MOLECULAR ECOLOGY RESOURCES
Molecular Ecology Resources (2010) doi: 10.1111/j.1755-0998.2010.02877.x

DNA BARCODING

Barcoding aphids (Hemiptera: Aphididae) of the Korean Peninsula: updating the global data set

WONHOON LEE,*[1] HYOJOONG KIM,*[1] JONGOK LIM,* HWAL-RAN CHOI,* YEYEUN KIM,* YANG-SU KIM,† JEONG-YEON JI,† ROBERT G. FOOTTIT‡ and SEUNGHWAN LEE*
*Insect Biosystematics Laboratory, Research Institute for Agricultural and Life Sciences, Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Korea, †National Plant Quarantine Service, Incheon International Airport Office, Incheon 400-430, Korea, ‡National Environmental Health Program, Invertebrate Biodiversity, Agriculture and Agri-Food Canada, Ottawa, Canada

Abstract

DNA barcode (mitochondrial *COI*) sequences are provided for species identification of aphids from the Korean Peninsula. Most (98%) of the 154 species had distinct *COI* sequences (average 0.05% intraspecific pairwise divergence) relative to the degree of sequence divergence among species (average value 5.84%). For species in common with other regions, barcodes for Korean samples fell near or within known levels of variation. Based on these results, we conclude that DNA barcodes can provide an effective tool for identifying aphid species in such applications as pest management, monitoring and plant quarantine.

Keywords: Aphididae, COI, DNA barcode, Korea

Received 9 March 2010; revision received 13 April 2010; accepted 23 April 2010

Introduction

As international trade has expanded, quarantine and plant protection activities require accurate and timely identification of pests (Miller & Foottit 2009), especially of small insects such as aphids. However, taxonomic keys using morphological characteristics usually treat only certain life stages or genders and are often difficult for the nonexpert to use.

DNA barcoding is based on the use of a short standardized sequence to characterize species (Hajibabaei *et al.* 2006). In animals, the selected region is a 650 base pair fragment of the 5′ end of mitochondrial *cytochrome c oxidase subunit I* (*COI*) (Hebert *et al.* 2003). Currently, 'DNA barcoding' has been applied in pest monitoring and quarantine (Armstrong & Ball 2005; Ratnasingham & Hebert 2007), and its usefulness has been confirmed in several hexapod orders: Coleoptera (Löbl & Leschen 2005), Diptera (Scheffer *et al.* 2006), Ephemeroptera (Ball *et al.* 2005), Hemiptera (Foottit *et al.* 2008), Hymenoptera (Smith *et al.* 2008) and Lepidoptera (Hajibabaei *et al.* 2006). Species identification is achieved by comparing the sequence of an unknown sample to a reference database through similarity methods such as BLAST (Altschul *et al.* 1990). The reliability of identification depends on the extent of taxonomical coverage of the group of interest and an understanding of the degree of variation within species.

Detecting exotic aphid pests is very important to protect domestic agricultural and/or horticultural crops in the quarantine system because aphids inflict economic damage on agriculture and horticulture by direct feeding on plant phloem or by vectoring numerous plant diseases (Blackman & Eastop 2000). Generally, aphids have been identified using morphological characters. However, their small size, polymorphism, environmentally induced variation and reduction in morphological characters can make identification difficult (Miller & Foottit 2009). Foottit *et al.* (2008) provided a survey of DNA barcode data for over 300 species in more than 130 genera. Other studies related to DNA barcoding of aphids have been restricted to analysis of single genera or treated only a few species (Valenzuela *et al.* 2007; Coeur d' Acier *et al.* 2008; Foottit *et al.* 2009a; Wang & Qiao 2009). Thus, reference barcodes are currently available for <10% of the 4700 aphid species in the world (Remaudière & Remaudière 1997).

Correspondence: Seunghwan Lee, Fax: +82 2 880 4691; E-mail: seung@snu.ac.kr
[1]These authors contributed equally to this work.

© 2010 Blackwell Publishing Ltd

In this study, we present *COI* barcodes for 249 individuals of 154 species collected from the Korean Peninsula, and compare our results to sequence 334 aphid species archived in GenBank.

