Molecular Ecology Resources (2009) **9** (Suppl. 1), 188–195 doi: 10.1111/j.1755-0998.2009.02644.x

BARCODING ARTHROPODS

DNA barcodes to identify species and explore diversity in the Adelgidae (Insecta: Hemiptera: Aphidoidea)

R. G. FOOTTIT,* H. E. L. MAW,* N. P. HAVILL,†§ R. G. AHERN‡ and M. E. MONTGOMERY§
*National Environmental Health Program, Invertebrate Biodiversity, Agriculture and Agri-Food Canada, K. W. Neatby Bldg, 960 Carling Avenue, Ottawa, Ontario, Canada K1A 0C6, †Department of Ecology and Evolutionary Biology, Yale University, New Haven, CT 06520, USA, ‡Department of Entomology, Michigan State University, East Lansing, MI 48824, USA, §Northern Research Center, United States Department of Agriculture, Forest Service, Hamden, CT 06514, USA

Abstract

The Adelgidae are relatively small, cryptic insects, exhibiting complex life cycles with parthenogenetic reproduction. Due to these characteristics, the taxonomy of the group is problematic. Here, we test the effectiveness of the standard 658-bp barcode fragment from the 5′-end of the mitochondrial cytochrome *c* oxidase 1 gene (COI) in differentiating among 17 species of Adelgidae, in associating life-cycle stages, and in assessing patterns of geographical variation in selected species. Species of Adelgidae are well-differentiated by DNA barcodes, enabling the identification of different morphological forms, immature stages and individuals on different hosts and at different periods of the life cycle. DNA barcodes have uncovered cryptic diversity within taxa and, in other cases, a lack of sequence divergence in species pairs previously separated by life-cycle characteristics, indicating a need for further taxonomic analysis.

Keywords: Adelgidae, COI, DNA barcoding, life cycles, mitochondrial DNA, parthenogenesis, species identification

Received 14 October 2008; revision received 18 December 2008; accepted 20 January 2009

Introduction

The Adelgidae, or conifer woolly adelgids, comprise a small group of about 70 species within the Aphidoidea (Insecta: Hemiptera) that includes destructive pests of forest ecosystems. The taxonomy, evolution and biology of the group were recently reviewed by Havill & Foottit (2007).

Species recognition has traditionally been based on the shape and distribution of the dorsal sclerites, wax plates and wax glands of the first instar stage. Adult morphological characters have proved useful in some cases. However, the small size of adelgids (0.4–2 mm), requires microscope-slide mounts for examination. Morphological plasticity due to environmental and host plant effects makes simple identification keys difficult to develop and use (Foottit & Mackauer 1980; Foottit 1997).

The Adelgidae undergo complex life cycles which can take 2 years to complete and include up to five morphological forms (Havill & Foottit 2007) (Fig. 1). These life cycles can include a sexual generation followed by a series of asexual generations (holocyclic) or can be entirely parthenogenetic (anholocyclic). Holocyclic and some anholocyclic species (e.g. *Pineus floccus* (Patch), Walton 1980) are host-alternating, with a fundatrix (offspring of sexual forms) and a gall-forming generation on spruce (*Picea* species) and non-gall forming parthenogenetic generations on other conifers (*Abies*, *Larix*, *Pinus*, *Pseudotsuga* or *Tsuga* species). Non-alternating species may be found on spruce, where they may or may not form galls, or they may be restricted to another species of conifer. Certain species pairs (for example *Pineus orientalis* and *P. pini*) are distinguished primarily by life-cycle characteristics, specifically as a holocyclic host-alternating species (*P. orientalis*) and an anholocyclic non-alternating species (*P. pini*).

As a result of the above factors, accurate documentation of all species and association of various morphological forms within the life cycle of adelgid species is still incomplete. Some species, such as the balsam woolly adelgid (*Adelges piceae* Ratzeburg) and hemlock woolly adelgid

Correspondence: Robert G. Foottit, Fax: (613)759 1701;
E-mail: foottitrg@agr.gc.ca

