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Molecular phylogeny of Pemphiginae (Hemiptera: Aphididae) inferred from nuclear gene EF-1α sequences

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Abstract

Three traditional tribes of Fordini, Pemphigini and Eriosomatini comprise Pemphiginae, and there are two subtribes in Fordini and Pemphigini, respectively. Most of the species in this subfamily live heteroecious holocyclic lives with distinct primary host specificity. The three tribes of Pemphigini (except Prociphilina), Eriosomatini and Fordini use three families of plants, Salicaceae (*Populus*), Ulmaceae (*Ulums*) and Anacardiaceae (*Pistacia* and *Rhus*), as primary hosts, respectively, and form galls on them. Therefore, the Pemphigids are well known as gall makers, and their galls can be divided into true galls and pseudo-galls in type. We performed the first molecular phylogenetic study of Pemphiginae based on molecular data (EF-1α sequences). Results show that Pemphiginae is probably not a monophylum, but the monophyly of Fordini is supported robustly. The monophyly of Pemphigini is not supported, and two subtribes in it, Pemphigina and Prociphilina, are suggested to be raised to tribal level, equal with Fordini and Eriosomatini. The molecular phylogenetic analysis does not show definite relationships among the four tribes of Pemphiginae, as in the previous phylogenetic study based on morphology. It seems that the four tribes radiated at nearly the same time and then evolved independently. Based on this, we can speculate that galls originated independently four times in the four tribes, and there is no evidence to support that true galls are preceded by pseudo-galls, as in the case of thrips and willow sawflies.

Keywords: Pemphiginae, molecular phylogeny, EF-1α, tribe, radiation, gall

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Introduction

Pemphiginae (*sensu* Remaudière & Remaudière, 1997; Pemphigidae *sensu* Heie, 1980) are composed of three tribes: Pemphigini, Eriosomatini and Fordini. According to their primary host association and some important morphological characters, Fordini was further divided into Melaphidina and Fordina, and Pemphigini into Pemphigina and Prociphilina. Pemphigids are mostly distributed in the Holarctic and Oriental regions, represented by about 310 species in the world (Remaudière & Remaudière, 1997).

The life histories of aphids in this subfamily are complicated, including holocyclic and anholocyclic types. Anholocyclic species lose their primary hosts and are parthenogenetic on secondary host all the year round. However, most of the species are heteroeciously holocyclic with distinct primary host specificity. The three tribes of Pemphigini, Eriosomatini and Fordini use three families of plants, Salicaceae (*Populus*), Ulmaceae (*Ulums*) and Anacardiaceae (*Pistacia* and *Rhus*), as their primary hosts. In a typical life history, apterous fundatrices produce second and third generation alatae (fundatrigeniae) (on Anacardiaceae, Salicaceae, Ulmaceae), which migrate (virginoparae) to the

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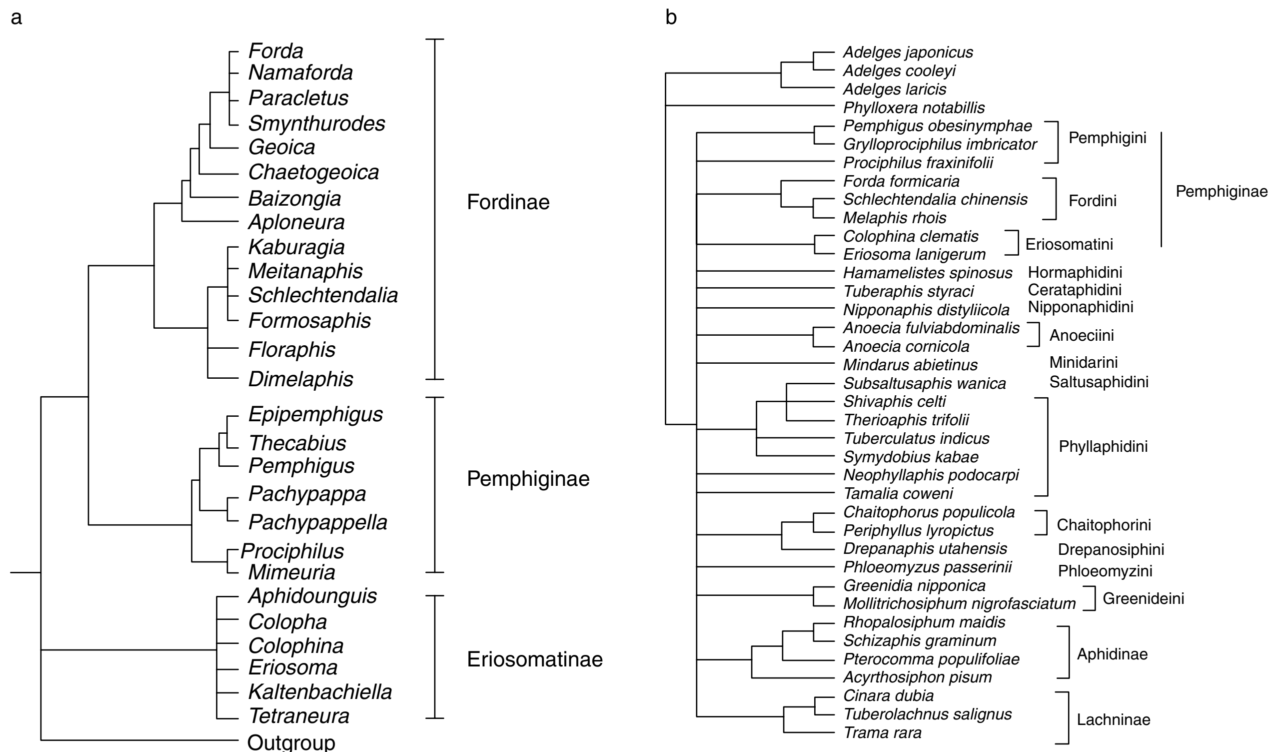

