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ORIGINAL ARTICLE

Phylogeny of *Rhus* gall aphids (Hemiptera : Pemphigidae) based on combined molecular analysis of nuclear EF1α and mitochondrial COII genes

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Abstract

Rhus gall aphids (Fordinae : Melaphidini) have a disjunct distribution in East Asia and North America and have specific host plant relationships. Some of them are of economic importance and all species form sealed galls which show great variation in shape, size, structure, and galling-site. We present a phylogeny incorporating ten species and four subspecies of *Rhus* gall aphids based on 1694 base pairs of nuclear elongation factor-1α (EF1α) and mitochondrial cytochrome oxidase subunit II (COII) DNA sequence data. The results suggest that Melaphidini is monophyletic and at the genus level, *Schlechtendalia*, *Nurudea,* and *Floraphis* were each monophyletic. *Kaburagia* and *Meitanaphis* were not monophyletic and therefore inconsistent with the current classification. The North American sumac gall aphid, *Melaphis rhois,* was most closely related to the East Asian *Floraphis* species, although this was poorly supported. The conservation of gall morphology with respect to aphid phylogeny rather than their host plants suggests that gall morphology is largely determined by the aphids. While there is no evidence of strict co-speciation between the aphids and their primary host plants, switching between recently diverged host plants may be involved in the speciation process in Melaphidini.

Key words: gall evolution, Melaphidini, molecular phylogeny, taxonomy.

INTRODUCTION

The *Rhus* gall aphids (Fordinae : Melaphidini) are a small group of insects in the Pemphigidae (Hemiptera : Aphidoidea). They have complex life cycles with cyclical parthenogenesis and multiple generations with alternative hosts. According to the current classification, *Rhus* gall aphids are divided into six genera, ten species, and four subspecies (Zhang *et al*. 1999). Each species or subspecies form sealed, sac-like galls which show great variation in shape, size, structure, and galling-site (Zhang *et al*. 2006). The galls have been used for medicine and chemical purposes for more than two thousand years because they are rich in tannins. These products are still important as traditional resources in China and as commercial exports (Zhang *et al*. 1999; Zhang *et al*. 2008). The intimate relationships between gall-forming insects and their host plants has attracted the attention of ecologists and evolutionary biologists (e.g. Inbar *et al*. 2004). Although the mechanism of gall formation remains largely unknown, it has been suggested that insects are more important than host plants for determining gall morphology (Stern 1995; Crespi & Worobey 1998). Compared with other groups of gall forming insects (Price 2005), *Rhus* gall aphids have a high diversity of gall morphologies and are therefore a good model for studying gall evolution and plant-insect co-evolution. *Rhus* gall aphids can also contribute to our knowledge of historical biogeography because of

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they have a disjunct distribution in East Asia and North America (von Dohlen *et al.* 2002).

Reconstructing phylogenetic relationships within *Rhus* gall aphids has been difficult because of a lack of a comprehensive sample of the group. Zhang and Chen (1999) analyzed the phylogeny of Pemphigidae using morphological and ecological data and found Melaphidini (Schlechtendalini) to be monophyletic, however the relationships among *Kaburagia*, *Meitanaphis,* and *Schlechtendalia* were unclear. Zhang and Qiao (2007) examined the phylogenetic relationships of three genera of *Rhus* gall aphids using nuclear EF1α and mitochondrial COI genes. Their results suggested that these three genera are monophyletic and their galls probably evolved towards a better ability to manipulate their host plant, induce strong nutrient sinks, and gain high reproductive success. Their analysis did not include the two additional Asian genera or the monotypic American genus.

The aim of this study is to reconstruct the phylogeny of Melaphidini using sequence data from the nuclear elongation factor-1α (EF1α) and mitochondrial cytochrome oxidase subunit II (COII) genes. Ten species and four subspecies were included, including the rare species, *Floraphis choui,* which produces flower-like galls, and the North American sumac aphid, *Melaphis rhois*. We discuss implications for aphid taxonomy and gall evolution.