Materials and methods

Taxon sampling

We analysed *COI* sequences from 249 individuals of 154 species belonging to 72 genera and 11 subfamilies within Aphididae (Table S1). All aphid samples were collected at several regions in the Korean Peninsula from 2003 to 2009 and stored in 80% ethanol for slide voucher specimens and 99% ethanol at −20 °C for extracting genomic DNA. Identification of each aphid was based on exterior morphology of slide-mounted specimens, and all samples and voucher specimens were preserved in the Insect Collection of the College of Agriculture and Life Sciences (Seoul National University, Korea).

DNA extraction, amplification, sequencing and alignment

Total genomic DNA extraction was performed using DNeasy® Blood & Tissue Kit (QIAGEN Inc., Dusseldorf), according to the manufacturer's protocol. Samples for extraction consisted of single or several individuals from the same colony. The target 658-bp fragment of *COI* was amplified by polymerase chain reaction (PCR) using AccuPower® PCR PreMix (Bioneer Corp., Daejeon, Korea) and universal primer pair, LCO1490 (5′–GGT CAA CAA ATC ATA AAG ATA TTG G–3′) and HCO2198 (5′–TAA ACT TCA GGG TGA CCA AAA AAT CA–3′) (Folmer *et al.* 1994). We used the following thermal cycle parameters for 20 μL amplification reactions: initial denaturation for 5 min at 94 °C, followed by 34 cycles of 1 min at 94 °C, 1 min at 45.2 °C, and 1 min at 72 °C and a subsequent final extension at 72 °C for 5 min. PCR products were tested by electrophoresis on an agar gel and, if a single band was observed, were purified using a QIAquick® PCR purification kit (QIAGEN Inc). PCR products were directly sequenced in both directions by the automated sequencer (ABI Prism 3730 XL DNA Analyzer) in the NICEM (the National Instrumentation Center for Environment Management, Seoul National University, Republic of Korea). Resulting chromatograms were evaluated for miscalls and ambiguities and assembled into contigs in SeqMan™Pro (version 7.1.0, 2006; DNAstar™ Inc., Madison, WI, USA). The sequences were individually checked by eye, verified for protein coding frame-shifts to avoid pseudogenes (Zhang & Hewitt 1996). Consensus files were aligned using Clustal X 1.83 (Thompson *et al.* 1997).

All sequences are deposited in GenBank (accession numbers given in Table S1).

Data analysis

Pairwise sequence divergences were calculated using a Kimura 2-parameter (K2P) distance model (Kimura 1980) and analysed at species, genus and family level. In addition, for 31 species for which published barcodes are available (Valenzuela *et al.* 2007; Coeur d' Acier *et al.* 2008; Foottit *et al.* 2008; Wang & Qiao 2009), we examined the effect of introducing Korean samples on overall intraspecific divergence in these species (Table S2).

Neighbour-joining (NJ) analyses were conducted independently for two data sets, Korean species data set and combined data set, in MEGA 4.0 (Tamura *et al.* 2007).

Results and discussion

Species collected in the Korean Peninsula

One hundred and seventeen species were represented by single individual, and the remaining 37 species characterized by more than two samples. The neighbour-joining tree for Korean samples is given in Fig. 2. Replicated species form tight clusters, with average pairwise genetic divergences among conspecific individuals (Fig. 1; Table 1) of 0.05% (range 0.00–1.00%). Mean pairwise divergence between specimens of congeneric species was 5.84% (range. 0.00–14.04%); of the 1694 species pair comparisons, 1595 (94.15%) showed genetic divergences more than 2%. Mean pairwise divergences between specimens of different genera belong to same subfamily was 8.86 (range 1.60–19.00%), and mean pairwise divergence between specimens of different subfamilies belong to Aphididae was 12.66 (range. 7.40–