Fig. 1. (a) Phylogeny of Pemphiginae based on morphology (Pemphiginae was regarded as family Pemphigidae; Zhang & Chen, 1999); (b) Phylogenetic relationships of Aphididae inferred from the mitochondrial ribosomal DNA (partial 12S and 16S) sequence (von Dohlen and Moran, 2000).

roots of secondary hosts (Graminae, Dicots and Conifers) and produce apterae exules. The apterous generations continue for sometime, and later alate sexuparae migrate back to the primary host and produce small arostrate apterous males and apterous oviparous females. These, after copulation, produce an egg. The entire cycle, in Pemphigini and Eriosomatini, is annual, while, in Fordini, the cycle takes two years to be completed (Ghosh, 1984).

The forming galls or not, and the position, morphology and structure of galls are an extended phenotype of aphids, and are helpful for species identification and diagnosis (Zhang *et al*., 2006). Most of the Pemphigids can produce galls on their primary hosts. Their galls may be: (i) simple pseudo-leaf gall, formed by rolling along the length of leaf or sac-like, pear-like irregular, hairy or smooth (Eriosomatini); (ii) pocket-gall or pyriform, vesicular closed galls or spiral galls (Pemphigini); or (iii) bag-like galls of elongated cylindrical horn-like structure formed on a single leaflet, irregular spherical gall on the underside of leaflet blade or bizarrely shaped galls arising from resting buds (Fordini). The galls are believed to provide the specific nutrient tissue for the growing larva (Stone & Schönrogge, 2003; Inbar *et al*., 2004), but the origin and evolution of galls in the whole subfamily hitherto have not been discussed.

Phylogenetic relationships of Pemphiginae have been proposed, based on morphological and ecological characters, by Zhang & Chen (1999) (fig. 1a). Pemphiginae was raised to the family Pemphigidae in their analysis. The monophyly of traditional Fordini and Pemphigini was supported, while Eriosomatini were found to be a paraphyletic group. Moreover, Fordini and Pemphigini had closer relationships with each other than with Eriosomatini. However, until now, there has been no molecular phylogenetic study carried out on Pemphiginae. The relationships among these three tribes of Pemphiginae were outlined coarsely, only in the molecular phylogenetic inference of Aphididae, because of sparse sampling. von Dohlen & Moran (2000) reconstructed the phylogeny of aphids based on the mitochondrial 12S and 16S rDNA (fig. 1b). Except for the monophyly of two subfamilies, Aphidinae and Lachninae, there was little well-supported phylogenetic structure at levels deeper than tribes. Three tribes of Pemphiginae were parallel branches in the tree topology. Phylogenetic results of Ortiz-Rivas *et al*. (2004), based on nuclear gene long-wavelength opsin (LWO), suggested some new insights into aphid phylogeny, but the monophyly of Pemphiginae still was not supported. So, a relatively thorough molecular phylogenetic analysis needs to be constructed, and the phylogenetic relationship of Pemphiginae needs to be tested.

Nuclear gene elongation factor-1alpha (EF-1α) has been extensively used in phylogenetic analyses of aphids and has been proven useful in producing reliable phylogenetic relationships at genus level and above (see e.g. Normark, 2000; Rokas *et al*., 2002; von Dohlen & Teulon, 2003; von Dohlen *et al*., 2006; Zhang & Qiao, 2006, 2007a,b). The purposes of this study are to: (i) reconstruct the phylogeny of Pemphiginae; (ii) revise the taxonomic system of Pemphiginae; and (iii) discuss the origin and evolution of galls in Pemphiginae in the context of its molecular phylogeny.

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Materials and methods

Taxon sampling

Taxa examined in this study include representatives of three historically recognized lineages, Eriosomatini, Pemphigini and Fordini. Outgroups were chosen from Hormaphidinae (Aphididae), Adelgidae and Phylloxeridae because Hormaphidinae is the sister group of Pemphiginae (Wojciechowski, 1992; Zhang *et al*., 1999; Ortiz-Rivas *et al*., 2004), and Adelgidae and Phylloxeridae are the most ancient aphidine lineages (Heie, 1987; Wojciechowski, 1992; Zhang *et al*., 1999; Ortiz-Rivas *et al*., 2004) used here to root the tree. Information on each species and the GenBank accession numbers are listed in table 1.

