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An aphid lineage maintains a bark-feeding niche while switching to and diversifying on conifers

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Abstract

Lachnine aphids are unusual among phytophagous insects because they feed on both leafy and woody parts of both angiosperm and conifer hosts. Despite being piercing-sucking phloem-feeders, these aphids are most speciose on woody parts of coniferous hosts. To evaluate the significance of this unusual biology on their evolution, we reconstructed the ancestral host and feeding site of the lachnine aphids and estimated important host shifts during their evolution. We sampled 78 species representing 14 of the 18 genera of Lachninae from Asia and North America. We performed parsimony, Bayesian and likelihood phylogenetic analyses of combined mitochondrial *Cox1*, *Cox2*, *CytB* and nuclear *EF1a1* DNA sequences. We dated the resulting phylogram's important nodes using Bayesian methods and multiple fossil and secondary calibrations. Finally, we used parsimony and Bayesian ancestral state reconstruction to evaluate ancestral feeding ecology. Our results suggest the lachnine common ancestor fed on a woody part of an angiosperm host in the mid-Cretaceous. A shift to conifer hosts in the Late Cretaceous is correlated with a subsequent increased diversification in the Palaeogene, but a switch to leafy host tissues did not engender a similar burst of diversification. Extant lachnine lineages exhibit the full range of historical association with their hosts: some appeared before, some concomitant with and some after the appearance of their hosts. We conclude our study by placing all the lachnine genera in five tribes.

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Introduction

Lachnine aphids (Insecta: Hemiptera: Aphididae: Lachninae) represent a lineage with a significant number of bark phloem feeders in a family almost entirely composed of piercing and sucking specialists on leafy tissue. They also include the single largest radiation of extant aphids on conifer hosts. Determining the ancestral feeding condition of Lachninae, whether on woody or leafy tissue on angiosperm or conifer hosts, is important to understanding the host-associated evolution of aphids specifically and phytophagous insects more generally.

Insects are the most speciose group of multicellular life and phytophagous insects represent a dominant proportion of that diversity (Strong et al., 1984). The most dramatic explosions of phytophagous insect species diversity are correlated with the arrival and diversification of angiosperm plants, either concomitant in the Cretaceous or, more often, immediately thereafter in the Palaeogene (Farrell, 1998; Grimaldi and Engel, 2005). Although shifts from gymnosperm to angiosperm hosts led to the historical development of hyperdiverse lineages of phytophagous insects, the flow of novel host acquisition need not be unidirectional: instances of switching from gymnosperm to angiosperm hosts and back again have been documented (Sequeira et al., 2000; Farrell et al., 2001).

Aphids are a family of over 5000 extant phloem-feeding insect species (Favret, 2015). As with many

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other phytophagous groups, aphids experienced a marked diversification correlated with the appearance and radiation of angiosperms. Aphid diversification of extant lineages began in the Late Cretaceous and continued during the Palaeogene (Von Dohlen and Moran, 2000). Most of the aphid subfamilies with largely plesiomorphic morphological character states include species with coniferous hosts (e.g. Eriosomatinae, Hormaphidinae, Mindarinae), as do the Adelgidae, one of the putative sister families of the Aphididae (Peccoud et al., 2010). This phylogenetic evidence, and especially the fossil record, have led some to infer a gymnosperm host for the ancestral aphid (Heie, 1987; Shaposhnikov, 1987). However, the character-state of a sister group cannot, on its own, inform the ancestral condition of the ingroup (Crisp and Cook, 2005), nor can the conifer-feeding habit of an extant aphid tell us that of its ancestor (Von Dohlen and Moran, 2000). Several modern phylogenetic hypotheses place the Lachninae as sister to most of the rest of the aphids (Von Dohlen and Moran, 2000; Ortiz-Rivas et al., 2004; Ortiz-Rivas and Martínez-Torres, 2010) (but see Nováková et al., 2013). This placement leaves unresolved the question of ancient aphid–host associations.

Normark (2000) presented a summary of the arguments regarding the ancestral feeding habit of Lachninae, submitting that the predominant consensus was for ancestral feeding on angiosperms, with subsequent colonization of conifers. However, he provided support, albeit limited, for an ancestral conifer-feeding habit; he suggested conifer-feeding may represent the plesiomorphic condition, also present in the other conifer-feeding Aphidomorpha lineages (e.g. Adelgidae, Mindarinae) (Normark, 2000). The lachnine fossil record, exclusively from Miocene deposits (Heie and Wegierek, 2011), does not contribute to resolving the question.

The Lachninae are also interesting because they include a particularly diverse lineage on conifer hosts. A majority of the 400 species of Lachninae belong to a single genus, *Cinara* Curtis, the 246 species of which feed exclusively on trees of the coniferous pine and cypress families (Pinaceae and Cupressaceae). The marked success of *Cinara* on conifers presents two phylogenetic hypotheses and consequences: (i) with ancestral conifer-feeding, the radiation of *Cinara* may have pre-dated the appearance of other lachnine species, or (ii) with ancestral angiosperm-feeding, the colonization and subsequent radiation on conifers would be a more derived condition.

All species of the tribe to which *Cinara* belongs, Eulachnini, feed on conifers. Whereas various *Cinara* species feed on the bark of the woody parts of their hosts (i.e. roots, trunks, branches, twigs and shoots, but not needles; Fig. 1), the species of the other four eulachnine genera are known to feed only on the

Fig. 1. Diversity of feeding sites of *Cinara* spp. (including *Schizolachnus* [b]), on (a) twigs, (b) needles, (c) branches, (d) trunks and roots, and (e) shoots. All photos by C.F.

needles of members of the pine family (Table 1). Feeding-site specificity in *Cinara* has been suggested as a means towards reproductive isolation and thus speciation in the genus (Favret and Voegtlin, 2004; Jousselin et al., 2013). The feeding site (e.g. branch, twig, shoot) is a synapomorphy of at least some *Cinara* species groups (Favret and Voegtlin, 2004): needle-feeding might thus be a synapomorphy of the other four eulachnine genera. Alternatively, needle-feeding may have evolved more than once. The morphology of the needle-feeding *Schizolachnus* Mordvilko led Mamontova (2008) to place the genus in its own subtribe, but other work hints that *Schizolachnus* may have arisen from within *Cinara* (Nováková et al., 2013; Henry et al., 2015; Meseguer et al., 2015). A paraphyletic *Cinara* would suggest that needle-feeding arose at least twice in the Eulachnini: once in *Schizolachnus* and once in the common ancestor of the other needle-feeding eulachnine genera.

The tribe Lachnini contains far fewer species than the Eulachnini (78, compared with 291), although they are more diverse in terms of host-plant utilization (Table 1). Most Lachnini species feed on a range of woody angiosperm plants with at least one species known to colonize the trunks of conifers (Fig. 2). Lachnini is the least supported Lachninae tribe: Normark (2000) recovered it as paraphyletic. The third lachnine tribe is the biologically distinct Tramini, consisting of 31 species, largely asexual, feeding on roots of herbaceous angiosperms (composites) (Fig. 2b; Table 1). Due to its particular biology, the monophyly of Tramini has never been in doubt, although its phylogenetic position may render the Lachnini paraphyletic (Normark, 2000).

In the last 20 years, several competing classifications of Lachninae have been proposed, each presenting contrasting levels of hierarchical complexity. The simplest, with three subdivisions as presented above, is the most frequently used (Heie, 1995; Remaudière and Remaudière, 1997; Nieto Nafría et al., 2011; Favret, 2015). Others have included five (Normark, 2000), six (Heie and Wegierek, 2009) or seven (Mamontova, 2008) subdivisions of varying complexity. Normark's (2000) is the only cladistic classification.

The main objective of our study was to build a robust phylogeny to test the contributions to lachnine species diversification of host identity and feeding site preference. In particular, we posited the two following questions. (i) Did the most recent ancestor of Lachninae feed on angiosperms or conifers and did it feed on woody or leafy host tissue? (ii) Is the relative evolutionary success of various Lachninae lineages, especially the diverse genus *Cinara* on woody parts of conifer hosts, correlated with the adoption of a novel host or a novel tissue type? Secondarily, we sought to provide a stable, cladistic, higher classification of the subfamily.

