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Ancient association with Fagaceae in the aphid tribe Greenideini (Hemiptera: Aphididae: Greenideinae)

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Abstract. Aphids are intimately associated with their host plants. Evolutionary lability of host association is common within heteroecious aphid lineages, whereas our knowledge of host-use evolution in non-host-alternating aphids is limited. In the present study, we construct the first detailed molecular phylogeny of the monoecious aphid tribe Greenideini based on three mitochondrial genes (*COI*, *COII* and *Cytb*) and one nuclear gene (*EF-1α*), and investigated its history of host association. Maximum likelihood and Bayesian phylogenies strongly support the monophyly of Greenideini and most constituent genera. Divergence time estimates and character reconstructions suggest that Greenideini may have originated during the Late Cretaceous to early Paleogene, which accompanies the origin of its ancestral host, members of the family Fagaceae. Colonisation of novel host plants has occurred multiple times during the evolutionary history of Greenideini, thereby leading to current patterns of host association. We suggest that directly shifting to novel hosts, together with expanding host range onto pre-existing, unused plants, has probably promoted diversification in this tribe.

Introduction

Aphids have very intimate associations with their host plants, which provide them with nutrients (via phloem feeding) and habitat. The host plants are thereby commonly assumed to have had a great influence on aphid diversification (Peccoud *et al.*, 2010). Three major evolutionary radiations of aphids are all closely linked with variations of dominant flora elements and subsequent changes in host use: (i) the Mesozoic aphid radiation accompanies the diversification of gymnosperms, (ii) the Late Cretaceous radiation coincides with the origin and diversification of woody angiosperms, and (iii) the late Tertiary radiation in aphids is contemporaneous with the great development of herbaceous angiosperms (Heie, 1987, 1996; von Dohlen & Moran, 2000).

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Host associations within heteroecious (i.e. host-alternating) aphid lineages seem to be evolutionarily labile. Gain and loss of heteroecy are believed to have played a crucial role in shaping the host-association patterns of certain aphid groups and in their diversification (Moran, 1988, 1990, 1992; Guldemond, 1990; Jousselin *et al.*, 2010). The most species-rich subfamily Aphidinae is supposed to have originated from the ancestor on a woody rosaceous host, and then diversified in the late Tertiary accompanying the acquisition of host alternation to herbaceous hosts (Hille Ris Lambers, 1950; Heie, 1987, 1996; von Dohlen *et al.*, 2006; Kim *et al.*, 2011). Afterwards some genera such as *Uroleucon* Mordvilko became monoecious on herbaceous plants through losing their primary hosts (Moran, 1992; Blackman & Eastop, 2000), and radiated extensively on the previous secondary host plants, thus leading to very high species richness (Moran, 1990). Repeated switches among different host taxa might have driven speciation in these genera (Moran *et al.*, 1999), whereas in *Brachycaudus* van der Goot, reacquisition of heteroecy is revealed to have occurred several times and life-cycle changes have probably promoted the diversification of some *Brachycaudus* species (Jousselin *et al.*, 2010). Studies attempting to elucidate host-association

history in other host-alternating aphid groups have also been conducted, such as the studies on the Eriosomatini (Eriosomatinae) (Sano & Akimoto, 2011) and Hormaphidinae (Huang *et al.*, 2012).

For non-host-alternating aphid lineages, however, our knowledge of their host-association histories is relatively limited. Previous studies have mostly focused on the subfamily Lachninae. Nevertheless, there has not yet been an agreement on whether conifer feeding is ancestral or derived in lachnid aphids (Heie, 1987; Zhang & Chen, 1999; Normark, 2000). Frequent host shifts are considered to have played a prominent role in the evolutionary radiation of its largest genus *Cinara* Curtis (Favret & Voegtlin, 2004; Durak *et al.*, 2014).

The aphid tribe Greenideini Baker, 1920 (Hemiptera: Aphididae: Greenideinae) is monoecious on woody plants (Dixon, 1985; Ghosh & Agarwala, 1993), feeding on young leaves and shoots, with holocyclic (Takahashi & Sorin, 1959; Sugimoto, 2011) or anholocyclic (Raychaudhuri, 1956; Ghosh & Agarwala, 1993) life cycles. It comprises more than 140 species within eight genera worldwide (Remaudière & Remaudière, 1997; Zhang & Qiao, 2007b; Favret, 2014). This group of aphids is characterised by siphunculi densely covered with long setae and is distributed mainly in South and South-east Asia (Raychaudhuri, 1956; Ghosh & Agarwala, 1993). More than half of the greenideine species feed on a single plant family, whereas other species, particularly several widely distributed species (e.g. *Greenidea ficicola* Takahashi and *G. psidii* van der Goot), tend to have a wider host range and colonise plants from different families. Plants belonging to approximately 30 families have been recorded to serve as their hosts (Ghosh & Agarwala, 1993; Blackman & Eastop, 1994, 2000, 2006; Zhang *et al.*, 2012). Fagaceae is the most dominant host, harbouring almost 70% of Greenideini species. Mordvilko (1934) speculated that the subfamily Greenideinae might have arisen on the plants of Fagaceae and other primitive host plants. Fagaceae is also supposed to be the ancestral host plant for *Mollitrichosiphum* species (Zhang *et al.*, 2012). In the present study, we investigated the possible evolutionary scenarios for host association within the Greenideini and addressed the following questions: (i) What is the ancestral host-association state in this tribe? (ii) Are the dominant Fagaceae trees the ancestral host plants for Greenideini species and, if so, when did they establish such association? (iii) How did current host-association patterns in Greenideini take shape? (iv) What are the consequences of evolutionary changes in host association for aphids?

We reconstructed a detailed phylogeny of Greenideini and its close relatives using one nuclear gene (*EF-1α*) and three mitochondrial genes (*COI*, *COII* and *Cytb*), and then estimated the divergence times of key clades and performed ancestral character reconstruction. The results of this study could increase our knowledge about the history of host plant association within monoecious aphids. Exploring the patterns in different lineages will help us get a clear picture of the evolutionary scenarios for aphid–plant associations. The implications for aphid speciation were also discussed.

Material and methods

Taxon sampling

A total of 51 species were included in this study. Some published sequences were taken from Kim *et al.* (2011), Zhang *et al.* (2011), Chen *et al.* (2012), Huang *et al.* (2012) and Liu *et al.* (2013). Specimens for slide mounting were stored in 75% ethanol, and other specimens for molecular studies were preserved in 95 or 100% ethanol. Species identification was conducted by Ge-Xia Qiao based on the exterior morphology of slide-mounted specimens, via following the keys in authoritative monographs and literatures (e.g. Ghosh & Agarwala, 1993; Noordam, 1994; Quednau & Martin, 2006), and by comparison with previously identified specimens and the original morphological descriptions. All samples and voucher specimens were deposited in the National Zoological Museum of China, Institute of Zoology, Chinese Academy of Sciences, Beijing, China (NZMCAS). Collection information for all samples is shown in Table S1. Thirty-two species belonging to six genera of Greenideini were used as ingroups. Most species were sampled from three large genera, *Eutrichosiphum*, *Greenidea* and *Mollitrichosiphum.* One or two exemplar species were chosen from the other genera.

Based on the widely accepted classification of Greenideinae (Remaudière & Remaudière, 1997; Favret, 2014) and the current morphological phylogenetic hypothesis for Aphididae (Wojciechowski, 1992; Heie & Wegierek, 2009), eight species of the other two tribes in Greenideinae (i.e. Cervaphidini and Schoutedeniini), one species of the subfamily Aiceoninae, and one species of Anoeciinae were selected as outgroups. Nine species of Aphidinae, Lachninae, Adelgidae and Phylloxeridae were also employed for calibration in the dating analysis. Adelgidae and Phylloxeridae were used to constrain the age of the Aphidoidea crown clade. The subfamilies Aphidinae and Lachninae were selected for calibrating the age of the Aphididae crown clade. Six species of Aphidini and Macrosiphini were included for setting the Aphidinae calibration point.

DNA sequencing and alignment

Total DNA was extracted from single aphids preserved in 95 or 100% ethanol using DNeasy Blood & Tissue Kit (QIAGEN, Dusseldorf, Germany) according to the manufacturer's protocol. All primers used in this study are listed in Table 1. Typical polymerase chain reactions were prepared in a 30-μL volume containing 10 × EasyTaq DNA Polymerase Buffer ($+Mg^{2+}$), 2 U EasyTaq DNA Polymerase, 3 mM each dNTP (all from TransGen Biotech, Beijing, China), 5 pmol each primer and 3 μL DNA extract. PCR was performed under the following conditions: an initial 95°C denaturation for 5 min, followed by 35 cycles of 95°C denaturation for 30–60 s, 42–52°C for 30–60 s, 72°C for 60–90 s, and a 10-min final extension at 72°C. The primer-specific annealing temperatures of each primer set were 52°C for *COI*, 48°C for *Cytb*, 42°C for *COII* and 50°C for *EF-1α*. PCR products were purified using EasyPure Quick Gel Extraction Kit (TransGen Biotech) and then directly sequenced

Table 1. Primers used in this study.