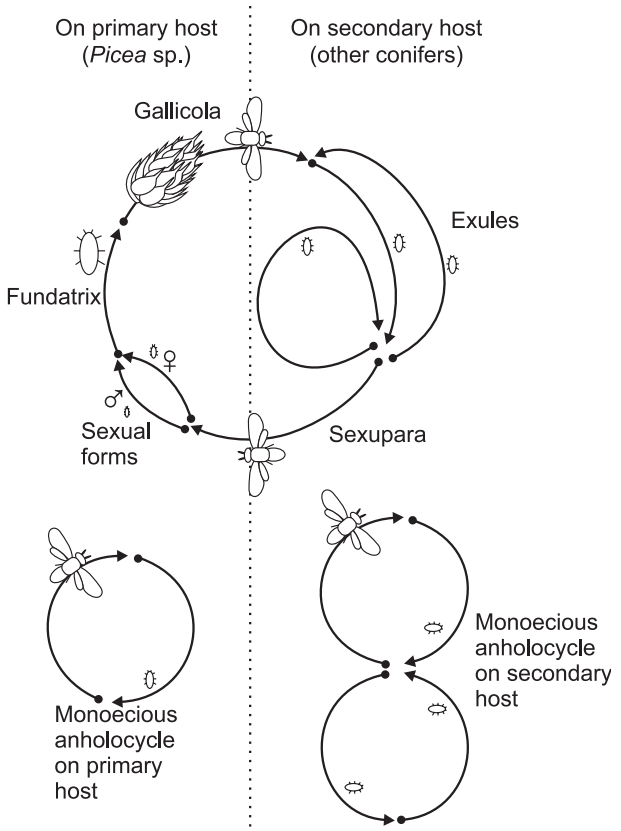

Fig. 1 Life-cycle variation among Adelgidae.

(*Adelges tsugae* Annand), are serious threats to forest ecosystems and are subject to monitoring and quarantine restrictions (Havill & Foottit 2007). Pest significance of other species may increase with increased trade. Effective management and regulation requires timely and accurate identification of species, morphological forms and life-cycle stages. The addition of molecular characters will aid in the resolution of taxonomic problems in the group.

DNA barcoding using the 5′-terminus of the mitochondrial cytochrome *c* oxidase subunit 1 gene (COI) (Hebert *et al*. 2003) has been demonstrated to be an effective standardized approach to the characterization of a wide range of organisms (Hajibabaei *et al*. 2007) including insects (Floyd *et al*. 2009). A previous study (Foottit *et al*. 2008) examined the utility of COI barcodes for the identification of species of the related family Aphididae. This study examines the utility of DNA barcodes to accurately identify adelgid species, including linking morphological forms and life-cycle stages. We also examine geographical variation within selected species.

Materials and methods

Samples were collected into 95% ethanol between 1991 and 2008. Vouchers from each collection were slide-mounted and deposited in the Canadian National Collection of Insects (Agriculture and Agri-Food Canada, Ottawa). Taxon assignment follows the list of described species, life cycles, secondary hosts and native ranges as provided in Havill & Foottit (2007). Samples were identified to species by the authors based on morphological and life-history criteria. The identification of members of pairs of species distinguished by life-cycle characters was deduced from the host association (presence on primary or secondary host) and distribution (which determines availability of the alternate host). A total of 581 samples representing 17 species were analysed. An additional 23 undetermined samples representing undescribed species or undescribed forms of named but incompletely described species were also included. The number of samples for each species and geographical origin are given in Table 1. Detailed collection information and voucher identifiers are available from the website of Barcode of Life Data System (BOLD; http://www.barcodinglife.org, 'Published projects/Barcoding the Adelgidae' links, Ratnasingham & Hebert 2008).

For the majority of samples, single specimens were transferred to coded tubes in a Matrix box (TrakMates microplate system; Matrix Technologies), and sent to the Biodiversity Institute of Ontario (BIO) for DNA extraction and sequencing. A few samples were processed by the third author (NPH) through the Yale University DNA Analysis Facility on Science Hill. Standard protocols (Hajibabaei *et al*. 2005; deWaard *et al*. 2007) were employed for DNA extraction and amplification, sequencing of the COI barcode region, sequence editing and alignment. Total DNA was extracted from individual specimens and the primer pairs LepF and LepR or M13-tailed alternates, LCO1490_t1 and HCO2198_t2 (primer sequences are available from BOLD, 'Published projects/View all primers' links) were used to amplify an approximately 700-bp DNA fragment of mitochondrial COI which was subsequently sequenced in both directions using either LepF and LepR or M13F and M13R (primer sequences from BOLD). All sequences obtained in this study have been deposited in GenBank [Accession nos EF73061 to EF73119 (from Havill *et al*. 2007), EU786119 to EU786388 (from Ahern *et al*. 2009), and FJ502356 to FJ502638], and are also accessible from BOLD.

Electropherograms for the COI gene were edited and aligned with Sequencher (version 4.5; Gene Codes Corporation). Nucleotide sequence divergences were calculated using the Kimura 2-parameter model of base substitution (Kimura 1980), the best model for species-level analysis with low distances (Hebert *et al*. 2003) as exhibited by some species clusters in this data set. Neighbour-joining (NJ) analysis (Saitou & Nei 1987), as implemented by the Taxon ID Tree function in BOLD, was used to provide a graphic representation of the phenetic distance matrix, and should not be interpreted as a phylogenetic hypothesis.

