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Phylogenetic relationships in the "*grossulariae*" species group of the genus *Aphis* (Hemiptera: Sternorrhyncha: Aphididae): Molecular evidence

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Abstract. Phylogenetic relationships among Palaearctic *Ribes* and/or Onagraceae inhabiting *Aphis* species from five countries were examined using mitochondrial gene cytochrome oxidase I (*CO-I*) and nuclear gene elongation factor 1 α (*EF-1*α) sequences. There was no major conflict between the trees obtained from two data sets; nodes with strong bootstrap support from one analysis never contradicted those strongly supported by the other analysis. Palaearctic species of the subgenus *Bursaphis* (= "*grossulariae*" species group of the genus *Aphis*) form a monophyletic group within the genus *Aphis*. All these analyses indicated that *Aphis grossulariae* and *A. schneideri* are close relatives, which is supported by the information on experimental and probably also natural hybridisation. Our data indicate the independent colonisation of *Ribes* spp. by two species groups of the genus *Aphis*: *A. triglochinis* (subgenus *Aphis* s. str.), and *A. grossulariae* and *A. schneideri* (subgenus *Bursaphis*). Once the subgenus *Bursaphis* (and other subgenera) is accepted, the subgenus *Aphis* s. str. will require further subdivision.

INTRODUCTION

A group of species of the genus *Aphis* L. associated with *Ribes* spp. and/or Onagraceae, in which the ultimate rostral segment has 5 or more additional hairs were initially called the "*grossulariae*" group (Eastop, 1979; Stroyan, 1984; Heie, 1986). There are seven Palaearctic species in this group (Table 1). Two of them are monoecious holocyclic on currants and gooseberries (*Ribes* spp.), one facultatively alternates between *Ribes* spp. and Onagraceous herbs, two are monoecious holocyclic on various species of the genus *Epilobium* (Onagraceae), one is anholocyclic monoecious on *E. hirsutum* and one is monoecious anholocyclic on *Oenothera* spp. (Onagraceae). All these species (except *A. popovi*) are currently in the subgenus *Bursaphis* McVicar Baker, 1934 (Remaudière, 1993; Remaudière & Remaudière, 1997). *A. popovi* should also be in this subgenus as it is very similar to *A. schneideri* (Rakauskas, 1996; G. Remaudière, pers. commun.). Another six Palaearctic species resemble the "*grossulariae*" group in their host specificity, but have fewer additional hairs on their ultimate rostral segment (Table 1). In this group, one species exploits *Ribes* spp. as winter hosts, three species live on *Epilobium* spp. (including *Chamaenerion*) in summer and two are monoecious holocyclic on *Epilobium* spp. All these species are currently assigned to the subgenus *Aphis* s. str. (Remaudière & Remaudière, 1997).

The above information supposes independent colonisation of *Ribes* spp. and/or Onagraceae by two phylogenetic lineages in separate subgenera, *Bursaphis* and *Aphis* s. str. of the genus *Aphis*. This is based on a morphological difference (number of additional hairs on the ultimate rostral segment). Furthermore, multiple independent adoptions of Onagraceous hosts by different lineages of the genus *Aphis* might be advocated. On the contrary, a single ancestral occupation of these hosts, followed by morphological divergence might also be argued. For further analysis, independent sources of information, such as molecular evidence, are needed.

Mitochondrial cytochrome oxidase I (*CO-I*) and nuclear elongation factor 1 α (*EF-1*α) genes have already been used in phylogenetic analyses of insects (Crozier et al., 1989; Simon et al., 1994; Moran et al., 1999; Sanchis et al., 2001; Cruickshank et al., 2001). The aim of this study was to infer phylogenetic relationships of the Palaearctic *Ribes* and/or Onagraceae-inhabiting *Aphis* species by means of a DNA sequence analysis of mitochondrial *CO-I* and nuclear *EF-1*α genes. The focus of this study was to compare DNA sequence data with the morphologically and ecologically based differences of the Palaearctic *Aphis* species inhabiting *Ribes* spp. and/or Onagraceae.

MATERIAL AND METHODS

Aphid samples

Twenty-five samples of seven species originating from five countries were used in this study (Table 2). Samples from 9 aphid clones of 4 species were also included (Table 3). Detailed information on the aphid cloning methods used is published in Rakauskas (1993). Microscope slides were prepared for the morphological identification of the aphids. Keys of H.L.G. Stroyan (1984), O.E. Heie (1986), R. Rakauskas (1998) and J. Holman (1990) were used for morphological identification of the aphid material. Microscope slides are deposited at the Department of Zoology of Vilnius University (Lithuania).

When establishing aphid clones, colonies were initiated from a single parthenogenetic female and expected to contain DNA

TABLE 1. Species of the genus *Aphis* L. associated with *Ribes* spp. and/or Onagraceae in the Palearctic (after Müller, 1974; Martin, 1982; Stroyan, 1984; Heie, 1986; Holman, 1990; Rakauskas, 1996, 1998; Buga & Rakauskas, 2003).