Materials and methods

Taxon sampling

We aimed to maximize the representation of Lachninae at the generic level: we collected 150 individuals of 78 species representing 14 of the 18 lachnine genera from Asia and North America (Table S1). Only *Pseudessigella* Hille Ris Lambers (a monotypic genus),

Table 1
List of Lachninae genera and their feeding ecology (Blackman and Eastop, 2015; Favret, 2015)

Tribe: standard classification	Genus	Principal hosts	Feeding site
Eulachnini	*Cinara*	Cupressaceae & Pinaceae	Bark
	Essigella	Pinaceae	Leaves (needles)
	Eulachnus	Pinaceae	Leaves (needles)
	Pseudessigella	Pinaceae	Leaves (needles)
	Schizolachnus	Pinaceae	Leaves (needles)
Lachnini	*Lachnus*	Angiosperm trees	Bark—stem & branch
	Longistigma	Angiosperm trees	Bark—stem & branch
	Maculolachnus	Rosaceae	Bark—stem & branch
	Neonippolachnus	Betulaceae	
	Nippolachnus	Rosaceae	Leaves
	Pterochloroides	Rosaceae	Bark—stem & branch
	Pyrolachnus	Rosaceae	Bark—stem & branch
	Sinolachnus	Elaeagnaceae	Bark—stem & branch
	Stomaphis	Angiosperm & gymnosperm trees	Bark—trunk & root
	Tuberolachnus	Rosaceae & Salicaceae	Bark & leaves
Tramini	*Eotrama*	Tamaricaceae	Root
	Protrama	Asteraceae	Root
	Trama	Asteraceae	Root

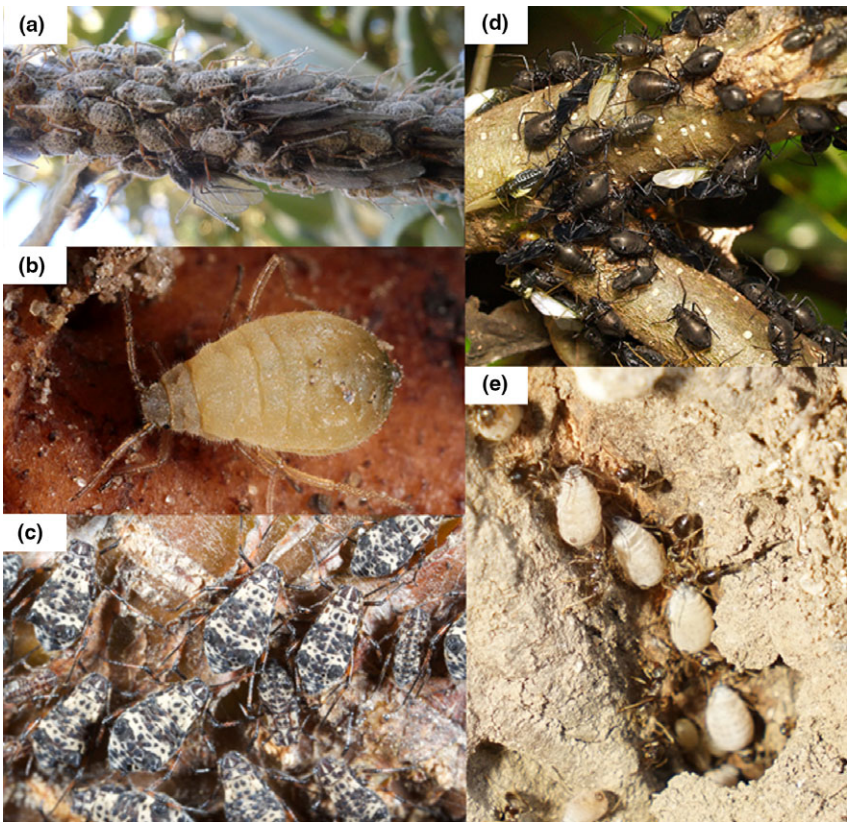

Fig. 2. Relatively stable feeding sites within genera in the rest of the Lachninae: (a) *Pyrolachnus* on branches and small trunks (photo by R.C.), (b) *Trama* on roots (photo by Claude Pilon, used with permission), (c) *Pterochloroides* on branches and trunks (photo by C.F.), (d) *Lachnus* on branches and small trunks (photo by Nigel Stott, Natural-Japan.net, used with permission), (e) *Stomaphis* on trunks (photo by R.C.).

Neonippolachnus Shinji (monotypic), *Sinolachnus* Hille Ris Lambers (two species) and *Eotrama* Hille Ris Lambers (four species) were absent from the study. Because the monophyly of certain genera was not fully established, we included as many species as possible for genera with fewer than 40 species. For the largest genus, *Cinara*, species were selected from different host taxa, including one species found on *Abies* Miller and *Taxus* Linnaeus, one from *Pseudotsuga* Carrière, one from *Cedrus* Trew, one from *Metasequoia* Hu & Cheng, one from Cupressaceae, two from *Larix* Miller, four from *Picea* A. Dietrich and 12 from *Pinus* Linnaeus. In some cases, additional effort to cover the geographical distribution at the species level was made to ensure genetic variation between populations.

On the basis of current phylogenetic hypotheses for Aphididae (Heie, 1987; Wojciechowski, 1992; Zhang, 1999; Ortiz-Rivas et al., 2004; Ortiz-Rivas and Martínez-Torres, 2010), 12 species were chosen to serve as outgroups. *Pineus armandicola* (Zhang) (Adelgidae) and *Phylloxerina salicis* (Lichtenstein) (Phylloxeridae) represent lineages that diverged prior to the common ancestor of extant Aphididae. *Mindarus keteleerifoliae* Zhang (Mindarinae) represents a lineage on conifers. *Anoecia fulviabdominalis* (Sasaki) (Anoeciinae) shares some similar features with Tramini, such as root feeding, and was placed in the Lachnini in the past (Baker, 1920). Eight species in Aphidinae represent the most diverse aphid subfamily (Heie, 1967, 1987, 1994), including *Aphis kurosawai* Takahashi, *A. gossypii* Glover, *Toxoptera aurantii* (Boyer de Fonscolombe), *Rhopalosiphum padi* (Linnaeus) and *Hyalopterus pruni* (Geoffroy) within the tribe Aphidini, and *Brevicoryne brassicae* (Linnaeus), *Macrosiphoniella yomogifoliae* (Shinji) and *Lipaphis pseudobrassicae* (Davis) in the Macrosiphini.

All samples were collected directly into 95 or 100% ethanol and stored in the laboratory at −80 °C. Preserved aphid colonies were examined prior to preparation to ensure that they did not consist of multiple species. DNA from one to three individuals per sample was isolated for molecular studies and three to five individuals per sample were mounted on microscope slides. Voucher specimens for each sample were identified by G.Q. based on morphological diagnostic features using standard literature-based keys (Blackman and Eastop, 1994) and by comparison with previously identified specimens in the National Zoological

Museum of China, Beijing. Voucher specimens were deposited in this same museum. The complete list of taxa and collection data, including host plants and collection localities and dates, is provided in Supporting Information, Table S1.

DNA extraction, amplification and sequencing

We targeted five molecular markers: mitochondrial cytochrome oxidase c subunit I (*Cox1*), cytochrome oxidase c subunit II (*Cox2*), and cytochrome oxidase b (*CytB*), and nuclear elongation factor-1α1 (*EF1a1*) and long-wavelength opsin genes (*LWO*). Mitochondrial genes were selected to provide resolution at lower taxonomic levels (generic and specific) (Coeur d'acier et al., 2007, 2008; Kim and Lee, 2008; Zhang et al., 2011), whereas nuclear genes were used to provide resolution deeper within the subfamily (Normark, 2000; Ortiz-Rivas et al., 2004; Von Dohlen et al., 2006; Zhang and Qiao, 2008; Ortiz-Rivas and Martínez-Torres, 2010).

Total genomic DNA was extracted from single aphids using a modified CTAB protocol (Doyle and Doyle, 1987). PCR primers are listed in Table 2. Typical PCRs were prepared in a 25-μL volume containing 10 × EasyTaq DNA Polymerase Buffer ($+Mg^{2+}$) (TransGen Biotech, Beijing, China), 1.5 U EasyTaq DNA Polymerase (TransGen Biotech), 2.5 mM each dNTP (TransGen Biotech), 5 pmol of each primer and 1 μL whole genomic extract. The PCR thermal regime was as follows: 5 min initial denaturation at 95 °C, followed by 35 cycles of 95 °C for 30–60 s, 48–52 °C for 30–60 s, 72 °C for 1–1.5 min and a 10-min final extension at 72 °C. The primer-specific annealing temperatures of each primer set were 52 °C for *Cox1*, 46 °C for *Cox2*, 48 °C for *CytB*, 50 °C for *EF1a1* and 48 °C for *LWO*. For amplification of the *Cox1* gene, two pairs of primers were used to obtain the majority of the gene. For amplification of the *LWO* gene, a second nested PCR was necessary for some samples, using primers OPSETF2 and OPSETR2 (Table 2) on a 1-μL aliquot from the first PCR. Conditions were identical except for an increase of the annealing temperature to 50 °C. PCR products were purified using an EasyPure Quick Gel Extraction Kit (TransGen Biotech) and sequenced directly. Sequencing reactions were performed using the corresponding PCR primers from both directions with BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and run on an ABI 3730 automated sequencer (Applied Biosystems). In some cases, cloning of nuclear genes was necessary, using the pMD19-T Vector System (TaKaRa, Dalian, China) and Trans5α Chemically Competent Cell (TransGen Biotech) following the manufacturers' instructions. At least three clones were sequenced in each case.