Table 1 Summary of materials sampled. Detailed collection data are available from the Barcode of Life Data System at http://www.barcodinglife.org, 'Barcoding the Adelgidae' project. (c, central; e, eastern; s, southern; w, western)

Species	Region	No. of samples
Adelges		
abietis (Linaeus)	e USA, Switzerland, Poland	21
cooleyi Gillette	e USA, w USA, w Canada, UK, Poland, Portugal	309
glandulae Zhang	Japan, China	13
japonicus Monzen	Japan, Russian Far East	5
lariciatus (Patch)	w, c Canada	11
laricis Vallot	e North America, Europe	24
nordmannianae (Eckstein)	Denmark, Switzerland, Slovakia, Turkey, Georgia, s Russia	14
pectinatae (Cholodkovsky)	Poland, Japan	12
piceae (Ratzeburg)	e USA, Switzerland	13
tsugae Annand	e, w North America, Japan, China	120
viridis (Ratzeburg)	Slovakia	1
Undetermined *Adelges*	Japan, China, North America	15
Pineus		
armandicola Zhang	w China	5
cembrae (Cholodkovsky)	Switzerland, Poland, Japan, Russian Far East	6
coloradensis (Gillette)	w Canada, w USA	7
orientalis (Dreyfus)	Poland, Turkey, s Russia	6
pini (Macquart)	w Canada, Switzerland, Poland, Israel	5
strobi (Hartig)	e USA, Poland	9
Undetermined *Pineus*	Japan, China, Israel	8

Results

Comparisons among determined samples

Pairwise distances (as per cent sequence divergence) between samples are summarized in Table 2A–C, and shown graphically as a neighbour-joining tree in Figs 2–5.

Maximum pairwise intersample distances within species (values on diagonals in Table 2A, B) range from 0.33 to 8.87%, (mean 1.425%, but the distribution strongly trimodal: 11 species with a maximum within-species divergence of less than 2%, 5 species between 2.8% and 4.6%, and *Adelges tsugae* at 8.87%).

Distances between samples of different congeneric species range from 0 to 16.30% (mean 11.33%). Three of these species pairs have between-species sequence divergences of less than 1%. The single sample of *A. viridis*, which is host-alternating between spruces and larches, differs from samples of the morphologically similar *A. abietis*, restricted to spruces, by 0 to 0.46%. Samples of *A. nordmannianae*, host-alternating between spruces and firs, differ from samples of *A. piceae*, non-alternating on fir, by 0 to 0.90%. Samples of *Pineus orientalis*, alternating between spruces and pines, and samples of *P. pini*, feeding only on pines, differ by only 0 to 0.33%. Two of these three pairs, in addition to the life-cycle differences, are reported to have subtle morphological differences in some life stages, but other stages are indistinguishable, while *P. orientalis* and *P. pini* are not reliably separable at any stage. The minimum interspecies distances among all species, when these pairs are pooled, is 3.2% (*Adelges laricis* vs. *A. japonicus*). Distances between *A. tsugae* and other *Adelges* species exceed all other interspecies differences. If *A. tsugae* is excluded and the above pairs pooled, the congeneric species distances range from 3.2 to 12.71% (mean 9.64%).

Intergeneric distances (Table 2C) range from 8.29 to 16.63%, with a mean of 11.95%. The mean intergeneric distance is thus similar to the mean intrageneric distances if *A. tsugae* is included (11.33%), but considerably higher than that distance if *A. tsugae* is excluded (9.64%).

Adelges species feeding on *Larix* (or non-alternating *Picea*-feeding counterparts of species alternating to larch) form a relatively tight cluster in which the nearest neighbour is another larch-associated species (Fig. 2; Table 2A). These species also share strong morphological similarities.

Intraspecific variation

Two species were sampled extensively over their known geographical range: *Adelges tsugae*, with 120 samples, and *A. cooleyi*, with 309 samples.

As noted above, variation within *A. tsugae* is comparable to the between-species distances exhibited by other adelgid species. Furthermore, neighbour-joining analysis groups samples into three major distinct clusters (Fig. 4), one consisting of samples from mainland China, a second (comprising a single sample) from Taiwan, and the third from Japan and North America. Internal variation within

Table 2 Summary of pairwise distances (per cent sequence divergence) between samples of identified adelgid species. A, ranges of pairwise distances between samples among *Adelges* species (off diagonal) and maximum within-species divergence (on diagonal). B, same, for *Pineus* species. C, ranges of pairwise sample divergence between samples of *Adelges* and *Pineus* species pairs

A		*Adelges*										
		abietis	*cooleyi*	*glandulae*	*japonicus*	*lariciatus*	*laricis*	*nordmannianae*	*pectinatae*	*piceae*	*tsugae*	*viridis*
Adelges	*abietis*	0.62	8.66–10.76	8.66–12.41	7.91–9.30	9.27–10.46	7.13–8.99	11.38–12.16	8.51–10.21	11.12–11.99	13.06–16.15	0–0.46
	cooleyi		3.49	8.44–11.56	8.07–12.03	9.31–12.42	8.79–11.67	7.13–9.71	5.76–9.29	7.57–10.69	11.25–15.17	9.11–10.53
	glandulae			3.96	7.97–9.97	8.87–10.57	7.42–9.88	9.51–11.23	7.43–10.94	9.50–10.78	9.40–14.48	9.84–12.22
	japonicus				0.46	6.99–8.17	3.20–4.31	10.57–11.06	7.48–9.02	10.56–11.41	11.91–14.95	8.34–8.75
	lariciatus					1.26	6.80–7.79	10.69–11.85	8.97–10.02	10.50–11.50	12.12–16.30	9.83–10.41
	laricis						0.93	10.88–12.71	7.66–9.14	10.76–11.66	11.64–14.91	8.28–8.99
	nordmannianae							0.72	6.95–9.00	0–0.90	11.26–14.71	11.73–12.10
	pectinatae								1.57	6.95–8.61	9.86–13.66	8.97–9.98
	piceae									0.46	11.26–14.33	11.73–11.97
	tsugae										8.87	13.41–16.04