Species	Life-cycle	Winter host	Summer hosts (* indicates occasional hosts)	No. of additional hairs on ultimate rostral segment
subg. *Bursaphis* (= "*grossulariae*" species group)				
schneideri (Borner, 1940)	holocyclic, monoecious	*Ribes* spp.		5–11
grossulariae Kaltenbach, 1843	holocyclic, facultatively heteroecious	*Ribes* spp.	*Epilobium* spp., *Chamaenerion angustifolium*, **Oenothera* spp., **Godetia* spp., **Fuchsia* spp.	4–13
popovi Mordvilko, 1932	? holocyclic, monoecious	*Ribes* spp.		5–6
epilobii Kaltenbach, 1843	holocyclic, monoecious		*Epilobium montanum*, **E. lanceolatum*	6–9
epilobiaria Theobald, 1927	holocyclic, monoecious		*Epilobium hirsutum*, *E. palustre*	6–12
oenotherae Oestlund, 1887	anholocyclic, monoecious		*Oenothera* spp., **Epilobium* spp., **Chamaenerion angustifolium*, **Godetia* spp., **Fuchsia* spp., **Gaura* spp.	5–10
fluvialis Martin, 1982	anholocyclic, ? monoecious		*Epilobium hirsutum*	10–12
subg. *Aphis* s. str.				
triglochinis Theobald, 1927	holocyclic, heteroecious	*Ribes* spp.	Brassicaceae, Scrophulariaceae, Asteraceae	2–3 (4)
praeterita Walker, 1849	holocyclic, monoecious		*Epilobium hirsutum*, **E. palustre*, **E. parvifolium*	2
pollinaria (Borner, 1952)	? holocyclic, monoecious		*Epilobium obscurum*, *E. parviflorum*	2
spiraephaga F.P. Muller, 1961	holocyclic, heteroecious	*Spiraea* spp.	*Epilobium montanum*	2–3
frangulae frangulae Kaltenbach, 1845	holocyclic, heteroecious	*Frangula alnus*	*Chamaenerion angustifolium*, *Capsella bursa pastoris*, *Lysimachia* spp.	2
salicariae Koch, 1855	holocyclic, heteroecious	*Cornus* spp.	*Chamaenerion angustifolium*	2

from genetically homogenous individuals. Field samples were taken from a single colony. Aphids were frozen at –80° C or stored in 96% ethanol. Voucher specimens (96% ethanol samples) are deposited at Vilnius University, Department of Zoology (Lithuania).

DNA extraction

DNA was extracted using DNAeasy kit (Qiagen Nordic, Crawley, UK), which involved at least a 2 h digestion of tissue with proteinase K.

Sequences

The target sequences were 592 bp from the mitochondrial gene encoding cytochrome oxidase subunit I (CO-I) and 499 bp of the nuclear gene encoding elongation factor 1 α (*EF-1α*). To amplify and sequence partial sequences of *CO-I* and *EF-1α* genes, new primers were designed by the authors, using related data from GenBank. PCR amplifications for both gene regions were carried out in a thermal cycler in 50 µl volumes containing 1 µl genomic DNA, 5 µl of each primer (10 µM), 5 µl of PCR-reaction buffer, 20 µl of dNTP mix (GATC 0.5 mM each), 10 µl ddH_2O, 2 µM $MgCl_2$ and 0.25 µl of Gold *Taq* polymerase (5 U/µl). The cycling parameters for both fragments were as follows: denaturation at 95°C for 10 min (1 cycle), denaturation at 95°C for 30 s, primer annealing temperatures were optimized for the different fragments: 49°C for *CO-I* and 57°C for *EF-Iα* (30 s), and extension at 72°C for 30 s; 32 cycles total. Primers are given in Table 4. Electrophoresis of the PCR products was done on 2% NuSieve gel, stained with ethidium bromide and sized against a ΦX 174 DNA ladder (Boeringen Manheim, Mannheim, Germany) under UV light. PCR products were cleaned with QIAquick PCR Purification Kit (Qiagen). Cyclic sequencing was carried out using a Perkin Elmer/ABI Dye Terminator Cyclic Sequencing Kit (PerkinElmer, Boston, USA) and run on the thermocycler. Cyclic sequencing products were cleaned using ethanol precipitation and sequenced using a Perkin Elmer ABI377 Automated Sequencer (Applied Biosystems Inc., Foster City, California). DNA sequences for each specimen were confirmed with both sense and anti-sense strands and aligned in the program Sequencher (Gene Codes Corporation, Ann Arbor, Michigan). The mtDNA sequences were tested for stop codons and none were found.

Phylogenetic analysis

Phylogenetic reconstructions were obtained by maximum parsimony method (Swofford et al., 1996). Unweighted parsimony analyses were performed using PAUP*4.0b10 (Swofford, 1998) in combination with Mac Clade 3.05 (Maddison & Maddison, 1992). Heuristic searches were carried out with twenty random taxon addition replicates. To evaluate the strength of internal branches in the trees based on parsimony, the bootstrap procedure in PAUP was used. Bootstrap values were generated from 1000 replicates, each with ten random-addition heuristic searches. Parsimony analysis resulted in one most parsimonous

TABLE 2. Field collected material of the genus *Aphis* L. studied. Morph abbreviations: apt. – apterous viviparous females; al. – winged viviparous females; ov. – oviparous females.

