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# Evolution of Delphacidae (Hemiptera: Fulgoroidea): combined-evidence phylogenetics reveals importance of grass host shifts

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**Abstract.** The planthopper family Delphacidae is a speciose lineage of phloem-feeding insects, with many species considered as pests of economic significance on essential world food commodities (including rice, maize, wheat, barley and sugar cane). Despite their economic importance, evolutionary relationships among delphacids, particularly those within the speciose tribe Delphacini, are largely unknown. Presented here are the results of a phylogenetic investigation of Delphacidae based on DNA nucleotide sequence data from four genetic loci (*18S* rDNA, *28S* rDNA, *wingless* and *cytochrome oxidase I*) and 132 coded morphological characters. The resulting topologies are used to test the higher classification of Delphacidae and to examine evolutionary patterns in host–plant associations. Our results generally support the higher classifications of Delphacidae proposed by Asche, Emeljanov and Hamilton, and suggest that the rapid diversification of the Delphacini was associated with host shifts to, and within, Poaceae, and specifically from C3 to C4 grasses.

## Introduction

The insect family Delphacidae (Hemiptera: Fulgoroidea), including approximately 2100 described species, is the most speciose and economically important of the ~20 planthopper families. Delphacids occur in most terrestrial habitats worldwide (excluding Antarctica, but including oceanic islands), and are phloem feeders that are typically associated with monocots. In particular, delphacids are often associated with grasses in moist/wet habitats (Wilson *et al*., 1994) including, notably, rice. Rice planthoppers [such as the brown planthopper *Nilaparvata lugens* (Stål) and the white-backed planthopper *Sogatella furcifera* (Horvath)] have caused intermittent rice famines in Japan and Korea for centuries (Dyck & Thomas, 1979). More recently, they have been identified as a major threat to rice production throughout Asia, with significant infestations in 2009 reportedly occurring in Bangladesh, China, Malaysia, Philippines, Thailand and Vietnam (Heong, 2009). Within Delphacidae, 85 species are recognized as economically significant pests, incurring damage to approximately 25 plant crops (Wilson & O'Brien, 1987; Wilson, 2005). Of these pest species, most incur direct damage to plants through feeding and oviposition. However, approximately 30 delphacid species also serve as vectors of 28 plant pathogens, including viruses that damage several of the world's most important food crop commodities (rice, maize, wheat, barley and sugar cane; O'Brien & Wilson, 1985; Wilson & O'Brien, 1987; Wilson, 2005; Hogenhout *et al*., 2008), and one phytoplasma that damages sugar cane (Arocha *et al*., 2005).

Given such agricultural importance, it is unsurprising that the most damaging species have been investigated broadly, particularly with respect to their physiology, genetics, pesticide resistance and roles as plant disease vectors. Delphacid ecology has been broadly examined: several factors contribute to their pestiferous nature on cereal crops. For example, Wilson *et al*. (1994) assembled nearly 500 host plant records for delphacids, and found that the majority of worldwide records (65%)

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concerned monocot feeding. However, when host plant records from oceanic islands (where dicot feeding predominates) are ignored, 92% of continental mainland records associate delphacids with monocot hosts.

The physically small size of delphacids allows some species to undergo annual 'migrations' in which thousands of individuals fly in the jet stream for long distances across the South China Sea (Otuka *et al*., 2005; Bentur & Viraktamath, 2008). This intermixing of insects from populations in the Philippines, Taiwan, Vietnam, China and Japan contributes to damaging planthopper outbreaks, as populations appear to vary with respect to insecticide resistance and the strains of rice-infecting viruses that they carry (Otuka *et al*., 2005; Bentur & Viraktamath, 2008). Life-history traits also play a role, as many delphacid species exhibit wing dimorphism (Denno & Roderick, 1990; Denno & Peterson, 2000). Nymphs develop into either macropterous or brachypterous adults, depending on a number of factors, including their density on the host plant and the quality of the host plant in terms of nitrogen availability (Denno *et al*., 1985; Denno & Peterson, 2000). Nymphs receiving sufficient nitrogen levels develop into brachypters, which have higher reproductive success than macropters. When nitrogen levels within the host plant are depleted, typically from prior damage by the feeding activity of delphacids, nymphs develop into macropters capable of dispersing to as-yet undepleted host plants (Denno *et al*., 1985; Langellotto *et al*., 2000).

Although these studies paint an intriguing picture of delphacids as uniquely adapted pests of grasses (or grassland habitats), such adaptations and host–plant associations are best explored within an evolutionary context, although phylogenetic relationships within Delphacidae remain largely unexplored. The monophyly of the family Delphacidae is generally accepted, and is well supported (among other synapomorphies) by the presence of a moveable spur (the 'calcar') on the metathoracic tibiae. However, the origin of the family remains in some doubt: several studies based on either morphological (Muir, 1923; Asche, 1985; Ceotto & Bourgoin, 2008) or molecular (Urban & Cryan, 2007; Ceotto *et al*., 2008) evidence discussed the possibility that Delphacidae arose from within the planthopper family Cixiidae. Although this question has yet to be definitively resolved, clearly Delphacidae and Cixiidae are among the most anciently derived of the extant planthoppers (Asche, 1988; Emeljanov, 1991; Urban & Cryan, 2007).

The taxonomic classification of Delphacidae was addressed in several studies that respectively divided Delphacidae into two subfamilies (Asiracinae and Delphacinae; Muir, 1915, 1930; Metcalf, 1943), three subfamilies (Ugyopinae, Asiracinae and Delphacinae; Emeljanov, 1996), four subfamilies (Asiracinae, Tropidocephalinae, Megamelinae and Delphacinae; Haupt, 1929) or nine subfamilies (Asiracinae, Kelisiinae, Jassidaeinae, Stirominae, Achorotilinae, Delphacinae, Chlorioninae, Stenocraninae and Megamelinae; Wagner, 1963).