Forward and reverse chromatograms were analysed and assembled with Seqman in the DNAStar* software package (DNASTAR, Inc., Madison, WI, USA) to obtain a single consensus sequence. Multiple alignments were conducted with ClustalX (Excoffier et al., 2005) and subsequently reduced to 1228 bp (*Cox1*), 668 bp (*Cox1*), 730 bp (*CytB*), 779 bp (*EF1a1*) and 557 bp (*LWO*). No stop codons or indels that would indicate the presence of nuclear pseudogenes (Sorenson and Quinn, 1998) were found in the mitochondrial protein-coding genes. Introns in *EF1a1* and *LWO* sequences contained a large number of variably sized indels that significantly reduced the confidence of our alignments; we therefore located and removed introns prior to further analysis. We confirmed the sequences by testing whether they could be appropriately translated

Table 2
List of PCR primers

Gene	Primer	Sequence	Reference
Cox1	LepF	5′-ATTCAACCAATCATAAAGATATTGG-3′	Foottit et al. (2008)
	LepR	5′-TAAACTTCTGGATGTCCAAAAAATCA-3′	
	CIS	5′-ACCAGTTTTAGCAGGTGCTATTAC-3′	Favret and Voegtlin (2004)
	CIA	5′-GTATATCGACGAGGTATACCATTT-3′	
Cox2	2993	5′-CATTCATATTCAGAATTACC-3′	Stern (1994)
	3772	5′-GAGACCATTACTTGCTTTCAGTCATCT-3′	
CtyB	CP1	5′-GATGATGAAATTTTGGATC-3′	Harry et al. (1998)
	CP2	5′-CTAATGCAATAACTCCTCC-3′	
	CB2	5′-ATTACACCTCCTAATTTATTAGGAAT-3′	Jermiin and Crozier (1994)
EF1a1	EF3	5′-GAACGTGAACGTGGTATCAC-3′	Von Dohlen et al. (2002)
	EF6	5′-TGACCAGGGTGGTTCAATAC-3′	
	EF2	5′-ATGTGAGCAGTGTGGCAATCCAA-3′	Palumbi (1996)
LWO	OPSETF1	5′-GGYRTYACNATTTTYTTCTTRGG-3′	Designed B. Ortiz-Rivas
	OPSETR1	5′-GANCCCCADATYGTNAATAAYGG-3′	Ibid.
	OPSETF2	5′-ATGTGYCCRCCRATGGTNTGGA-3′	Ibid.
	OPSETR2	5′-GGWGTCCANGCCATRAACCA-3′	Ibid

into proteins with Editseq (DNASTAR, Inc.). All sequences were deposited in GenBank. Taxon sampling was expanded by including additional DNA sequences from GenBank. Sequence accession numbers are given in Table S1.

Phylogenetic analyses

To estimate congruence between datasets, we performed 100 replicates of the partition homogeneity test (Farris et al., 1994) as implemented in PAUP*4.0 (Swofford, 2002). The results indicated that *LWO* was incongruent with the other four genes ($P = 0.01$), whereas the sequence data for the other four genes were congruent ($P > 0.01$). Our combined analyses therefore omitted *LWO*. Phylogenetic inferences were conducted individually on each of the five genes and the combined four-gene dataset (*Cox1*+ *Cox2*+ *CytB*+ *EF1a1*) using maximum-parsimony (MP), Bayesian and maximum-likelihood (ML) methods.

Datasets were analysed with MP under equal weights using TNT v1.1 (Goloboff et al., 2008). New technology searches were applied consisting of 10 000 random addition sequence replicates, each employing default sectorial, ratchet, drift and tree-fusing parameters. The best trees were then resubmitted for tree bisection and reconnection (TBR) branch swapping to check for additional most parsimonious trees. Clade support was assessed with 1000 replicates of the bootstrap (Felsenstein, 1985).

For Bayesian analysis, the best-fit model of nucleotide substitution was selected for each gene using jModelTest 0.1.1 (Guindon and Gascuel, 2003; Posada, 2008): GTR+G for *Cox1*, and GTR+I+G for *Cox2*, *CytB*, *EF1a1* and *LWO*. Phylogenetic reconstruction was carried out in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). We ran two analyses for each gene, each with four Markov chains, three heated and one cold. Each run started from a random tree and proceeded for three to eight million Markov chain Monte Carlo (MCMC) generations, sampling chains every 100 generations. A plot of sampled log-likelihood scores against generation time was used to determine the stationarity of the chains. The trees recovered prior to stationarity were discarded as burn-in samples: for *Cox1*, *EF1a1* and *LWO*, 7500 trees; for *Cox2*, 20 000 trees; for *CytB*, 17 500 trees. For the combined data analysis, 15 000 trees were discarded as burn-in samples. The remaining trees from the concurrent runs were used to compute a majority-rule consensus tree with posterior probabilities (PP).

ML analyses were implemented on the five individual genes and the combined dataset in RAxML 7.2.6 (Stamatakis, 2006; Stamatakis et al., 2008) with the GTR + I + G model and the same model parameters as in the Bayesian analyses. The combined dataset was also analysed with PhyML 3.0 (Guindon et al., 2010) under the optimal substitution model obtained from ModelTest and model parameter values estimated during the analysis. Branch support for all ML analyses was assessed with the bootstrap with 1000 replicates.

Molecular dating

A Bayesian uncorrelated lognormal clock model with multiple calibration points was used to estimate divergence times in BEAST v.1.7.5 (Drummond and 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 analyses described above. A Yule prior was used on the tree to simulate the speciation process. Chains were analysed for 400 million generations, sampling every 1000 generations. Tracer v.1.5.0 (Rambaut and Drummond, 2009) was used to verify convergence and stability, to decide on the appropriate number of generations to discard as burn-in, and to confirm that the effective sample size of the posterior and all major clades reached > 200. The samples were summarized 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 intervals. The results were visualized using FigTree 1.4 (Rambaut, 2012).

When estimating dates for nodes in a molecular phylogeny, the choice of calibration points and the way they are represented can have a large influence on node ages and confidence intervals (Inoue et al., 2010). To place a relatively accurate time scale on the lachnine phylogeny and prevent introduction of additional biases, we used multiple calibration points including fossil specimens and secondary calibrations. Based on fossil evidence, the most recent common ancestor of the Aphididae, Adelgidae and Phylloxeridae (together composing the Aphidomorpha) was inferred to have occurred between the Late Jurassic and the Early Cretaceous (120–150 Ma) (Heie, 1987; Havill et al., 2007). Therefore, a normally distributed calibration prior with a mean of 135 Ma and a standard deviation of 9.09 Ma was specified for the age of the Aphidomorpha crown. The Aphididae crown was set from 80 to 100 Ma, derived from previous time-calibrated phylogenies based on aphid molecular data (Von Dohlen and Moran, 2000) and fossil remains of extant subfamilies in Aphididae from Upper Cretaceous deposits (Heie, 1987, 1999; Heie and Wegierek, 2011). The fossil record of Aphidinae is restricted to the Late Cretaceous and Palaeogene (Heie, 1987; Hong, 2002), indicating the possible age of the common ancestor of Aphidinae lineages 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. A fossil of the extant species *Longistigma caryae* (Harris) was found in Iceland and dated to 8 Ma (Heie and Friedrich, 1971; Heie, 1987), and we therefore assigned a uniform age prior to this genus crown (lower bound: 8 Ma; upper bound: 1.0E100 Ma). One fossil species of *Stomaphis* Walker (*S. eupetes* Wegierek & Mamontova) and three fossil species of *Cinara* (*C. elegans* Zhang, *C. limnogena* Zhang and *C. reconditivenia* Zhang) were found in Europe and China and dated to the Middle Miocene (11.608 ± 0.05–15.97 ± 0.05 Ma) (Wegierek and Mamontova, 1993; Heie and Wegierek, 2011), suggesting that these two genera are at least this old. We therefore assigned a uniform age prior to each genus crown (lower bound: 11.6 Ma; upper bound: 1.0E100 Ma). The time calibration points are summarized in Table 3.