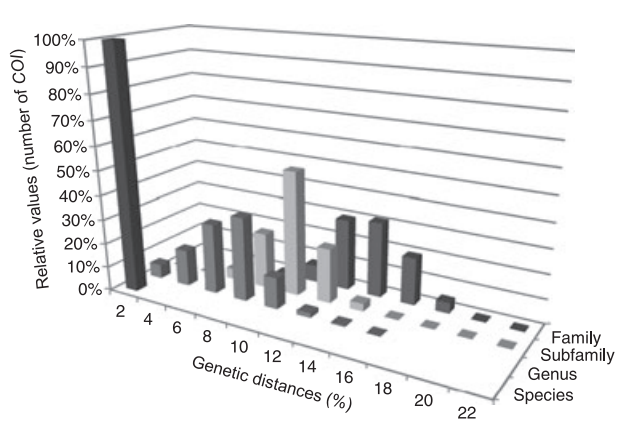

Fig. 1 Distribution of genetic divergences based on the Kimura-2 parameter for *cytochrome c oxidase subunit I* sequences according to taxonomic levels.

© 2010 Blackwell Publishing Ltd

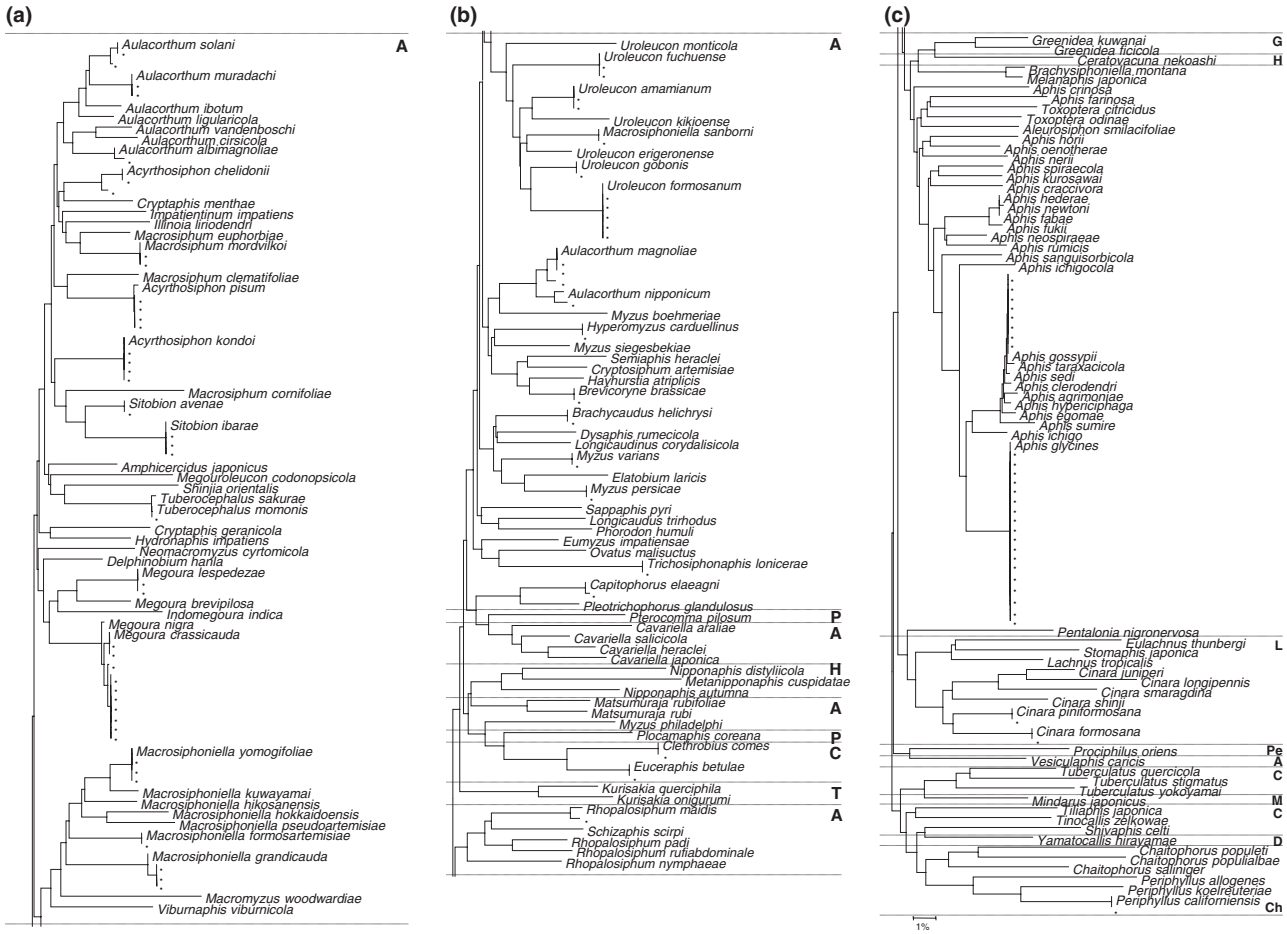

Fig. 2 A neighbour-joining tree using 249 *cytochrome c oxidase subunit I* sequences from 154 Korean Aphididae species: A, Aphidinae; C, Calaphidinae; Ch, Chaitophorinae; D, Drepanosiphinae; G, Greenideinae; H, Hormaphidinae; L, Lachninae; M, Mindarinae; Pe, Pemphiginae; P, Pterocommatinae; T, Thelaxinae.

Table 1 Genetic divergences according to different taxonomic levels within the family Aphididae

Comparison	Number of comparisons	Average (%)	Minimum (%)	Maximum (%)	SE
Within species	362	0.05	0.00	1.00	0.001
Within genus, between species	1694	5.84	0.00	14.04	0.023
Within subfamily, between genera	19603	8.86	1.60	19.00	0.017
Within family, between subfamilies	9924	12.66	7.40	20.90	0.020

20.90%). This is similar to values previously reported for aphids (Foottit *et al.* 2008, 2009b). Thus, in general, species are clearly differentiated from each other. There are, however, exceptions. Specimens identified as *Aphis hederae*, *Aphis fabae* and *Aphis newtoni* cluster closely together, and *Megoura nigra* is not clearly distinguished from *Megoura crassicauda*.

Among genera represented by several species, the species tend to group together: *Macrosiphoniella* species (except *M. sanborni*), *Aphis* species (except *A. crinosa* and *A. farinosa*), *Aulacorthum* species (except *A. magnoliae* and *A. nipponicum*), *Cavariella* species, *Megoura* species (with inclusion of *Indomegoura*), *Sitobion* species, *Uroleucon* species (with inclusion of *Macarosiphoniella sanborni*) and *Rhopalosiphum* species (with the inclusion of *Schizaphis scirpi*) each form a cluster. On the other hand, *Acyrthosiphon* and *Macrosiphum* species do not form cohesive groups. At higher levels, the Lachninae and Chaitophorinae form single groups. Thus, barcodes seem to reflect some higher level associations but are not adequate for

© 2010 Blackwell Publishing Ltd

Table 2 Comparison of regions of distribution and interspecific genetic divergences of the 24 species pairs of 33 species showing exceptionally close similarity (<1% sequence divergence)