Species	Place and date	Host plant	Morphs	Abbrev. in Figs
oenotherae	Skirgiskes, Vilnius distr., Lithuania, 2002.08.03	*Oenothera biennis*	Apt.	0282
	Skirgiskes, Vilnius distr., Lithuania, 2002.08.03	*Oenothera biennis*	Apt., al.	0284
	Naujaneriai, Vilnius, Lithuania, 2003.07.05	*Oenothera biennis*	Apt.	103
	Gangwon-Dunnae, South Korea, 2003.06.27	*Oenothera lamarckiana*	Apt., al.	Kor
	Dabrowa Gornicza, Katowice distr., Poland, 2003.10.18	*Oe. rubricaulis*	Apt., nym.	125
	Czeladz, Katowice distr., Poland, 2003.10.19	*Oenothera* sp.	Apt.	129
	Puvociai, Varena distr., Lithuania, 2002.06.28	*Oenothera* sp.	Apt.	AG10
	Puvociai, Varena distr., Lithuania, 2002.06.28	*Oenothera* sp.	Apt.	AG15
praeterita	Ceske Vrbne, South Bohemia, Czech Rep., 2003.09.27	*Epilobium hirsutum*	Apt., ov., males	180
	Ceske Vrbne, South Bohemia, Czech Rep., 2003.10.12	*Oenothera* sp.	Apt., ov., males	186
	Prachatice, South Bohemia, Czech Rep., 2003.10.16	*Oenothera* sp.	Apt.	196
fabae	Skirgiskes, Vilnius distr., Lithuania, 2002.08.24	*Myosotis palustris*	Apt.	*fabae*
epilobiaria	Skirgiskes, Vilnius distr., Lithuania, 2002.09.25	*Epilobium palustre*	Apt., al.	E101
	Skirgiskes, Vilnius distr., Lithuania, 2002.09.25	*Epilobium palustre*	Apt., al., ov.	E102
	Ceske Vrbne, South Bohemia, Czech Rep., 2003.09.27	*Epilobium hirsutum*	Apt., ov., males	179
	Prachatice, South Bohemia, Czech Rep., 2003.10.16	*Oenothera* sp.	Apt.	197
schneideri	Vilnius, Kairenai, 2001.07.27	*Ribes rubrum*	Al, apt	S19
	Vilnius, Kairenai, 2001.07.04	*Ribes nigrum*	Al, apt	S7
	Vilnius, Kairenai, 2001.07.04	*Ribes nigrum*	Al, apt	S8
grossulariae	Vilnius, Kairenai, 2001.07.21	*Ribes rubrum*	Al, apt	G15
	Bo, Norway, 2003.06.28	*Epilobium* sp.	Al, apt	eN
	Vilnius, Kairenai, 2003.07.02	*Epilobium* sp.	Al, apt	100
	Vilnius, Kairenai, 2002.05.28	*Ribes nigrum*	Al, apt	AG8
	Bo, Norway, 2003.06.28	*Ribes alpinum*	Al, apt	aN
salicariae	Skirgiškės, Vilniaus raj., Lietuva, 2002.06.27	*Chamaenerion angustifolium*	Al.	*salicariae*

tree. Performing *CO-I* dataset analysis *Acyrthosiphon pisum* (GenBank Accesion N AF077776) and *Schizaphis rotundiventris* (GenBank Accesion N AF220511) were included as outgroups in order to establish the rooting of the *Aphis* + *Bursaphis* clade. *Acyrthosiphon pisum* is situated in the subtribe Macrosiphina of the tribe Macrosiphini, inhabiting leguminous plants all over the World (Heie, 1994). *Schizaphis rotundiventris* belongs to the subtribe Rhopalosiphina of the tribe Aphidini, living mostly on *Cyperus* spp. in Old World tropical and warm temperate regions (Blackman & Eastop, 2000). The genus *Aphis* is situated in subtribe Aphidina, which belongs to the tribe Aphidini, together with the subtribe Rhopalosiphina. Consequently, of the two species used as outgroups, one (*Schizaphis rotundiventris*) represents a sister subtribe of the same tribe Aphidini that the genus *Aphis* belongs to, whilst the other (*Acyrthosiphon pisum*) is a more distantly related species representing a different tribe, the Macrosiphini. Both tribes are expected to be sister groups in the subfamily Aphidinae of the family Aphididae sensu Shaposhnikov, 1964. It was not possible to obtain the same outgroup species for the *EF-1*α analyses. *Acyrthosiphon pisum* was not used as an outgroup in this analysis due to the considerable length differences in the introns. *A. spiraecola* (GenBank Accesion N AY219725) was used as an outgroup. After the Blast search, a sequence of the genus *Casimira* (GenBank Accesion N AY219742, Von Dohlen & Teulon, 2003) available at the GenBank (identities 475/499, 95%) was taken for comparative analysis. The type of the genus *Casimira* was originally described as *Aphis canberrae* from Canberra, Australia (Eastop, 1961). Later on, a separate genus *Casimira* was established. *Casimira* resembles *Aphis* and *Toxoptera*, but differs from both in the absence of lateral tubercles on the seventh abdominal segment and the presence of only 2 hairs on all the first tarsal segments. It also differs from *Aphis* in the media of the forewing being only branched once. Noticeably, *C. canberrae* usually has four additional hairs on the ultimate rostral segment and is only reported from *Epilobium junceum* (Eastop, 1961, 1966). Recently, a new species of *Casimira* was reported from New Zealand (von Dohlen & Teulon, 2003) living on the endemic New Zealand host plant, *Ozothamnus leptophylus* of the *Asteraceae* family. Unfortunately, the species identity of the *Casimira* used for the molecular analysis is unknown. Part of the *EF-1*α (introns) of *Acyrthosiphon pisum* varied considerably in length and only minor parts of the introns could be aligned to *Aphis*. An artificial outgroup was made for the combined analysis: *CO-I* partial sequences from *Acyrthosiphon pisum* and *EF-1*α partial sequences from *A. spiraecola* (GenBank Accesion N AY219725).