Ancestral state reconstruction

To evaluate the evolution of host association and feeding sites in Lachninae, 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. The host association for the sampled lachnine species is summarized in Table S1. First, we evaluated the evolution of host association at the insects' tribal level. The following host-association character states were identified: (0) Fagaceae, (1) select woody angiosperm plants, (2) *Cedrus*, (3) *Larix*, (4) *Picea*, (5) *Pyrus* Linnaeus or *Eriobotrya* Lindley, (6) *Pinus*, (7) *Juglans* Linnaeus, (8) *Liquidambar* Linnaeus, (9) Cupressaceae, (A) *Abies* or *Taxus*, (B) *Metasequoia*, (C) Asteraceae, (D) *Pseudotsuga* and (E) Rosaceae. Two character states were selected to focus specifically on the feeding condition of the most recent ancestor: (0) conifers and (1) angiosperms. The following feeding sites character states, presented in Table 1, were identified: (0) leaves (including conifer needles), (1) green bark (i.e. conifer shoots), (2) thin bark (bark of twigs and small branches), (3) thick bark (bark of large branches and trunks) and (4) roots.

Parsimony reconstruction was conducted in Mesquite 2.75 (Maddison and Maddison, 2011), using the "trace character over trees" option and unordered character state transformations. For Bayesian ancestral state reconstruction, we used a reverse jump Markov chain Monte Carlo method (Pagel and Meade, 2006) as implemented in BayesTraits v2.0 (Pagel, 1994). Reverse jump MCMC (RJ-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%. For host-association character states, 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. For the feeding condition of the most recent ancestor and feeding sites character states, three independent runs were performed for a total of 1 010 000 iterations, sampling every 1000 iterations after a burn-in of 100 000 iterations, respectively.

Results

Parsimony, Bayesian and likelihood methods produced similar topologies in the combined four-gene analyses. The model-based methods provided stronger support than MP for a number of nodes: here we use the consensus tree from the combined four-gene ML analyses to summarize the results (Figs 3 and 4). RAxML and PhyML analyses displayed the same topology, although bootstrap support of PhyML (Fig. S6) was generally weaker than that of RAxML. The four-gene combined analyses provided a well-resolved phylogram. Average node support for clades above the genus level was 90.9/87.5/1/88.7 (RaxML bootstrap/PhyML bootstrap/Bayesian posterior probability/MP bootstrap). For those genera with more than one species in the analysis, average node support was also strong (99.7/99.2/1/96.1) although two genera (*Cinara* and *Protrama* Baker) were recovered as paraphyletic.

Table 3
Time calibration points

Taxon	Age (Ma)	Source	Reference(s)
Aphidomorpha	120–150	Fossils	Heie, 1987
Aphididae	80–100	Time-calibration	Von Dohlen and Moran (2000)
		Fossils	Heie (1999, 1987), Heie and Wegierek (2011)
Aphidinae	60–80	Fossils	Heie (1987), Hong (2002)
Longistigma	> 8	Fossils	Heie (1987), Heie and Friedrich (1971)
Stomaphis	> 11.6	Fossils	Heie and Wegierek (2011), Wegierek and Mamontova (1993)
Cinara	> 11.6	Fossils	Heie and Wegierek (2011), Wegierek and Mamontova (1993)

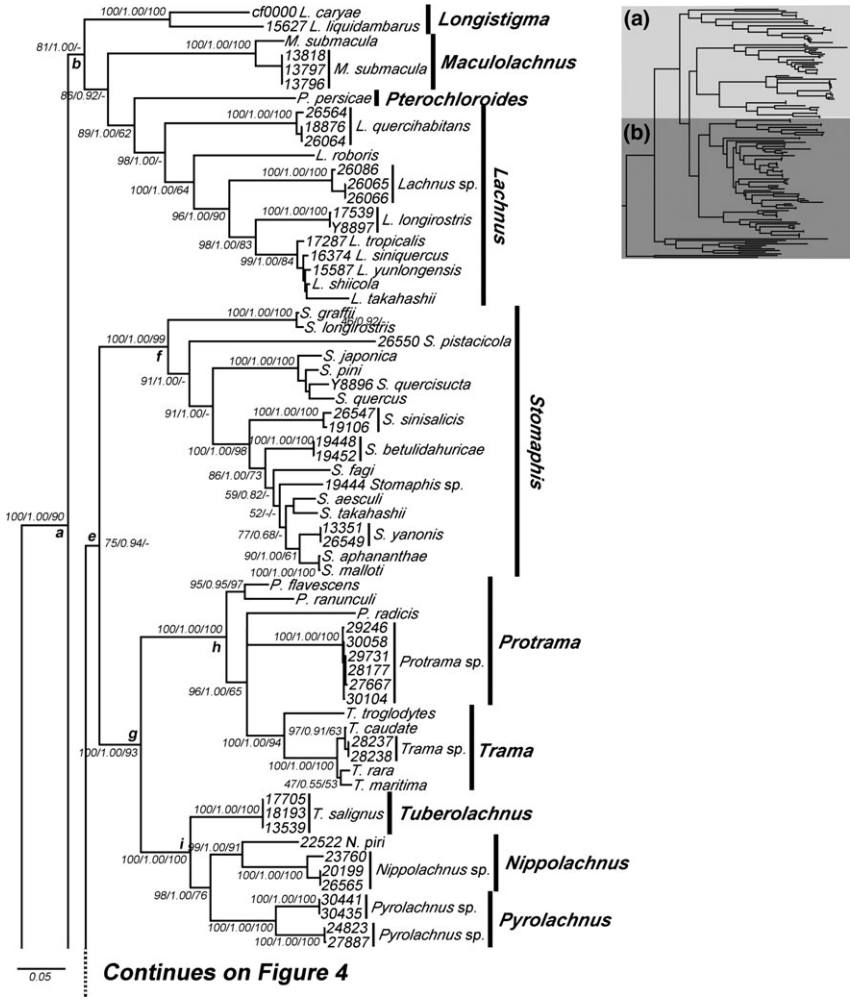

Fig. 3. First half of ML phylogram from the analyses of the Lachninae based on the combined dataset. The four numbers near nodes refer, in order, to RAxML bootstrap support, PhyML bootstrap support, Bayesian posterior probability and MP bootstrap support. Top right miniature represents an overview of the full tree: (a) part of phylogram shown here (lighter background); (b) part of phylogram shown in Fig. 4. Key nodes are labelled with letters a, b, and e–i, and are referred to in the text and tables. Terminals are labelled with identifiers for the specimens sequenced and the aphid species name. Terminals without identifiers represent taxa with GenBank data only. Clades are labelled with aphid genera (see Discussion).

The species belonging to the subfamily Lachninae formed a monophyletic group with high bootstrap support under ML, Bayesian and MP analyses (100/100/1/90, Fig. 3, node a). Lachnini (Fig. 3, node b, 81/65/1/<50), consisting of the genera *Longistigma* Wilson, *Maculolachnus* Gaumont, *Pterochloroides* Mordvilko and *Lachnus* Burmeister, formed the sister-group to the rest of the Lachninae (Fig. 4, node c, 67/61/1/<50). The Lachninae other than Lachnini consisted of Eulachnini (Fig. 4, node d, 78/80/1/<50) sister to Stomaphidini + Tramini + Tuberolachnini (Fig. 3, node e, 75/58/0.94/<50). This latter clade (node e) was the least supported of the deeper-level clades although its four constituent clades all had near-perfect support (100/100/1/93–100) (Fig. 3, nodes f, g, h and i). Stomaphidini (node f) included all *Stomaphis* representatives. The remaining genera of Lachnini, *Tuberolachnus* Mordvilko + *Nippolachnus* Matsumura + *Pyrolachnus* Basu & Hille Ris Lambers (Tuberolachnini, node i), and *Protrama* + *Trama* von Heyden (Tramini, node h), also grouped together (node g). The Eulachnini (node d) consisted of sister groups *Cinara* + *Schizolachnus* (node j; 99/98/1/52) and *Essigella* Del Guercio + *Eulachnus* Del Guercio (node k; 100/100/1/97) (Fig. 4). The *Pinus*-needle-feeding *Schizolachnus* species clustered within a group of *Pinus*-feeding *Cinara* species, rendering the latter paraphyletic.

