9.71% (table 4). Partial *COI* sequences of *R. rufiabdominale* were most similar to those of *R. padi* and *R. insertum* (table 4).

EF-1α fragment

The analyzed region of *EF-1α* consisted of two parts of three exons and two introns, which were not removed before the further analysis. 44 partial *EF-1α* sequences were collapsed into 12 haplotypes: 4 of *R. padi*, 4 of *R. nymphaeae*, 2 of *R. insertum* and 2 of *R. rufiabdominale* (table 5). The maximum parsimony (MP) analysis of partial *EF-1α* sequences representing 12 haplotypes resulted in 62 equally parsimonious trees (length = 86, CI = 0.94, RI = 0.97). ML tree (T92+G model) showed similar topology, the same as MP, NJ (K2P model) and

Table 5. *EF-1α* haplotypes of four *Rhopalosiphum* species revealed by construction of haplotype network using TCS 1.21 software (Clement *et al*., 2000). Sample information is given in table 1.

Haplotype number	Number of sequences	Sample numbers
	R. padi (n = 20)	
1	15	12-09;12-35; 04-09; 13-77; 13-115; 13-151; B08-27; 9S3; 2P8; 13S5; 22T6; 30V5; 41V9; B1; B6
2	1	13-61
3	2	13-56 ; 13-132
4	2	EU358936; AY219719
	R. rufiabdominale (n = 3)	
1	2	13-21; 13-6a
2	1	EU358937
	R. insertum (n = 4)	
1	3	13-10; 13-31; 13-32
2	1	13-37
	R. nymphaeae (n = 17)	
1	12	12-8a; 12-10a; 12-29c; 12-31; 12-33a; 12-40; 11-29; 11-36; J13-110; 13-46a; 13-47a; 13-48
2	1	06-110
3	3	13-53; 13-54a; 13-93
4	1	EU358935

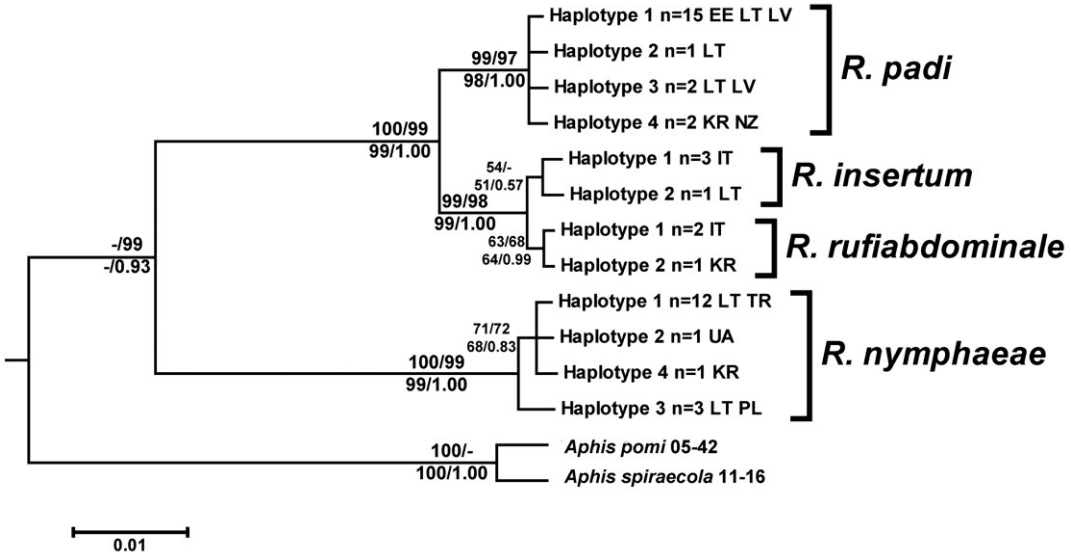

Figure 2. Bayesian Inference (BI) tree showing phylogenetic relationships among four *Rhopalosiphum* species based on haplotypes of partial sequences of nuclear *EF-1α* (506 positions in final set). Numbers above branches indicate support of NJ (left, > 50%) and MP (right, > 50%) bootstrap test with 1,000 replicates, and numbers below branches indicate support of ML (left, > 50%) bootstrap test with 1,000 replicates and posterior probabilities of BI analysis (right, > 0.50). The number of sequences representing particular haplotype is given next to its label. Sample numbers / sequence accession numbers are presented in table 5. EE - Estonia, IT - Italy, KR - Korea, LV - Latvia, LT - Lithuania, NZ - New Zealand, PL - Poland, TR - Turkey, UA - Ukraine.