Asche (1985, 1990) presented the first cladistic investigation of the higher delphacid taxa, stating that previous studies were unsatisfactory because their '... results were either only phenetical groupings for diagnostic purposes... [or] an artificial system based on so-called "anagenic trends"... leading to perfect confusion' (Asche, 1985). Asche (1985) recognized six delphacid clades, including the paraphyletic Asiracinae (comprising the tribes Asiracini and Ugyopini) and four monophyletic subfamilies (Kelisiinae, Stenocraninae, Plesiodelphacinae and Delphacinae), with the later addition of the subfamily Vizcayinae (Asche, 1990). Asche's (1985, 1990) hypothesis of delphacid phylogeny is shown in Fig. 1a. The largest subfamily, Delphacinae, comprised three tribes: Saccharosydnini (with three genera and nine species), Tropidocephalini (with 31 genera and ~150 species) and Delphacini (with approximately 296 genera and ~1600 species). Morphological evidence putatively supports the monophyly of Delphacini, with the tribe's primary

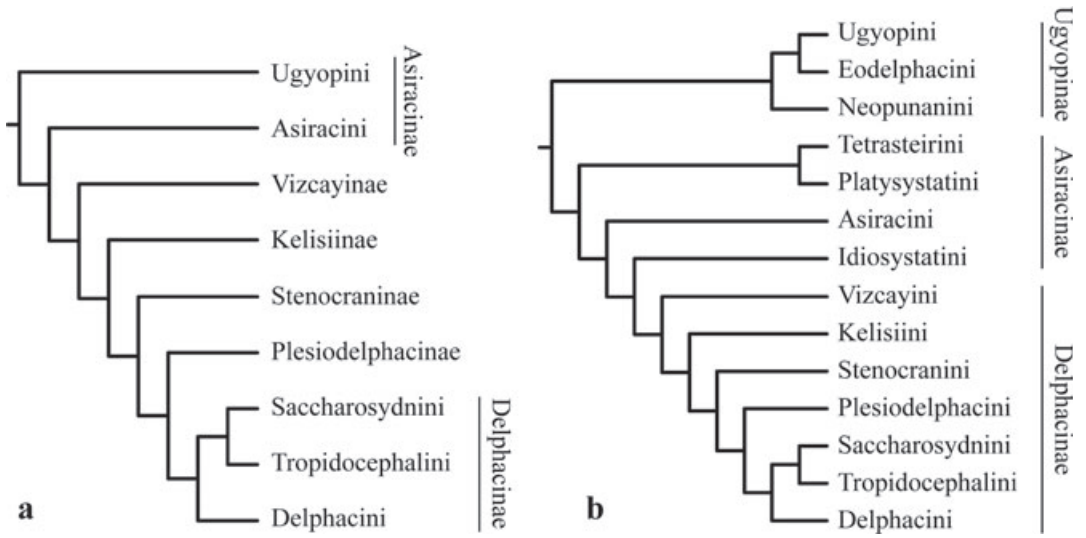


**Fig. 1.** Previous hypotheses of Delphacidae phylogeny: (a) Asche (1985, 1990); (b) Emeljanov (1996).

 

synapomorphies being the presence of a suspensorium (derived from the phallobase and linking abdominal segment 10 to the phallus) often shaped like a Y, U or ring (Asche, 1985, 1990), and a unique forewing venation [viz. media joined with radius + subcosta at base; Hamilton, 2006]. Relationships among the numerous genera of Delphacini remain unresolved: the only attempt to discern internal relationships, by Wagner (1963), was deemed unsatisfactory by Asche (1985) because of its use of '. . . the doubtful method of "dynamic taxonomy". . .'.

Since Asche (1985, 1990), two other studies have recommended revisions to the higher classification of Delphacidae. Emeljanov (1996) investigated immature stages of Delphacidae and proposed that the asiracine tribes be raised to subfamily status, and defined several new tribes within Asiracinae (Idiosystatini, Platysystatini, Tetrasteirini and Asiracini) and Ugyopinae (Eodelphacini, Neopunanini and Ugyopini) (Fig. 1b). All remaining higher taxa (Vizcayini, Kelisiini, Stenocranini, Plesiodelphacini, Delphacini, Tropidocephalini and Saccharosydnini) were treated as tribes of Delphacinae (derived within the Asiracinae, as presented) with the same phylogenetic relationships as suggested by Asche (1985, 1990). Recently, Hamilton (2006) followed Emeljanov's (1996) scheme, but treated Kelisiini as a subtribe of Stenocranini, and Saccharosydnini (Delphacinae) as a subtribe of Tropidocephalini.

A phylogenetic investigation of Delphacidae was performed by Yang *et al*. (1987), who coded 62 characters associated with external morphology, genitalia and host plants for *Ugyops* (Asiracinae) and nine Asian genera of Tropidocephalini. Their analyses placed *Ugyops* as sister to Tropidocephalini, with the genus *Tropidocephala* placed as sister lineage to the remainder of the tribe. More recently, two studies have investigated the higher phylogeny of Delphacidae using mitochondrial DNA nucleotide sequence data. Dijkstra *et al*. (2003) presented an analysis of 16 delphacid species based on 504 bp of *cytochrome oxidase I* (*COI*), and Dijkstra *et al*. (2006) published an analysis of 11 delphacids based on 352 bp of *12S* rDNA. Taken together, these molecular studies recovered Asiracinae consistently as sister to the remaining subfamilies, and Kelisiinae and Stenocraninae forming a monophyletic clade [although in the analyses of *COI* (Dijkstra *et al*., 2003), three genera of Delphacinae were also included in that clade].

Although these phylogenetic studies provide some insight into higher level relationships within Delphacidae, those based on molecular data employed limited taxonomic and data sampling. The major lineages identified by Asche (1985, 1990) and Emeljanov (1996) have not been tested quantitatively, and phylogenetic relationships within the tribe Delphacini (comprising 80% of all described Delphacidae) remain essentially unexplored.

Clearly the tribe Delphacini, with its cosmopolitan distribution, has undergone a substantial radiation in comparison with the other tribes, which are significantly less speciose and are typically geographically restricted. Possibly host–plant associations may have played a key role in this radiation, whether through coevolution or through evolutionarily advantageous host shifts. Delphacid feeding is rather host specific, in that host records indicate that most delphacids either feed on a single plant species, or feed on multiple congeneric (i.e. closely related) plant species (Wilson *et al.,* 1994). Wilson *et al.* (1994) reported that monocots within the family Cyperaceae are the predominant hosts for delphacids in Asiracini and Kelisiinae, whereas species in Stenocraninae and Delphacinae (including Delphacini) tend to feed on Poaceae. Increased diversification within Delphacini, therefore, may reflect a shift to grass feeding, or to host shifts within Poaceae (perhaps from grasses with C3–C4 photosynthetic pathways). Evaluation of these hypotheses requires a better understanding of evolutionary relationships within Delphacidae.