	Species (No. of *COI* sequences)	Region [REF]	Species (No. of *COI* sequences)	Region [REF]	Pairwise distances (%) Ave. (Min.–Max.)
Previously reported species pairs	*Aphis varians* (3)	N [1]	*Aphis manitobensis* (1)	N [1]	0.61 (0.46–0.77)
	Aphorophora rubicumberlandi (1)	N [1]	*Amphorophora agathonica* (1)	N [1]	0.92 (0.92–0.92)
	Brachycaudus ballotae (1)	W [3]	*Brachycaudus lamii* (1)	W [5]	0.16 (0.16–0.16)
	Brachycaudus lychnidis (3)	W [5]	*Brachycaudus lychnicola* (1)	W [5]	0.33 (0.33–0.33)
	Brachycaudus lychnidis (3)	W [5]	*Brachycaudus populi* (1)	W [5]	0.17 (0.17–0.17)
	Brachycaudus lychnicola (1)	W [5]	*Brachycaudus populi* (1)	W [5]	0.50 (0.50–0.50)
	Brachycaudus prunicola (1)	W [5]	*Brachycaudus tragopogonis* (3)	W [5]	0.23 (0.00–0.34)
	Brachycaudus prunicola (1)	W [5]	*Brachycaudus schwartzi* (3)	W [5]	0.00 (0.00–0.00)
	Brachycaudus schwartzi (3)	W [5]	*Brachycaudus tragopogonis* (3)	W [5]	0.23 (0.00–0.34)
	Brachycaudus lateralis (5)	W [5]	*Brachycaudus cardui* (4)	N, W [1, 5]	0.24 (0.00–0.70)
	Ericaphis wakibae (8)	N [1]	*Aulacorthum dorsatum* (3)	N [1]	0.10 (0.00–0.15)
	Myzocallis punctata (1)	N [1]	*Myzocallis asclepiadis* (2)	N [1]	0.15 (0.15–0.15)
	Uroleucon nigrotibium (4)	N [1]	*Uroleucon eupatoricolens* (1)	N [1]	0.54 (0.46–0.61)
Newly observed species pairs	*Acyrthosiphon caraganae* (1)	N [1]	*Acyrthosiphon kondoi* (1)	E [7]	0.31 (0.31–0.31)
	Aphis fabae (14)	N, E [1, 2, 7]	*Aphis newtoni* (1)	E [7]	0.25 (0.17–0.33)
	Aphis fabae (14)	N, E [1, 2, 7]	*Aphid hederae* (1)	E [7]	0.08 (0.00–0.17)
	Aphis newtoni (1)	E [7]	*Aphid hederae* (1)	E [7]	0.16 (0.16–0.16)
	Aphis gossypii (103)	N, E, A [1, 3, 4, 7]	*Aphis clerodendri* (1)	E [7]	0.08 (0.00–0.71)
	Aphis gossypii (103)	N, E, A [1, 3, 4]	*Aphis sedi* (1)	E [7]	0.32 (0.24–0.95)
	Aphis clerodendri (1)	E [7]	*Aphis sedi* (1)	E [7]	0.23 (0.23–0.23)
	Macrosihpum daphnidis (1)	N [1]	*Macrosiphum hellebore* (2)	A [4]	0.31 (0.31–0.31)
	Megoura litoralis (1)	W [6]	*Megoura crassicauda* (14)	E [6, 7]	0.18 (0.17–0.34)
	Megoura viciae (1)	W [6]	*Megoura crassicauda* (14)	E [6, 7]	0.18 (0.17–0.34)
	Megoura viciae (1)	W [6]	*Megoura litoralis* (1)	W [6]	0.00 (0.00–0.00)

Ave, Average; Min, Minimum; Max, Maximum; N, Nearctic region; W, Western Palearctic region, E, East Palearctic region; A, Australian, [1] Foottit *et al.* (2008), [2] Traugott & Symondson (2008), [3] Carletto *et al.* (2009), [4] Valenzuela *et al.* (2007), [5] Coeur d' Acier *et al.* (2008), [6] Kim & Lee (2008) and [7] this study. COI, cytochrome c oxidase subunit I.

reliable identification at these levels, especially when taxon sampling is inadequate.

Combined world data set

The addition of the Korean material brings to number of aphid species with published barcodes to 457 in 167 genera (Fig. S1). Among these, 33 species diverge from their nearest neighbour by <1% (13 added in this study, Table 2) even though they are morphologically and biologically well-delineated species (Coeur d' Acier *et al.* 2008; Foottit *et al.* 2008; Kim & Lee 2008).