DNA extraction, PCR, and sequencing

Aphids were collected into 95% or 100% ethanol for DNA extraction. Voucher specimens were collected in 75% ethanol and deposited in the Zoological Museum of the Institute of Zoology, Chinese Academy of Sciences, Beijing. Samples for extraction consisted of individuals from the same colony. Tissue homogenates were incubated at 55°C in lysis buffer (30 mM Tris-HCl (pH 8.0), 200 mM EDTA, 50 mM NaCl, 1%SDS, and 100 $\mu g\ ml^{-1}$ Proteinase K) for 5–7 h, followed by a standard phenol-chloroform-isoamylalcohol (PCI) extraction with little improvement (Sambrook *et al*., 1989). DNA was precipitated from the supernatant with two volumes of cold ethanol, centrifuged, washed, dried and dissolved in 15–20 µl TE buffer, then stored at −20°C until used.

We used primers EF3 (5′-GAA CGT GAA CGT GGT ATC AC-3′) and EF2 (5′- ATG TGA GCA GTG TGG CAA TCC AA-3′) (Palumbi, 1996) or EF6 (5′-TGA CCA GGG TGG TTC AAT AC-3′: von Dohlen *et al*., 2002) to amplify a portion of EF-1α.; PCRs were performed in a total volume of 50 µl and contained 5 µl 10 × PCR buffer, 1.25 U *Taq* DNA polymerase, 200 µM dNTPs (Takara Biosystems, Dalian, China), 0.2 µM primers (Sangon Biotech, Shanghai, China) in a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). Amplification was implemented with denaturing at 95°C for 5 min, 35 cycles of denaturing at 94°C for 1 min, annealing at 49–51°C for 1 min, and extension at 72°C for 1 min, followed by the final extension at 72°C for 10 min.

Sequencing reactions were performed with the corresponding amplifying primers from both directions with BigDye Terminator Cycle Sequencing Kit v. 2.0 (Applied Biosystems, Foster City, CA, USA) and run with ABI 3730 automated sequencer (Applied Biosystems).

Alignment and sequence properties

Chromatograms, including sense and antisense, were edited and assembled using DNASTAR 5.0 (DNASTAR, Madison, Wisconsin, USA, Inc.) to obtain single consensus sequences. Intron splicing junctions were then identified by the GT-AG rule and by comparison with the cDNA sequence of *Schizaphis graminum* (Rondani) (GeneBank accession number AF068479). Introns were removed prior to the phylogenetic analysis (von Dohlen *et al*., 2006). The nucleotide sequences were translated into amino acid sequences to check for the presence of stop codons that might indicate that pseudogenes had been amplified (Sanders *et al*., 2006). Multiple alignments were done with Clustal_X (Thompson *et al*., 1997) and verified by eye.

Aligned sequence data were imported into MEGA3.1 (Kumar *et al*., 2004) for analyses of nucleotide composition. Nucleotide saturation was analyzed by plotting the number of transitions and transversions on each codon position against the Tamura & Nei (1993) (TN93) genetic distance using DAMBE (Xia & Xie, 2001). Saturation was considered to have occurred if the scatter of points showed leveling off mutations as sequence divergence increased.

Phylogenetic analysis

All phylogenetic analyses were performed with PAUP*4.0b10 (Swofford, 2003) and MrBayes 3.1.1 (Ronquist & Huelsenbeck, 2003). A maximum parsimony (MP) analysis was carried out first, with all sites weighted equally, gaps treated as missing data and 1000 random addition sequences and tree bisection reconnection (TBR) branch swapping. The command of 'contree' was used to yield the strict consensus tree. To assess the support for branching events, non-parametric bootstrapping was performed with 1000 pseudo-replicates under the heuristic search strategy and 100 random addition sequences in each pseudo-replicate. A node was interpreted as strongly supported if the bootstrap percentage (BP) was ≥70% (Hillis & Bull, 1993).

ModelTest 3.06 (Posada & Crandall, 1998) was used to select the best-fit nucleotide substitution model under the criterion hLRTs for maximum likelihood (ML) analysis. ML analysis was performed in PAUP* with the selected optimal model under the heuristic search strategy with ten random addition sequences and TBR branch swapping. Bootstrap analysis was performed under the same model, with 100 pseudo-replicates, ten random addition sequences per replicate and TBR branch swapping.

Bayesian analysis was conducted using MrBayes3.1.1, based on the model selected by ModelTest3.06. Model parameter values were treated as unknown variables with uniform prior probabilities and were estimated during the analysis. Four chains (three heated and one cold) were run, starting from a random tree and proceeding for 1,000,000 Markov chain Monte Carlo generations, sampling the chains every 200 generations. Two independent runs were conducted to verify results. For all runs, 1000 trees were discarded as burn-in samples. Remaining trees were used to generate a majority-rule consensus tree, in which the percentage of trees recovering a clade portrayed the clade's posterior probability (PP) (Huelsenbeck *et al*., 2001) or the probability that the clade is correct, given the data and the model parameters. Probabilities ≥95% were considered indicative of significant support (Reeder, 2003; Zkharov *et al*., 2004).