The goal of the present study is to investigate the phylogeny of the major lineages of Delphacidae in the context of a broad sample of taxa and data sources, and specifically to reconstruct evolutionary relationships among delphacid subfamilies, evaluate phylogenetic support for the higher-level classifications of Delphacidae, as proposed by Asche (1985, 1990) and Emeljanov (1996), and evaluate the phylogenetic support for Wagner's (1963) classification of Delphacini, and finally to evaluate and interpret host–plant associations of Delphacidae in light of our new phylogenetic hypotheses. Additionally, by including exemplars of most major cixiid lineages in our analyses, we can examine whether Delphacidae arose from within the planthopper family Cixiidae. Results are based on quantitative analyses of DNA nucleotide sequence data from four genetic loci [*18S* rDNA, *28S* rDNA, *wingless* (*Wg*) and *COI*] combined with a morphological data matrix of 132 coded characters.

## Materials and methods

### *Taxon sampling*

Insect specimens (Table S1) collected and immersed in 95–100% ethanol are stored at −80°C at the New York State Museum's Genome Bank (NYSM, Albany, NY). The 109 ingroup specimens represent all six subfamilies of Delphacidae recognized by Asche (1985, 1990), and eight of the nine subfamilies recognized by Wagner (1963) (only the monotypic Jassidaeinae was unavailable for inclusion in the present study). Exemplars of 19 species of nondelphacid Hemiptera were included as out-groups. Of these, 14 were exemplars of Cixiidae, the putative sister family to Delphacidae (Urban & Cryan, 2007). The remaining out-groups represented Cicadomorpha, Coleorrhyncha, Heteroptera and Sternorrhyncha (Table S1).

### *Morphological data*

One hundred and thirty two coded multistate characters emphasizing external anatomy were compiled using DELTA 1.04 for WINDOWS (Dallwitz, 1980; Dallwitz *et al*., 1999). Morphological character states and coded characters for each taxon are given in Tables S2 and S3, respectively.

*Molecular data*

Nucleotide sequence data were generated from two nuclear ribosomal genes (*18S* and *28S* rDNA), one nuclear protein coding gene (*Wg*) and one mitochondrial protein coding gene (*COI*). Jian *et al*. (2008) demonstrated that a combination of slow- and fast-evolving genes perform in a complementary manner when used to resolve ancient rapid diversifications. The loci used vary in evolutionary rate, and have been used in reconstructing phylogenetic relationships within Fulgoroidea (Urban & Cryan, 2007, 2009) and other closely related insect groups (Cryan *et al*., 2000, 2004; Dietrich *et al*., 2001; Cryan, 2005).

DNA was extracted from either thoracic or leg muscle tissue using FastDNA Extraction Kits (Qbiogene Inc., Carlsbad, CA) or Qiagen DNEasy Kits (Qiagen, Inc., Valencia, CA). Polymerase chain reactions (PCRs) were conducted in 25-μL volumes using AmpliTaq DNA polymerase (PE Applied Biosystems, Foster City, CA) with the following cycling protocol: 30 s at 94°C, 30–35 cycles of 1 min at 40–60°C, 1 min at 72°C, ending with 7 min incubation at 72°C. Negative controls were included in all PCR reactions to detect contamination.

Oligonucleotide primers used in PCR reactions (Table S4) were synthesized by Wadsworth Laboratories (NY Department of Health, Albany, NY) or by Integrated DNA Technologies, Inc. (Coralville, IA). Amplified DNA was visualized using 1–2% agarose gel electrophoresis with ethidium-bromide staining. DNA products were purified using GeneClean (BIO 101, Vista, CA) or ExoSAPIT (Affymetrix, Cleveland, OH). Sequences were obtained from complementary strands using dRhodamine terminator cycle sequencing on ABI Prism 3 3100/3700 or ABI 3730XL DNA sequencers at Wadsworth Laboratories and the High-Throughput Genomics Unit at the University of Washington.

All chromatography data were inspected, edited and assembled into contiguous sequences using SEQUENCHER 4.8 for Windows (GeneCodes Corp., Ann Arbor, MI). Multiple sequence alignments were performed manually, and were improved using the sequence alignment program MAFFT (Katoh *et al*., 2005). Highly variable regions of *18S* (five noncontiguous segments with a combined length of 208 bp) and *28S* (12 noncontiguous segments with a combined length of 1133 bp) that differed in base composition and sequence length among taxa were excluded from phylogenetic analysis because of extreme ambiguity of alignment. Multiple sequence alignments of *Wg* and *COI* were unambiguous, but each contained one gap that did not interrupt or shift the reading frame. Codon position for the two protein coding genes was determined by SEQUENCHER and by comparison with translated sequences available on GenBank.

*Phylogenetic analyses*

Phylogenetic analyses were conducted under the optimality criteria of maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference. Gaps were treated as missing data under all reconstruction methodologies.

**MP analyses**

Maximum parsimony analyses of the five individual data matrices, the 'DNA' data matrix (i.e. including data from all four genes combined) and the 'TOTAL' data matrix (i.e. including data from all four genes and morphology combined), were conducted using PAUP* 4.0b10 (Swofford, 2001). Heuristic tree searches were performed using 1000 random-addition search replicates with the tree bisection and reconnection (TBR) option. Because of the excessive computational time required for heuristic searches of each of the separate data partitions, the 'nchuck' and 'chuckscore' options were used in PAUP* in order to limit the number of trees saved (to no more than 100) of a particular score in each of the 1000 replicates. Because MP analysis of the 'DNA' matrix required excessive computational time, the parsimony ratchet (Nixon, 1999) was used with the program PAUPrat (Sikes & Lewis, 2001) in conjunction with PAUP*. Multiple ratchet runs were conducted such that 10, 15 and 25% of characters were upweighted (i.e. given a weight of 2) for each of 200 search iterations using TBR branch swapping. The best trees from each run were used as starting trees for subsequent TBR branch swapping searches, with maxtrees = 1000. To ensure that these analyses converged on the best tree, nine ratchet runs (three at each of the 10, 15 and 25% of characters reweighted) were conducted, each with subsequent swapping on the best tree. MP analysis of the 'TOTAL' data matrix did not require excessive computational time to complete 1000 replicates of the heuristic search using TBR branch swapping, and therefore did not require the use of the ratchet or nchuck/chuckscore options. Estimates of nodal support for the 'TOTAL' data matrix topology were computed with bootstrap (100 standard replicates), Bremer value (Bremer, 1988, 1994) and partitioned Bremer value (Baker & DeSalle, 1997) analyses. Bremer and partitioned Bremer analyses were performed using TREEROT 2 (Sorenson, 1999) and PAUP*.