B		*Pineus*					
		armandicola	*cembrae*	*coloradensis*	*orientalis*	*pini*	*strobi*
Pineus	*armandicola*	1.86	5.09–6.81	5.09–7.59	8.29–9.82	8.11–10.01	8.28–9.61
	cembrae		4.64	5.25–7.28	7.10–8.67	6.93–8.58	6.41–7.42
	coloradensis			2.85	7.25–8.27	7.08–8.16	7.25–7.76
	orientalis				0.33	0–0.33	6.41–7.54
	pini					1.26	6.58–7.60
	strobi						0.77

C		*Adelges*								
		abietis + virids	*cooleyi*	*glandulae*	*japonicus*	*lariciatus*	*laricis*	*nordmannianae + piceae*	*pectinatae*	*tsugae*
Pineus	*armandicola*	11.73–12.73	9.79–12.69	11.28–12.37	10.38–11.58	10.51–11.22	10.32–11.59	10.70–13.57	9.85–14.61	12.63–15.88
	cembrae	11.91–14.03	10.49–13.39	9.47–13.10	10.90–13.04	11.14–12.31	10.90–12.31	10.33–12.45	8.97–12.50	11.91–13.82
	coloradensis	10.36–12.47	9.27–12.87	9.12–12.73	10.37–12.66	10.85–12.49	10.14–11.79	8.93–12.05	8.97–12.12	9.46–14.15
	orientalis + pini	10.16–14.58	10.34–15.40	10.48–13.08	10.44–12.67	11.04–13.61	9.47–12.86	10.21–13.93	8.42–13.88	11.38–16.63
	strobi	13.51–14.39	11.38–13.95	10.88–12.18	10.90–12.85	12.12–13.04	11.39–12.49	11.76–12.90	10.02–13.36	12.45–16.63

the multisample clusters is similar in magnitude to that found within other species. Within the Japanese–American cluster, western North American material groups together, while eastern North American samples share haplotypes with Japanese samples. The observed pattern mirrors that obtained by Havill *et al*. (2006) using 1520 base pairs of data from portions of various mitochondrial genes other than COI.

Although overall variation among *A. cooleyi* specimens is substantially less than that seen in *A. tsugae*, there is still obvious structure within the sample set (Fig. 5). Again, there are three distinct relatively uniform clusters: one group from Arizona and Colorado, a second found from Utah to Arizona, and a third comprising the majority of samples, ranging from Alaska south through British Columbia and Alberta to California and Colorado. *A. cooleyi* was introduced into eastern North America during the latter part of the 19th century, and all specimens collected in introduced ranges share COI haplotypes with insects found in the central Rocky Mountains, suggesting this area as the source of introduced insects (Ahern *et al*. 2009).

Several other species show geographical or host separation of haplotype clusters (see Figs 2 and 3), although the limited sample size does not preclude the effects of sampling artefacts. For example, the four samples of *Adelges lariciatus* from the interior of British Columbia differ from the seven specimens from across the boreal forest zone ranging from Ontario to Alberta. Two specimens of *Adelges glandula*e from Yunnan Province of China differ from the remaining 13 samples from Sichuan, Yunnan and Japan. *Pineus coloradensis* samples from *Pinus contorta* differ from those collected on haploxylon pines (*Pinus monticola* and *P. lambertiana*). *Pineus cembrae* from Europe differs from *P. cembrae* in East Asia.