Analyses of different single-gene trees yielded no major incongruence, with the exception of *LWO* (Figs S1–S5). Support values for particular clades are summarized in Table 4. In all single-gene analyses, the relationships at higher levels are unresolved, while

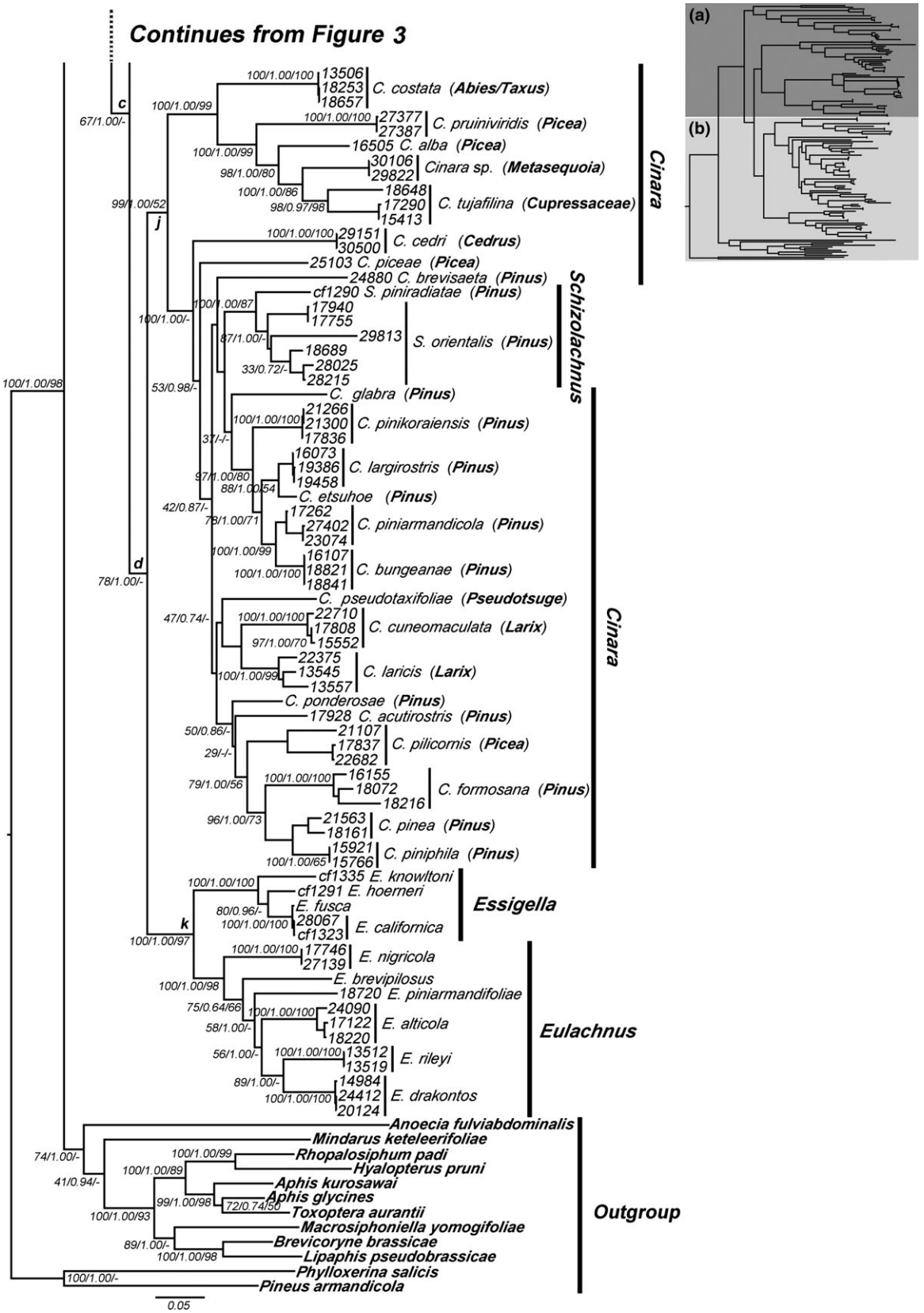

Fig. 4. Second half of ML phylogram from the analyses of the Lachninae based on the combined dataset. Top right miniature represents an overview of the full tree: (a) part of phylogram shown here (lighter background); (b) part of phylogram shown in Fig. 3. See legend to Fig. 3.

supporting the monophyly of almost all genera apart from *Cinara* and *Protrama*. Stomaphidini (node f), Tramini (h) and Tuberolachnini (i) were retrieved as monophyletic in all single-gene analyses whereas Lachnini was paraphyletic for three mitochondrial genes and Eulachnini was paraphyletic or unresolved for all genes except *Cox1*. In contrast to the mitochondrial results, the monophyly of Lachnini was well supported with *EF1a1* under ML and Bayesian analysis, albeit not under MP (80/1/<50). The branch support for *EF1a1* was generally equal to or greater than that of the mitochondrial genes.

The consensus phylogram based on *LWO* (Fig. S5) was incongruent with the other four genes, although Lachninae was recovered as monophyletic (100/1/100). The monophyly of Lachnini, Stomaphidini, Tramini and Tuberolachnini was supported with high or moderate support. Eulachnini remained paraphyletic and basal to the other tribes, with *Cinara* in particular forming several lineages branching from basal nodes. As with the other genes, *Schizolachnus* was nested within *Cinara*, but unlike the other genes, some *Cinara* species clustered with *Eulachnus* and *Essigella*. Tramini + Tuberolachnini was placed as sister to Lachnini + Stomaphidini. In general, *LWO* showed higher genus-level clade support than the other genes. The branch lengths for all but one *Cinara* species are very short and there is no support for any of the four basal branches causing Eulachnini paraphyly (ML bootstrap/Bayesian posteriors/no parsimony support: 24/0.52, 26/0.54, 27/0.65, 30/0.56) (Fig. S5).

Divergence times and character evolution

The most recent common ancestor of Lachninae dates to 95.45 Ma (95% HPD: 85.43–105.47 Ma), during the mid-Cretaceous (Fig. 5). The Lachnini crown was estimated to have arisen at 90.92 Ma (95% HPD: 80.18–101.67 Ma) and the mean age estimate for the divergence between Eulachnini and its sister group (node c) was 87.55 Ma, with a variance of 77.65–97.45 Ma (95% HPD). These two clades (nodes (f + g) and d) began diversifying dating back to 62.13 Ma (95% HPD: 51.37–72.90 Ma), 58.02 Ma (95% HPD: 47.54–68.51 Ma) and 78.39 Ma (95% HPD: 68.29–88.49 Ma), respectively. Within Lachninae, most living genera arose between the Early Paleocene and Early Oligocene, and most species-level divergences occurred from the Late Oligocene through the Miocene (Fig. 5).

Parsimony and RJ-MCMC analyses both gave strong evidence that the lachnine common ancestor fed on an angiosperm host (Fig. 6, PP = 0.999). A species of Asteraceae was shown as the ancestral host for Tramini (Fig. 5, node 5, PP = 0.999), and feeding on *Pyrus* or *Eriobotrya* was the ancestral state for Tuberolachnini (node 6, PP = 0.615). The ancestral hosts for Lachnini (node 1) and Eulachnini (node 9) remain unclear under parsimony, although RJ-MCMC hinted at *Pinus* as the ancestral host of Eulachnini (PP = 0.553). The ancestral host of *Cinara* + *Schizolachnus* (node 8) is unresolved, but other major nodes in the phylogeny can be assigned ancestral host associations: *Pterochloroides* + *Lachnus* on Fagaceae (node

Table 4
Summary of clade support (RaxML bootstrap, Bayesian PP, and MP bootstrap) from single-gene phylogenetic analyses

Taxa, genes	*Cox1* (Fig. S1)	*Cox2* (Fig. S2)	*CytB* (Fig. S3)	*EF1a1* (Fig. S4)	*LWO* (Fig. S5)
Lachninae	42/0.86/U	52/0.99/57	33/0.81/U	61/0.99/U	100/1.00/100
Lachnini (b)	P	P	P	89/1.00/U	78/0.91/U
Longistigma	74/0.95/U	99/1.00/68	99/1.00/92	100/1.00/98	100/1.00/100
Lachnus	23/U/U	95/1.00/U	95/1.00/U	55/0.79/U	96/1.00/88
Node c	6/U/U	16/U/U	P	P	P
Node e	P	P	P	P	P
Stomaphidini (f)	90/0.99/U	81/1.00/54	54/0.84/U	99/1.00/91	100/1.00/100
Tramini + Tuberolachnini (g)	93/1.00/76	88/1.00/U	49/0.72/U	75/0.93/U	100/1.00/100
Tramini (h)	100/1.00/96	100/1.00/94	96/0.95/85	100/1.00/93	70/0.97/85
Protrama	100/0.99/100	P	100/1.00/100	P	82/0.98/84
Trama	95/0.98/66	81/0.99/U	81/0.93/U	100/1.00/99	100/1.00/99
Tuberolachnini (i)	99/1.00/76	99/1.00/98	79/1.00/61	58/0.97/U	86/1.00/64
Nippolachnus	79/0.99/U	64/0.82/U	85/1.00/U	93/1.00/88	100/1.00/100
Pyrolachnus	100/1.00/99	100/1.00/94	–	98/1.00/96	100/1.00/97
Eulachnini (d)	4/U/U	P	P	P	P
Cinara + *Schizolachnus* (j)	32/0.89/U	P	P	56/0.87/U	P
Cinara	P	P	P	P	P
Schizolachnus	91/1.00/56	51/0.93/U	91/1.00/U	95/1.00/77	97/1.00/94
Essigella + *Eulachnus* (k)	95/1.00/U	P	82/0.99/U	87/1.00/U	94/1.00/70
Essigella	100/1.00/97	82/0.99/89	82/1.00/63	100/1.00/99	100/1.00/99
Eulachnus	96/1.00/54	37/0.62/U	58/0.85/U	95/1.00/89	100/1.00/100

P = paraphyletic, U = unresolved. Lower case letters "a" to "i" refer to nodes labelled in the figures.