The sequence data for all species have been submitted to the GenBank Data Libraries under the Accesion No. DQ418809-418875.

RESULTS

Both analyzed DNA fragments (mitochondrial *CO-I* and nuclear *EF-1*α) are protein coding genes. *EF-1*α is known as a conservative gene and has been used in phy-

TABLE 3. Clonal material of the genus *Aphis* L. studied. Morph abbreviations the same as in Table 2; fx – fundatrix.

Species	Collected	Reared on	Life cycle	Sample date	Abbrev. in Figs
oenotherae	Vilnius, Lithuania, 2002.07.07, *Oenothera biennis*, apt.	*Oenothera biennis*	Anholocyclic, monoecious	2002.07.07	M
	Vilnius, Lithuania, 2002.09.04, *Oenothera biennis*, apt	*Oenothera biennis*	Anholocyclic, monoecious	2002.09.25	L
grossulariae	Skirgiskes, Vilnius distr., Lithuania, 2002.05.28, *Ribes* sp. cult "Hollandische Rote", fx	*Ribes* sp. cult "black", *Epilobium adenocaulon*	Holocyclic, facultatively heteroecious	2002.08.03	F
	Skirgiskes, Vilnius distr., Lithuania, 2002.05.28, *Ribes* sp. cult "black", fx	*Ribes* sp. cult "black", *Epilobium adenocaulon*	Holocyclic, facultatively heteroecious	2002.09.01	A
	Skirgiskes, Vilnius distr., Lithuania, 2002.05.28, *Ribes* sp. cult "black", fx	*Ribes* sp. cult "black", *Epilobium adenocaulon*	Holocyclic, facultatively heteroecious	2002.06.26	C
	Vilnius, 1996.05.10, *Ribes nigrum*	*Ribes* sp. cult "black", *Epilobium palustre*	Holocyclic, facultatively heteroecious	1996.06.05	GT
triglochinis	Puvočiai, Varėnos distr., Lithuania, 1995.05.18, *Ribes* sp. cult. „black"	*Ribes* sp. cult "black", *Rorippa amphibia*	Holocyclic, heteroecious	1995.06.19	T1
	Skirgiskes, Vilnius distr., Lithuania, 1996.05.19, *Ribes* sp. cult. "black", fx	*Ribes* sp. cult "black", *Rorippa austriaca*	Holocyclic, heteroecious	1996.06.10	T3
schneideri	Vilnius, Lithuania, 1997.05.12	*Ribes* sp. cult "black"	Holocyclic, monoecious	1997.07.03	ST

logenetic analysis at higher taxonomic levels (Pedersen, 2002).

Mitochondrial (*CO-I*) data

The segments of *CO-I* used for the alignment of partial *CO-I* sequences contained 591 sites. A total of 107 sites varied between taxa. 77 were parsimony informative. The sequences were heavily biased toward A and T nucleotides. The average base composition at the first codon position was 37.9% A, 12.6% C, 20.4% G and 29.5% T; second 18.3% A, 23.8% C, 14.7% G and 43.2% T, and third 48.7% A, 6.1% C, 0.8% G and 44.3% T. The ratio of transitions to transversions was 33/27 for all sites; for the first position 12/0, second 0/0 and third 21/27. The sequence is very rich in A+T at the third codon position: 93%.