**ML analyses**

MODELTEST 3.5 (Posada & Crandall, 1998) was used to determine the best-fitting model for the 'DNA' data matrix. Based on results of the Akaike information criterion (AIC; Akaike, 1974), the GTR + I + G model was chosen. ML analyses were conducted on the 'DNA' data matrix using GARLI v0.951 (Zwickl, 2006). Twenty independent search replicates were run, with each replicate run for 1 000 000 generations. Bootstrap support values for nodes on the ML topology were computed with GARLI by running 100 bootstrap replicates for each of 100 000 generations.

**Bayesian analysis**

Bayesian analysis of the 'TOTAL' data matrix was conducted using MRBAYES 3.1.2 (Ronquist & Huelsenbeck, 2003).

Because we expected (based on the results of Urban & Cryan, 2009) that the genes in our analysis are evolving at differing rates across locus and codon positions, a mixed-model Bayesian analysis was run. MODELTEST 3.5 was used to determine the best-fitting model for each of the four gene regions. When each gene region was tested separately, the results of the AIC indicated that the GTR + I + G model was the best-fitting model for the *18S*, *28S* and *Wg* matrices. For the *COI* data matrix, the best-fitting model was determined to be TvM + I + G, which differs from the GTR + I + G model only in that there is a single transition rate (i.e. b = e), rather than the two transition rates under the GTR model. Because of the close similarity of these models and to prevent the possibility of over-fitting the data (i.e. constraining the transition rates to equality), the GTR + I + G model was also employed for *COI*. Model parameters were estimated from the data for respective partitions. For the morphological data, a Markov *k* model + G was assigned (Lewis, 2001).

The mixed-model Bayesian analysis was run for 25 million generations, with model parameters unlinked and estimated independently across partitions. Two independent runs were performed, each with four chains (three heated and one cold), uninformative priors and trees sampled at intervals of 1000 generations. To determine stationarity, log-likelihood scores were plotted across generations and standard deviation of split frequencies between the two independent runs were examined for convergence. Of the 25 000 trees sampled in each run, the first 15 000 trees were discarded as burn-in, and the remaining 10 000 trees were used to construct a 50% majority-rule consensus tree. The harmonic mean of likelihoods was estimated (for the 10 000 post burn-in trees) using the *sump* command in MRBAYES.

### *Significance tests of Delphacidae classification*

The classifications of Delphacidae proposed by Asche (1985, 1990), Emeljanov (1996) and Wagner (1963) were tested by comparing topologies artificially constrained to each classification with the topologies resulting from the MP, ML and Bayesian analyses. Constrained searches were conducted under ML using GARLI 0.951 (Zwickl, 2006), with the tested groups constrained to monophyly. Four independent search replicates were run for each constrained search, with each replicate run for 1 000 000 generations. The resulting topology (i.e. the one with the best likelihood score across the four replicates) of each constrained search was compared with the MP (strict consensus), ML and Bayesian (50% consensus) topologies using Shimodaira–Hasegawa (SH) tests (Shimodaira & Hasegawa, 1999) in PAUP* with the resampling estimated log-likelihood (RELL) bootstrap and 1000 pseudoreplicates.

### *Host plant data*

Host plant records for Delphacidae were compiled from Holzinger & Remane (1994), Wilson *et al*. (1994), Nickel & Remane (2002), Wheeler & Bartlett (2006), Gonzon & Bartlett (2007) and Anderson *et al*. (2009). The determination of C3 versus C4 photosynthetic pathways was based on the findings of the Flora of North America Editorial Committee (2003, 2007), and grass taxonomy was based on the Grass Phylogeny Working Group (2001). Host–plant association records (Table S5) were available for 75 of the 109 species of Delphacidae represented. To explore evolutionary trends in host–plant associations, host plants were coded to depict their family (Cyperaceae, Heliconiaceae, Poaceae, Juncaceae, Asteraceae, Moraceae and Equisetaceae). The single taxon reportedly feeding on woody plants in multiple families [i.e. *Asiraca clavicornis* (Fabricius)] was coded as polyphagous. Because Wilson *et al.* (1994) hypothesized that more recent diversification events within Delphacidae may be associated with a shift to grass feeding, taxa feeding on multiple hosts that included both Poaceae and non-Poaceae (i.e. Cyperaceae or Juncaceae) were coded as feeding on Poaceae. For taxa associated with Poaceae, codes were also assigned to denote C3 or C4 photosynthetic pathways. To detect hypothesized shifting from C3 to C4 hosts, taxa feeding on both C3 and C4 plants were coded as C4 feeders. Host–plant character states were mapped onto the 50% consensus Bayesian topology under maximum parsimony using the program MACCLADE (Maddison & Maddison, 2000). The 50% Bayesian topology was used because it was based on the 'TOTAL' data matrix (whereas the ML topology was based on the DNA matrix), and because it incorporates models of molecular evolution (whereas the MP topology does not, making it more vulnerable to long branch attraction problems).

## Results

For *18S* and *28S*, data were amplified in two (18*S*) or three (28*S*) contiguous overlapping fragments, each comprised of approximately 600–700 bp. For *Wg* and *COI*, 375- and 540-bp fragments, respectively, were amplified in one fragment for each gene. After ambiguously aligned regions of *18S* and *28S* were excluded (as described above), a combined molecular data set (the 'DNA' matrix) of approximately 4.2 kb for each taxon was retained for analyses. The number of variable and parsimony informative sites (as well as additional descriptive information) for each gene and morphology are provided in Table S6.