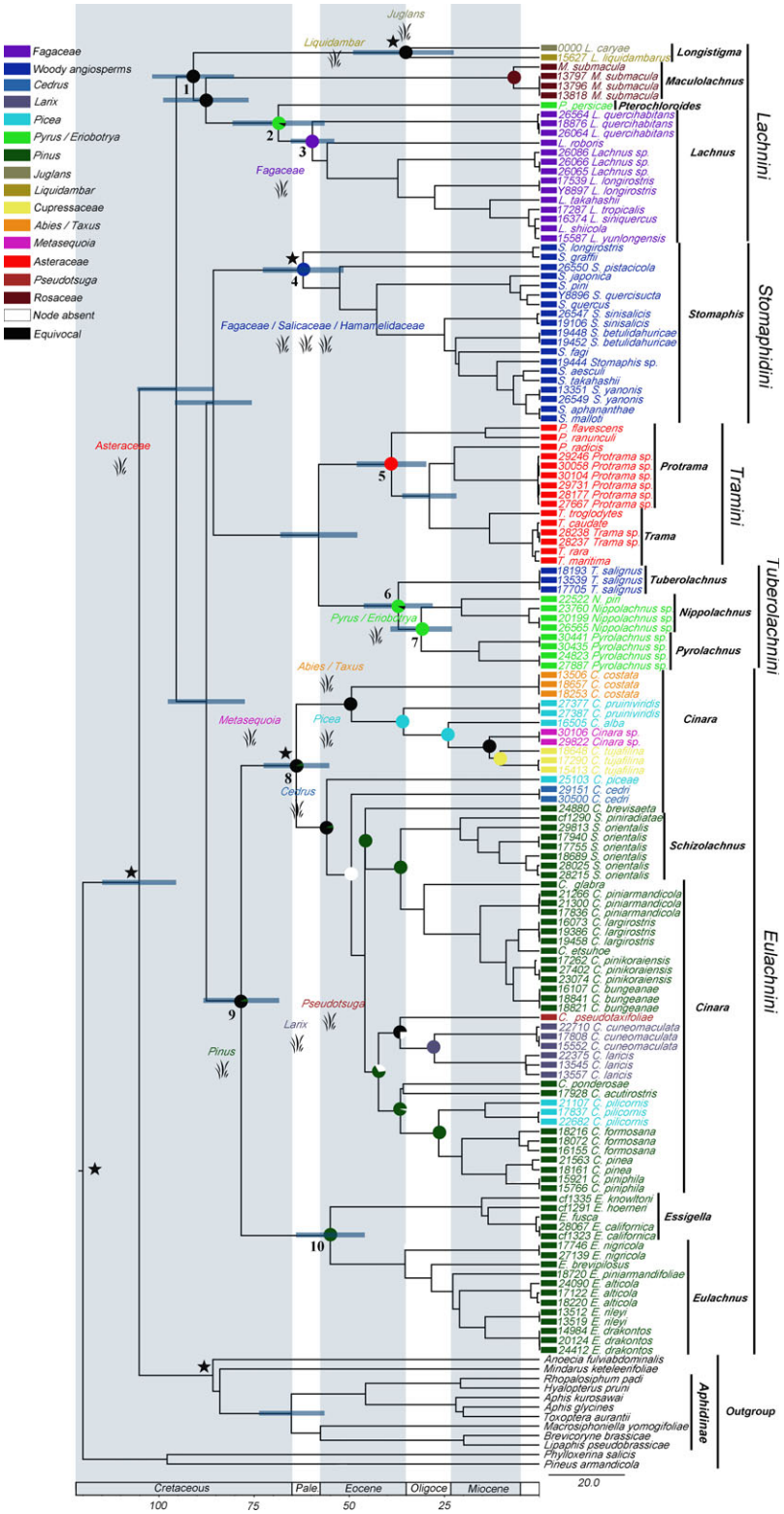

Fig. 5. Phylogram of Lachninae, time-calibrated with BEAST, with parsimony reconstruction for host association. Asterisks indicate time calibration points (see Table 3). Geological period (Cretaceous) and epochs (others) are coded in alternating grey and white. Date ranges for key nodes are presented with blue lines. Terminals are labelled aphid species, genera and tribes. Pie charts at nodes indicate the proportion of trees for which a given host association is considered the most parsimonious. Names of plant taxa are placed along the horizontal axis based on their appearance in the fossil record and along the vertical axis near the aphid groups that call them hosts.

2, PP = 0.592), *Lachnus* on Fagaceae (node 3, PP = 0.983), *Stomaphis* on angiosperm trees (node 4, PP = 0.984), *Nippolachnus* + *Pyrolachnus* on *Pyrus* or *Eriobotrya* (node 7, PP = 0.990) and *Essigella* + *Eulachnus* on *Pinus* (node 10, PP = 0.998). The results suggest there have been numerous transitions in host association within Lachninae. Parsimony reconstructions indicated at least two complete transitions from angiosperms to conifers, one in Eulachnini, the other in *Stomaphis pini* Takahashi.

Parsimony and RJ-MCMC analyses both provided strong support for a thick bark feeding site (i.e. the bark of trunks and large branches) as the ancestral condition in Lachninae (Fig. 6, PP = 0.999). At least five transitions from thick bark to other feeding sites took place during the evolution of lachnines on angiosperms: twice each in Lachnini and Tuberolachnini and once in Tramini. There have been numerous transitions in feeding sites for the sampled species within Eulachnini, with a thin bark feeding site recovered as the ancestral state for *Cinara* + *Schizolachnus*.

Discussion

Of our five analysed genes, only the *LWO* results were significantly incongruent from the others. Most relationships were broadly similar, except the Eulachnini were