Elongation factor 1 α

Five hundred bp sequenced, 67 sites were variable, 39 were phylogenetically informative. The analyzed region consists of two parts of two exons and two introns. Base composition was 31.7% A, 17.7% C, 20.2% G and 29.5% T. Distribution of nucleotides is more homogenous than in *CO-I*. Introns were included in alignment and in the phylogenetical analysis treated as the "fifth" base. Differences among species occur mostly in introns. In the aligned regions of the two exons, no deletions or insertions of bases were observed. The ratio of transitions to transversions was 12/17 for all sites.

Phylogenetic analyses

CO-I. The maximum parsimony analysis gave only one "shortest tree" of 171 steps. The cladogram (Fig. 1) is shown with branch lengths and bootstrap values (CI = 0.65, RI = 0.942). Aphidini appeared to be a monophyletic tribe when *Acyrthosiphon pisum* (Macrosiphini) was used as an outgroup. Phylogenetic analysis supported monophyly of the subgenus *Bursaphis*. Species of the subgenus *Aphis* s. str. used in the present study did not form a monophyletic group: namely, a node of *A. praeterita* is supported by a boostrap of 100% for maximum parsimony (Fig. 1.). Another clade consists only of *A. triglochinis* with a support of 100%. Subgenus *Aphis* s. str. contains two clades – *A. praeterita* together with *A. fabae*, and another clade – *A. triglochinis*, sister group of subgenus *Bursaphis*. The *Bursaphis* clade contains *A. epilobiaria*, *A. grossulariae*, *A.schneideri* and *A. oenotherae*. It is noteworthy that the *A. oenotherae* samples form a well supported separate clade at the base of the subgenus *Bursaphis*.

EF-1α. Maximum parsimony analysis of the *EF-1α* sequences yielded one tree. Fig. 2 shows a strict consensus cladogram with boostrap values (CI = 0.86, RI = 0.946); tree length – 83 steps. The bootstrap values are

TABLE 4. Primers used for PCR amplification of the cytochrome oxidase subunit I (*CO-I*) sequence and elongation factor 1α (*EF-1α*) sequence.

Genes (partial)	Forward primer	Reverse primer	Fragment size (portion sequenced)
CO-I	Aphis-L-465 5'-TCTTCTCTTTACATTTAGCAGGAAT-3'	Aphis-H-1068 5'-AATAGATGAATTAGCAAGAATTA-3'	591
EF-1a	EloaphisF 5'-TCACCTTGGGTGTAAAACAATTGA-3'	EloaphisR 5'-CAATAGACCAGTTTCAACACGACCT-3'	500

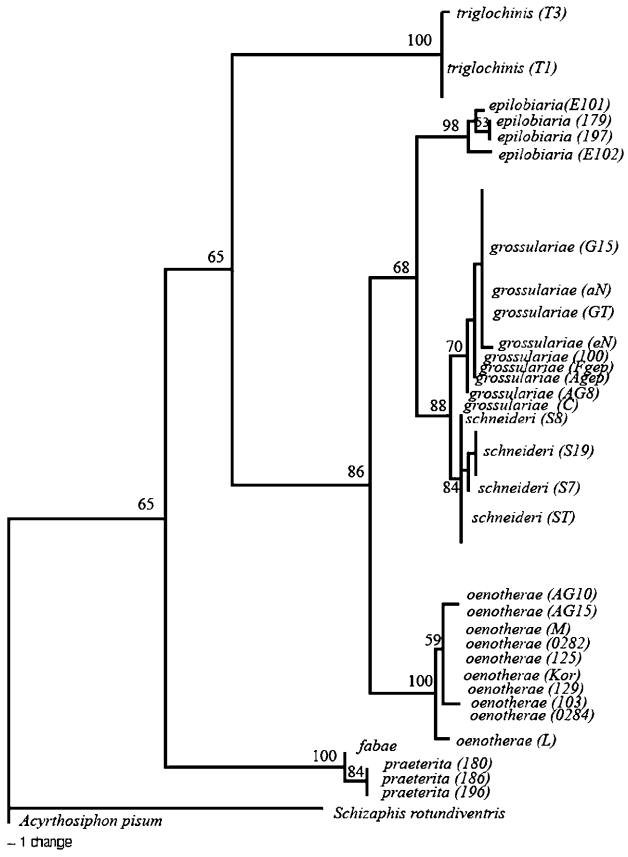

Fig. 1. Phylogeny of the genus *Aphis* based on a maximum parsimony analysis of a region of the mitochondrial *CO-I* gene. Bootstrap support based on 1000 replicates is indicated for nodes with greater than 50% support.