MATERIALS AND METHODS

Gall aphid and their host plants

The *Rhus* gall aphids are composed of five Asian genera and a monotypic American genus, *Melaphis* (Moran 1989; Zhang *et al.* 1999). Each species or subspecies induces a characteristic gall on its specific primary host plant (Anacardiaceae : *Rhus*) and alternates to distinct secondary hosts (mosses) (Moran 1989; Zhang *et al.* 1999). For example, *Schlechtendalia chinensis* alternates between *Rhus chinensis* where horned galls occur and *Plagiomnium maximoviczii* for overwintering (Zhang & Zhong 1983). Nine species and four subspecies of *Rhus* gall aphids were collected in China. The North American sumac gall aphid, *Melaphis rhois*, collected from Canada, is also included. Collection data are listed in Table 1. Outgroups were chosen from the Fordini (Pemphigidae : Fordinae) which is sister to Melaphidini based on morphological and molecular data (Zhang & Qiao 2008). A representative species from Prociphilini (Pemphigidae : Pemphiginae), *Pachypappa marsupialis*, is also included as a more distant outgroup (Zhang & Qiao 2007) because Pemphiginae is sister to Fordinae (Zhang & Chen 1999). Insect samples were collected from nearly mature galls between 2004 and 2007. The galls were opened in the laboratory and aphids were transferred to 100% ethanol and stored at –20°C until DNA extraction. Vouchers of winged aphids were mounted on microscope slides in Canada balsam and deposited at the Research Institute of Resource Insects (Chinese Academy of Forestry, Kunming, China).

DNA extraction, PCR amplifications and sequencing

Total genomic DNA was extracted from groups of five to ten aphid individuals derived from the same gall and preserved in 100% ethanol. Aphids were ground with a mortar and pestle and incubated at 56°C in 600 μL lysis buffer (50 mM Tris-HCl PH = 8.0, 100 mM NaCl, 100 mM EDTA, 5% SDS) including 1 mg proteinase K (Merck, Darmstadt, Germany) for 4 h (Yang *et al.* 2006). This was followed by a standard phenol/chloroform/isoamyl alcohol (25: 24: 1) extraction (Sambrook & Russell, 1989). PCRs were performed in 50 μL reactions with 1 × PCR buffer, 2.5 mM $MgCl_2$, 0.5 mM dNTPs, 2 U *Taq* DNA polymerase (Promega, Shanghai, China) and 0.5 μM of each primer. EF1α was amplified using the published primers EF2 (ATGTGAGCAGT GTGGCAATCCAA) and EF3 (GAACGTGAACGTGG TATCAC) (Palumbi 1996). The primers 2993+ (CATT CATATTCAGAATTACC) (Stern 1994) and A3772 (GAGACCATTACTTGCTTTCAGTCATCT) (Normark 1996) were used to amplify COII. Amplification reactions were performed in a PTC-200 programmable thermal cycler (MJ Research, Watertown, MA, USA). After an initial denaturing step of 4 min at 95°C, 35 cycles were performed with a denaturing for 1 min at 94°C, an annealing step of 1 min at 51°C, primer extension for 1 min 72°C, and a final elongation step of 10 min at 72°C. The amplified products were purified and sequenced in both directions on a CEQ2000XL capillary automated sequencer (Beckman Coulter, Brea, CA, USA). Chromatograms were analyzed and assembled with the program Seqman (DNAStar Inc. 1996). For EF1α, intron splicing junctions were identified by the GT-AG rule and by comparison with the cDNA sequence of *Pachypappa marsupialis* Koch (GenBank Accession No. DQ005135). Introns were included in analyses. Sequences were aligned using CLUSTAL_X version 1.81 (Thompson *et al.* 1997) and verified by eye. All new sequences are deposited in GenBank under accession numbers EU363657 to EU363680 and FJ215685 to FJ215686 (Table 1).

Phylogenetic analysis

Uncorrected pairwise sequence differences were calculated with the program MEGA4 (Tamura *et al.* 2007).