Gene	Primer	Sequence	References
COI	LepF	5′-ATTCAACCAATCA TAAAGATATTGG-3′	Foottit *et al.* (2008)
	LepR	5′-TAAACTTCTGGATG TCCAAAAAATCA-3′	
Cytb	CP1	5′-GATGATGAAA TTTTGGATC-3′	Harry *et al.* (1998)
	CP2	5′-CTAATGCAATA ACTCCTCC-3′	
	CB2	5′-ATTACACCTCCT AATTTATTAGGAAT-3′	Jermiin & Crozier (1994)
COII	mt2993+	5′-CATTCATATTCA GAATTACC-3′	Stern (1994)
	A3772	5′-GAGACCATTACTT GCTTTCAGTCATCT-3′	Normark (1996)
EF-1α	EF3	5′-GAACGTGAAC GTGGTATCAC-3′	von Dohlen *et al.* (2002)
	EF6	5′-TGACCAGGGT GGTTCAATAC-3′	
	EF2	5′-ATGTGAGCAGTG TGGCAATCCAA-3′	Palumbi (1996)

in both directions using the same amplifying primers by an ABI 3730 automated sequencer (Applied Biosystems, Foster City, CA, USA).

Raw sequences were assembled by SeqMan II (DNAStar, Madison, WI, USA) and verified for protein coding frame-shifts using EditSeq (DNAStar). The positions of introns in EF-1α sequences were determined by following the GT-AG rule and aligning sequences with the cDNA sequence from *G. ficicola* (GenBank accession no. JX273497). Introns were removed before further analysis. The GenBank accession numbers for sequences are listed in Table S1. Multiple alignments were performed with ClustalX 1.83 (Thompson *et al.*, 1997).

Phylogenetic analyses

Phylogenetic reconstructions were conducted based on the combined four-gene dataset using Bayesian inference and maximum likelihood (ML) methods. The partition-homogeneity test implemented in PAUP* v4.0b10 (Swofford, 2003) showed no significant incongruence between *COI*, *COII*, *Cytb* and *EF-1α* fragments ($P = 0.02$); hence, the four gene regions were concatenated into a single dataset. For the Bayesian analysis, the best-fit model of nucleotide substitution was selected for each gene using jModelTest 0.1.1 (Guindon & Gascuel, 2003; Posada, 2008). The Bayesian information criterion (BIC) (Schwarz, 1978) favoured TIM1 + I + G for *COI*, TIM2 + I + G for *COII*, TIM2 + I + G for *Cytb* and GTR + I + G for *EF-1α*. Bayesian inference was performed using MrBayes 3.1.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003) under default priors, with each partition unlinked for parameter estimations. Two independent runs were carried out, each with 4 million generations and four chains, sampling the chains every 500 generations. A plot of sampled log-likelihood scores against generation time was used to determine the stationarity of the chains. For all runs, the first 2000 trees were discarded as burn-in samples. The remaining trees were used to compute a majority-rule consensus tree with posterior probabilities (PP). ML analysis was conducted using RAxML v7.2.6 (Stamatakis, 2006; Stamatakis *et al.*, 2008) with the GTRCAT model for each gene partition. All model parameters were estimated during the ML analysis. A rapid bootstrapping algorithm was applied with 1000 replicates.

Molecular dating

A Bayesian uncorrelated lognormal relaxed clock model with multiple calibration points was used to estimate divergence times in BEAST v1.7.5 (Drummond & Rambaut, 2007; Drummond *et al.*, 2012). We partitioned the dataset by gene and applied a GTR + I + G model to each partition, as in the phylogenetic analysis described above. A Yule prior was used on the tree to simulate the speciation process. Thirteen independent analyses were run. Each run ranged from 10 to 100 million generations, sampling every 1000 generations. These independent runs were combined with LogCombiner v1.7.5 (Drummond *et al.*, 2012). Tracer v1.5.0 (Rambaut & Drummond, 2009) was used to verify the runs' convergence and stability, to determine the appropriate number of generations to discard as burn-in, and to confirm that effective sample size (ESS) values for the posterior and all major clades were greater than 200. The detailed information for each run is listed in Table S3. The samples were summarised onto the maximum clade credibility tree using TreeAnnotator v1.7.5 (Drummond *et al.*, 2012), listing the mean node age and 95% highest posterior density (HPD) intervals. The results were visualised using FigTree v1.4 (Rambaut, 2012).

Multiple calibration points were employed in the dating analysis. (i) The most recent common ancestor of the Aphididae, Adelgidae and Phylloxeridae was inferred to have occurred between the late Jurassic and Early Cretaceous (120–150 Ma) based on fossil evidence (Heie, 1987; Havill *et al.*, 2007). Thus a normally distributed calibration prior with a mean of 135 Ma and a standard deviation (SD) of 9.09 Ma was specified for the age of crown Aphidoidea. (ii) Based on previous molecular date estimates (von Dohlen & Moran, 2000) and fossil remains of extant subfamilies in Aphididae from Upper Cretaceous deposits (Heie, 1987, 1999; Heie & Wegierek, 1998), the age of the common ancestor of Aphididae was inferred to 80–100 Ma. We therefore adopted a normal age prior (mean = 90 Ma, SD = 6.08 Ma) to the Aphididae crown. (iii) Fossil records of Aphidinae are restricted to Upper Cretaceous and Paleocene deposits (Heie, 1987; Hong, 2002), indicating the possible age of the Aphidinae crown of approximately 60–80 Ma. A normal distribution (mean = 70 Ma, SD = 6.08 Ma) with 95% confidence interval covering this constraint was used for the calibration prior. (iv) One fossil species of *Mollitrichosiphum* and one species of *Greenidea* were found in Europe and dated to 18–19 Ma (Wegierek & Peñalver, 2002), suggesting that these two genera are at least this old. We therefore assigned a uniform age prior (lower bound: 19 Ma; upper bound: 1.0E100 Ma) to each genus crown.

Ancestral state reconstruction

In order to evaluate the evolution of host association within Greenideini, we performed ancestral state reconstruction using parsimony and Bayesian approaches. To account for phylogenetic uncertainty, 1000 randomly selected trees from the post-burn-in Bayesian trees were used. Based on the biological information from monographs, the literature and field observations (Ghosh & Agarwala, 1993; Blackman & Eastop, 1994, 2006; Noordam, 1994; Qiao *et al.*, 2006; Zhang & Qiao, 2007a, 2007b, 2008, 2010), the host association for the sampled greenideine species is summarised in Table S2. The following host-association character states were identified: (0) Lauraceae, (1) Fagaceae, (2) Betulaceae, (3) Sapindaceae, (4) Myrtaceae, (5) Moraceae, (6) Annonaceae, (7) Symplocaceae, (8) Sabiaceae, (9) Sonneratiaceae, (A) Euphorbiaceae, (B) Anacardiaceae, (C) Rosaceae, (D) Sapotaceae, (E) Vitaceae, (F) Hamamelidaceae, and (G) Proteaceae.

Parsimony reconstruction was conducted in Mesquite 2.75 (Maddison & Maddison, 2011), using the 'trace character over trees' option and character state transformations unordered. For Bayesian ancestral state reconstruction, we used a reverse jump Markov chain Monte Carlo method (Pagel & Meade, 2006), as implemented in BayesTraits 2.0 (Pagel & Meade, 2013). Ancestral states were estimated for all nodes which were specified using the 'AddMRCA' command. Reverse jump MCMC was used on an unrestricted model, with a hyper exponential prior seeded from a uniform on the interval 0–3. The rate deviation parameter was automatically tuned to achieve the recommended acceptance rates of 20–40%. Three independent runs were performed for a total of 5 050 000 iterations, sampling every 1000 iterations after a burn-in of 100 000 iterations. Results of the three runs were significantly similar; therefore, only one of them is reported here.

Results

Phylogenetic reconstructions

The final dataset used for phylogenetic analyses contained a total of 2809 bp (831 parsimony-informative sites), including 658 bp of *COI*, 705 bp of *COII*, 669 bp of *Cytb* and 777 bp of *EF-1α* exon sequences. ML analysis of the combined four-gene dataset resulted in a well-resolved phylogeny with most nodes highly supported (Fig. 1). Greenideinae was retrieved as monophyletic, with *Cervaphis* being sister to the remaining taxa. Cervaphidini was paraphyletic. The monophyly of Greenideini was well supported with a high bootstrap value (99%), and it was recovered as sister to *Schoutedenia* (Schoutedeniini). Within the clade of Greenideini, *Greenidea* formed the sister group to all remaining genera. *Mesotrichosiphum* was positioned as sister to the *Pentatrichosiphum* + *Allotrichosiphum* + *Eutrichosiphum* + *Mollitrichosiphum* clade. These four genera formed three distinct clades: *Pentatrichosiphum*, *Allotrichosiphum* + most species of *Eutrichosiphum*, and *Eutrichosiphum tattakanum* + *Mollitrichosiphum*. *Greenidea*, *Pentatrichosiphum* and *Mollitrichosiphum* were retrieved as monophyletic, whereas *Eutrichosiphum* was polyphyletic. The topology of the Bayesian tree was largely consistent with that of the ML tree, with little decrease in resolution (Figure S1).