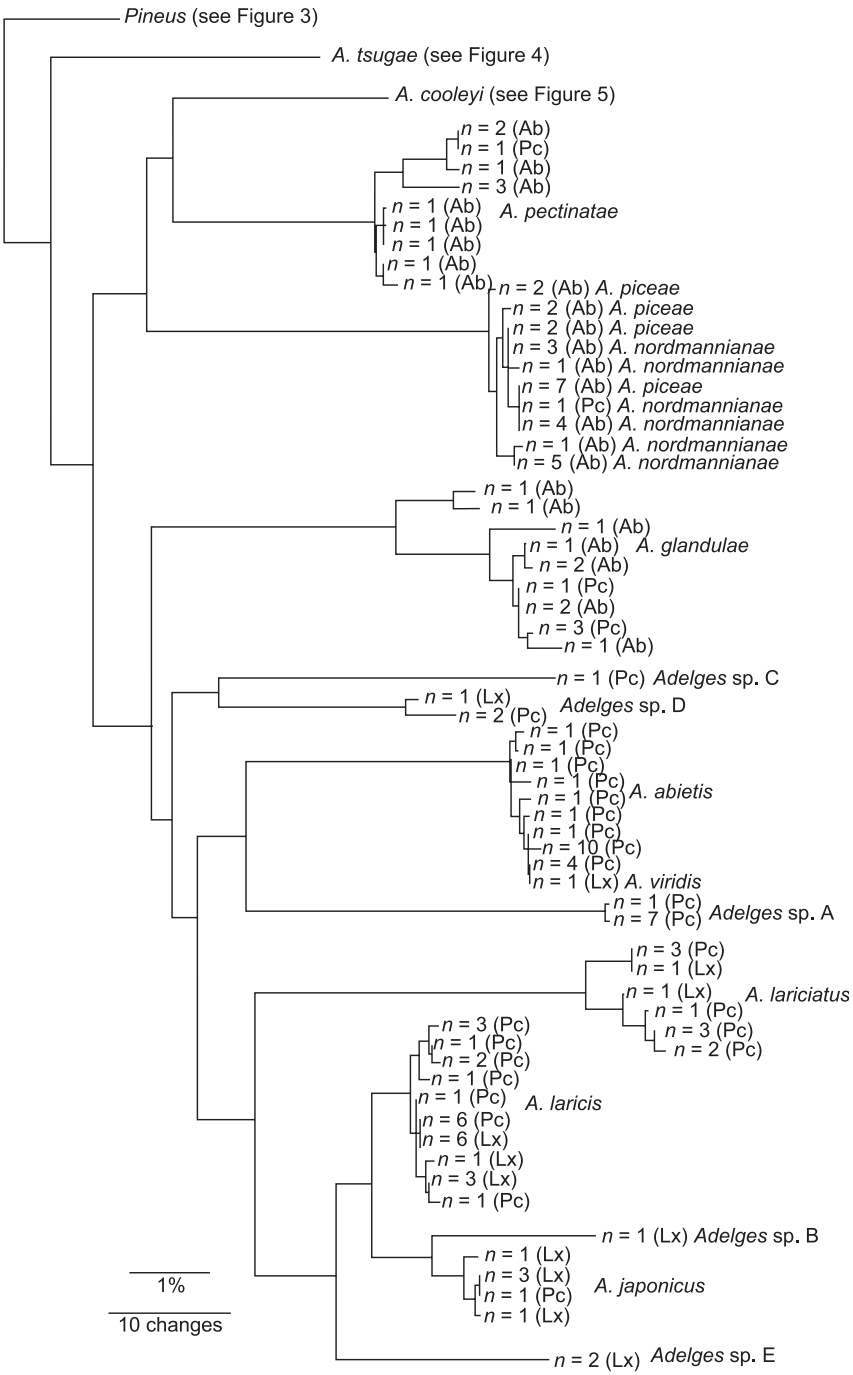

Fig. 2 Neighbour-joining tree for samples of Adelgidae, with host indicated. Samples with identical haplotypes and host-plant genus collapsed to a single terminal node (number of samples indicated). Samples of *Pineus* species, *Adelges tsugae*, and *Adelges cooleyi* are collapsed to the cluster root node and expanded in Figs 3, 4 and 5, respectively. Host codes: Pc, *Picea* spp.; Ab, *Abies* spp.; Lx, *Larix* spp.

Host alternation

Sequence similarity of collections from spruce and the secondary host confirm previously known host alternation in *Adelges laricis*, *A. lariciatus*, *A. nordmannianae*, *A. cooleyi*, *A. glandulae*, *A. pectinatae*, *Pineus orientalis* and Japanese populations of *A. tsugae*. In addition, Chinese collections of *A. tsugae* from spruce show that host alternation occurs in that region as well (see samples of *A. tsugae* from spruce indicated in Fig. 4). Host alternation of *Adelges japonicus*, previously reported to be restricted to spruce, has only been recently demonstrated (Sano *et al*. 2008), and is reflected in these data.

Undetermined samples

The 24 undetermined samples represented 15 haplotypes (15 *Adelges* samples with 7 haplotypes, 9 *Pineus* samples

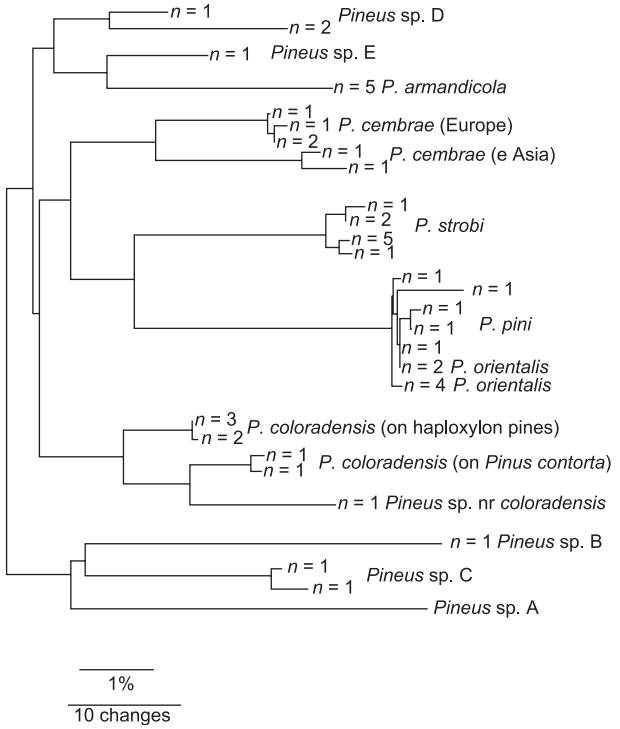

Fig. 3 Neighbour-joining tree for samples of *Pineus* species. Samples with identical haplotypes collapsed to a single terminal node (number of samples indicated).