Table 1 Information for tribe Melaphidini aphids examined in this study

Species (subspecies)	Primary host plant	Gall Shape	Cavity	Collection Locality and Data	GenBank Accession No. EF-1α / COII
Ingroup					
Floraphis meitanensis Tsai et Tang	*Rhus punjabensis* var. *sinica*	Flower-like	Multiple	Wanyuan, Sichuan, China. 30 Aug 2006	EU363669/ EU363666
Floraphis choui Xiang	*Rhus potaninii*	Flower-like	Multiple	Hanyin, Shaanxi, China. 5 Aug 2007	EU363668/ EU363665
Nurudea shiraii Matsumura	*Rhus chinensis*	Flower-like	Multiple	Eemei, Sichuan, China. 10 Sep 2004	EU363679/ AF454627†
Nurudea yanoniella (Matsumura)	*Rhus chinensis*	Flower-like	Multiple	Eemei, Sichuan, China. 17 Aug 2005	EU363680/ EU363667
Schlechtendalia chinensis (Bell)	*Rhus chinensis*	Horned	Single	Eemei, Sichuan, China. 10 Oct 2004	EU363670/ AF454628†
Schlechtendalia peitan (Tsai et Tang)	*Rhus chinensis*	Spherical	Single	Eemei, Sichuan, China. 17 Aug 2005	EU363671/ EU363658
Kaburagia rhusicola ovatirhusicola Xiang	*Rhus potaninii*	Spherical	Single	Xixiang, Shaanxi, China. 29 Jun 2005	EU363676/ EU363660
Kaburagia rhusicola ovogallis (Tsai et Tang)	*Rhus punjabensis* var. *sinica*	Spherical	Single	Eemei, Sichuan, China. 15 Jun 2005	EU363677/ EU363661
Kaburagia rhusicola rhusicola Takagi	*Rhus potaninii*	Spindle-like	Single	Eemei, Sichuan, China. 17 Jun 2005	EU363672/ EU363662
Kaburagia rhusicola ensigallis (Tsai et Tang)	*Rhus punjabensis* var. *sinica*	Spindle-like	Single	Renshou, Sichuan, China.18 Jun 2005	EU363675/ EU363659
Meitanaphis elongallis Tsai et Tang	*Rhus punjabensis* var. *sinica*	Pea-like	Single	Eemei, Sichuan, China. 15 Sep 2005	EU363678/ EU363657
Meitanaphis flavogallis Tang	*Rhus punjabensis* var. *sinica*	Pea-like	Single	Hanyin, Shaanxi, China. 8 Jul 2007	EU363673/ EU363663
Meitanaphis microgallis Xiang	*Rhus potaninii*	Pea-like	Single	Hanyin, Shaanxi, China. 8 Jul 2007	EU363674/ EU363664
Melaphis rhois Fitch	*Rhus glabra*	Spherical	Single	Canada; Ontario; Arden. 16 Sep. 1994	FJ215685/ FJ215686
Outgroup					
Pachypappa marsupialis Koch					DQ005135†/ DQ005162†
Smynthurodes betae Westwood					FM163598†/ AY227104†
Forda marginata Koch					FM163596†/ AY227098†
Baizongia pistaciae (Linnaeus)					FM163599†/ AY227093†
Aploneura lentisci (Passerini)					FM163601†/ AY227092†
Paracletus cimiciformis von Heyden					FM163597†/ AY227102†

†Retrieved from GeneBank.

Table 2 Characteristics for tRNA/COII gene and EF1a gene sequences used in this study

Gene name	Aligned Sites	A%	C%	G%	T%	Constant sites (%)	Variable sites (%)	Parsimony info. sites (%)	Ti : Tv Ratio	Mean pairwise distance with ingroup (%)	Gamma-shape parameter (α)
EF1α	1023	27.8	18.5	22.9	30.8	848 (82.9)	168 (16.4)	101 (9.9)	3.16	4.08 (0–6.4)	0.30
COII	671	39.9	12.4	7.3	40.4	506 (75.4)	165 (28.9)	110 (16.4)	2.37	8.98 (0.4–13.8)	0.11
EF1α + COII	1694	32.7	16.0	16.6	34.7	1351 (79.8)	336 (19.8)	215 (12.7)	2.04	6.11 (0.3–8.2)	0.16

Phylogenetic trees were reconstructed by Bayesian analysis (Larget & Simon 1999) using MrBayes 3.1.2 (Ronquist & Huelsenbeck 2003), and maximum parsimony (MP) and maximum likelihood (ML) analyses using PAUP*4.0 beta (Swofford 2002). Bayesian analyses started with randomly generated trees and four Markov chains under default heating values and were run for two million generations with sampling at intervals of 100 generations. A branch-and-bound search strategy was employed in MP analysis with all characters treated as equal weights and with gaps treated as missing data. To ensure that the analyses were not trapped on local optima, the data set was run three times independently. For Bayesian and ML analyses, the most appropriate nucleotide substitution model was evaluated using hierarchical likelihood ratio tests (hLRT) using ModelTest 3.06 (Posada & Crandall, 1998). The reliability of the topologies was evaluated using bootstrap support (Felsenstein 1985) with 1000 replicates for MP and 500 for ML. A partition homogeneity test (Farris *et al.* 1995) with 1000 replicates was performed in PAUP* to test for incongruence between COII and EF1α prior to conducting combined analyses.