Divergence times

The ultrametric tree with divergence time estimates resulting from the BEAST analysis is shown in Fig. 2. The most recent common ancestor of Greenideinae dates back to 83.55 Ma (95% HPD: 72.35–95.20 Ma). The mean age estimate for the divergence between Greenideini and Schoutedeniini was 64.87 Ma, with a variance of 54.57–75.63 Ma (95% HPD). The Greenideini crown was estimated to have arisen at 51.72 Ma (95% HPD: 43.58–60.57 Ma). Within Greenideini, most living genera arose during the middle Eocene to late Oligocene (26.25–47.05 Ma), and most species-level divergences occurred from the late Oligocene through the Miocene.

Character evolution

The results of ancestral state reconstruction for host association are shown in Fig. 3 and Table 2. Parsimony and Bayesian analyses both provided strong support for the Fagaceae as the ancestral host plant for Greenideini. In the parsimony analysis (Fig. 3), Fagaceae was highly favoured for all but one of the internal nodes. Lauraceae was clearly shown as the ancestral state for *Pentatrichosiphum*. Bayesian analysis yielded similar reconstructions to the parsimony method, except for nodes 23 and 25, of which the ancestral states were equivocal (Table 2).

Character reconstructions suggested that there have been numerous transitions in host association for the sampled species within the Greenideini. Parsimony reconstructions indicated that there have been approximately 20 host-association transitions (Fig. 3). At least six complete transitions from the Fagaceae to other host plants were identified with one in each of the following species: *Greenidea* sp. 2, *G. cayratiae*, *G. symplocosis*, *G. psidii* and *Mollitrichosiphum nigrum*, and at least one in the genus *Pentatrichosiphum*. At least 14 incomplete transitions (i.e. new host plants are acquired while the Fagaceae is still occupied) have occurred: 7 in *Greenidea*, 3 in *Eutrichosiphum* and at least 4 in *Mollitrichosiphum*. Bayesian reconstructions suggested a similar total number of host-association transitions to MP analysis (Table 2). Six complete and at least 13 incomplete transitions from the Fagaceae to other host plants were assumed to take place during the evolution of Greenideini.

Discussion

Phylogenetic relationships

No extensive phylogenetic analysis has ever been performed for Greenideinae at the molecular level. Qiao (1996) estimated the phylogeny of this subfamily based on morphological characters, but failed to reconstruct a monophyletic Greenideinae.

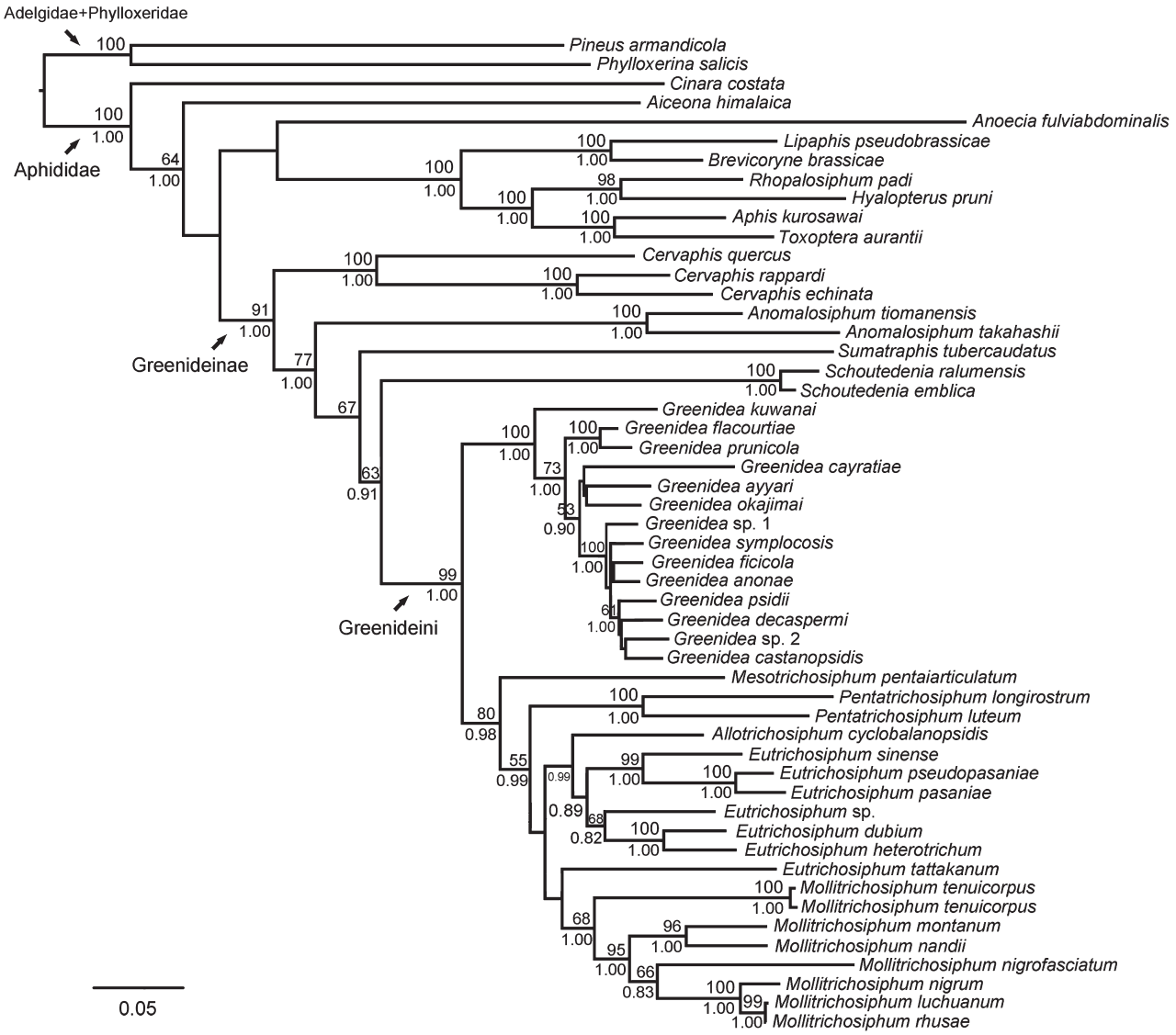

Fig. 1. Maximum likelihood tree showing phylogenetic relationships of aphids resulting from the ML analysis. ML bootstrap values (>50%) and Bayesian posterior probabilities (PP) values (>0.70) are shown above and below the branches, respectively.

Our study provided the first molecular test for the monophyly of Greenideinae. It was recovered as monophyletic with strong support in both ML and Bayesian analyses (Fig. 1, Figure S1), which is in agreement with the morphological point of view (Takahashi, 1931; Zhang & Zhong, 1983; Ghosh & Agarwala, 1993). The tribe Cervaphidini was paraphyletic in our study. However, this result is based on a small sample size of three genera; therefore, a much broader taxonomic sampling is needed in the future to test the monophyly of this tribe. Schoutedeniini, represented by *Schoutedenia* here, was placed as the sister group to Greenideini.

The tribe Greenideini is considered as a distinct assemblage by many taxonomists based on the absence of dorsal processi, and the presence of dense and long setae on siphunculi in adults (Raychaudhuri, 1956; Raychaudhuri & Chatterjee, 1980; Ghosh & Agarwala, 1993). In a high-level phylogenetic analysis of Aphididae, two sampled Greenideini species were grouped as a single clade (von Dohlen & Moran, 2000). Based on the wide sampling of this tribe, our study strongly confirmed the monophyly of Greenideini using the combined mitochondrial and nuclear gene dataset and different model-based approaches (Fig. 1, Figure S1).