Results

Sequences characteristics and saturation analysis

For all the taxa, excluding five downloaded sequences, approximately 1100 bp were sequenced for EF-1α. All sequences were submitted to GenBank (see table 1 for accession numbers). Except for the introns, the exons were assembled into a 726 bp sequence, yielding a data set of 37 sequences used for phylogenetic analysis. Of a total of 726 characters, 498 sites are conserved, 228 variable and 187 parsimony-informative (531 sites are constant, 195 variable and 154 parsimony-informative for ingroups only), and average base frequencies are well proportioned with

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Table 1. Information regarding species examined in this study.

Higher taxon	Species	Collection Locality	Collection Date	Host Plant	GenBank Accession No: (EF-1α)
Adelgidae	*Adelges laricis* Vallot	China: Tibet (Seqila)	02–07–2002	*Larix* sp.	DQ493827
	Adelges sp.	China: Heilongjiang (Fujin)	06–08–2005	*Picea* sp.	EF418793
Phylloxeridae	*Moritziella castaneivora* Miyazaki	China: Shandong (Rizhao)	29–08–2005	*Castanea crenata*	EF418795
Aphididae: Hormaphidinae	*Hormaphis betulae* (Mordvilko)	China:Jilin (Ji'an)	13–08–2004	*Hamamelis japonica*	DQ493864
	Hormaphis similibetulae Qiao et Zhang	China: Tibet (Linzhi)	05–07–2002	*Betula albo-sinensis*	DQ493849
	Hamamelistes betulinus (Horvath)	Japan: Tokyo, Okutamako	20–05–1999	*Hamamelis japonica*	AF454597*
	Nipponaphis distyliicola Monzen	Japan: Shinkiba, Tokyo	16–04–1999	*Quercus glauca*	AF454614*
	Thoracaphis quercifoliae Ghosh	China: Fujian (Wuyi Mountain)	20–07–2003	*Cinnamomum camphora*	DQ493851
Pemphiginae: Fordini	*Chaetogeoica* sp.	China: Shaanxi (Qishan)	14–07–2004	*Pistacia* sp.	EF418794
	Slavum wertheimae Hille Ris Lambers	Israel: Oranim	01–10–2005	*Pistacia atlantica*	DQ499616
	Aploneura lentisci (Passerini)	Israel: Oranim	01–10–2005	*Pistacia lentiscus*	DQ499605
	Baizongia pistacia (Linnaeus)	Israel: Oranim	01–10–2005	*Pistacia palaestina*	DQ499606
	Geoica wertheimae Brown et Blackman	Israel: Oranim	01–10–2005	*Pistacia palaestina*	DQ499610
	Forda marginata Koch	Israel: Oranim	01–10–2005	*Pistacia palaestina*	DQ499609
	Forda formicaria von Heyden	Israel: Oranim	01–10–2005	*Pistacia palaestina*	DQ499608
	Meitanaphis elongallis Tsai et Tang	China: Sichuan (Emei Mountain)	07–09–2005	*Rhus punjabensis* var.*sinica*	DQ499618
	Kaburagia rhusicola ovatirhusicola Xiang	China: Sichuan (Emei Mountain)	27–06–2004	*Rhus potaninii*	DQ499612
	Kaburagia rhusicola rhusicola Takagi	China: Sichuan (Emei Mountain)	27–06–2004	*Rhus potaninii*	DQ499614
	Kaburagia rhusicola ensigallis (Tsai et Tang)	China: Sichuan (Emei Mountain)	27–06–2004	*Rhus chinensis*	DQ499611
	Kaburagia rhusicola ovogallis (Tsei et Tang)	China: Sichuan (Emei Mountain)	16–06–2005	*Rhus javanica*	DQ499613
	Schlechtendalia peitan (Tsai et Tang)	China: Sichuan (Emei Mountain)	25–08–2004	*Rhus chinensis*	DQ499615
	Schlechtendalia chinensis (Bell)	China: Sichuan (Emei Mountain)	29–09–2004	*Rhus javanica*	DQ499619
Pemphigini	*Formosaphis micheliae* Takahashi	Japan: Ise. Mie Preb.	19–08–2005	*Michelia longifolia*	DQ779152
	Pachypappa marsupialis (Koch)	Japan: Moshiri, Hokkaido	27–07–1999	*Populus maximowiczii*	DQ005135*
	Epipemphigus niisimae (Matsumura)	China: Tibet (Baiba)	01–07–2002	*Populus* sp.	DQ779151
	Thecabius (*Thecabius*) *beijingensis* Zhang	China: Heilongjiang (Mohe)	31–07–2004	*Populus* sp.	DQ499617
	Pemphigus borealis Tullgren	China: Tibet (Nyingchi)	06–08–2003	*Populus* sp.	DQ779153
	Pemphigus mordwilkoi Cholodkovsky	China: Tibet (Bomi)	19–08–2003	*Populus* sp.	DQ779156
	Pemphigus tibetensis Zhang	China: Tibet (Lhasa)	23–08–2003	*Populus* sp.	DQ779158
	Pemphigus bursarius (Linnaeus)	USA: Illinois (Champaign)	13–06–2001	*Populus* sp.	DQ779154
	Pemphigus monophagus Maxon	USA: Illinois (Champaign)	13–06–2001	*Populus* sp.	DQ779155
	Pemphigus populitransversus Riley	USA: Illinois (Champaign)	01–06–2001	*Populus* sp.	DQ779157
	Prociphilus sp.	USA: NC, Elizabethtown	29–04–1999	*Pinus* sp.	DQ005136*
	Prociphilus caryae (Fitch)	USA: UT, Logan	11–07–1999	*Amelanchier alnifoliae*	DQ005161*
	Prociphilus pini Tao	China: Beijing	13–05–2005	*Crataegus* sp.	DQ779159
Eriosomatini	*Tetraneura chinensis* Mordvilko	China: Beijing	30–04–2005	*Ulums pumili*	EF063688
	Tetraneura sorini Hille Ris Lambers	China: Beijing	12–05–2005	*Ulums* sp.	EF063689