RESULTS

The COII data set consisted of 671 aligned characters; of which 506 (75.4%) were constant, 165 (28.9%) were variable, and 110 (16.4%) were parsimony informative. This region was highly A+T rich agreeing with previous studies (Simon *et al.* 1994; von Dohlen *et al.* 2002), averaging 80.3% A+T across the 14 ingroup sequences. The EF1α data set consisted of 1023 aligned characters; of which 848 (82.9%) were constant, 168 (16.4%) were variable, and 101 (9.9%) were parsimony informative. The base composition of the EF1α coding regions had an average base composition of 27.8% A, 18.5% C, 22.9% G and 30.8% T. The mean transition to transversion ratio (ti/tv ratio) was 2.37 for COII and 3.16 for EF1α (Table 2).

Mean pairwise sequence divergences among the 14 ingroup taxa was 8.98% for COII (range 0.40–13.80%), 4.08% for EF1α (range 0.00–6.40%), and 6.11% for the combined data set (range 0.30–8.20%) (Table 2).

The partition homogeneity test did not indicate significant conflict in phylogenetic signal between the COII and EF1α data sets ($P = 0.52$) allowing a total of 1694 bp characters to be combined for tree reconstruction. Trees resulting from analysis of COII and EF1α separately had similar topologies. Analyses using the combined data resulted in improved resolution and nodal support than either gene alone, especially regarding relationships among genera. The 50% majority rule consensus tree resulting from Bayesian analysis of the combined data set is shown in Figure 1. Melaphidini formed a monophyletic group with robust support. Species from *Nurudea, Floraphis*, and *Schlechtendalia* each clustered into clades with well-supported values that correspond to the current taxonomy based on aphid morphology, host plant use, and gall shape. *Meitanaphis* and *Kaburagia* were not monophyletic, but members of both genera formed a single clade with *M. elongallis* sister to the remaining species. The North American sumac gall aphid, *Melaphis rhois* was sister to *Floraphis* but with low support.

Maximum parsimony analysis of the combined data set produced two most parsimonious trees (Tree length = 1329 steps, Retention Index = 0.64, Consistency Index = 0.64) with shared topology, except for the positions of *Kaburagia rhusicola ovogallis* and *Meitanaphis microgallis*. The ML analysis employed a GTR model of nucleotide substitution as selected by hierarchical likelihood ratio tests (hLRTs). The ML and MP analyses resolved fewer clades but otherwise agreed with the topology of the Bayesian tree.

DISCUSSION

Systematics implications of phylogeny

Our analysis suggests that Melaphidini is monophyletic. Species of *Nurudea, Floraphis*, and *Schlechtendalia* each clustered into groups consistent with the traditional classification (Zhang & Chen 1999). Remaudiere and