Within the Greenideini, *Greenidea* was monophyletic and formed the sister lineage to all remaining greenideines. ML and Bayesian analyses revealed somewhat different interspecies relationships within this genus, and both left unresolved positions for several species (Fig. 1, Figure S1). Neither analysis supported the three subgenus classification (i.e. *Greenidea*, *Paragreenidea* and *Trichosiphum*) (Raychaudhuri, 1956; Remaudière & Remaudière, 1997), suggesting a need for

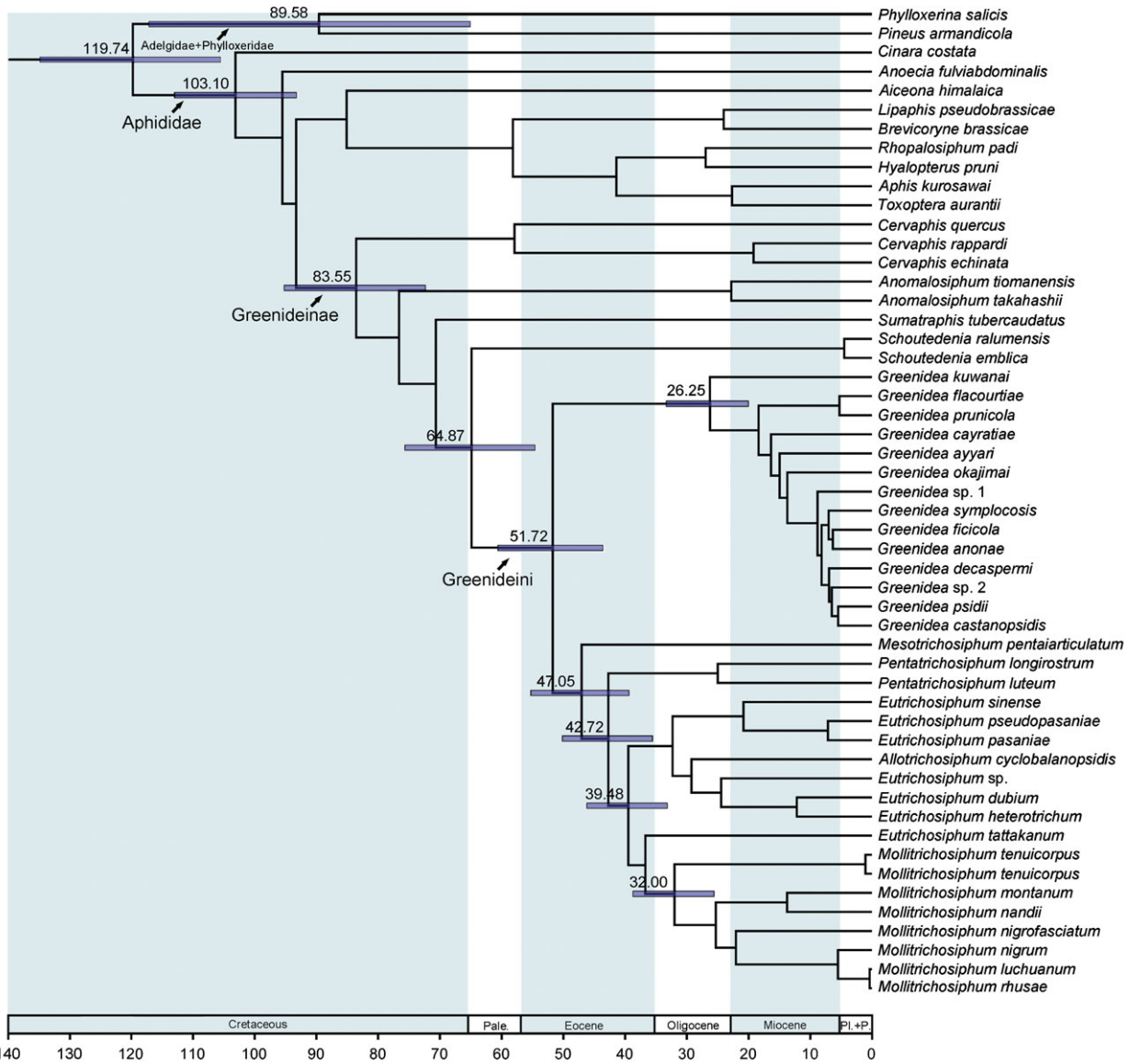

Fig. 2. Time-calibrated phylogeny resulting from the BEAST analysis. Horizontal bars indicate 95% highest posterior density (HPD) intervals of the estimated divergence times, with the mean ages shown above each bar. Pale., Palaeocene; Pl., Pliocene; P., Pleistocene.

taxonomic re-evaluation of *Greenidea*. In the ML tree (Fig. 1), *Allotrichosiphum* + *Eutrichosiphum* (excl. *E. tattakanum*) and *E. tattakanum* + *Mollitrichosiphum* were clustered into a sister group, and *Pentatrichosiphum* was placed as sister to them. However, the bootstrap values were very low, leaving this pattern of relationships unreliable. In Bayesian analysis, the relationships among these three clades were unresolved (Figure S1). *Eutrichosiphum* was polyphyletic with *E. tattakanum* being a sister group to *Mollitrichosiphum*, whereas the remaining *Eutrichosiphum* species were split into two clades. *Mollitrichosiphum* was retrieved as monophyletic as well as the two morphologically defined subgenera, *Metatrichosiphon* and *Mollitrichosiphum*, which is consistent with a previous study (Zhang *et al.*, 2011).

Ancient association with Fagaceae in Greenideini

Fagaceae trees are the main host plants for modern Greenideini species. Based on the available data in our study, parsimony and Bayesian reconstructions both strongly suggested that the Fagaceae could be the ancestral host for Greenideini. Furthermore, the ancestral character states for most internal nodes in the phylogenetic tree were also reconstructed to be Fagaceae (Fig. 3, Table 2), thus implying a long history of Fagaceae association within Greenideini.

The earliest unequivocal megafossils of Fagaceae are the oldest remains of the two constituent subfamilies, Castaneoideae and Fagoideae, discovered in western Tennessee (Crepet & Nixon, 1989). They occurred at the Paleocene/Eocene

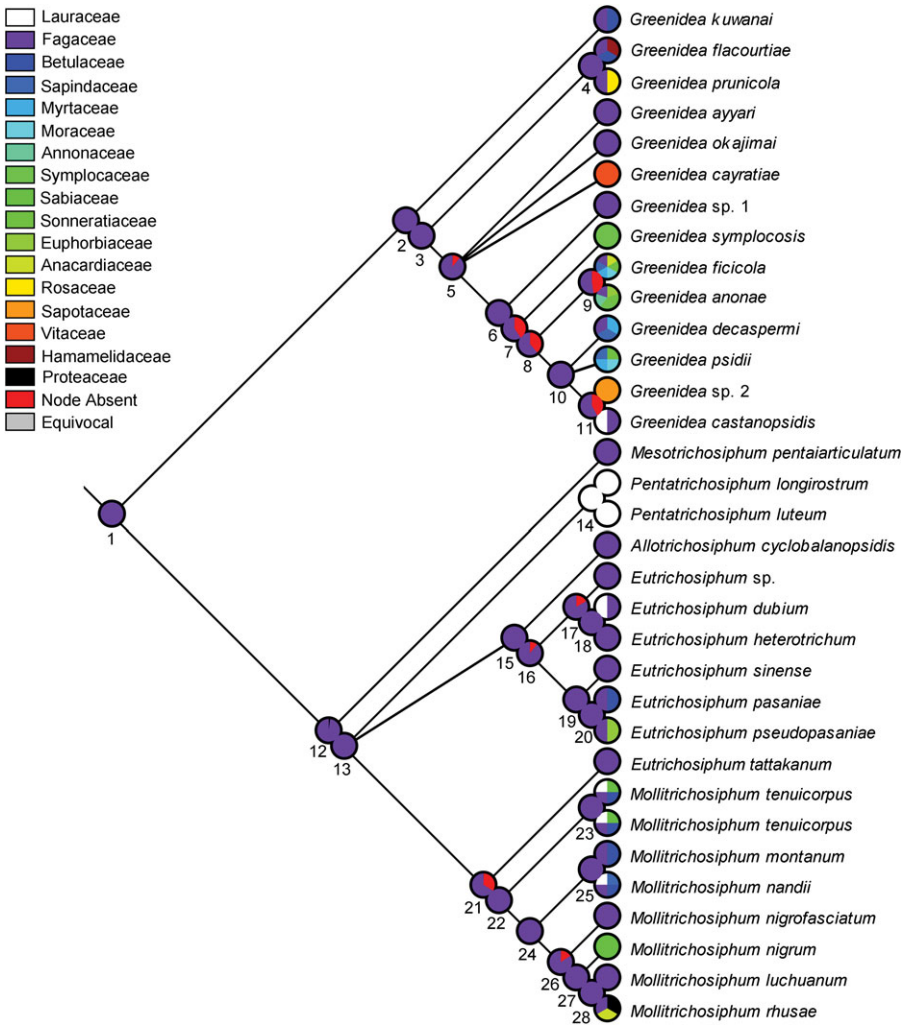

Fig. 3. Parsimony reconstructions for host association shown on the Bayesian consensus tree. Pie charts at nodes show the percentages of trees for which a given state is reconstructed as the uniquely best state for that node.

boundary, indicating a divergence of these two lineages as certainly no later than Paleocene. Putative castaneoid pollen was found in Upper Cretaceous sediments (Chmura, 1973). Proto-Nothofagaceae-Fagaceae fossils were recorded from the Upper Cretaceous deposits of central Georgia (Herendeen *et al.*, 1995; Sims *et al.*, 1998). Thus, Fagaceae might have originated during the Late Cretaceous (Crepet & Nixon, 1989; Zhou, 1993, 1999). Molecular dating in our study indicated that Greenideini diverged from its sister group, Schoutedeniini, during the Late Cretaceous to early Paleogene (Fig. 2), which is coincident with the origin of Fagaceae and diversification of its main lineages. Tribal diversifications in aphids usually occur contemporaneously with the appearances of their host plants. In Hormaphidinae, three tribes are inferred to have arisen in the Late Cretaceous, corresponding well with the appearances of their specific primary host plants (Huang *et al.*, 2012). On a larger scale, modern aphids are thought to have undergone a rapid radiation at the tribal level during the Late Cretaceous, accompanying the origin and diversification of woody angiosperm plants (Heie, 1996; von Dohlen & Moran, 2000). Our results suggest that the Greenideini may have originated from a common ancestor on a primitive taxon of Fagaceae when it emerged in the Late Cretaceous, and then evolved in concert with its host plants.