The majority of previously reported barcodes are for North American material (Foottit *et al.* 2008). We selected 22 species with *COI* sequences available from more than one geographic region and examined the effect of adding Korean samples on the previously reported intraspecific divergence (Table 3). Maximum pairwise intraspecific distances increased in eight species, *Acyrthosiphon pisum*, *Myzus persicae*, *Rhopalosiphum padi*, *Toxoptera citricidus*, *Rhopalosiphum maidis*, *Hayhustia atriplicis*, *Aulacorthum solani* and *Aphis farinosa* with the addition of the Korean samples. However, with the possible exception of *Rh. maidis*, for which the maximum distance among samples is 2.04%, these species remain distinct. The variation within *Rh. maidis* is high compared to most other aphid species and suggests that this species should be reassessed to determine whether it is simply unusually variable, or if there are cryptic taxa. In the other species, the Korean samples fell within the known range of variation for the species. In one species, *A. spiraecola*, the unusually high maximum divergence value of 3.43% is because of a single sequence from Australia (Valenzuela *et al.* 2007). In contrast, the range of variation otherwise shown by this species among numerous samples from a broad geographic range, including Australia, New Zealand, Hawaii and across North America (Foottit *et al.* 2009a) and from Korea, is quite narrow.

We have provided additional *COI* sequences for nine species not previously replicated (Table 3). Of these, only one species, *Aphis oenotherae*, had *COI* sequence identical to that previously reported. The range of variation exhibited by these species is well within expected limits.

© 2010 Blackwell Publishing Ltd

Table 3 The intraspecific genetic divergences of species replicated in the combined data set for 31 species present in the Korean data set

	Among all regions			Among other regions excluding Korea			Between Korea and other regions		
Species	No. of individuals	Ave. (%)	(Min.(%) –Max.(%))	No. of individuals	Ave.	(Min.–Max.)	No. of individuals (Other region/ Korea)	Ave.	(Min.–Max.)
Acyrthosiphon pisum	14	0.02	(0.00–0.17)	9	0.00	(0.00–0.00)	9/5	0.03	(0.00–0.17)
Aphis craccivora	9	0.17	(0.00–0.32)	8	0.17	(0.00–0.32)	8/1	0.16	(0.16–0.16)
Aphis fabae	14	0.13	(0.00–0.33)	13	0.13	(0.00–0.33)	13/1	0.09	(0.00–0.17)
Aphis farinosa	4	0.86	(0.24–1.48)	3	0.65	(0.24–0.98)	3/1	1.07	(0.74–1.48)
Aphis glycines	24	0.00	(0.00–0.00)	5	0.00	(0.00–0.00)	5/19	0.00	(0.00–0.00)
Aphis gossypii	103	0.08	(0.00–1.15)	93	0.07	(0.00–1.15)	93/10	0.12	(0.00–1.15)
Aphis nerii	4	0.31	(0.00–0.61)	3	0.41	(0.00–0.61)	3/1	0.20	(0.00–0.61)
Aphis spiraecola	51	0.18	(0.00–3.43)	50	0.18	(0.00–3.43)	50/1	0.09	(0.00–2.95)
Aulacorthum solani	10	0.24	(0.00–0.68)	7	0.05	(0.00–0.17)	7/3	0.42	(0.34–0.68)
Brachycaudus helichrysi	13	1.43	(0.00–2.67)	11	1.26	(0.00–2.67)	11/2	1.93	(1.65–2.05)
Brevicoryne brassicae	5	0.07	(0.00–0.17)	3	0.11	(0.00–0.17)	3/2	0.08	(0.00–0.17)
Hayhurstia atriplicis	4	0.15	(0.00–0.30)	3	0.00	(0.00–0.00)	3/1	0.30	(0.30–0.30)
Illinoia liriodendri	4	0.49	(0.00–0.97)	3	0.65	(0.16–0.97)	3/1	0.32	(0.00–0.81)
Macrosiphum euphorbiae	6	0.58	(0.00–1.25)	5	0.69	(0.00–1.25)	5/1	0.37	(0.00–0.93)
Megoura lespedezae	4	0.00	(0.00–0.00)	3	0.00	(0.00–0.00)	3/1	0.00	(0.00–0.00)
Megoura nigra	3	0.00	(0.00–0.00)	2	0.00	(0.00–0.00)	2/1	0.00	(0.00–0.00)
Megoura brevipilosa	2	0.00	(0.00–0.00)	1	0.00	(0.00–0.00)	1/1	0.00	(0.00–0.00)
Myzus persicae	14	0.04	(0.00–0.16)	12	0.00	(0.00–0.00)	12/2	0.16	(0.16–0.16)
Rhopalosiphum maidis	5	1.12	(0.31–2.04)	3	1.04	(0.62–1.25)	3/2	1.22	(0.31–2.04)
Rhopalosiphum padi	8	0.65	(0.00–1.55)	7	0.50	(0.00–1.39)	7/1	1.10	(0.15–1.55)
Rhopalosiphum rufiabdominale	3	0.20	(0.00–0.31)	2	0.31	(0.31–0.31)	2/1	0.16	(0.00–0.31)
Toxoptera citricidus	4	0.08	(0.00–0.16)	3	0.00	(0.00–0.00)	3/1	0.16	(0.16–0.16)
Aphis ichigo	2	0.48	(0.48–0.48)	1	–	–	1/1	0.48	(0.48–0.48)
Aphis oenotherae	2	0.00	(0.00–0.00)	1	–	–	1/1	0.00	(0.00–0.00)
Aphis rumicis	2	0.36	(0.36–0.36)	1	–	–	1/1	0.36	(0.36–0.36)
Capitophorus elaeagni	3	0.11	(0.00–0.17)	1	–	–	1/2	0.08	(0.00–0.17)
Hyperomyzus carduellinus	3	0.10	(0.00–0.16)	1	–	–	1/2	0.16	(0.16–0.16)
Myzus varians	3	0.12	(0.00–0.17)	1	–	–	1/2	0.17	(0.17–0.17)
Rhopalosiphum nymphaeae	2	0.00	(0.00–0.00)	1	–	–	1/1	0.00	(0.00–0.00)
Shivaphis celti	2	0.46	(0.46–0.46)	1	–	–	1/1	0.46	(0.46–0.46)
Sitobion avenae	3	1.35	(1.09–1.72)	1	–	–	1/2	1.48	(1.25–1.72)