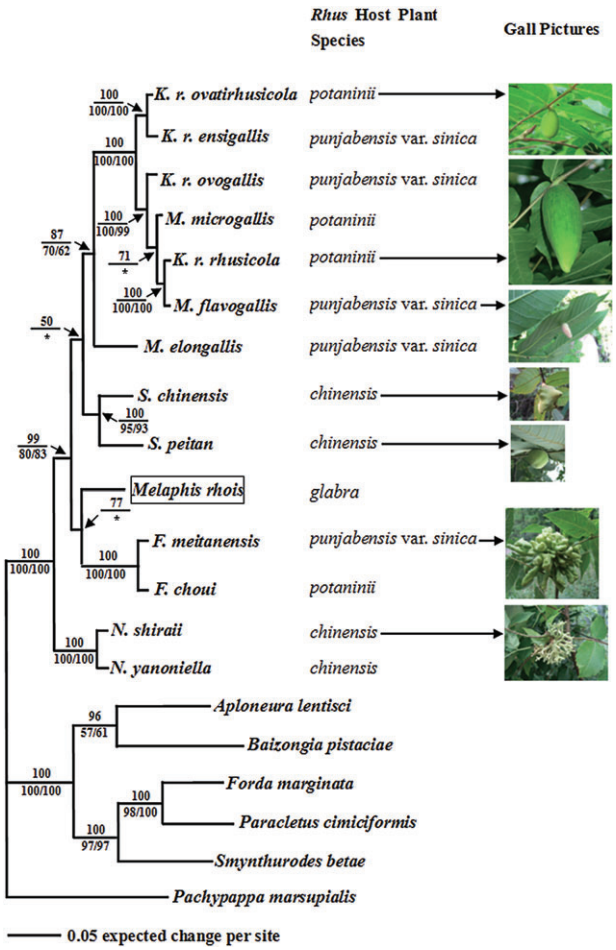

Figure 1 Bayesian 50% consensus tree of Melaphidini based on a combined data set of COII and EF1α DNA sequences. Posterior probabilities are above and maximum parsimony and maximum likelihood bootstrap values >50% are below branches. An asterisk indicates a bootstrap value less than 50%. Gall pictures go with select species with arrows are shown on the right. Absolute scaling among photos was not maintained.

Remaudiere (1997) suggested that *Nurudea* and *Floraphis*, and *Schlechtendalia* and *Meitanaphis* should be synonymised. These changes were not supported by our results. *Nurudea* and *Floraphis* formed a single clade with weak support based on COII analysis alone, but this was not the case for EF1α and the combined data set. These two genera form similar flower-like galls, but *Nurudea* uses *R. chinensis* as its primary host, and *Floraphis* uses *R. punjabensis* var. *sinica* and *R. potaninii*. Furthermore, *Nurudea* has five antennal segments and short pterostigmas, while *Floraphis* has six antennal segments and extended pterostigmas (Yang *et al*. 2009). Likewise, *Kaburagia* and *Meitanaphis* did not form a monophyletic group. *Meitanaphis flavogallis* and *M. microgallis* nested within *Kaburagia*. *Meitanaphis flavogallis* and *M. microgallis* share many characteristics with *Kaburagia* such as antennal structure, host selection, and gall shape, so it seems reasonable to include them in *Kaburagia*. According to the results of Zhang and Qiao (2007), *Meitanaphis elongallis* is sister to a clade consisting of *Kaburagia* and *Schlechtendalia*. In contract, our results show that *M. elongallis* is sister to *Kaburagia* plus all the remaining species of *Meitanaphis*. *Meitanaphis elongallis* shows distinct differences in the ultrastructure of antennal sensilla to *Kaburagia* and the other two species of *Meitanaphis* (Yang *et al*. 2009). This suggests that retaining *M. elongallis* in the genus *Meitanaphis* may be appropriate.

Another of our results that contrasts with historic views is the position of the North American species, *Melaphis rhois*. Baker (1920) placed it in the East Asian genus *Schlechtendalia*. Tsai and Tang (1946) speculated further that it may be synonymous with *Schlechtendalia peitan* based on their similar morphology and gall types. *Melaphis rhois* did not cluster with *Schlechtendalia* as expected, rather it was sister to *Floraphis* with weak support. The phylogenetic position of *M. rhois* is therefore still not clear and may require additional data.

Gall evolution and host association

Galls can be considered extended phenotypes of the insects that induce them (Stern 1995; Stone *et al*. 2002; Kurosu *et al*. 2008). Studies of other gall-forming insects have suggested a close linkage between insect phylogeny and gall morphology (Stern 1995; Crespi *et al*. 1998; Stone & Cook 1998; Nyman *et al*. 2000). For example, Stern (1995) suggested that gall morphology is determined more by the aphids than the host plants in the Cerataphidini because gall morphology was more conserved with respect to aphid phylogeny than plant taxonomy as shown by the existence of host plant switches where the aphids retained their ancestral gall morphology.

Although aphid galls have diverse shapes and sizes, they can be divided into two major groups: single-cavity and multiple-cavity. Within the Cerataphidini, multiple-cavity galls probably had a single origin and were derived from single-cavity galls (Fukatsu *et al*. 1994; Stern 1995). In our molecular phylogeny, the four species forming multiple-cavity galls were not monophyletic. The placement of *M. rhois*, which forms single-cavity galls, was also not well-resolved and the remaining species that form single-cavity galls cluster into a separate group with weak support values. Therefore, whether there was a single origin of multiple-cavity galls in Melaphidini is still not clear.