History of host association in Greenideini

Character reconstructions revealed the occurrence of numerous transitions in host association within Greenideini during the course of its evolution. Multiple independent host shift (i.e. complete transition from the Fagaceae to other plants) and host expansion (i.e. broadening host range to other plants while still occupying the Fagaceae) events were suggested to have occurred in different genera (Fig. 3, Table 2). The occurrence of host expansions indicates that gaining the ability to colonise new plants does not require the loss of ancestral Fagaceae association. The common ancestor of *Pentatrichosiphum* has

Table 2. Mean of posterior probabilities (PPs) for host-association states estimated in Bayesian reconstructions.

Node	0	1	2	3	4	5	6	7	8
1	0.000120749	**0.999004508**	0.000037400	0.000029100	0.000132995	0.000072100	0.000056600	0.000049800	0.000070100
2	0.000141458	**0.995531385**	0.002292872	0.000083200	0.000242129	0.000157142	0.000132930	0.000112187	0.000157930
3	0.000023100	**0.998971683**	0.000018900	0.000011400	0.000050400	0.000022200	0.000021100	0.000016800	0.000022100
4	0.000037600	**0.985345619**	0.004162154	0.000024400	0.000047700	0.000047300	0.000037000	0.000024600	0.000039400
5	0.000191328	**0.985228065**	0.000128254	0.000107699	0.000521501	0.000146381	0.000182415	0.000156650	0.000157371
6	0.000044600	**0.990119653**	0.000017700	0.000076900	0.000323707	0.000042100	0.000038200	0.008951755	0.000044000
7	0.000275472	**0.924507452**	0.000075600	0.006681770	0.009525348	0.000466411	0.000228025	0.048939271	0.000167272
8	0.000139653	**0.956488044**	0.000004000	0.008383550	0.013412910	0.000447649	0.000168012	0.007456234	0.000011400
9	0.000116454	**0.654053450**	0.000044300	0.006759799	0.008678206	0.003710513	0.003284276	0.048258686	0.000093400
10	0.024022559	**0.538482946**	0.000107091	0.189244314	0.211538805	0.008015468	0.000199666	0.000113548	0.000211338
11	0.188214302	**0.450220617**	0.000712243	0.052376814	0.059939298	0.012741424	0.001339237	0.000716429	0.001415176
12	0.002346881	**0.994818894**	0.000093300	0.000105416	0.000387958	0.000214022	0.000207032	0.000178602	0.000236417
13	0.036650907	**0.957408890**	0.000230841	0.000294612	0.000517606	0.000414737	0.000503830	0.000375052	0.000612614
14	**0.992452274**	0.000463489	0.000324767	0.000442740	0.000343536	0.000440822	0.000763466	0.000274587	0.000645925
15	0.000052400	**0.999297161**	0.000020100	0.000023200	0.000096800	0.000053400	0.000043400	0.000038400	0.000052300
16	0.000031600	**0.999610782**	0.000010600	0.000011300	0.000057300	0.000028500	0.000022600	0.000020100	0.000028600
17	0.000232001	**0.998175469**	0.000052400	0.000057000	0.000232550	0.000125730	0.000105363	0.000094900	0.000131527
18	0.005573009	**0.993037576**	0.000048700	0.000056400	0.000167389	0.000101058	0.000105774	0.000080200	0.000123393
19	0.000191894	**0.997102903**	0.000132952	0.000093200	0.000349893	0.000199346	0.000166995	0.000151141	0.000207620
20	0.000066100	**0.986861113**	0.004306885	0.000052700	0.000113001	0.000085100	0.000069600	0.000057500	0.000083400
21	0.001460805	**0.996529910**	0.000148751	0.000065100	0.000249506	0.000137954	0.000125734	0.000106839	0.000294575
22	0.003716082	**0.983063209**	0.004222063	0.000231423	0.000574737	0.000379587	0.000375512	0.000298805	0.004344811
23	0.249811697	0.249882590	0.250334543	0.000000532	0.000000959	0.000000902	0.000000836	0.000000490	0.249960516
24	0.000112515	**0.996724529**	0.001663907	0.000060600	0.000127392	0.000064800	0.000050100	0.000042200	0.000717637
25	0.005320777	0.484312364	0.502361207	0.004757079	0.000295916	0.000275627	0.000213424	0.000161415	0.000259038
26	0.000408784	**0.980669082**	0.000212734	0.000215947	0.000882444	0.000390168	0.000326393	0.000302309	0.014057282
27	0.001129884	**0.708223709**	0.000476708	0.000592317	0.002124181	0.000850869	0.000861935	0.000771522	0.278669046
28	3.48E-08	**0.999711717**	9.30E-09	1.72E-08	8.85E-08	5.20E-08	3.48E-08	2.79E-08	6.91E-08

Node	9	A	B	C	D	E	F	G
1	0.000032400	0.000064400	0.000040200	0.000053700	0.000036700	0.000074400	0.000089400	0.000035300
2	0.000082500	0.000158027	0.000101743	0.000171935	0.000105433	0.000201509	0.000237424	0.000090000
3	0.000011300	0.000018300	0.000016100	0.000032200	0.000015800	0.000690390	0.000046500	0.000011200
4	0.000022800	0.000032200	0.000028800	0.004633326	0.000030800	0.000046200	0.005418063	0.000021600
5	0.000096500	0.000119680	0.000131945	0.000165435	0.000157257	0.012233983	0.000175815	0.000099400
6	0.000120955	0.000031400	0.000040800	0.000030200	0.000020100	0.000036400	0.000039600	0.000021400
7	0.008105707	0.000206083	0.000225284	0.000111356	0.000127257	0.000117997	0.000138544	0.000100883
8	0.013103865	0.000174958	0.000109549	0.000006680	0.000006630	0.000008330	0.000011500	0.000070800
9	0.267930332	0.003583387	0.003135043	0.000065400	0.000072800	0.000072400	0.000089600	0.000051900
10	0.005769933	0.000197984	0.000138434	0.000178540	0.021087970	0.000269825	0.000248731	0.000172760
11	0.007904213	0.001094065	0.000749577	0.000998807	0.218308459	0.001461881	0.001083889	0.000723508
12	0.000112253	0.000210521	0.000138957	0.000178483	0.000123724	0.000248819	0.000277601	0.000120921
13	0.000263579	0.000401133	0.000280298	0.000363321	0.000266958	0.000635323	0.000478805	0.000301219
14	0.000344881	0.000434577	0.000287550	0.000398068	0.000284791	0.001311641	0.000393424	0.000393281
15	0.000025500	0.000048700	0.000031200	0.000040900	0.000027600	0.000055100	0.000065400	0.000028100
16	0.000012700	0.000028700	0.000016500	0.000021100	0.000014500	0.000031200	0.000038400	0.000015200
17	0.000062100	0.000120272	0.000078700	0.000099200	0.000069200	0.000135923	0.000158253	0.000069100
18	0.000057200	0.000097500	0.000069300	0.000089400	0.000062100	0.000131559	0.000134848	0.000064400
19	0.000100518	0.000329588	0.000126749	0.000160526	0.000112055	0.000211780	0.000252228	0.000110443
20	0.000054800	0.007771004	0.000052400	0.000101738	0.000057800	0.000085600	0.000136734	0.000044500
21	0.000067100	0.000131024	0.000083800	0.000112910	0.000074800	0.000149458	0.000185120	0.000076500
22	0.000235868	0.000369466	0.000299197	0.000405776	0.000276369	0.000442984	0.000519799	0.000244238
23	0.000000504	0.000000811	0.000000610	0.000000949	0.000000602	0.000001090	0.000001160	0.000000567
24	0.000031500	0.000064200	0.000038300	0.000065100	0.000039100	0.000070700	0.000094200	0.000032900
25	0.000150104	0.000260513	0.000160485	0.000351204	0.000199506	0.000312415	0.000444182	0.000164620
26	0.000238617	0.000364022	0.000306243	0.000310849	0.000255264	0.000392237	0.000436955	0.000230454
27	0.000613180	0.000694492	0.001213275	0.000681798	0.000643343	0.000762503	0.000819197	0.000872015
28	1.56E-08	4.35E-08	0.000155706	3.48E-08	1.33E-08	4.67E-08	6.65E-08	0.000131572

Node numbers refer to those in Fig. 3.

Bold numbers indicate PPs of the optimal states.