BI (GTR+G model) analyses. NJ, MP and ML bootstrap values over 50% together with BI posterior probabilities over 0.50 are given at respective nodes of the same tree in figure 2. Out of 3 partial *EF-1α* sequences of *R. rufiabdominale* analyzed in our study, two haplotypes were identified (table 5), which made up one cluster in all phylogenetic trees (figure 2). Remaining sequences of three other *Rhopalosiphum* species formed clearly defined clusters in the trees constructed using *EF-1α* fragment (figure 2). Interspecific pairwise sample *EF-1α* sequence divergences between these four species ranged from 0.20 to 7.82%. Partial *EF-1α* sequences of *R. rufiabdominale* were most similar to those of *R. padi* and *R. insertum* (table 4). Noticeably, the difference between *R. rufiabdominale* and *R. insertum* did not exceed 0.60%.

Morphology

On the macroscopic level, *R. rufiabdominale* can be easily confused with the common European *Prunus*-inhabiting species *R. nymphaeae* due to similar body shape and coloration of live aphids (figure 3D). On the

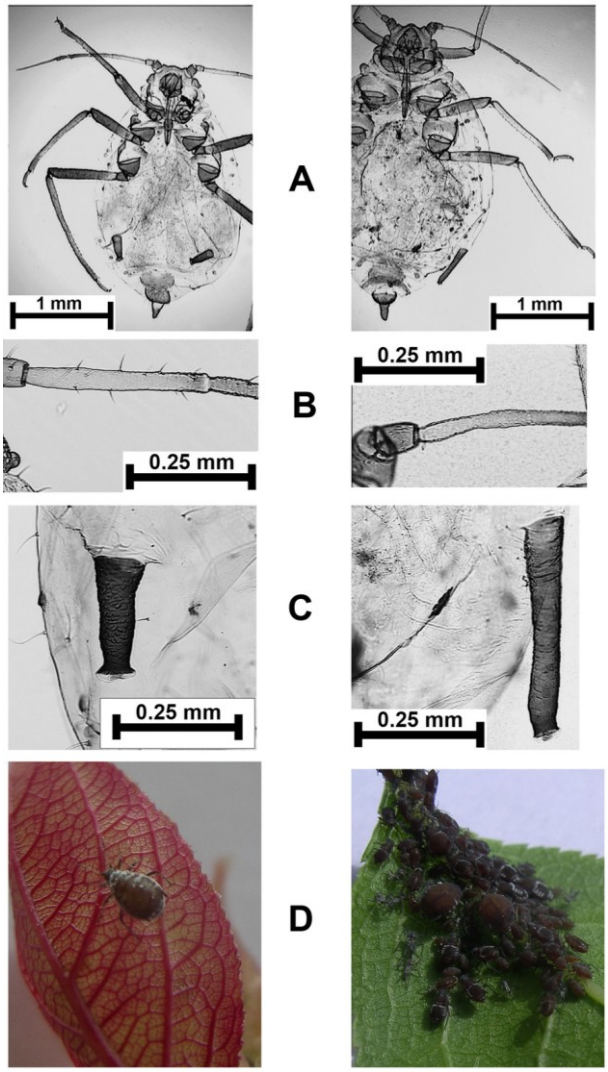

Figure 3. Apterous viviparous females (fundatrigeniae) of *R. nymphaeae* (on the right, specimen from sample 13-47a) and *R. rufiabdominale* (on the left, specimen from sample 13-21): (A-C) mounted specimens; (A) body and appendages, (B) hairs on antennal segment III, (C) siphuncle, (D) live aphids. Sample information is given in table 1.

microscopic level, fundatrigenia of both species differ by the coloration of appendages (figure 3A), length of the antennal (figure 3B) and body hairs, shape and length of siphuncles (figure 3C). Discriminative morphological characters are summarized in table 6 (for more details, see Doncaster, 1956; Torikura, 1991; Blackman and Eastop, 2000).