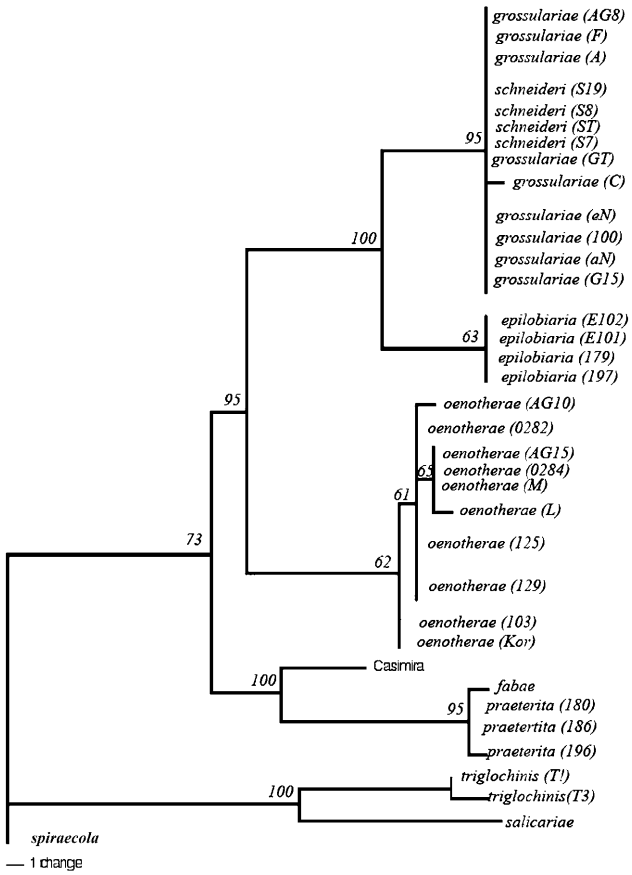

Fig. 2. Phylogeny of the genus *Aphis* based on a maximum parsimony analysis of a region of the nuclear *EF-1*α gene. Bootstrap support based on 1000 replicates is indicated for nodes with greater than 50% support.

percentages based on 1000 bootstrap replicates. When compared with the mitochondrial *CO-I*, no conflict seems to exist between the terminal groupings and the division of subgenera, although support for some clades seems weak. Deeper nodes have stronger support from *EF-1*α than mitochondrial *CO-I* (Fig. 2). *A. spiraecola* (GenBank Accesion N AY219725) was used as an outgroup. *A. salicariae* was used in this analysis only, because we did not succeed in amplifying *CO-I* fragments of this sample. Analyses of *EF-1*α gave a different topology with deeper nodes when compared with *CO-I*. *A. triglochinis* (with *A. salicariae*) formed the basal branch, while in the phylogeny based on *CO-I* (Fig. 1), the *A. fabae – A. praeterita* node was at the base. Bootstrap analysis under MP method supported many of the relationships in the tree, including the monophyly of *Bursaphis.* In contrast, the monophyly of the subgenus *Aphis* s. str. is not supported.

There was no major conflict between the trees obtained from the two data sets; nodes with strong bootstrap support in one analysis were similarly strongly supported in the other analysis. However, the two data sets differ in the level of resolution of particular nodes (Figs 1, 2).

Combined analysis. Phylogeny based on both mitochondrial and nuclear sequences, totaling 1091 nucleotides with 107 parsimony informative sites, yielded one tree (Fig. 3); tree length – 200 (CI = 0.815, RI = 0.948). It seems that the majority of the variable characters in the closely related taxa are in *CO-I*. *CO-I* contains more variable sites, while the variability in *EF-1*α was due to the presence of introns.

DISCUSSION

Molecular data definitely show Palaearctic representatives of the subgenus *Bursaphis* as a clearly defined monophyletic group. Inside this clade, the Nearctic species, *A. oenotherae,* seems to form a sister group to the remaining species of the subgenus. Noticeably, *A. oenotherae* is the only Nearctic representative of *Bursaphis* used in this study. It is reported as being introduced into Europe in the second half of the 20th century (for wider discussion, see Rakauskas, 2004; Buga & Rakauskas, 2003). This might mean that Nearctic and Palaearctic representatives of *Bursaphis* are sister groups of sibling vicariant species. This is just a hypothesis, because more *Bursaphis* species from Nearctic (*A. manitobensis*, *A. mimuli*, *A. varians*, *A. solitaria*) should be included in the analysis.

Molecular data clearly show that European *A. oenotherae* differ from *A. grossulariae*, which are often misidentified, as they are morphologically very similar.