Character states are scored as follows: 0 = Lauraceae; 1 = Fagaceae; 2 = Betulaceae; 3 = Sapindaceae; 4 = Myrtaceae; 5 = Moraceae; 6 = Annonaceae; 7 = Symplocaceae; 8 = Sabiaceae; 9 = Sonneratiaceae; A = Euphorbiaceae; B = Anacardiaceae; C = Rosaceae; D = Sapotaceae; E = Vitaceae; F = Hamamelidaceae; G = Proteaceae.

shifted to feeding on Lauraceae after the split from the cluster of *Allotrichosiphum*, *Eutrichosiphum* and *Mollitrichosiphum*, whereas the ancestors of other sampled genera were all reconstructed to be associated with Fagaceae. Within *Greenidea*, *Eutrichosiphum* and *Mollitrichosiphum*, transitions from the Fagaceae trees towards plants of other families were estimated to have occurred multiple times at the species level. Betulaceae, Lauraceae, Sapindaceae and other plants appear to have been colonised independently.

Fossil findings of Greenideini from the early Miocene of southern Europe indicate that this tribe used to have a much wider range (Wegierek & Peñalver, 2002). The early to middle Miocene flora of southern Europe is rich and diverse in thermophilous elements; representatives of the host plants of modern Greenideini species such as Fagaceae, Betulaceae, Lauraceae, etc. were widespread (Barrón & Diéguez, 2001; Ivanov *et al.*, 2011). Thus, it seems likely that some ancient European greenideine lineages have switched to plants other than Fagaceae during that time. Drastic climate and paleogeographic changes in the late Miocene greatly affected the floristic composition of the forests in Europe (Mai, 1989; Jiménez-Moreno *et al.*, 2010; Ivanov *et al.*, 2011). Vegetation changes may have led to the extinction of greenideine aphids in Europe and their distribution being restricted to South-east Asia (Wegierek & Peñalver, 2002), where thermophilous flora was conserved and developed due to ample summer monsoon rain (Sun, 2002; Sun & Li, 2003). The micro- and megafossil plant record suggests that plants serving as hosts of modern greenideines such as Fagaceae, Betulaceae, Annonaceae, Lauraceae, Sapindaceae, Sonneratiaceae and Myrtaceae are very rich in the late Tertiary flora of South and South-east Asia (Lakhanpal, 1970; WGCPC, 1978; Wang, 1992; Li, 1995; Li & Zhang, 1998). Fagaceae fossils are quite dominant, which is consistent with the assumption of a primitive Fagaceae association for Greenideini. Character reconstructions and divergence time estimates revealed that most host-association transitions in extant Greenideini aphids happened during and probably after the Miocene. Considering the abundant plant resources in South and South-east Asia, it is highly likely that occasional acquisition of new host plants commonly took place. As stated above, we may hypothesise that in the evolutionary history of Greenideini colonisation of novel host plants has occurred many times due to floristic changes, thereby resulting in the current patterns of host association.

Consequences of host-association changes for aphid speciation

Previous studies in host-alternating aphid lineages have demonstrated that host shift and life-cycle changes have played important roles in aphid diversification. Within the galling aphid tribe Fordini and the genus *Uroleucon* whose ancestor has a heteroecious life cycle, switching to new plants, followed by host specialisation, is supposed to have driven the speciation events (Moran *et al.*, 1999; Inbar *et al.*, 2004). The genera *Cryptomyzus* and *Brachycaudus* seem to have diversified via shifting to a previously unused herbaceous host, and through gain or loss of the heteroecious life cycle (Guldemond, 1990; Jousselin *et al.*, 2010). For monoecious aphids, the few previous studies reveal that host shift might be the main mode of speciation in the genus *Cinara* (Favret & Voegtlin, 2004; Durak *et al.*, 2014). Our study in the monoecious aphid tribe Greenideini suggests that acquisition of new hosts, whether directly switching to novel hosts or expanding host range onto pre-existing, unexploited plants, has probably promoted the diversification of some Greenideini species.

Molecular dating results showed that species divergences in *Greenidea* occurred mainly during the Miocene (Fig. 2). Short internal branch lengths, unresolved positions for several species (Fig. 1, Figure S1), and high morphological similarities among species suggest that this genus has undergone a rapid diversification during this short period. Transitions in host association were estimated to have occurred 11 times in *Greenidea*, which is much more frequent than in other genera. Four sampled species were inferred to have shifted to feeding on plants other than Fagaceae. Closely related species (e.g. *G. okajimai* and *G. cayratiae*) were found to colonise different host plants. Host shift is recognized as a key component in speciation of aphids (Moran *et al.*, 1999; Favret & Voegtlin, 2004; Durak *et al.*, 2014) and other phytophagous insects (Bush, 1969; Funk *et al.*, 1995; Percy *et al.*, 2004; Winkler & Mitter, 2008; Winkler *et al.*, 2009; Fordyce, 2010). Shifting to new plant species can provide a barrier to gene flow between parental and daughter populations, and may then lead to host-associated adaptation, reproductive isolation and eventually speciation (Bush, 1975; Jermy, 1984; Feder *et al.*, 1988). Seven *Greenidea* species were suggested to have expanded their host ranges onto plants of Betulaceae, Myrtaceae, Sapindaceae, etc. Most assumed host expansions appear to have led to polyphagy. The incorporation of new plants into the repertoire is likely to have been an important evolutionary phase that has contributed to the diversification in phytophagous insects (Weingartner *et al.*, 2006; Janz & Nylin, 2008). A broader host range may facilitate speciation through forming specialised host races, and also may allow an aphid species to increase its geographical distribution and speciate via subsequent local adaptation and population fragmentation (Janz *et al.*, 2006; Janz & Nylin, 2008). Multiple transitions in host association were also observed in the genus *Mollitrichosiphum* whose representatives were limited in this study. In a multigene phylogenetic study with a much broader sampling, it has been proposed that geographical isolation, coupled with expansions in host plant range, might account for species differentiation in this genus (Zhang *et al.*, 2012).

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12100

Figure S1. Fifty percent majority-rule consensus tree inferred from the Bayesian analysis. Posterior probabilities (PP) values (>0.70) are shown above the branches.

Table S1. Voucher information and GenBank accession numbers of aphid species used in this study.

Table S2. Host associations of the sampled Greenideini species in this study.

Table S3. Summary of individual BEAST runs.

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References

Barrón, E. & Diéguez, C. (2001) Estudio macroflorístico del Mioceno inferior lacustre de la Cuenca de Rubielos de Mora (Teruel, España). *Boletín Geológico y Minero*, **112**, 13–56.

Blackman, R.L. & Eastop, V.F. (1994) *Aphids on the World's Trees: An Identification and Information Guide*. CAB International, Wallingford.

Blackman, R.L. & Eastop, V.F. (2000) *Aphids on the Word's Crops: An Identification and Information Guide*, 2nd edn. John Wiley & Sons, Chichester.

Blackman, R.L. & Eastop, V.F. (2006) *Aphids on the World's Herbaceous Plants and Shrubs*. John Wiley & Sons, Chichester.

Bush, G.L. (1969) Sympatric host race formation and speciation in frugivorous flies of the genus *Rhagoletis* (Diptera, Tephritidae). *Evolution*, **23**, 237–251.

Bush, G.L. (1975) Modes of animal speciation. *Annual Review of Ecology and Systematics*, **6**, 339–364.

Chen, R., Jiang, L.Y. & Qiao, G.X. (2012) The effectiveness of three regions in mitochondrial genome for aphid DNA barcoding: a case in Lachininae. *PLoS ONE*, **7**, e46190.

Chmura, C.A. (1973) Upper Cretaceous (Campanian-Maastrichtian) angiosperm pollen from the western San Joaquin Valley, California, USA. *Paleaontographica Abteilung B*, **141**, 89–171.

Crepet, W.L. & Nixon, K.C. (1989) Earliest megafossil evidence of Fagaceae: phylogenetic and biogeographic implications. *American Journal of Botany*, **76**, 842–855.

Dixon, A.F.G. (1985) *Aphid Ecology*. Blackie, Glasgow.

von Dohlen, C.D. & Moran, N.A. (2000) Molecular data support a rapid radiation of aphids in the Cretaceous and multiple origins of host alternation. *Biological Journal of the Linnean Society*, **71**, 689–717.

von Dohlen, C.D., Kurosu, U. & Aoki, S. (2002) Phylogenetics and evolution of the eastern Asian–eastern North American disjunct aphid tribe, Hormaphidini (Hemiptera: Aphididae). *Molecular Phylogenetics and Evolution*, **23**, 257–267.

von Dohlen, C.D., Rowe, C.A. & Heie, O.E. (2006) A test of morphological hypotheses for tribal and subtribal relationships of Aphidinae (Insecta: Hemiptera: Aphididae) using DNA sequence. *Molecular Phylogenetics and Evolution*, **38**, 316–329.

Drummond, A.J. & Rambaut, A. (2007) BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology*, **7**, 214.

Drummond, A.J., Suchard, M.A., Xie, D. & Rambaut, A. (2012) Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution*, **29**, 1969–1973.