Ave., Average; Min., Minimum; Max., Maximum.

Conclusion

In this study, we added *COI* sequences for 124 aphid species, most endemic to the Eastern Palearctic region. The majority (92.34%) of the 457 species in the combined data set were characterized by distinct *COI* sequences or clusters of closely similar sequences. Our results confirm that DNA barcodes are a highly effective identification tool for species of the family Aphididae and thus provide promise for applications in pest management and plant quarantine.

Acknowledgement

This research was supported by Korea Ministry of Environment as 'The Eco-technopia 21 project', a grant from by Agricultural R&D Promotion Center, and the project on survey and excavation of Korean indigenous species of the National Institute of Biological Resources under the Ministry of Environment, Korea. We wish to gratefully acknowledge the technical assistance of Eric Maw (Agriculture and Agri-Food Canada, Ottawa).

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. *Journal of molecular biology*, **215**, 403–410.

Armstrong KF, Ball SL (2005) DNA barcodes for biosecurity: invasive species identification. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, **360**, 1813–1823.

© 2010 Blackwell Publishing Ltd

Ball SL, Hebert PDN, Burian SK, Webb JM (2005) Biological identifications of mayflies (Ephemeroptera) using DNA barcodes. *Journal of the North American Benthological Society*, **24**, 508–524.

Blackman RL, Eastop VF (2000) *Aphids on the World's Crops: an Identification and Information Guide*, 2nd edn. John Wiley & Sons Ltd., England.

Carletto J, Blin A, Vanlerberghe-Masutti F (2009) DNA-based discrimination between the sibling species *Aphis gossypii* Glover and *Aphis frangulae* Kaltenbach. *Systematic Entomology*, **34**, 307–314.

Coeur d' Acier A, Cocuzza G, Jousselin E, Cavalieri V, Barbagallo S (2008) Molecular phylogeny and systematic in the genus *Brachycaudus* (Homoptera: Aphididae): insights from a combined analysis of nuclear and mitochondrial genes. *Zoologica scripta*, **37**, 175–193.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, **3**, 294–299.

Foottit RG, Maw HEL, von Dohlen CD, Herbert PDN (2008) Species identification of aphids (Insecta: Hemiptera: Aphididae) through DNA barcodes. *Molecular Ecology Resources*, **8**, 1189–1201.