### *Phylogenetic analyses*

#### MP analyses

Separate analyses of data from each partition (i.e. each of the four gene regions and morphology) yielded topologies with little resolution. Of the nine separate parsimony ratchet analyses performed on the 'DNA' matrix, six converged on the same topology (length = 8178 steps). The remaining three runs each yielded trees with higher scores (8180, 8181 and 8184 steps)

that were similar in topology but were less resolved. The strict consensus topology of the 1000 equally parsimonious trees from each of the six most parsimonious runs all recovered an unusual paraphyletic assemblage of delphacids and cixiids at the base of the phylogeny. *Neopunana* + *Burnilia* (our single exemplar of Plesiodelphacinae) formed a sister clade to the remaining Delphacidae and Cixiidae. Cixiidae was recovered as a monophyletic clade arising from within a paraphyletic assemblage of Asiracinae (exemplars of the genera *Copicerus*, *Asiraca* and *Pentagramma*) and Ugyopinae (exemplars of the genus *Ugyops*). Beyond these placements, the topology of the rest of the tree was very similar to that obtained with the 'TOTAL' data matrix, in recovering Vizcayinae ((Kelisiinae + Stenocraninae) ((Tropidocephalini + Saccharosydnini) (Delphacini))). Relationships among taxa within Delphacini were also similar to those obtained with the 'TOTAL' data matrix.

Maximum parsimony analysis of the 'TOTAL' matrix yielded 12 equally parsimonious trees (the strict consensus of which is shown in Figure S1), of length = 9721 steps. Bootstrap, Bremer and partitioned Bremer node support values, listed in Table S7, were relatively high for the most ancient and the most recent diversifications of delphacids. However, support values were generally low for intermediate diversifications. Delphacidae was placed as a monophyletic lineage arising from within a paraphyletic assemblage of Cixiidae. Neither Asiracinae nor Ugyopinae (*sensu* Emeljanov, 1996) was recovered as monophyletic. Plesiodelphacinae was placed as the sister group to *Neopunana*. The remaining higher level relationships were generally consistent with the hypotheses of Asche (1985, 1990) and Emeljanov (1996), in terms of the relative order of diversification events. However, inconsistent with those hypotheses, Kelisiinae and Stenocraninae were recovered together as a monophyletic lineage, whereas Saccharosydnini and Tropidocephalini were not placed together within a monophyletic lineage. Furthermore, the exemplar of the genus *Phrictopyga* was placed in Tropidocephalini rather than with the otherwise monophyletic Delphacini. Within Delphacini, the monophyly of several genera was not supported: *Nothodelphax*, *Paraliburnia* and *Delphacodes* (the latter has often been implicated as polyphyletic, e.g. Wagner, 1963; Asche & Remane, 1983; Asche, 1985).

### ML analyses

The topology of the tree with the best score (Fig. 2) was nearly identical to those obtained in seven of the 20 ML search replicates. All 20 searches yielded trees with similar topologies, and −ln scores ranging from 43927.86 to 43936.41. As with the results of the MP analyses (see above), the ML topology recovered Delphacidae as arising from within Cixiidae, and Asiracinae and Ugyopinae were not recovered as monophyletic lineages. The relative branching order of the major lineages was similar to that recovered in the MP topology, but showed greater agreement with the hypotheses of Asche (1985, 1990) and Emeljanov (1996) in that Plesiodelphacinae was placed as sister to a monophyletic ((Saccharosydnini + Tropidocephalini) + Delphacini). Some relationships within Delphacini differed compared with the MP topology: *Bakerella* was placed as sister to the remaining Delphacini and *Nothodelphax* was recovered as a monophyletic genus (however, *Euides*, *Tagosodes* and *Sogatella* were not). As under MP, *Paraliburnia* was paraphyletic and *Delphacodes* was polyphyletic. Bootstrap support values were relatively high for the most recent diversifications, and were generally low for the most ancient diversifications. As under MP, support was low for intermediate diversifications within Delphacini.

### Bayesian analyses

Two identical 50% consensus trees (shown in Fig. 3 with posterior probabilities for each clade given above the corresponding node) with a harmonic mean of $-\ln = 49594.11$ were obtained from the two independent runs of the mixed-model Bayesian analysis. Relationships among Cixiidae, and the position of Cixiidae relative to Delphacidae, were unresolved. As under MP and ML, Delphacidae was recovered as monophyletic, but relationships among exemplars of Asiracinae and the two exemplars of *Ugyops* were unresolved. The ugyopine *Neopunana* was placed as sister to the remaining delphacids. The relative branching order of the higher groupings was similar to that recovered under ML, with the exception that Plesiodelphacinae was placed at a more basal subtending node in the topology. Kelisiinae and Stenocraninae were recovered as predicted by Asche (1985, 1990) and Emeljanov (1996), but not as a monophyletic clade (as was reconstructed by MP and ML). As in the ML analyses, a monophyletic Saccharosydnini + Tropidocephalini was recovered, and *Bakerella* was placed as sister to the remaining Delphacini. Relationships within Delphacini differed somewhat from the MP and ML topologies: *Nothodelphax*, *Tagosodes* and *Sogatella* were recovered as monophyletic genera, whereas *Euides*, *Paraliburnia* and *Delphacodes* were not. Posterior probabilities generally showed higher support for the most recent diversifications in the topology, although some of the intermediate nodes within Delphacini showed much higher support than in the MP or ML topologies.

### *Significance tests of Delphacidae classification*

The taxonomic classifications of Delphacidae (Fig. 1) proposed by Asche (1985, 1990) and Emeljanov (1996) were tested with respect to the monophyly of the higher level groupings recognized in each classification scheme. Although these two schemes differ in the taxonomic rank afforded to certain lineages (i.e. a particular clade recognized as either tribe or subfamily), both recognize the following as monophyletic lineages: Ugyopini/Ugyopinae (represented here by *Ugyops* and *Neopunana*), Asiracini/Asiracinae (represented here by *Pentagramma*, *Asiraca*, and *Copicerus*), Saccharosydnini + Tropidocephalini and Delphacini (represented here by *Phrictopyga* + the remaining Delphacini). As summarized in Table S8, the monophyly of Ugyopini/Ugyopinae and of