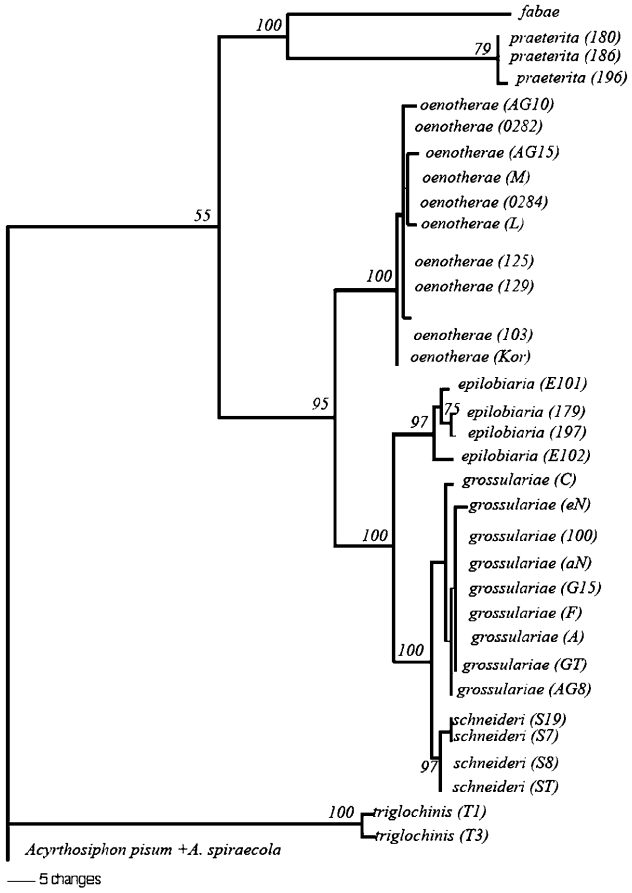

Fig. 3. Phylogeny of the genus *Aphis* based on a maximum parsimony analysis of both mitochondrial and nuclear sequences. Bootstrap support based on 1000 replicates is indicated for nodes with greater than 50% support.

Together with ecological features (*A. grossulariae* alternates between *Ribes* spp. and *Epilobium* spp. and only occasionally occurs on *Oenothera* spp., whilst *A.oenotherae* in Europe inhabits mainly *Oenothera* spp.) the DNA data strongly corroborates the viewpoint that *A. oenotherae* is a separate species and not an anholocyclic derivative of *A. grossulariae*.

The present data indicate that the subgenus *Aphis* s. str. is not monophyletic. Consequently, it should be broken up into more subgenera, or *Bursaphis* should be included in *Aphis* s. str. Phylogenetic analysis of both genes and the combined analysis supported monophyly of the subgenus *Bursaphis*. All the species of the subgenus *Bursaphis* appear to be closely related; every species has strong bootstrap support values from the *CO-I* and combined analyses (Figs 1 and 3). Therefore, the phylogenetic signal for the terminal branches is probably swamped by the greater amount of information yielded by *CO-I* with its faster mutation rate. Phylogenetic studies using *EF-1*α have shown this marker to be excellent for solving problems at higher taxonomic levels (Damgaard et al., 2001; Pedersen, 2002).

Of the four species of *Aphis* s. str. used in the present *EF-1*α analysis, *A. fabae* and *A. praeterita* are situated closer to *Bursaphis* than *A. triglochinis* and *A. salicariae*: the former two species seem to have had a more recent mutual ancestor in common with the *Bursaphis* species analysed, when compared with the *A. triglochinis* and *A. salicariae* clade (Fig. 2). For *CO-I*, the opposite is shown (Fig. 1). This supports the view that the subgenus *Aphis* s. str. is an artificial taxon, which includes all species of the genus *Aphis* that do not fit into any other subgenus of *Aphis*. The only feature shared by these species seems to be that they do not have the diagnostic characters of any other subgenus in the genus *Aphis*. A more detailed analysis including representatives of all subgenera is needed to clarify this matter.

A sequence of the *EF-1*α gene from an unidentified species of the genus *Casimira* (Eastop, 1966), taken from the GenBank for comparative analysis, appeared in the same clade as *A. fabae* and *A. praeterita* in our *EF-1*α analysis (Fig. 2). Phylogenetic analysis of New Zealand indigenous Aphidines (von Dohlen & Teulon, 2003) based on *EF-1*α gene grouped *Casimira* sp. with the two northern species, *A. fabae* and *A. spiraecola*, which could reflect a northern origin. Our data strongly support the sister-group relationship between the *Casimira* sp. and Palearctic *A. fabae* and *A. praetaerita* (Fig. 2). Considering molecular and host-specificity data, *Casimira* might be an Australian derivative of some Palaearctic *Epilobium*-inhabiting *Aphis* species (close to *A. praeterita*), or might have had a mutual ancestor with *A. fabae* and *A. praeterita*. Changes in morphology might be explained as an adjustment to the Australian environment and an exotic host plant species. Certain morphological characters are rather variable in the Aphidina, which have resulted in debates among aphid taxonomists and several different infrageneric systems proposed for the genus *Aphis* (cf. Stroyan, 1984; Heie, 1986; Remaudière, 1993; Remaudière & Remaudière, 1997). For example, the presence of marginal abdominal tubercles appeared to be variable enough within the same species (Rakauskas, 1998), making the distinction between *Aphis* and *Paradoxaphis* uncertain (Carver, 2000). Thus the separation of *Casimira* (presumably also *Paradoxaphis*) from the genus *Aphis* is ambiguous.