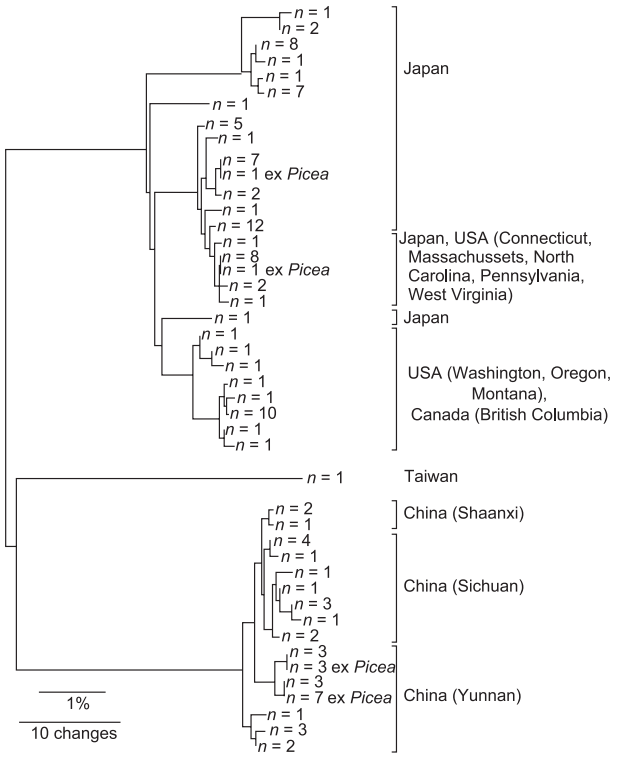

Fig. 4 Neighbour-joining tree for samples of *Adelges tsugae*. Samples with identical haplotypes collapsed to a single terminal node (number of samples indicated).

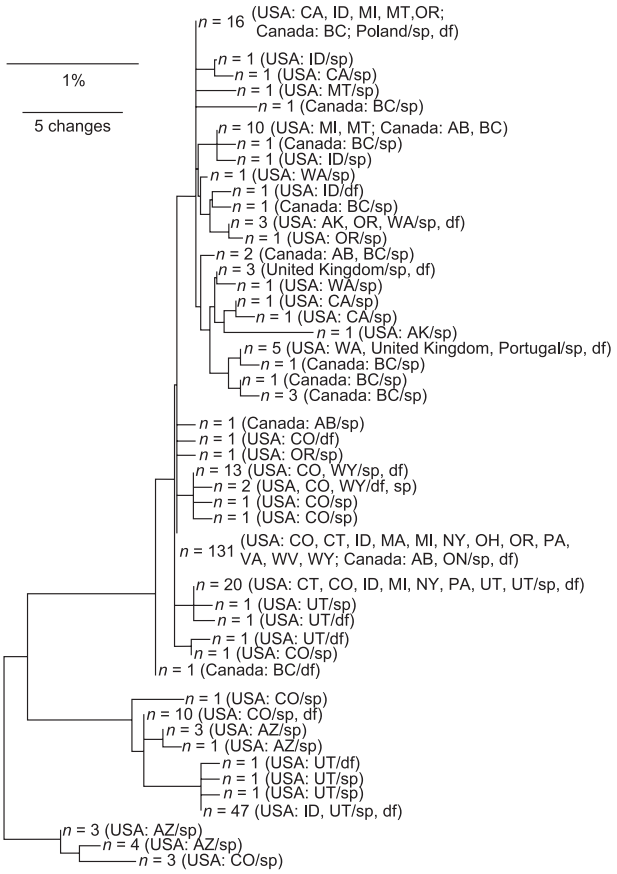

Fig. 5 Neighbour-joining tree for samples of *Adelges cooleyi*. Samples with identical haplotypes collapsed to a single terminal node with indication of number of samples, geographical origin, and hosts represented. States of USA: AK, Alaska; AZ, Arizona; CA, California; CO, Colorado; CT, Connecticut; ID, Idaho; MA, Massachusetts; MI, Michigan; MT, Montana; NY, New York; OH, Ohio; OR, Oregon; UT, Utah; VA, Virginia; WV, West Virginia; WY, Wyoming. Provinces of Canada: AB, Alberta; BC, British Columbia; ON, Ontario. Host abbreviations: sp., spruces (*Picea* species); df, Douglas fir (*Pseudotsuga menziesii*).

with eight haplotypes). Ranges of distances between each haplotype and determined congeners, as well as distance to the nearest noncongeneric sample are given in Table 3A, B.