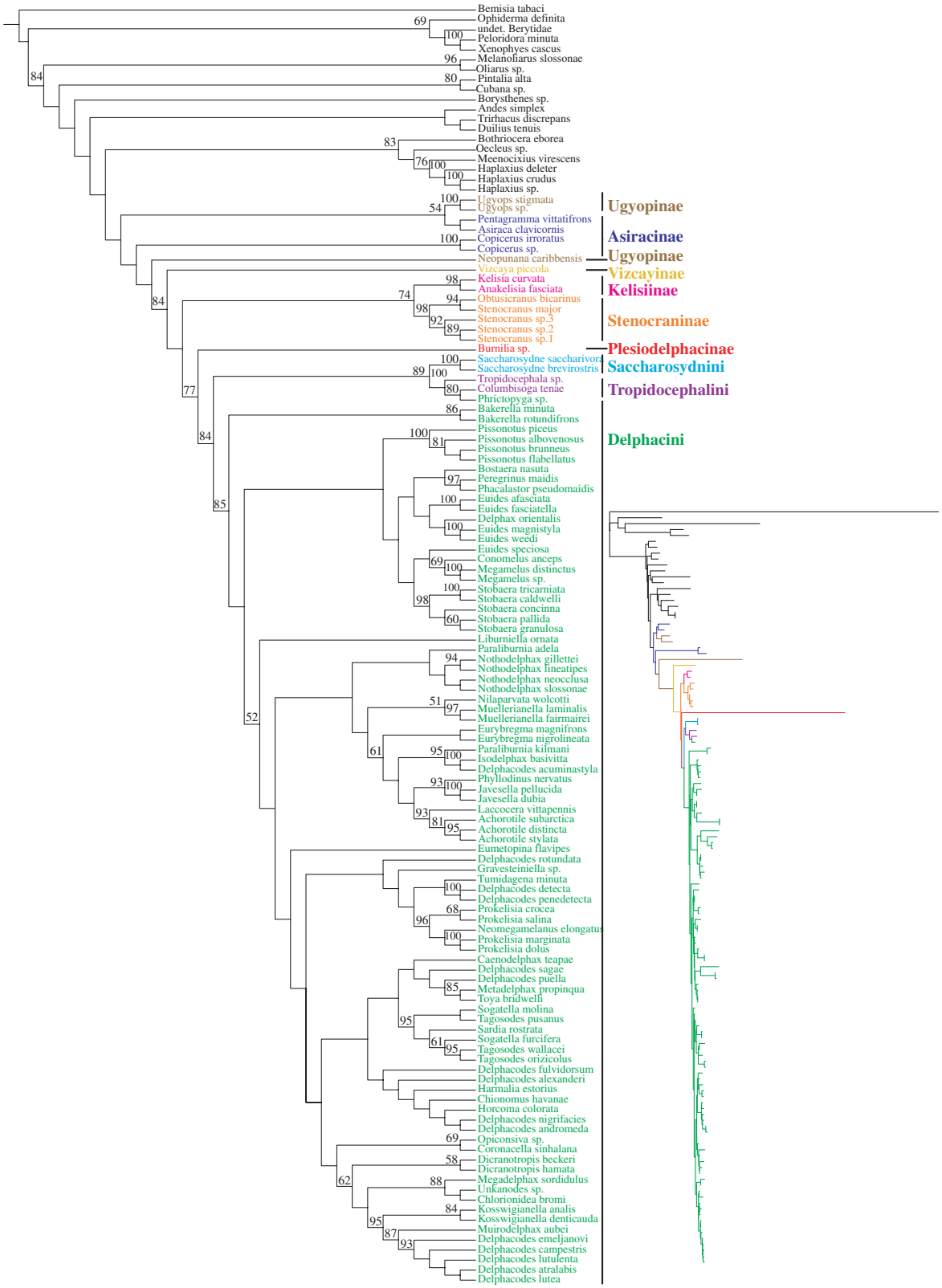


**Fig. 2.** Legend on next page.

Asiracini/Asiracinae were each significantly rejected in the SH test using the ML topology, but not in the tests of the MP and Bayesian topologies. Saccharosydnini + Tropidocephalini was recovered as monophyletic in the ML and Bayesian topologies. However, the SH test using the MP topology failed to reject the monophyly of this lineage. The monophyly of Delphacini was significantly rejected in the SH test of the ML topology, but not in tests of the MP or Bayesian topologies.

Emeljanov (1996) further recognized Asiracini (to include only *Asiraca* and *Copicerus*) and Delphacinae (to include Vizcayinae, Kelisiinae, Stenocranini, Plesiodelphacini, Saccharosydnini, Tropidocephalini and Delphacini). Asiracini was recovered as monophyletic in the Bayesian analysis (Fig. 3) and was not statistically rejected in SH tests of the MP or ML topologies. Delphacinae was recovered as monophyletic in the ML (Fig. 2) and Bayesian (Fig. 3) topologies, and was not statistically rejected in the SH test of the MP topology.

To test the hypothesized placement of Plesiodelphacini relative to the other major lineages (based on Asche 1985, 1990; Emeljanov 1996), the monophyly of Plesiodelphacini + Tropidocephalini, Saccharosydnini and Delphacini was tested. Monophyly of this lineage was supported in the ML topology, but was significantly rejected in SH tests of the MP and the Bayesian topologies.

To examine further relationships among the Delphacini, three groupings proposed by Wagner (1963) were also tested. Wagner's classification was tested as it is the only hypothesis for grouping the numerous taxa within Delphacini, and to examine Asche's (1985) assertion that Wagner's (1963) groupings were not monophyletic. Taxa included in the present study allowed us to test Wagner's proposed Achorotilinae (represented here by the genera *Achorotile* and *Bakerella*), Delphacinae (represented here by *Conomelus*, *Delphax* and *Euides*) and Megamelinae (represented here by *Delphacodes*, *Dicranotropis*, *Chlorionidea*, *Gravesteiniella*, *Kosswigianella*, *Megamelus*, *Metadelphax*, *Muellerianella*, *Muirodelphax* and *Paraliburnia*). The monophyly of Achorotilinae was not statistically rejected in tests of the MP or Bayesian topologies, but was rejected in tests of ML topology. Tests of all topologies failed to reject Wagner's Delphacinae, whereas Megamelinae was significantly rejected in SH tests of all topologies.

Because previous studies have suggested that Delphacidae may have arisen from within Cixiidae, the monophyly of Cixiidae was also tested. SH tests of all topologies failed to reject the monophyly of Cixiidae.

### *Mapping of host–plant associations*

Host–plant associations mapped on the Bayesian topology under MP (Fig. 3) suggested that a host shift from Cyperaceae to Poaceae occurred in the common ancestor of Stenocraninae + Delphacinae. Although this analysis suggests Cyperaceae as the ancestral host for Delphacidae, many host–plant associations are unavailable for taxa placed at the base of the topology, thus leaving the identity of the actual ancestral host (i.e. prior to Cyperaceae) an open question. Within Delphacini, mapping under MP suggested numerous host shifts from Poaceae to the families Asteraceae, Cyperaceae, Juncaceae or Equisetaceae (Fig. 3). For Poaceae feeders, mapping of the photosynthetic pathway of the host plant (C3 vs C4) indicated that the common ancestor of Stenocraninae + Delphacinae fed on C3 grasses, with multiple subsequent shifts to C4 grasses occurring within Delphacini. Indeed this analysis suggests that C4 grass feeding is likely to be the most derived feeding condition within Delphacini (Fig. 3).