Our data advocate independent colonisation of *Ribes* spp. by two species groups of the genus *Aphis*: *A. triglochinis* (subgenus *Aphis* s. str.) and *A. grossulariae* with *A. schneideri* (subgenus *Bursaphis*). The latter two species are very close in the molecular cladograms (Figs 1–3), are similar in morphology (Rakauskas, 1998) and hybridize in the laboratory (Rakauskas, 1999) and presumably in nature (Rakauskas, 2003). Thus it is possible that *Ribes* spp. was colonized by a single mutual ancestor of *A. grossulariae* and *A. schneideri*. In contrast, in our cladograms (Figs 1–3), *A. triglochinis* is in a separate group, clearly indicating an independent colonisation of currants by this aphid species compared to *A. grossulariae* and *A. schneideri*. Futher analysis is needed to determine whether Nearctic *Ribes*-inhabiting *Aphis* species of the genus *Bursaphis* colonised currants and gooseberries independently from their Palaearctic relatives, or are derivatives of Palaearctic species (or vice-versa). Phy-

logeographic analysis, together with an analysis of the phylogeography of the genus *Ribes*, are also needed.

Three independent switches to Onagraceous host plants are evident from the molecular cladograms (Figs 1–3). *Epilobium* species seem to have been independently colonised by *A. salicariae*, *A. praeterita* (both in *Aphis* s. str.) and an *A. epilobiaria-epilobii-grossulariae* complex in the subgenus *Bursaphis*. The latter species group might be subdivided into two branches – *A. epilobii-epilobiaria* and *A. grossulariae*. Together with *Casimira canberrae* (which might in fact also belong to the genus *Aphis* – see above), 8 aphid species of the genus *Aphis* use *Epilobium*-species as host plants (Table 1). They represent three clusters of species that are monoecious on *Epilobium hirsutum* (*A. epilobiaria, A. praeterita, A. fluvialis*), *E. montanum* (*A. epilobii*) and *Epilobium junceum* (*Casimira canberrae*), respectively. Four more species exploit *Epilobium* and/or *Chamaenerion* as temporary or obligate summer hosts and *Ribes* (*A. grossulariae*), *Spiraea* (*A. spiraephaga*), *Cornus* (*A. salicariae*) and *Frangula* (*A. frangulae*), respectively, as winter hosts. This might indicate several independent colonisations of onagraceous herbs by host switching or host capture in different lineages of the genus *Aphis*. Genus *Oenothera* (evening primrose) seems to have a specific host plant status: only one aphid species (*A. oenotherae*) is well adapted for feeding on it. Other species colonize evening primrose only occasionally (Table 1). This might be due to the specific biochemical compounds in *Oenothera*-species (Rostanski et al., 2004). Nevertheless, attempts to colonize *Oenothera* still occur. Recently, parthenogenetic apterous and winged females, together with oviparae and males of *A. epilobiaria* and *A. praeterita* were found on *Oenothera* spp. in the Czech Republic (R. Rakauskas, unpubl.). The colonies were thriving and winter eggs were deposited in great numbers. However, the fundatrices of these species were unable to feed on *Oenothera* in spring. This indicates that *Aphis* species do colonize novel host plants.

Present analysis does not support the traditional view of a common evolutionary pathway in aphids. As the ancient angiosperms were woody plants (Takhtajan, 1966), the evolution of host specificity in aphids is presented as a transition from trees to shrubs and subsequently herbs (Shaposhnikov, 1956; Guldemond, 1990; Shaposhnikov et al., 1998; Rakauskas, 2000). Thus, the primeval aphid life cycle should be holocyclic monoecy on trees and/or shrubs. If so, *A. schneideri* should be the stem species in the *Bursaphis* clade, followed by heteroecious *A. grossulariae* and herbophilous species. This is not supported by these analyses (Figs 1–3). Instead, anholocyclic monoecious *A. oenotherae* is at the base, followed by monoecious *A. epilobiaria*, whilst *A. grossulariae* and *A. schneideri* form the terminal buds. This indicates the opposite scenario: *Bursaphis* emerged as feeders on herbaceous plants and transferred later on to currant and gooseberry bushes. This accords with the viewpoint (Heie 1994, 1996) that the genus *Aphis* is one of the youngest genera of the Aphididae; it might have radiated with the introduction of herbaceous angiosperms at the end of Tertiary. If so, dendrophilous species of *Aphis* might have emerged later than their herbophilous relatives. This is speculative. More species need to be analysed, including *A. epilobii* and Nearctic representatives of *Bursaphis*, to test this suggestion.

CONCLUSIONS

Summarising the results of the present study, based on the analysis of the mitochondrial *CO-I* and nuclear *EF-1α* genes, the following conclusions emerge. (i) Palaearctic species of the subgenus *Bursaphis* (= "*grossulariae*" species group of the genus *Aphis*) form a monophyletic group within the genus *Aphis*. (ii) *Aphis grossulariae* and *A. schneideri* are close relatives, which is in accordance with their ability to hybridize in the laboratory and possibly in nature (Rakauskas, 2003). (iii) Independent multiple colonisation of *Ribes* spp. and Onagraceae herbs by the *Aphis* genus might have occurred several times. (iv) The genus *Casimira* (Eastop, 1966) might be an atypical species within the genus *Aphis* L. (v) Nominal subgenus *Aphis* s. str. might be an artificial taxon within the genus *Aphis* L. Once the subgenus *Bursaphis* is accepted, the subgenus *Aphis* s. str. should be subdivided.

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