Discussion and conclusions

Attribution of two Bagnolo Mella (Brescia province, Italy) samples to *R. rufiabdominale* species was confirmed both morphologically and by the application of two molecular markers, partial sequences of mitochondrial *COI* and nuclear *EF-1α*. *COI* sequences of *R. rufiabdominale* from Italian samples were identical with those collected in Canada and Australia from herbaceous hosts and in Korea from *Prunus* sp., although Korean sample had unique *EF-1α* haplotype differing by 1 substitution (tables 1, 3, 5; figures 1-2). Finding of aphids on winter hosts early in the season (April 25 and 30 respectively, table 1), strongly suggests sampled lineages having complete life cycles. Therefore, this study gives the first evidence for *R. rufiabdominale* undergoing complete life cycle, including bisexual generations and overwintering on *Prunus* spp., outside the East Asian region. For *R. rufiabdominale* this means the existence of bisexual generation producing overwintering eggs on *Prunus* spp., with subsequent migration to the underground parts of numerous species of herbaceous plants in the summer (Doncaster, 1956; Kindler *et al*., 2004). Holocyclic lineages of *R. rufiabdominale* are understood to be a rather recent phenomenon in Italy, where the aphid, though quite common in several regions on its secondary host plants (Barbagallo *et al*., 2008; 2011; 2014), has been not yet recorded here on primary hosts (Prunoideae). This species could hardly have been overlooked as inhabiting *Prunus* spp. as Italy has highly experienced long lasting aphid research traditions, particularly concerning orchard pests (Barbagallo *et al*., 1997; Patti and Barbagallo, 1998; Barbagallo *et al*., 2009).

Table 6. Morphological characters for the discrimination between *Prunus*-inhabiting *R. nymphaeae* and *R. rufiabdominale* (after Doncaster, 1956; Torikura, 1991; Blackman and Eastop, 2000).

Character name	*R. nymphaeae*	*R. rufiabdominale*
Length and shape of siphuncles	> 0.3 mm, swollen proximal to subapical constriction	< 0.3 mm, without any discernible subapical swelling
The colour of appendages	Light brown	Dark brown
Number of setae on abdominal tergite VIII	2	3-9
The position of marginal tubercles	On I-VII abdominal tergites	Normally on I and VII abdominal segments only
Length and shape of hairs on abdominal tergites I-IV	Less than 0.04 mm long	More than 0.04 mm long
Length of antennal hairs	Hairs on antennal joint III shorter than the articular diameter of the same joint	Hairs on antennal joint III up to twice or more the articular diameter of the same joint

Obligate parthenogenetic lineages, species and higher taxa have been reported to be successful invaders due to broad dispersal and large population sizes that compensate the evolutionary cost of long-term abstinence from sexual reproduction (Fontaneto *et al*., 2007; 2008). Therefore, aphids are generally understood as successful invaders. First, aphids are mobile insects both due to their biological pecularities and as a result of aphid-related (although indirect in most cases) human activities. Aphids can produce winged individuals when facing the need to colonize new host plants. During migration flights, these tiny insects might overcome large distances by means of air currents (Irwin *et al*., 2007). Humans commonly introduce aphids together with exotic plant material, on the other hand, introduced native plants may also be contaminated with exotic aphid species (Holman, 1971; Coeur d'acier *et al*., 2010). Second, parthenogenetic mode of aphid reproduction favours invasivity, because very few introduction events (even introduction of a single parthenogenetic female) might lead to the establishment of an alien species (Coeur d'acier *et al*., 2010). Therefore, genetic variation (in terms of broad-sense heritability) in fitness might appear higher in asexual (permanently anholocyclic) aphid genotypes compared with sexual ones (Carter *et al*., 2012). Asexual aphid populations can demonstrate higher allelic richness per locus than sexual populations and might consist of a few predominant clones that appear considerably differentiated from one another (Kanbe and Akimoto, 2009). All this enables phenotypic plasticity that has often been cited as a life-history trait favoring colonization of new areas (Sakai *et al*., 2001). Yet coexistence of asexual and sexual populations has been also reported as increasing adaptive plasticity, also invasiveness of aphids (Kanbe and Akimoto, 2009; Carter *et al*., 2012).

As a consequence, coexistence of *R. rufiabdominale* lineages propagating both by obligate or facultative parthenogenesis and bisexually, might considerably increase adaptive plasticity and invasiveness of this species, as has been shown for aphid species *R. padi* (Hulle *et al*., 1999; Delmotte *et al*., 2003; Carter *et al*., 2012). This might substantially endanger graminaceous (possibly also stone-fruit) crops not only in subtropical but also in temperate regions of Europe.

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