* Downloaded sequence

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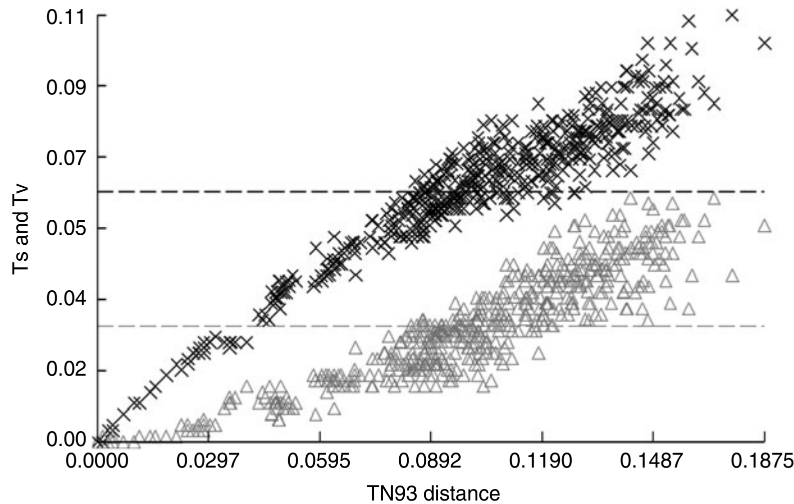

Fig. 2. Saturation plots for the nuclear gene EF-1α. The number of transitions and transversions of each pairwise comparison of taxa are plotted against the TN93 model corrected distance and the broken lines show the mean value of transition and transversion, respectively (X, T_s; △, T_v).

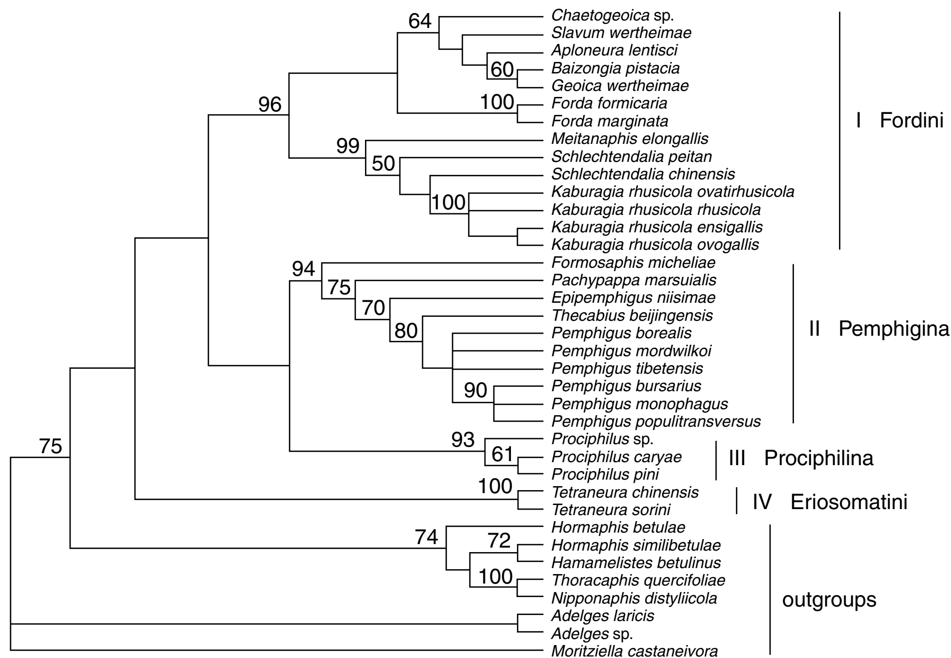

Fig. 3. The strict consensus tree of Pemphiginae generated by MP analysis based on the nuclear EF-1α sequences (tree length = 723; CI = 0.438451, RI = 0.694277, RC = 0.304405 for all sites). Numbers at the nodes denote the bootstrap percentages of 1000 replicates (only those ≥50% are shown).

25.9% T, 21.4% C, 27.5% A and 25.2% G. Nucleotide frequencies average Ti/Tv ratio = 2.2.

Because transitions and transversions in the nuclear EF-1α were accumulated linearly and showed no saturation patterns at any position (fig. 2), all nucleotide positions were employed in the subsequent analysis.