Durak, R., Lachowska-Cierlik, D. & Bartoszewski, S. (2014) Relationships within aphids *Cinara* (*Cupressobium*) (Hemiptera) based on mitochondrial and nuclear DNA sequences. *Journal of Applied Genetics*, **55**, 89–96.

Favret, C. (2014) *Aphid Species File. Version 5.0/5.0* [WWW document]. URL http://Aphid.SpeciesFile.org [accessed on 7 May 2014].

Favret, C. & Voegtlin, D.J. (2004) Speciation by host-switching in pinyon *Cinara* (Insecta: Hemiptera: Aphididae). *Molecular Phylogenetics and Evolution*, **32**, 139–151.

Feder, J.L., Chilcote, C.A. & Bush, G.L. (1988) Genetic differentiation between sympatric host races of the apple maggot fly *Rhagoletis pomonella*. *Nature*, **336**, 61–64.

Foottit, R.G., Maw, H.E.L., von Dohlen, C.D. & Hebert, P.D.N. (2008) Species identification of aphids (Insecta: Hemiptera: Aphididae) through DNA barcodes. *Molecular Ecology Resources*, **8**, 1189–1201.

Fordyce, J.A. (2010) Host shifts and evolutionary radiations of butterflies. *Proceedings of the Royal Society B*, **277**, 3735–3743.

Funk, D.J., Futuyma, D.J., Orti, G. & Meyer, A. (1995) A history of host associations and evolutionary diversification for *Ophraella* (Coleoptera: Chrysomelidae): new evidence from mitochondrial DNA. *Evolution*, **49**, 1008–1017.

Ghosh, A.K. & Agarwala, B.K. (1993) *The Fauna of India and the Adjacent Countries. Homoptera, Aphidoidea, Part 6, Subfamily Greenideinae*. Zoological survey of India, Calcutta.

Guindon, S. & Gascuel, O. (2003) A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Systematic Biology*, **52**, 696–704.

Guldemond, J.A. (1990) *On Aphids, their Host Plants and Speciation: A Biosystematic Study of the Genus Cryptomyzus*. Ponsen & Looijen, Wageningen.

Harry, M., Solignac, M. & Lachaise, D. (1998) Molecular evidence for parallel evolution of adaptive syndromes in fig-breeding *Lissocephala* (Drosophilidae). *Molecular Phylogenetics and Evolution*, **9**, 542–551.

Havill, N.P., Foottit, R.G. & von Dohlen, C.D. (2007) Evolution of host specialization in the Adelgidae (Insecta: Hemiptera) inferred from molecular phylogenetics. *Molecular Phylogenetics and Evolution*, **44**, 357–370.

Heie, O.E. (1987) Palaeontology and phylogeny. *Aphids: Their Biology, Natural Enemies, and Control*, Vol. 2A. (ed. by A. K. Minks and P. Harrewijn), pp. 367–391. Elsevier, Amsterdam.

Heie, O.E. (1996) The evolutionary history of aphids and a hypothesis on the coevolution of aphids and plants. *Bollettino di Zoologia agraria e di Bachicoltura, Series II*, **28**, 149–155.

Heie, O.E. (1999) Aphids of the past (Hemiptera, Sternorrhyncha). *Proceedings of the First International Palaeoentomological Conference*. Moscow 1998, pp. 49–55. AMBA Projects, Bratislava.

Heie, O.E. & Wegierek, P. (1998) A list of fossil aphids (Homoptera: Aphidinea). *Annals of the Upper Silesian Museum (Entomology)*, **8–9**, 159–192.

Heie, O.E. & Wegierek, P. (2009) A classification of the Aphidomorpha (Hemiptera: Sternorrhyncha) under consideration of the fossil taxa. *Redia*, **92**, 69–77.

Herendeen, P.S., Crane, P.R. & Drinnan, A.N. (1995) Fagaceous flowers, fruits, and cupules from the Campanian (Late Cretaceous) of central Georgia, USA. *International Journal of Plant Sciences*, **156**, 93–116.

Hille Ris Lambers, D. (1950) Host plants and aphid classification. *Proceedings of the 8th International Congress of Entomology.* Stockholm, 1948, pp. 141–144.

Hong, Y.C. (2002) *Amber Insects of China*. Beijing Science and Technology Press, Beijing.

Huang, X.L., Xiang-Yu, J.G., Ren, S.S., Zhang, R.L., Zhang, Y.P. & Qiao, G.X. (2012) Molecular phylogeny and divergence times of Hormaphidinae (Hemiptera: Aphididae) indicate Late Cretaceous tribal diversification. *Zoological Journal of the Linnean Society*, **165**, 73–87.

Huelsenbeck, J.P. & Ronquist, F. (2001) MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics*, **17**, 754–755.

Inbar, M., Wink, M. & Wool, D. (2004) The evolution of host plant manipulation by insects: molecular and ecological evidence from gall-forming aphids on *Pistacia*. *Molecular Phylogenetics and Evolution*, **32**, 504–511.

Ivanov, D., Utescher, T., Mosbrugger, V., Syabryaj, S., Djordjević-Milutinović, D. & Molchanoff, S. (2011) Miocene vegetation and climate dynamics in Eastern and Central Paratethys (Southeastern Europe). *Palaeogeography, Palaeoclimatology, Palaeoecology*, **304**, 262–275.

Janz, N. & Nylin, S. (2008) The oscillation hypothesis of host-plant range and speciation. *Specialization, Speciation, and Radiation: The Evolutionary Biology of Herbivorous Insects* (ed. by K. J. Tilmon), pp. 203–215. University of California Press, Berkeley, CA.

Janz, N., Nylin, S. & Wahlberg, N. (2006) Diversity begets diversity: host expansions and the diversification of plant-feeding insects. *BMC Evolutionary Biology*, **6**, 4.

Jermiin, L.S. & Crozier, R.H. (1994) The cytochrome b region in the mitochondrial DNA of the ant *Tetraponera rufoniger*: sequence divergence in Hymenoptera may be associated with nucleotide content. *Journal of Molecular Evolution*, **38**, 282–294.

Jermy, T. (1984) Evolution of insect/plant relationships. *The American Naturalist*, **124**, 609–630.

Jiménez-Moreno, G., Fauquette, S. & Suc, J.P. (2010) Miocene to Pliocene vegetation reconstruction and climate estimates in the Iberian Peninsula from pollen data. *Review of Palaeobotany and Palynology*, **162**, 403–415.

Jousselin, E., Genson, G. & Coeur d'acier, A. (2010) Evolutionary lability of a complex life cycle in the aphid genus *Brachycaudus*. *BMC Evolutionary Biology*, **10**, 295.

Kim, H., Lee, S. & Jang, Y. (2011) Macroevolutionary patterns in the Aphidini aphids (Hemiptera: Aphididae): diversification, host association, and biogeographic origins. *PLoS ONE*, **6**, e24749.

Lakhanpal, R.N. (1970) Tertiary floras of India and their bearing on the historical geology of the region. *Taxon*, **19**, 675–694.

Li, X.X. (1995) *Fossil Floras of China through the Geological Ages*. Guangdong Science and Technology Press, Guangzhou.

Li, J.G. & Zhang, Y.Y. (1998) Neogene palynofloras from east offshore, Hainan Island. *Acta Micropalaeontologica Sinica*, **15**, 323–330.

Liu, Q.H., Jiang, L.Y. & Qiao, G.X. (2013) DNA barcoding of Greenideinae (Hemiptera: Aphididae) with resolving taxonomy problems. *Invertebrate Systematics*, **27**, 428–438.

Maddison, W.P. & Maddison, D.R. (2011) *Mesquite: A Modular System for Evolutionary Analysis. Version 2.75* [WWW document]. URL http://mesquiteproject.org [accessed on 15 March 2013].

Mai, D.H. (1989) Development and regional differentiation of the European vegetation during the Tertiary. *Plant Systematics and Evolution*, **162**, 79–91.

Moran, N.A. (1988) The evolution of host-plant alternation in aphids: evidence for specialization as a dead end. *The American Naturalist*, **132**, 681–706.

Moran, N.A. (1990) Aphid life cycles: two evolutionary steps. *The American Naturalist*, **136**, 135–138.

Moran, N.A. (1992) The evolution of aphid life cycles. *Annual Review of Entomology*, **37**, 321–348.

Moran, N.A., Kaplan, M.E., Gelsey, M.J., Murphy, T.G. & Scholes, E.A. (1999) Phylogenetics and evolution of the aphid genus *Uroleucon* based on mitochondrial and nuclear DNA sequences. *Systematic Entomology*, **24**, 85–93.

Mordvilko, A.K. (1934) On the evolution of aphids. *Archiv für Naturgeschichte*, **3**, 1–60.

Noordam, D. (1994) Greenideinae from Java (Homoptera: Aphididae). *Zoologische Verhandelingen*, **296**, 1–284.

Normark, B.B. (1996) Phylogeny and evolution of parthenogenetic weevils of the *Aramigus tessellatus* species complex (Coleoptera: Curculionidae: Naupactini): evidence from mitochondrial DNA sequences. *Evolution*, **50**, 734–745.