Thirteen delphacid species sampled in the present study are regarded as economically important pests (Wilson & O'Brien, 1987; Wilson *et al*., 1994; Wilson, 2005). These species, indicated in Fig. 3 with the crop(s) they damage, and the virus type and disease vectored (full names of viruses are listed with abbreviations in Table S9), are placed in distantly related lineages across the topology, although all occur within the Poaceae-feeding lineage. Of these 13 pest species, 11 are documented plant virus vectors, and one is a vector of a phytoplasma. Whereas some viruses are vectored by distantly related taxa (e.g. maize rough dwarf virus, MRDV, and oat sterile dwarf virus, OSDV), the European wheat striate mosaic virus (EWSMV) and the *Phleum* green stripe virus (PGSV) are each vectored by two closely related delphacid species.

## Discussion

Although analyses of the individual data partitions yielded topologies with little resolution, analyses of the 'DNA' data matrix and of the 'TOTAL' data matrix yielded topologies with greater resolution. The differences observed, especially with respect to the basal nodes within delphacid evolution, may be better understood in light of the phylograms of the ML (Fig. 2, inset) and Bayesian (not shown) topologies. In both, taxa arising from earliest nodes (e.g. *Neopunana* in Ugyopinae, *Copicerus* in Asiracinae and *Burnilia* in Plesiodelphacinae) are represented by relatively long branches. Among all in-group taxa the mean uncorrected pairwise distance for the 'DNA' data matrix was 0.028, whereas distances computed for *Neopunana*, *Copicerus* and *Burnilia* with each of the remaining in-group taxa had a mean value of 0.092. Furthermore, internode lengths within Cixiidae and branch lengths in the backbone of each topology are relatively short. Such a pattern is characteristic of ancient rapid radiations, which are notoriously difficult to reconstruct by any method, but can be particularly problematic for MP-based reconstructions (Whitfield & Kjer, 2008). In rapid diversifications, a relatively small level of change occurs in a narrow time span, and subsequent change within lineages often obscures earlier changes (Whitfield & Lockhart, 2007). Reconstruction methodology incorporating evolutionary models (ML and Bayesian) are necessary to enhance the

**Fig. 2.** Maximum likelihood (ML) topology (likelihood, −43927.86) resulting from 20 independent GARLI analyses of the 'DNA' data matrix (*18S*, *28S*, *Wg* and *COI*). Bootstrap node support values are indicated above each node; ML phylogram is inset to show branch lengths.

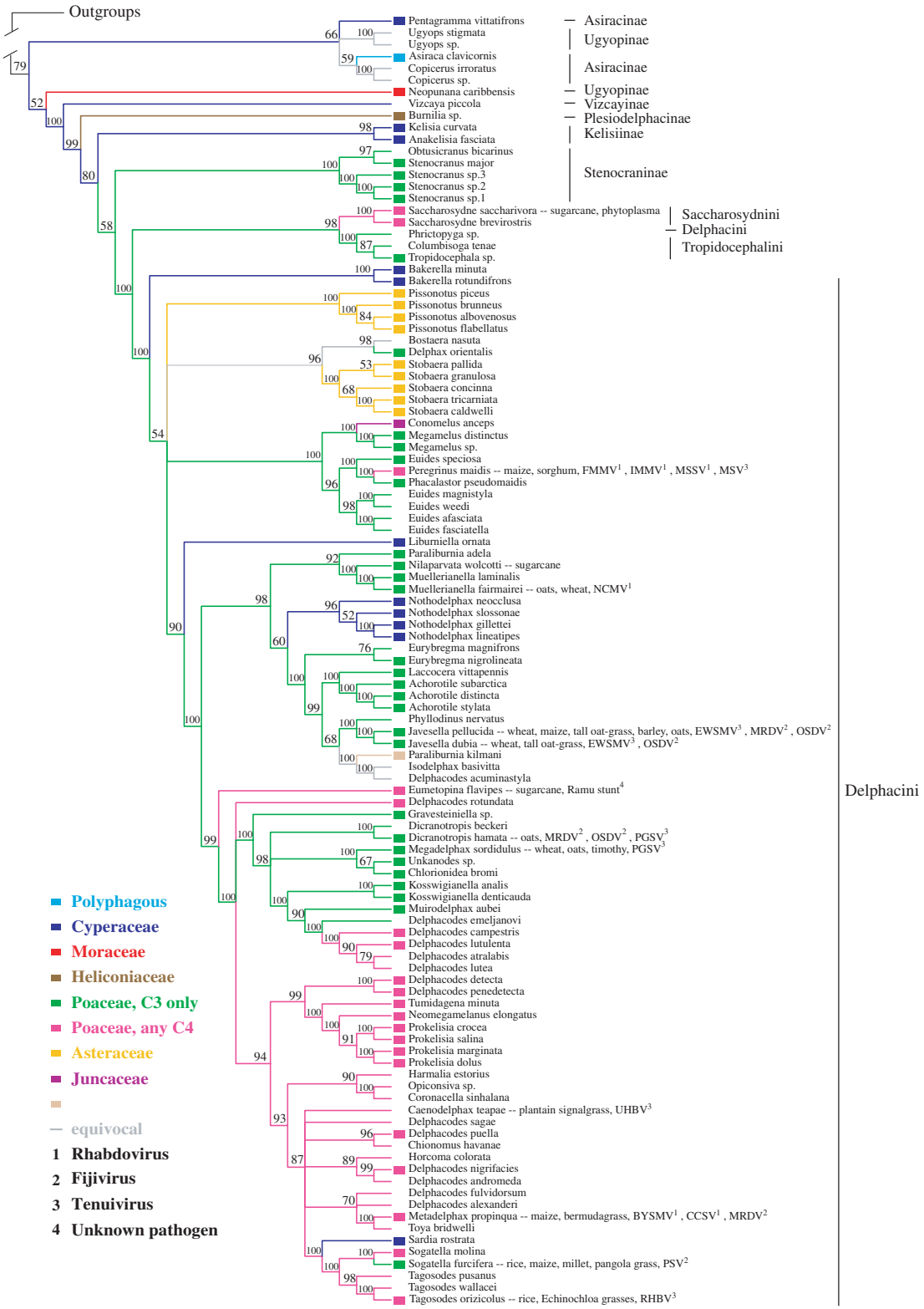


**Fig. 3.** Mixed model Bayesian analysis topology (harmonic mean of −ln = 49594.11) resulting from two independent analyses of the 'TOTAL' data matrix (including data from *18S*, *28S*, *Wg*, *COI* and morphology). Posterior probabilities are indicated above each node. Host–plant associations were mapped under maximum parsimony using MacClade.

phylogenetic signal, and reduce 'noise' (e.g. homoplastic similarity between long branches) in the reconstruction (Whitfield & Lockhart, 2007; Whitfield & Kjer, 2008). Therefore, the following interpretations and conclusions reflect a greater reliance on the ML and Bayesian topologies (Figs 2 and 3) than on the MP topology (Figure S1).