Foottit RG, Lowery DT, Maw HEL, Smirle MJ, Lushai G (2009a) Identification, distribution, and molecular characterization of the apple aphids *Aphis pomi* and *Aphis spiraecola* (Hemiptera: Aphididae: Aphidinae). *The Canadian Entomologist*, **141**, 478–495.

Foottit RG, Maw HEL, Pike KS (2009b) DNA barcodes to explore diversity in aphids (Hemiptera Aphididae and Adelgidae). *Redia*, **92**, 87–91.

Hajibabaei M, Janzen DH, Burns JM, Hallwachs W, Hebert PD (2006) DNA barcodes distinguish species of tropical Lepidoptera. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 968–971.

Hebert PD, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. *Proceedings. Biological sciences/The Royal Society*, **270**, 313–321.

Kim H, Lee S (2008) Molecular systematics of the genus *Megoura* (Hemiptera: Aphididae) using mitochondrial and nuclear DNA sequences. *Molecules and cells*, **25**, 510–522.

Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *Journal of molecular evolution*, **16**, 111–120.

Löbl I, Leschen RAB (2005) Demography of Coleopterists and Their Thoughts on DNA Barcoding and the Phylocode, with Commentary. *The Coleopterists Bulletin*, **59**, 284–292.

Miller GL, Foottit RG (2009) The taxonomy of crop pests: the aphids. In: *Insect Biodiversity: Science and Society* (eds Foottit RG & Adler PH), pp. 463–473. Wiley-Blackwell, Oxford.

Ratnasingham S, Hebert PDN (2007) BOLD: the Barcode of Life Data System (http://www.barcodinglife.org). *Molecular Ecology Notes*, **7**, 355–364.

Remaudière G, Remaudière M (1997) *Catalogue des Aphididae du monde/ Catalogue of the World's Aphididae. Homoptera Aphidoidea*. INRA Editions, Versailles, France.

Scheffer SJ, Lewis ML, Joshi RC (2006) DNA Barcoding Applied to Invasive Leafminers (Diptera: Agromyzidae) in the Philippines. *Annals of the Entomological Society of America*, **99**, 204–210.

Smith MA, Rodriguez JJ, Whitfield JB *et al.* (2008) Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. *Proceedings of the National Academy of Sciences of the United States of America*, **105**, 12359–12364.

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Molecular biology and evolution*, **24**, 1596–1599.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research*, **25**, 4876–4882.

Traugott M, Symondson WOC (2008) Molecular analysis of predation on parasitized hosts. *Bulletin of entomological research*, **98**, 223–231.

Valenzuela I, Hoffmann AA, Malipatil MB, Ridland PM, Weeks AR (2007) Identification of aphid species (Hemiptera: Aphididae: Aphidinae) using a rapid polymerase chain reaction restriction fragment length polymorphism method based on the cytochrome oxidase subunit I gene. *Australian Journal of Entomology*, **46**, 305–312.

Wang J-F, Qiao G-X (2009) DNA barcoding of genus *Toxoptera* Koch (Hemiptera: Aphididae): identification and molecular phylogeny inferred from mitochondrial *COI* sequences. *Insect Science*, **16**, 475–484.

Zhang D-X, Hewitt GM (1996) Nuclear integrations: challenges for mitochondrial DNA markers. *Trends in Ecology and Evolution*, **11**, 247–251.

Supporting Information

Additional supporting information may be found in the online version of this article.

Table S1 The list of 249 individuals of 154 species, belong to 72 genera, and 11 subfamilies, within the family Aphididae.

Table S2 The list of 773 *COI* sequences of species, belong to 334 species, 131 genera, and 15 subfamilies, within the family Aphididae achieved in GenBank.

Fig. S1 A neighbour-joining tree using 1022 *COI* sequences from 456 species, 167 genera and 16 subfamilies.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any supporting information supplied by the authors. Any queries (other than missing material) should be directed to the corresponding author for the article.

© 2010 Blackwell Publishing Ltd