As with Cerataphidini, the conservation of gall morphology with respect to aphid phylogeny rather than their host plants suggests that gall morphology is largely determined by the aphids. Each primary host plant species supports both single-cavity and multiple-cavity galls, except *R. glabra* which only has single-cavity galls (Table 1). *Rhus chinensis*, *R. potaninii,* and *R. punjabensis* var. *sinica* each map onto disparate branches of the aphid phylogeny with single-cavity and multiple-cavity galls. This suggests multiple host switches by the aphids but conservation of gall morphology across host plants.

On the other hand, aphid species within Pemphigidae are remarkably specific to their primary host plants which makes host use an important character for taxonomic division (Zhang & Chen 1999). While there is no evidence of a strict co-speciation between the aphids and their primary host plants, is may be more likely for aphids to switch between hosts that are more recently diverged than those that are not closely related. All of the primary hosts of *Rhus* gall aphids are in the subgenus *Rhus*. *Rhus potaninii* and *R. punjabensis* var. *sinica* are closely related sister species that are part of an Asian/Hawaiian clade along with *R. chinensis*, while *R. glabra* is part of a separate North American clade (Yi *et al*. 2007). *Rhus potaninii* and *R. punjabensis* var. *sinica* also share a very similar morphology that can hardly be distinguished by external appearance. Four aphid taxa that have *R. punjabensis* var. *sinica* as a host and three that have *R. potaninii* as a host form a recently derived clade of closely related species. This suggests that host switching between recently diverged host plants may be involved in the speciation process in Melaphidini.

Conclusions and future directions

Like most taxa of aphids, the historic classifications of Melaphidini are inconsistent because of the reduced and convergent nature of aphid morphology (Remaudiere & Remaudiere 1997; Zhang *et al*. 1999). The generic divisions by Zhang *et al*. (1999) are mostly supported by our molecular analysis, but the relationships among genera, the phylogenetic position of *M. rhois*, and the identity of *Kaburagia* and *Meitanaphis* are still unclear. Additional DNA sequence data combined with morphological and ecological data are needed to further clarify phylogenetic relationships of Melaphidini, and the taxonomy of the group may need to be revisited in future studies.

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REFERENCES

Baker AC (1920) Generic classification of the hemipterous family Aphididae. *United States Department of Agriculture Bulletin* **826,** 1–109.

Crespi B, Worobey M (1998) Comparative analysis of gall morphology in Australian gall thrips: the evolution of extended phenotypes. *Evolution* **52,** 1686–1696.

Crespi B, Carmean DA, Mound LA, Worobey M, Morris D (1998) Phylogenetics of social behavior in Australian gall-forming thrips: evidence from mitochondrial DNA sequence adult morphology and behavior, and gall morphology. *Molecular Phylogenetics and Evolution* **9,** 163–180.

Farris JS, Kallersjo M, Kluge AG, Bult C (1995) Constructing a significance test for incongruence. *Systematic Biology* **44,** 570–572.

Felsenstein J (1985) Phylogenies and the comparative method. *The American Naturalist* **125,** 1–15.

Fukatsu T, Aoki S, Kurosu U, Ishikawa H (1994) Phylogeny of Cerataphidini aphids revealed by their symbiotic microorganisms and basic structure of their galls: implications for host-symbiont coevolution and evolution of sterile soldier castes. *Zoological Science* **11,** 613–623.

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.

Kurosu U, Kutsukake M, Aoki S, Wang C (2008) Galls of *Cerataphis bambusifoliae* (Hemiptera, Aphididae) found on *Styrax suberifolius* in Taiwan. *Zoological Studies* **47,** 191–199.

Larget B, Simon DL (1999) Markov chain monte carlo algorithms for the Bayesian analysis of phylogenetic trees. *Molecular Biology and Evolution* **16,** 750–759.

Moran NA (1989) A 48-million-year-old aphid-host plant association and complex life cycle: biogeographic evidence. *Science* **245,** 173–175.

Normark BB (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.