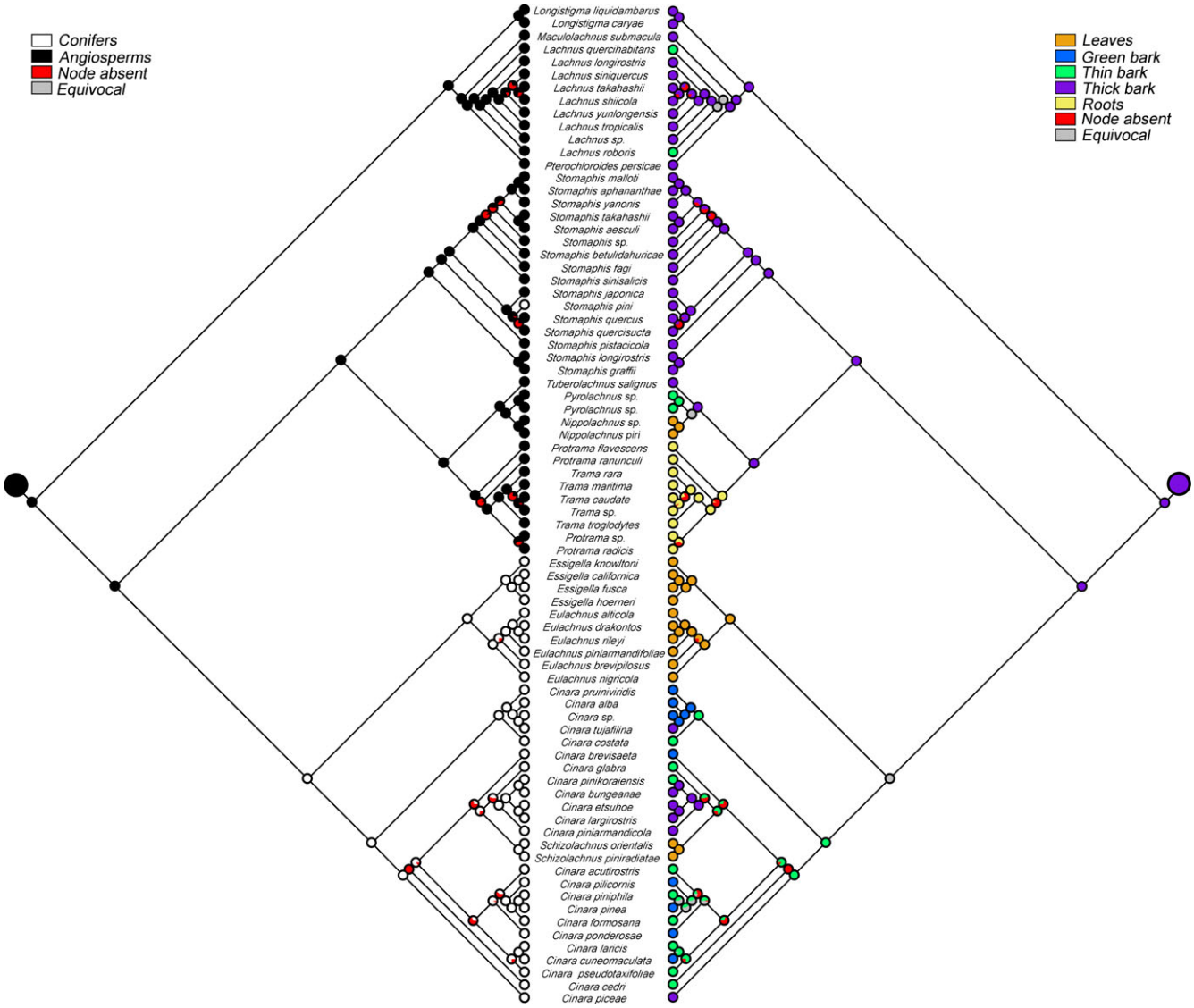

Fig. 6. Parsimony reconstruction for host association (angiosperms or conifers), left, and feeding sites (leaves, roots, or green, thin or thick bark), right, projected onto the Bayesian consensus cladogram. Small pie charts at nodes show the proportion of trees for which a given host association or feeding site is reconstructed as the most parsimonious character state. Large pie charts at root nodes show the marginal probability of ancestral states for a given host association or feeding site, as derived from RJ-MCMC reconstructions.

recovered as paraphyletic and positioned basally to the rest of the Lachninae. Ortiz-Rivas et al. (2004) also recovered a short branch for *Cinara* relative to *Lachnus* and *Eulachnus*, as well as a paraphyletic Eulachnini, although the sampling in their *LWO* analysis was limited to three lachnine species. It seems likely that *LWO* experiences a depressed rate of evolution in *Cinara*. This fact, the complete lack of branch support in our phylogram and a well-established morphological consensus for a monophyletic *Cinara* (with the exception of *Schizolachnus*) (Heie, 1988) leads us to view our *LWO* results with skepticism. However, as very few nuclear loci have been developed for aphid phylogenetics, and as our two yielded discordant results, future work should aim to acquire additional nuclear sequence data. The combined analyses will be referred to for the remainder of the discussion.

A switch to and diversification on conifers

The relationship of the Lachninae with regard to the outgroup taxa, sister to the rest of the Aphididae but more recent than the other Aphidomorpha families (i.e. Adelgidae and Phylloxeridae), is in line with previous nuclear and mitochondrial DNA studies (Von Dohlen and Moran, 2000; Ortiz-Rivas and Martínez-Torres, 2010). The basal divergence of the angiosperm-feeding species of Lachnini and the more derived position of the conifer-feeding species of Eulachnini suggest an angiosperm host for the common ancestor to all Lachninae (Fig. 6). Positing a conifer-feeding ancestor would require a minimum of three steps: a switch to angiosperm hosts in Lachnini and Stomaphidini + Tramini + Tuberolachnini and a reversion to conifers in the pine-feeding *Stomaphis* species. In contrast, positing an angiosperm-feeding ancestor would require two steps to colonize conifers, once each in Eulachnini and pine-feeding *Stomaphis*. Based on a limited taxon sampling outside *Cinara*, Meseguer et al. (2015) also suggested an angiosperm host for the ancestral Lachninae. However, they dated the lachnine crown to the Late Cretaceous, 70 Ma, whereas we placed it 25 Ma before that, in the Middle Cretaceous.

Given an ancestral angiosperm host, the increased diversification seen in Eulachnini thus correlates with a switch to conifers. Although gymnosperms arose much earlier (Middle Devonian, 365 Ma; Richardson and Rundel, 2000), fossil evidence indicates that most or all of the extant conifer families were established and diversified only from the Early Cretaceous onward. For example, *Pinus* appeared during the Early Cretaceous and the current lineages originated during the Oligocene–Miocene expansions, *Cedrus* and *Larix* appeared before the Palaeogene, and *Abies*, *Picea*, *Taxus* and *Pseudotsuga* appeared only during or recent to the Palaeogene (Miller, 1988; Serbet, 1997; Millar, 1998; Richardson and Rundel, 2000; Smith and Stockey, 2001, 2002; Stockey et al., 2005; Taylor et al., 2009; Leslie et al., 2012; Wang and Wang, 2014). Climate oscillation in the Palaeogene led to the extinction of ancient conifer species (Millar, 1993, 1998) and the diversification of modern conifer lineages (Li, 1995; Wang and Wang, 2014). The extant conifer families were thus diversifying contemporaneously with temperate angiosperm families (e.g. Juglandaceae, Betulaceae, Fagaceae). As modern conifer species were flourishing, eulachnine species experienced a concomitant diversification. Our results showed that the most recent common ancestor of Eulachnini dates to the Late Cretaceous (78.39 Ma). Species divergence mainly occurred during the Palaeogene (Fig. 5), accompanying the origin and diversification of modern conifer species. The ancestral *Cinara* is likely to have appeared during the Late Cretaceous or Palaeocene. Our results place the origin of *Cinara* further back than those of Meseguer et al. (2015), who posited an Eocene origin. Although there are important host-based clades within *Cinara* (e.g. *Schizolachnus* + its *Cinara* sister-group on *Pinus*), interspersed host-associations suggest that a significant amount of host-switching took place during the diversification of Eulachnini (Fig. 5). We did not explicitly test whether that host-switching caused speciation, however (Favret and Voegtlin, 2004; Peccoud et al., 2010; Jousselin et al., 2013).

The lachnine ancestor fed on the woody parts of its angiosperm host. Feeding on leaves is a relatively derived condition in Lachninae, found only in certain genera of Tuberolachnini and Eulachnini (*Nippolachnus*, *Schizolachnus*, *Essigella* and *Eulachnus*; Table 1, Fig. 6). Feeding on the woody parts of plants presents particular anatomical challenges to piercing and sucking phloem feeders and is thus relatively rare in aphids. To feed on bark, Lachninae have evolved the longest mouthparts among aphids, reaching their greatest lengths in the trunk-feeding *Stomaphis* (Blackman and Eastop, 1994). The three most diverse lachnine genera are those that arose and diversified on ligneous feeding sites in concert with their hosts: *Cinara* (253 spp.) on Pinaceae and Cupressaceae, *Stomaphis* (32 spp.) on several plant families and *Lachnus* (25 spp.) on Fagaceae. The Eulachnini were particularly well suited to exploit and hence diversify following a shift to their coniferous host: *Eulachnus* (24 spp.) on needles, but especially *Cinara* on ligneous feeding sites.

The plesiomorphic condition of feeding on angiosperm bark, in combination with a possible basal divergence of Lachninae among Aphididae, raises the question as to the ancestral feeding ecology for aphids in general. Some species of Adelgidae and Phylloxeridae feed on the woody parts of plants (Blackman and Eastop, 1994), as do many Coccoidea (Hardy, 2008),

the probable sister-group to the Aphidomorpha. Is it possible that the ancestral aphid fed on angiosperm bark? A broader taxon sampling, including other groups of Sternorrhyncha, will be necessary to address this question.