In all cases, the nearest neighbour among determined specimens to an undetermined sample was a member of a congeneric species. However, the nearest *Pineus* sample to the undetermined *Adelges* samples was always closer than *A. tsugae*, and in most cases, closer than at least one other *Adelges* species. This reflects the greater diversity among *Adelges* species as compared to the relatively cohesive genus *Pineus*.

One sample from spruce falls close to *P. coloradensis*, differing by 3% sequence divergence, a level less than the minimum observed interspecies difference of 3.2% (ignoring biological species pairs) and only a little above the

Table 3 Ranges of pairwise distances (per cent divergence) between undetermined specimens and determined adelgid specimens in same genus, and distance to nearest sample in the other genus. A, *Adelges* species; B, *Pineus* species

A	*Adelges*									
	abietis + *viridis*	*cooleyi*	*glandulae*	*japonicus*	*lariciatus*	*laricis*	*nordmannianae* + *piceae*	*pectinatae*	*tsugae*	Nearest *Pineus* sample
Adelges sp. A sample 1	7.93–8.50	9.64–12.15	8.27–9.69	7.76–7.86	10.02–10.31	8.10–8.63	10.88–11.12	8.95–10.02	10.84–14.34	9.80 (*P. coloradensis*)
Adelges sp. A ($n = 7$)	7.76–8.32	9.64–12.15	8.27–9.69	7.76–7.86	10.02–10.32	8.10–8.63	10.88–11.12	8.95–10.02	10.84–14.51	9.98 (*P. coloradensis*)
Adelges sp. B	7.96–8.85	9.87–12.03	8.28–9.82	2.35–2.84	8.35–9.42	4.40–5.15	12.41–12.71	8.85–9.68	11.46–14.15	10.73 (*P. coloradensis*)
Adelges sp. C	9.08–9.62	9.43–10.75	8.26–9.60	8.17–8.35	9.47–9.66	7.98–8.18	10.50–10.89	9.84–11.09	12.73–15.55	9.20 (*P. coloradensis*)
Adelges sp. D samples 1, 2	7.95–8.97	8.28–10.19	9.27–11.00	7.12–7.77	8.81–9.21	8.28–8.81	9.11–9.64	8.99–10.54	11.89–14.53	9.62 (*P. coloradensis*)
Adelges sp. D sample 3	7.76–8.48	7.73–9.57	8.75–10.43	6.94–7.29	8.63–9.03	8.11–8.63	9.28–9.51	8.47–10.00	12.10–14.95	8.92 (*P. coloradensis*)
Adelges sp. E	7.56–8.80	9.15–10.75	7.76–9.17	5.31–5.79	7.86–8.69	4.62–4.98	9.87–10.52	8.14–9.51	10.67–13.83	9.32 (*P. coloradensis*)

B	*Pineus*					
	armandicola	*cembrae*	*coloradensis*	*orientalis* + *pini*	*strobi*	Nearest *Adelges* sample
Pineus sp. A	7.55–8.50	6.45–8.71	6.82–7.37	6.71–7.17	8.12–9.09	8.80 (*A. glandulae*)
Pineus sp. B	9.74–9.85	8.33–10.50	7.82–8.73	8.14–8.97	10.37–10.9	11.21 (*A. laricis*)
Pineus sp. C sample 1	7.43–9.05	7.25–8.30	7.44–8.16	8.46–9.22	7.97–8.50	9.91 (*A. japonicus*)
Pineus sp. C sample 2	7.08–8.66	6.91–8.30	6.92–7.81	7.93–9.01	7.62–8.14	9.85 (*A. japonicus*)
Pineus sp. D samples 1, 2	4.61–5.67	4.78–6.12	5.27–5.62	6.93–8.15	7.60–8.01	9.49 (*A. pectinatae*)
Pineus sp. D sample 3	4.27–6.11	4.93–6.27	4.59–5.78	7.08–8.07	7.58–7.80	8.73 (*A. cooleyi*)
P. sp. nr *coloradensis*	6.27–7.59	6.60–8.16	3.00–4.15	7.75–8.27	7.76–8.63	10.52 (*A. piceae*)
Pineus sp. E	4.29–4.68	7.14–7.99	6.96–7.17	8.98–10.29	8.47–9.35	10.02 (*A. japonicus*)

intraspecific distances seen within *P. coloradensis*. Were it not for the fact that *P. coloradensis* is believed to be non-alternating on pines, it would be reasonable to tentatively classify this sample in that species. Clearly, the taxonomic diversity encompassed by this name requires further investigation.

A sample of *Adelges* from *Larix* collected in Yunnan Province in western China is very similar to *A. japonicus* (less than 2.84% COI sequence divergence), but this species is otherwise known only from Japan and adjacent areas in far eastern Russia.

Other undetermined samples diverged by at least 4.4% from identified specimens and thus unlikely to belong to any of the identified species included here.