Nyman T, Widmer A, Roininen H (2000) Evolution of gall morphology and host-plant relationships in willow-feeding sawflies (Hymenoptera : Tenthredinidae). *Evolution* **54,** 526–533.

Palumbi SR (1996) Nucleic acids II: the polymerase chain reaction. *Molecular Systematics* **2,** 205–247.

Posada D, Crandall KA (1998) MODELTEST: testing the model of DNA substitution (version 3.06). *Bioinformatics* **14,** 817–818.

Price PW (2005) Adaptive radiation of gall-inducing insects. *Basic and Applied. Ecology* **6,** 413–421.

Remaudiere G, Remaudiere M (1997) *Catalogue Des Aphididae Du Monde*. INRA, Paris.

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

Sambrook J, Russell DW (1989) *Molecular Cloning: A Laboratory Manual*. **1**. Cold Spring Harbor Laboratory Press, New York.

Simon C, Frati F, Beckenbach A, Crespi B, Liu H, Flook P (1994) Evolution, weighting, and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Annals of the Entomological Society of America* **87,** 651–701.

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

Stern DL (1995) Phylogenetic evidence that aphids, rather than plants, determine gall morphology. *Proceedings of the Royal Society of London Series B-Biological Sciences* **260,** 85–89.

Stone GN, Cook JM (1998) The structure of cynipid oak galls: patterns in the evolution of an extended phenotype. *Proceedings of the Royal Society of London Series B-Biological Sciences* **265,** 979–979.

Stone GN, Schonrogge K, Atkinson RJ, Bellido D (2002) The population biology of oak gall wasps (Hymenoptera-: Cynipidae). *Annual Review of Entomology* **46,** 633–668.

Swofford DL (2002) *PAUP*: Phylogenetic Analysis Using Parsimony (* and Other Methods) 4.0 Beta*. Sinauer Associates, Sunderland, USA.

Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution* **24,** 1596–1599.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG (1997) The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **24,** 4876–4882.

Tsai PH, Tang C (1946) The classification of the Chinese gall aphids with description of three genera and six species from Meitan, Kweichow. *Transactions of the Royal Entomological Society of London* **97,** 405–418.

von Dohlen CD, 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.

Yang ZX, Chen XM, Feng Y, Miao YC (2006) Method improvement for extraction of genomic DNA from aphids. *Chinese Bulletin of Entomology* **43,** 880–884. (In Chinese.).

Yang ZX, Chen XM, Ying F, Hang C (2009) Morphology of the antennal sensilla of *Rhus* gall aphids (Hemiptera : Aphidoidea : Pemphiginae): a comparative analysis of five genera. *Zootaxa* **2204,** 48–54.

Yi TS, Miller AJ, Wen J (2007) Phylogeny of *Rhus* (Anacardiaceae) based on sequences of nuclear Nia-i3 intron and chloroplast trnC-trnD. *Systematic Botany* **32,** 379–391.

Zhang CX, Tang XD, Cheng JA (2008) The utilization and industrialization of insect resources in China. *Entomological Research* **38,** S38–S47.

Zhang GX, Zhong TS (1983) *Economic Insect Fauna of China. 25 Homoptera: Aphidinea*. Science Press, Beijing China. (In Chinese.)

Zhang GX, Chen XL (1999) Study on the phylogeny of Pemphigidae (Homoptera : Aphidinea). *Acta Entomologica Sinica* **42,** 176–183. (In Chinese.)

Zhang GX, Qiao GX, Zhong TS, Zhang WY (1999) *Fauna Sinica Insecta Vol. 14 Homoptera: Mindaridae and Pemphigidae*. Science Press, Beijing China. (In Chinese.)

Zhang HC, Qiao GX (2007) Molecular phylogeny of Fordini (Hemiptera : Aphididae : Pemphiginae) inferred from nuclear gene EF-1 α and mitochondrial gene COI. *Bulletin of Entomological Research* **97,** 379–386.

Zhang HC, Qiao GX (2008) Molecular phylogeny of Pemphiginae (Hemiptera : Aphididae) inferred from nuclear gene EF-1α sequences. *Bulletin of Entomological Research* **98,** 499–507.

Zhang HC, Qiao GX, Zhang GX (2006) A Study on diversity of aphid's galls in Pemphigidae. *Acta Zootaxonomica Sinica* **31,** 48–54. (In Chinese.)