Normark, B.B. (2000) Molecular systematics and evolution of the aphid family Lachnidae. *Molecular Phylogenetics and Evolution*, **14**, 131–140.

Pagel, M. & Meade, A. (2006) Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. *The American Naturalist*, **167**, 808–825.

Pagel, M. & Meade, A. (2013) *BayesTraits, version 2.0*. University of Reading. [WWW document]. URL http://www.evolution.rdg.ac.uk [accessed on 6 May 2013].

Palumbi, S.R. (1996) Nucleic acids II: the polymerase chain reaction. *Molecular Systematics* (ed. by D. M. Hillis, C. Moritz and B. K. Mable), pp. 205–247. Sinauer, Sunderland, MA.

Peccoud, J., Simon, J.C., von Dohlen, C., Coeur d'acier, A., Plantegenest, M., Vanlerberghe-Masutti, F. & Jousselin, E. (2010) Evolutionary history of aphid-plant association and their role in aphid diversification. *Comptes Rendus Biologies*, **333**, 474–487.

Percy, D.M., Page, R.D.M. & Cronk, Q.C.B. (2004) Plant-insect interactions: double-dating associated insect and plant lineages reveals asynchronous radiations. *Systematic Biology*, **53**, 120–127.

Posada, D. (2008) jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution*, **25**, 1253–1256.

Qiao, G.X. (1996) *Studies on the Phylogeny of Hormaphididae, Thelaxidae, Chaitophoridae and Greenideidae etc. 7 Families.* PhD Thesis, Chinese Academy of Sciences, Beijing.

Qiao, G.X., Jiang, L.Y. & Martin, J.H. (2006) Notes on the genus *Allotrichosiphum* (Hemiptera: Aphididae: Greenideinae), with the description of one new species from Hong Kong, China. *Zootaxa*, **1225**, 57–68.

Quednau, F.W. & Martin, J.H. (2006) Descriptions of two new species of *Anomalosiphum* (Hemiptera: Aphididae, Greenideinae), including a winged ovipara with pedunculate eggs. *Zoological Journal of the Linnean Society*, **146**, 239–249.

Rambaut, A. (2012) *FigTree version 1.4.0* [WWW document]. URL http://tree.bio.ed.ac.uk/software/figtree [accessed on 29 March 2013].

Rambaut, A. & Drummond, A.J. (2009) *Tracer v1.5.0* [WWW document]. URL http://tree.bio.ed.ac.uk/software/tracer [accessed on 30 November 2009].

Raychaudhuri, D.N. (1956) Revision of *Greenidea* and related genera (Homoptera, Aphididae). *Zoologische Verhandelingen*, **31**, 1–106.

Raychaudhuri, D.N. & Chatterjee, M. (1980) Subfamily Greenideinae. *Aphids of North-East India and Bhutan* (ed. by D. N. Raychaudhuri), pp. 314–358. The Zoological Society, Calcutta.

Remaudière, G. & Remaudière, M. (1997) *Catalogue des Aphididae du Monde. Homoptera Aphidoidea*. Institut National de la Recherche Agronomique, Paris.

Ronquist, F. & Huelsenbeck, J.P. (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, **19**, 1572–1574.

Sano, M. & Akimoto, S.I. (2011) Morphological phylogeny of gall-forming aphids of the tribe Eriosomatini (Aphididae: Eriosomatinae). *Systematic Entomology*, **36**, 607–627.

Schwarz, G. (1978) Estimating the dimension of a model. *The Annals of Statistics*, **6**, 461–464.

Sims, H.J., Herendeen, P.S. & Crane, P.R. (1998) New genus of fossil Fagaceae from the Santonian (Late Cretaceous) of central Georgia, USA. *International Journal of Plant Sciences*, **159**, 391–404.

Stamatakis, A. (2006) RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, **22**, 2688–2690.

Stamatakis, A., Hoover, P. & Rougemont, J. (2008) A rapid bootstrap algorithm for the RAxML web servers. *Systematic Biology*, **57**, 758–771.

Stern, D.L. (1994) A phylogenetic analysis of soldier evolution in the aphid family Hormaphididae. *Proceedings of the Royal Society of London B: Biological Sciences*, **256**, 203–209.

Sugimoto, S. (2011) Two new species of the genus *Eutrichosiphum* Essig *et* Kuwana (Hemiptera: Aphididae: Greenideinae) on *Lithocarpus edulis* (Fagaceae) from Japan. *Insecta Matsumurana. Series Entomology. New Series*, **67**, 23–32.

Sun, H. (2002) Tethys retreat and Himalayas-Hengduanshan Mountains uplift and their significance on the origin and development of the Sino-Himalayan elements and alpine flora. *Acta Botanica Yunnanica*, **24**, 273–288.

Sun, H. & Li, Z.M. (2003) Qinghai-Tibet plateau uplift and its impact on Tethys flora. *Advance in Earth Science*, **18**, 852–862.

Swofford, D.L. (2003) *PAUP*. Phylogenetic Analysis Using Parsimony (*and Other Methods). Version 4*. Sinauer Associates, Sunderland, MA.

Takahashi, R. (1931) Aphididae of Formosa, Part 6. *Report of the Department of Agriculture Government Research Institute of Formosa*, **53**, 1–127.

Takahashi, R. & Sorin, M. (1959) Biology of the aphid, *Paratrichosiphum kashicola* (Kurisaki) (Aphididae, Homoptera). *Insect Ecology*, **7**, 129–132.

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

Wang, W.M. (1992) Palynofloristic changes in the Neogene of South China. *Acta Micropalaeontologica Sinica*, **9**, 81–85.

Wegierek, P. & Peñalver, E. (2002) Fossil representatives of the family Greenideidae (Hemiptera, Aphidoidea) from the Miocene of Europe. *Geobios*, **35**, 745–757.

Weingartner, E., Wahlberg, N. & Nylin, S. (2006) Dynamics of host plant use and species diversity in *Polygonia* butterflies (Nymphalidae). *Journal of Evolutionary Biology*, **19**, 483–491.

WGCPC (The Writing Group of Cenozoic Plants of China) (1978) *Cenozoic Plants from China, Fossil Plants of China*, Vol. **3**. Science Press, Beijing.

Winkler, I.S. & Mitter, C. (2008) The phylogenetic dimension of insect-plant interactions: a review of recent evidence. *Specialization, Speciation, and Radiation: The Evolutionary Biology of Herbivorous Insects* (ed. by K. J. Tilmon), pp. 240–263. University of California Press, Berkeley, CA.

Winkler, I.S., Mitter, C. & Scheffer, S.J. (2009) Repeated climate-linked host shifts have promoted diversification in a temperate clade of leaf-mining flies. *Proceedings of the National Academy of Sciences of the United States of America*, **106**, 18 103–18 108.

Wojciechowski, W. (1992) *Studies on the Systematic System of Aphids (Homoptera, Aphidinea)*. Uniwersytet Slaski, Katowice.

Zhang, G. & Chen, X. (1999) Studies on the phylogeny and host plant association of Lachnidae (Homoptera: Aphidinea). *Entomologia Sinica*, **6**, 193–208.

Zhang, D. & Qiao, G. (2007a) Review of the subgenus *Paragreenidea* Raychaudhuri of *Greenidea* Schouteden with description of one new species (Hemiptera: Aphididae: Greenideinae). *Annales Zoologici*, **57**, 497–504.

Zhang, D. & Qiao, G. (2007b) *Pentatrichosiphum* (Sternorrhyncha: Aphididae), a generic account with the description of a new species from Hainan, China. *Journal of Natural History*, **41**, 2419–2431.

Zhang, D. & Qiao, G. (2008) *Mesotrichosiphum* Calilung (Hemiptera: Aphididae: Greenideinae), a newly recorded genus from China, with the description of a new species. *Entomological News*, **119**, 232–238.

Zhang, D. & Qiao, G.X. (2010) *Mollitrichosiphum* Suenaga from China (Hemiptera: Aphididae), with the description of one new species. *Zootaxa*, **2608**, 1–24.

Zhang, G.X. & Zhong, T.S. (1983) *Economic Insect Fauna of China. Fasc. 25. Homoptera: Aphidinea, Part I*. Science Press, Beijing.

Zhang, R., Huang, X., Jiang, L. & Qiao, G. (2011) Phylogeny and species differentiation of *Mollitrichosiphum* spp. (Aphididae, Greenideinae) based on mitochondrial *COI* and *Cyt b* genes. *Current Zoology*, **57**, 806–815.

Zhang, R., Huang, X., Jiang, L., Lei, F. & Qiao, G. (2012) Species differentiation of Chinese *Mollitrichosiphum* (Aphididae: Greenideinae) driven by geographical isolation and host plant acquirement. *International Journal of Molecular Sciences*, **13**, 10441–10460.

Zhou, Z.K. (1993) The fossil history of *Quercus*. *Acta Botanica Yunnanica*, **15**, 21–33.

Zhou, Z.K. (1999) Fossils of the Fagaceae and their implications in systematics and biogeography. *Acta Phytotaxonomica Sinica*, **37**, 369–385.

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