Phylogenetic analysis

Parsimony analysis, using equal weights, yielded 16 MPTs (most parsimonious trees) (not shown). The strict consensus tree is shown in fig. 3. Representatives of Adelgidae and Phylloxeridae were used to root the tree. Hormaphidinae formed a monophylum with 74% bootstrap value, being the sister group of Pemphiginae in the tree. The monophyly of Pemphiginae is not supported because of lower BP. In Pemphiginae, four strongly supported major clades (I, II, III and IV) were formed, corresponding to traditional Fordini, Pemphigina, Prociphilina and Eriosomatini, respectively (BP = 96, 94, 93, 100, respectively). The monophyly of the traditionally recognized tribe Pemphigini was not supported (BP < 50, not shown).

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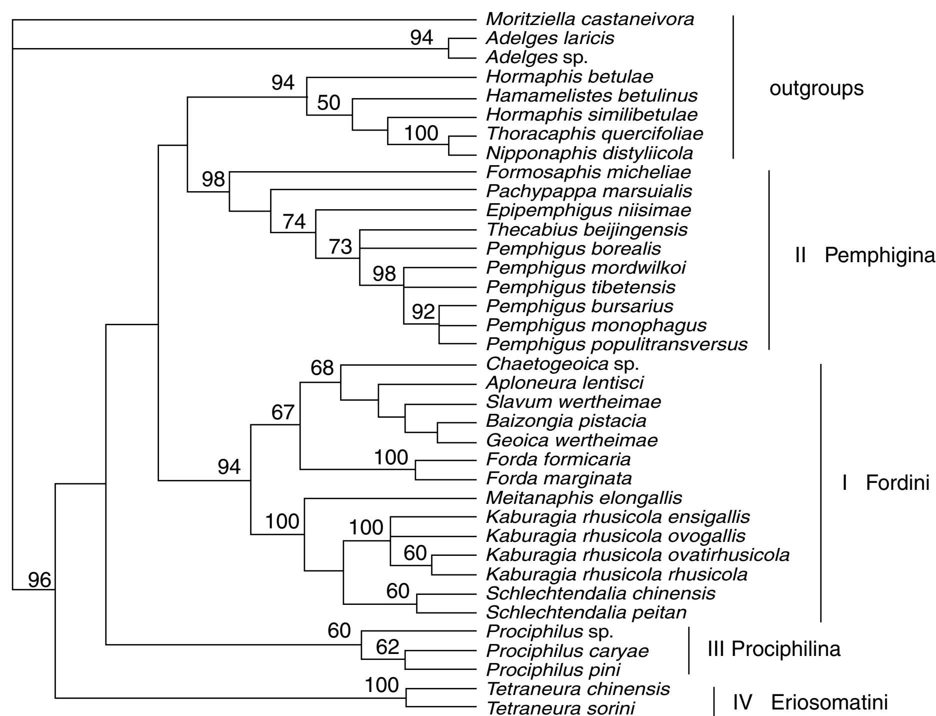

Fig. 4. ML tree of Pemphiginae based on the nuclear EF-1α sequences (best-fit model: TrN+I+G, I=0.5832, G=1.0465; −lnL=4427.1509). Numbers at the nodes denote the bootstrap percentages of 100 replicates (only those ≥50% are shown).

Deep level relationships among these four clades were not supported as the bootstrap values were lower than 50% (not shown).

The ML tree (fig. 4) was yielded, based on the optimal model TrN+I+G selected by hLRT in ModelTest3.06. The monophyletic Hormaphidinae nested in Pemphiginae. Three major well-supported clades (I, II, IV) in the MP tree were also reconstructed with high BPs (BP=94, 98 and 100, respectively) in the ML tree; the BP value of clade III was a little lower (60%). Pemphigina and Prociphilina did not cluster together, which was inconsistent with phylogeny based on morphology, and higher level relationships among these four clades were uncertain just as the MP tree indicated.

The topology of the Bayesian tree (fig. 5) was almost identical with that of the MP tree. Representatives of Adelgidae and Phylloxeridae were at the basal position, and Hormaphidinae was the sister group of Pemphiginae group with strong support (PP=1.00). Four major well-supported clades in the MP tree were four parallel branches in the Bayesian tree with PP=1.00, 1.00, 0.95 and 1.00, respectively; and the phylogenetic relationships within three clades (excluding Fordini) were completely identical with those uncovered by the MP tree.