Discussion

The present study has shown that DNA barcodes are generally consistent with current morphological species concepts in the Adelgidae and are therefore a useful tool for species identification in the group. This enables the identification and linking of morphological forms, including immature stages, of the life cycle on different hosts and at different periods of the life-cycle phenology. Unknown species and undescribed forms and stages may be reliably placed in the proper genus. DNA-based identification in this group thus has the potential to provide a practical approach to pest surveillance and interception activities that require timely and accurate identifications.

An integrated approach, with the addition of DNA barcodes, will provide a stable taxonomy for the Adelgidae. DNA barcodes have uncovered cryptic diversity within taxa, suggesting areas where further taxonomic analysis is required. For example, the current concept of *Adelges tsugae* may encompass as many as three species. On the other hand, the lack of divergence between species defined by life-cycle characteristics (*Pineus orientalis*/*pini*, *Adelges nordmannianae*/*piceae*, *Adelges viridis*/*abietis*) indicates that further taxonomic analysis of the status of these species is required. This study has provided perspectives concerning species delineation in the Adelgidae, which will be applied in future analysis and description of new taxa.

Acknowledgements

Specimens were provided by C. Bystrowski, C. Donahue, K. Glover, M. Hall, L. Jones, M. Kenis, G. Kohler, A. Lamb, A. Lynch, T. McAvoy, Z. Mendel,Y. Miyatake, K. Ripley, K. Ozaki, S. Salom, K. Saltonstall, M. Schultz, S. Stearns, S. Shiyake, J. Vakula, H. Y. Wang, B. Willhite, Guoyue Yu, G. Zilahi-Balogh, and M. Zubrik. This work is supported in part by funding provided by the USDA

Forest Service and USDA CSREES, and through funding to the Canadian Barcode of Life Network from Genome Canada (through the Ontario Genomics Institute), NSERC and other sponsors listed at www.BOLNET.ca.

Conflict of interest statement

The authors have no conflict of interest to declare and note that the funders of this research had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

Ahern RG, Hawthorne DJ, Raupp MJ (2009) Founder effects and phenotypic variation in *Adelges cooleyi*, an insect pest introduced to the eastern United States. *Biological Invasions*, **11**, 951–971.

Floyd RM, Wilson JJ, Hebert PDN (2009) DNA barcodes and insect biodiversity. Chapter 17, pp. 417–431. In: *Insect Biodiversity: Science and Society* (eds Foottit RG, Adler PH). Wiley-Blackwell. In press.

Foottit RG (1997) Recognition of parthenogenetic insect species. In: *Species. The Units of Biodiversity* (eds Claridge MF, Dawah HA, Wilson MR), pp. 291–307. Chapman & Hall, London.

Foottit RG, Mackauer M (1980) Morphometric variation between populations of the balsam woolly aphid, *Adelges piceae* (Ratzeburg) (Homoptera: Adelgidae), in North America. *Canadian Journal of Zoology*, **58**, 1494–1503.

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

Hajibabaei M, deWaard JR, Ivanova NV *et al*. (2005) Critical factors for assembling a high volume of DNA barcodes. *Philosophical Transactions of the Royal Society of London B: Biological Sciences*, **360**, 1959–1967.

Hajibabaei M, Singer GAC, Hebert PDN, Hickey DA (2007) DNA barcoding: how it complements taxonomy, molecular phylogenetics and population genetics. *Trends in Genetics*, **23**, 167–172.

Havill NP, Foottit RG (2007) Biology and evolution of the Adelgidae. *Annual Review of Entomology*, **52**, 325–349.

Havill NP, Montogomery ME, Yu G, Shiyake S, Caccone A (2006) Mitochondrial DNA from hemlock woolly adelgid (Hemiptera: Adelgidae) suggests cryptic speciation and pinpoints the source of the introduction in eastern North America. *Annals of the Entomological Society of America*, **99**, 195–203.

Havill NP, Foottit RG, von Dohlen CD (2007) Evolution of host specialization in the Adelgidae (Insecta: Hemiptera) inferred from molecular phylogenetics. *Molecular Phylogenetics and Evolution*, **44**, 357–370.

Hebert PDN, Cywinska A, Ball SL, deWaard JR (2003) Biological identifications through DNA barcodes. *Proceedings of the Royal Society B: Biological Sciences*, **270**, 313–322.

Kimura M (1980) A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*, **16**, 11–120.

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

Saitou N, Nei N (1987) The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*, **4**, 406–425.

Sano M, Tabuchi K, Ozaki K (2008) A holocyclic life cycle in a gall-farming adelgid, *Adelges japonicus* (Homoptera: Adelgidae). *Journal of Applied Entomology*, **132**, 557–565.

deWaard JR, Ivanova NV, Hajibabaei M, Hebert PDN (2008) Assembling DNA barcodes: analytical protocols. In: *Environmental Genomics*, *Methods in Molecular Biology, Vol. 410* (ed. Martin CC), pp. 275–293. Humana Press, Totowa, New Jersey.

Walton L (1980) Gall formation and life history of *Pineus floccus* (Homoptera: Adelgidae) in Virginia. *Virginia Journal of Science*, **31**, 55–60.