Host-associated evolution in other lachnine lineages

Lachnine lineages exhibit the full range of historical association with their hosts: some appeared before, some concomitant with and some after the appearance of their hosts. The principal hosts of *Lachnus* species are in the family Fagaceae, likely to have originated during the Late Cretaceous (Chmura, 1973; Jones, 1986; Crepet and Nixon, 1989; Zhou, 1993, 1999; Herendeen et al., 1995; Sims et al., 1998). Molecular dating in our study indicated that *Lachnus* had diverged from its sister genus, *Pterochloroides*, during the Late Cretaceous to Palaeogene (Fig. 5), which is coincident with the diversification of Fagaceae. Species divergence in *Longistigma* occurred during the Late Eocene to Oligocene, but the genus diverged from its Lachnini relatives in the Middle Cretaceous (Fig. 5). *Longistigma* thus probably had different ancestral and modern hosts given that the modern hosts, *Juglans* and *Liquidambar*, date to the late Eocene and Palaeogene (Kuprianova, 1960; Wolfe, 1977; Gregor, 1978; Tiffney, 1986; Stuchlik and Shatilova, 1987; Ferguson, 1989; Zhilin, 1989; Stanford et al., 2000). Extant *Maculolachnus* and *Pterochloroides* are found principally on species of Rosaceae, this plant family dating back to the Early Paleogene and having diversified during the Cenozoic (DeVore and Pigg, 2007; Töpel et al., 2012). However, these two aphid genera appear to have originated earlier, during the Late Cretaceous, and have a low extant diversity. Thus, as in *Longistigma*, it seems likely that host shifts from ancient to modern hosts and extinction events occurred in these genera.

Estimates placed the initial diversification of *Stomaphis* in and around the Palaeocene. Its main host plants, species of Fagaceae, Salicaceae and Hamamelidaceae, originated in the Palearctic dating back at least to the Palaeocene (Kuprianova, 1960; Wolfe, 1977; Gregor, 1978; Jones, 1986; Tiffney, 1986; Stuchlik and Shatilova, 1987; Crepet and Nixon, 1989; Ferguson, 1989; Zhilin, 1989; Ding, 1995). Similarly, *Pyrus* and *Eriobotrya* (Rosaceae), the main host plants for Tuberolachnini, originated in East Asia and diversified during the Palaeogene (Rubtsov, 1944; Martínez-Calvo et al., 2008), corresponding to the differentiation and distribution of their aphids. In contrast, either the older lineages of Tramini have gone extinct, or Tramini diversification followed a host shift onto an already-diversified Asteraceae. Our estimates show that species divergence in Tramini occurred recent to the Late Eocene, whereas Asteraceae, the family of Tramini hosts, originated at least in the Middle Cretaceous (Bremer and Anderberg, 1994).

Classification of the Lachninae Herrich-Schaeffer 1854

Schizolachnus rendered *Cinara* paraphyletic, in line with the phylogenetic analyses of Nováková et al. (2013) and Meseguer et al. (2015). *Schizolachnus* thus appears to represent the radiation of *Cinara* on pine needles. The largest aphid genera are extremely difficult to work with taxonomically and as a result systematists have had to deal with them on regional bases or by carving out species groups and subgenera. It is thus not uncommon to establish monophyletic subgenera, all the while recognizing that in doing so the parent genus becomes temporarily paraphyletic (e.g. Lagos et al., 2014). As such, we here transfer *Schizolachnus* to the rank of subgenus, *Cinara* (*Schizolachnus*) stat. nov.

As with other aphid subfamilies, Lachninae has experienced a number of changes in higher classification (Koch, 1854; Passerini, 1863; Buckton, 1881; Mordvilko, 1914; Baker, 1920; Takahashi, 1921; Börner, 1930, 1949, 1952; Mamontova, 1972, 2008; Normark, 2000; Heie and Wegierek, 2009). Given our phylogenetic results, we here adapt Normark's (2000) five-tribe classification, placing the lachnine genera not treated by him. The four genera not treated in our phylogenetic analysis are also placed with varying degrees of confidence. *Pseudessigella* has always been considered closely related to *Eulachnus* and *Essigella* (Hille Ris Lambers, 1966; Mamontova, 2008) and hence is placed in the Eulachnini. Likewise, *Eotrama* has strong ties to *Trama* and *Protrama* of the Tramini (Hille Ris Lambers, 1969; Czylok, 1990). *Sinolachnus* is placed in the Tuberolachnini based on its perceived proximity to *Eotrama* (Hille Ris Lambers, 1969), a member of Tramini the sister tribe to Tuberolachnini, its lack of root-feeding found in the Tramini, and its east-Asian distribution, in common with several other genera of the tribe. Lastly, *Neonippolachnus* has close ties to *Nippolachnus*, and indeed is a possible synonym of the latter (Blackman and Eastop, 2015), and hence its tentative placement in Tuberolachnini.

We do not further elaborate this hierarchical classification. First, in maintaining Normark's (2000) classification, the only other cladistic evaluation of the subfamily, we contribute to its stability. Second, despite support for Tramini + Tuberolachnini (node g, Fig. 3), we do not name it because three of the four genera missing from our analyses, *Eotrama*, *Neonippolachnus* and *Sinolachnus*, probably belong in this group. *Eotrama* was even proposed as a "missing link" between the Tramini and *Sinolachnus* (Hille Ris Lam-

bers, 1969), and thus it is not clear where each can confidently be placed. The eventual placement of these three genera may affect how we understand Tuberolachnini and Tramini. Third, Tramini species share a suite of ecological synapomorphies that make them biologically distinct (Czylok, 1990; Normark, 1999; Blackman et al., 2001). Subordinating the members of the Tuberolachnini within a "Trami_"-named taxon would contribute little to our biological understanding of the groups and would risk needlessly complicating future research.

Proposed five-tribe classification. **Lachnini** Herrich-Schaeffer 1854: *Lachnus* Burmeister 1835, *Longistigma* Wilson 1909, *Maculolachnus* Gaumont 1920, *Pterochloroides* Mordvilko, 1914; **Stomaphidini** Mordvilko, 1914: *Stomaphis* Walker 1870. **Tramini** Herrich-Schaeffer 1854: *Eotrama* Hille Ris Lambers, 1969; *Protrama* Baker, 1920; *Trama* von Heyden 1837. **Tuberolachnini** Mordvilko 1942: *Neonippolachnus* Shinji 1924, *Nippolachnus* Matsumura 1917, *Pyrolachnus* Basu & Hille Ris Lambers 1968, *Sinolachnus* Hille Ris Lambers 1956, *Tuberolachnus* Mordvilko 1909. **Eulachnini** Baker, 1920;: *Cinara* Curtis 1835, *Essigella* Del Guercio 1909, *Eulachnus* Del Guercio 1909, *Pseudessigella* Hille Ris Lambers, 1966.

Author contributions

Chen contributed fieldwork, performed most of the laboratory and analytical work, and participated in conceptual design and manuscript preparation. Favret contributed data and identified material, phylogenetic results and taxonomic interpretation, and participated in conceptual design and manuscript preparation. Jiang performed identifications and participated in conceptual design. Wang participated in analytical work. Qiao coordinated the study, contributed fieldwork, performed most of the identifications, and participated in conceptual design. All authors examined and approved the final version of the manuscript for publication.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Fig. S1. ML tree from the analyses of the Lachninae based on COI dataset. Numbers at nodes refer to ML bootstrap support/Bayesian posterior probability/MP bootstrap support. Unique identifiers for the specimens sequenced as part of this study are shown before the taxon name. Host plants of species in *Cinara* and *Schizolachnus* are shown after the taxon name.

Fig. S2. ML tree from the analyses of the Lachninae based on COII dataset. Numbers at nodes refer to ML bootstrap support/Bayesian posterior probability/MP bootstrap support. Unique identifiers for the specimens sequenced as part of this study are shown before the taxon name. Host plants of species in *Cinara* and *Schizolachnus* are shown after the taxon name.

Fig. S3. ML tree from the analyses of the Lachninae based on Cytb dataset. Numbers at nodes refer to ML bootstrap support/Bayesian posterior probability/MP bootstrap support. Unique identifiers for the specimens sequenced as part of this study are shown before the taxon name. Host plants of species in *Cinara* and *Schizolachnus* are shown after the taxon name.

Fig. S4. ML tree from the analyses of the Lachninae based on EF-1α dataset. Numbers at nodes refer to ML bootstrap support/Bayesian posterior probability/MP bootstrap support. Unique identifiers for the specimens sequenced as part of this study are shown before the taxon name. Host plants of species in *Cinara* and *Schizolachnus* are shown after the taxon name.

Fig. S5. ML tree from the analyses of the Lachninae based on LWO dataset. Numbers at nodes refer to ML bootstrap support/Bayesian posterior probability/MP bootstrap support. Unique identifiers for the specimens sequenced as part of this study are shown before the taxon name. Host plants of species in *Cinara* and *Schizolachnus* are shown after the taxon name.

Fig. S6. ML tree from the analyses of the Lachninae based on the combined dataset. The numbers at nodes refer to PhyML bootstrap support. Key nodes are labelled with letters a, b, and e–i, and are referred to in the text and tables. Terminals are labelled with identifiers for the specimens sequenced and the aphid species name. Terminals without identifiers represent taxa with GenBank data only. Clades are labelled with aphid genera and tribes (see Discussion).

Table S1. Detailed collection information and GenBank accession numbers of species used in this study.