Discussion

Molecular phylogeny compared with previous morphological hypotheses

There has been only one phylogenetic study on Pemphiginae carried out by Zhang & Chen (1999), which was based on morphology, and there has been no molecular phylogeny focused on this subfamily to date. In Zhang & Chen's study, in which Pemphiginae was raised to the family Pemphigidae, the monophyly of tribes Fordini and Pemphigini, and subtribes Fordina, Melaphidina and Pemphigina were recognized, while traditional Prociphilina and Tetraneurina were found to be paraphyletic groups. Moreover, in their phylogenetic tree, Fordini and Pemphigini clustered together first, then clustered with Eriosomatini. Based on this result, the relationships among these three tribes were speculated: Pemphigini and Fordini have a closer phylogenetic relationship with each other than with the third tribe, Eriosomatini. *Formosaphis* Takahashi nested in Fordini in their phylogenetic tree. In our molecular phylogeny based on nuclear gene EF-1α, four clades were supported robustly, corresponding to traditional Fordini (BP=96 in the MP tree and 94 in the ML tree, PP=1.00 in the Bayesian tree), Pemphigina (BP=94 in the MP tree and 98 in the ML tree, PP=1.00 in the Bayesian tree), Prociphilina (BP=93 in the MP tree and 60 in the ML tree, PP=0.95 in the Bayesian tree) and Eriosomatini (BP=100 in the MP tree and 100 in the ML tree, PP=1.00 in the Bayesian tree), respectively. This is consistent with the results based on morphology. However, the monophyly of Pemphigini is not supported. The relationships among tribes are unresolved with low BPs in MP and ML trees and comb-like topology in the Bayesian tree. Furthermore, in our phylogenetic analysis, including MP, ML analysis and Bayesian inference, *Formosaphis micheliae*, which represents the monotypic genus *Formosaphis*, undoubtedly clustered with the clade Pemphigina. Our molecular result coincides with the phylogeny based on morphology, in suggesting that Pemphiginae was probably not a monophyletic group.

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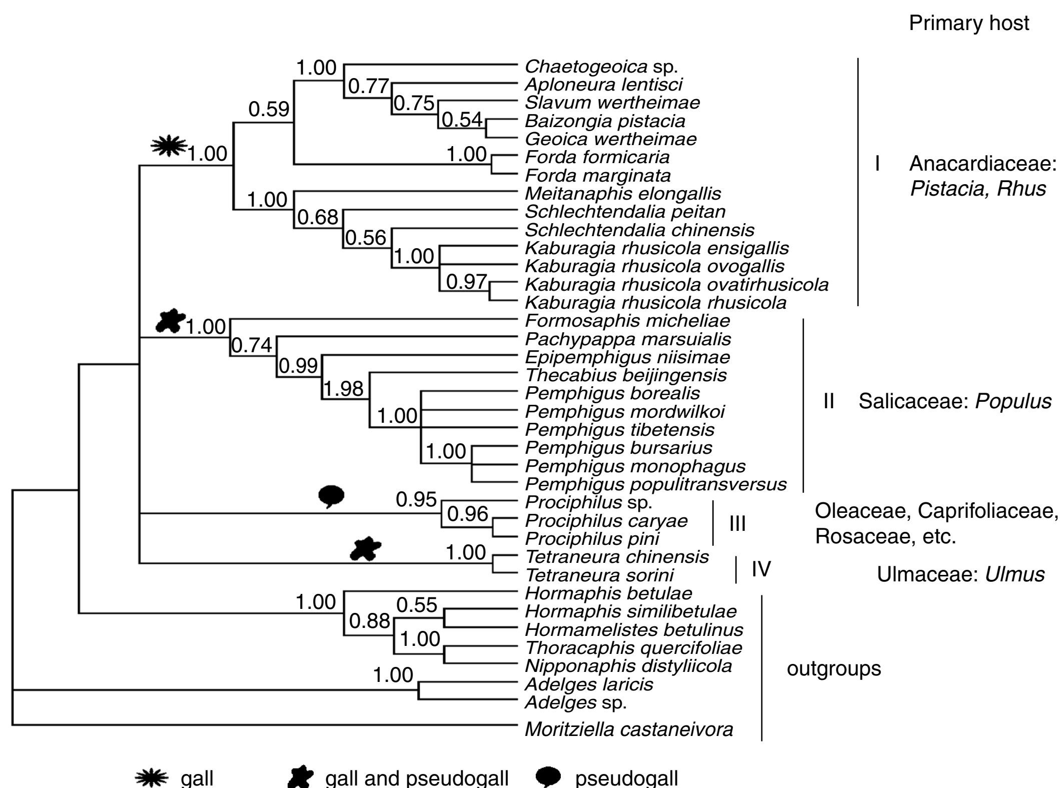

Fig. 5. Bayesian tree reconstructed from EF-1α sequences. Numbers above the branches denote the posterior probabilities (only those ≥0.50 are shown) with gall types and primary host mapped on.

Phylogenetic relationships in Pemphiginae

In the Bayesian phylogenetic tree (fig. 5), four clades (I, II, III and IV) correspond to classical Fordini, Pemphigina, Prociphilina and Eriosomatini. Tribe Fordini is monophyletic with strong support; and its two subtribes, Fordina and Melaphidina, were also monophyletic clades. Whether Eriosomatini was monophyletic or not was uncertain because of the sparse sampling (only two species of the same genus). However, the classical Pemphigini was probably not a monophylum because its two subtribes, Pemphigina and Prociphilina, were two parallel monophyletic clades in Bayesian analysis. Also, in the MP and ML trees, monophyly of Pemphigini was not supported either (low support value). The primary host plant and gall type were mapped onto the Bayesian phylogenetic tree (fig. 5). Except for clade III, all clades have high primary host specificity. The two traditional subtribes in Pemphigini did not cluster together with strong support, and their primary hosts and gall types were different from each other. Pemphigina have strong primary host specificity, only feeding on *Populus* of Salicaceae, whereas Prociphilina have a wider primary host range, such as Oleaceae, Caprifoliaceae, Rosaceae, etc. Galls of clade III all have curly leaves, which are different from galls or pseudo-galls of clade II. Additional evidence from morphology is that the empodia of the newly born viviparous nymph of Prociphilina is curved and its length is equal to or longer than its claws, the media of fore wing is usually once-branched, and the ultimate rostral segment bears 4–6 secondary hairs. Whereas, in Pemphigina, the empodia of the newly born viviparous nymph is straight and its length is often shorter than the claws, the media of fore wing does not branch, and the ultimate rostral segment bears fewer secondary hairs, usually one or two (Zhang *et al.*, 1999). Therefore, combining the biological and morphological characters with the molecular phylogenetic results, we think that subtribes Pemphigina and Prociphilina are too different to be included into one tribe, and suggest they are more appropriate to be raised to tribal level, *viz*. Pemphigini and Prociphilini, equal rank with Fordini and Eriosomatini in the subfamily Pemphiginae.

von Dohlen & Moran (2000) reconstructed the phylogeny of aphids based on mitochondrial ribosomal DNA (partial 12S and 16S) sequences and found that there was little well-supported phylogenetic structure at levels deeper than tribes, except for the monophyly of Aphidinae and Lachninae. Therefore, they argued that aphids experienced a rapid radiation at the tribal level, after host shifting from gymnosperms to angiosperms. This viewpoint is consistent with the aphid fossil record, which records the presence of few subfamilies in the late Cretaceous, but most extant tribes by the early Tertiary (Heie & Wegierek, 1998; Heie & Peñalver, 1999). Our nuclear EF-1α phylogeny shows that Pemphiginae probably radiated at the tribal level, so there were no definite evolutionary relationships among its four tribes. This coincides with the viewpoint of von Dohlen & Moran (2000).

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The origin of galls in Pemphiginae

Galls are an extended phenotype of aphids (Stone & Schönrogge, 2003). Among the whole Aphididae, only Pemphiginae, Hormaphidinae and some species of Aphidinae can produce galls (Blackman & Eastop, 1994; Wool, 2004). In Pemphiginae, most of the species induce species-specific galls on their primary host plants (Ghosh, 1984; Zhang *et al*., 1999). Galls of this subfamily can be divided into true galls and pseudo-galls. Pseudo-galls are formed by leaf rolling, folding or local bulging and varied in different shapes as dumpling, silkworm, etc. Compared with pseudo-galls, true galls are more closed and complicated in structure, and diverse in shape (Zhang *et al*., 2006). We mapped the gall type on the Bayesian tree (fig. 5). Fordini form big galls with different shapes, such as bag-like, spherical, come-like, etc. Pemphigini and Eriosomatini can produce true galls, as well as pseudo-galls, while Prociphilini only produce pseudo-galls caused by leaf rolling. Based on the phylogenetic relationship, it is likely that the galls originated independently four times in the four strongly supported groups corresponding to the four tribes in Pemphiginae, since strong specific associations were presented between gall-inducing fundatrices and primary host plants in each tribe. This result is consistent with the case of Hormaphidinae, another main gall-forming subfamily (Ren Shan-Shan *et al*., unpublished data).

In thrips (Crespi & Worobey, 1998) and willow sawflies (Price, 1992; Price & Roininen, 1993), galling was probably preceded by leaf folding. In psyllids, true galls presumably developed from simple pseudo-galls (Yang & Mitter, 1994). However, in Pemphiginae, this relationship was not indicated by the molecular phylogeny; true galls and pseudo-galls seemed to originate at the same time.

Conclusions and future work

We found discrepancies between some well-supported molecular-based relationships in this study and previous morphology-based relationships of Pemphiginae, as in the case of Aphidinae (von Dohlen *et al*., 2006). This showed the limit of morphological characters in aphids because of their reductive and convergent nature. Pemphiginae was probably not a monophyletic group, and Fordini was monophyletic with strong support. Pemphigini was not a monophyletic group, and we suggested its two subtribes of Pemphigina and Prociphilina be raised to tribal level, *viz*. Pemphigini and Prociphilini, the same rank as the other two tribes, Fordini and Eriosomatini.

The molecular phylogenetic analysis did not show definite relationships among the four tribes of Pemphiginae, as in the previous phylogeny study based on morphology. It seemed that the four tribes radiated at nearly the same time and then evolved independently. This is consistent with the viewpoint of von Dohlen & Moran (2000). Based on this, we can speculate that galls originated four times in the four tribes, and there was no evidence to support the hypothesis that true galls were preceded by pseudo-galls, as in thrips and willow sawflies.

Because of sparse sampling in the tribe Eriosomatini, it is a monophyletic group or a paraphyletic group as the phylogeny based on morphology indicated is uncertain. Future work will focus on this tribe and try to answer this question. Furthermore, other molecular markers should be applied to reconstruct the phylogenetic relationship of Pemphiginae in order to verify the result inferred from EF-1α or suggest some other new insights.

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