Taken together, the results of this investigation generally agree with the taxonomic classifications of Delphacidae proposed by both Asche (1985, 1990) and Emeljanov (1996) concerning the relative placement of the major delphacid lineages. That is, Ugyopinae and Asiracinae were placed as successive sisters, followed by Vizcayinae, Kelisiinae, Stenocraninae, Saccharaosydnini + Tropidocephalini and Delphacini. The placement of Plesiodelphacinae agreed with these classifications in the ML topology (Fig. 2), but not in the Bayesian topology (Fig. 3). However, this lineage was represented here by only one exemplar (the subfamily consists of two genera and seven species, all Neotropical), and thus additional sampling will be required to place this lineage unequivocally, especially given the particularly long branch length exhibited by the single exemplar of *Burnilia*.

Equivocal results were obtained concerning relationships among exemplars of the most anciently diversified lineages, Ugyopinae and Asiracinae. Neither the Asiracinae nor the Ugyopinae were supported consistently as monophyletic in our analyses. However, the lack of resolution in the Bayesian topology did not reject a monophyletic Asiracinae, and only under ML did SH tests significantly reject the monophyly of Asiracinae and of Ugyopinae. Examination of our morphological data indicates that all taxa in the Ugyopinae + Asiracinae clade in the Bayesian topology (Fig. 3; comprising exemplars of the genera *Pentagramma*, *Ugyops*, *Asiraca* and *Copicerus*, but excluding *Neopunana*) exhibit three or more lateral spines on the metatibiae (Table S2, character 63), whereas all other delphacids bear fewer spines. In considering 'basal' delphacid lineages, Asche (1985) discussed features of the calcar, the hind basitarsus and the genal carinae, emphasizing the apparent paraphyletic nature of the nonugyopine Asiracinae. Our results suggest that his caution was warranted, and that the relationships of these lineages suggested subsequently by Emeljanov (1996) require verification. Additional taxon sampling from these lineages, particularly from Emeljanov's (1996) Eodelphacini (Ugyopiniae), Tetrasteirini and Platysystatini (Asiracinae), is needed to evaluate Emeljanov's (1996) hypothesis more fully.

In all resulting topologies, the monophyly of Kelisiinae, Stenocraninae, Delphacinae (*sensu* Asche to include Saccharosydnini, Tropidocephalini and Delphacini), and Saccharosydnini was supported. Kelisiinae is supported by the presence of a subanal process (Table S2, character 123). Stenocraninae is supported by the presence of a projection at the apex of the phallobase (Table S2, character 117). Delphacinae is supported by the absence of a flagellum on the apex of the aedeagus (Table S2, character 108), a membranous sperm-conducting tube (Table S2, character 109) that is partially or wholly fused with the theca (Table S2, character 119), a short, immovable and rigid distal region of the theca (Table S2, character 118), and parameres that are dorsally directed (Table S2, character 124). Saccharosydnini is supported by the presence of a greatly elongated aedeagus that is coiled at its base (Table S2, character 111).

Hamilton (2006) proposed reducing Kelisiinae to a subtribe of Stenocranini based on modified wing venation. Our results were equivocal in this regard as Kelisiinae was recovered as sister to Stenocranini in our MP (Figure S1) and ML (Fig. 2) topologies, whereas our Bayesian (Fig. 3) results placed Kelisiinae as sister to (Stenocranini + Delphacinae). We intend to examine this issue further with increased sampling of these lineages from other biogeographic regions (e.g. Africa and the Neotropics).

Emeljanov's (1996) definition of Delphacinae (including, as tribes, Asche's Vizcayinae, Kelisiinae, Stenocraninae, Plesiodelphacinae, Saccharosydnini, Tropidocephalini and Delphacini) was supported. Morphological synapomorphies supporting this node are the presence of a male drumming organ on the second abdominal tergite with a prominent plate system (Table S2, character 90), apodemes of metapostnotum that are strongly elongate (Table S2, character 91) and projected caudad (Table S2, character 92). Equivocal results were obtained concerning the placement of *Phrictopyga*. *Phrictopyga* was recovered within Tropidocephalini in these analyses, but morphological evidence suggests that the genus belongs in Delphacini, as it possesses teeth on the posterior margin of a tectiform calcar, and has a distinct suspensorium. In contrast, the Tropidocephalini have a thickened calcar without teeth, and the anal segment is in close functional contact with the phallus. Because the available material (i.e. *Phrictopyga* specimens) was limited, we were unable to verify this placement independently by sampling and examining additional individuals. Further investigation of this genus is warranted to determine its appropriate placement.

The arrangement of genera within Delphacini remains largely unresolved, although some emerging phylogenetic trends are evident. At present, there is no phylogenetic hypothesis among the genera of Delphacini. Formerly recognized were the tribe Alohini (a collection of genera lacking teeth on the calcar, including most of the Hawaiian genera, plus *Stiroma*, *Stobaera*, *Vizcaya, Burnilia* and others; Metcalf, 1943) and the five delphacine subfamilies erected by Wagner (1963; viz. Stirominae, Achorotilinae, Delphacinae, Chlorioninae and Megamelinae), based on his investigation of the central European fauna. However, Asche (1985) subsumed all these taxa under Delphacini because of a lack of synapomorphies. Although our sampling of the central European fauna is incomplete, results of all our topologies significantly reject Wagner's Megamelinae. Our topology tests were equivocal with respect to the monophyly of Wagner's Achorotilinae, and failed to reject Wagner's Delphacinae. However, none of these groupings were recovered as distinct clades in any topology.

In addition to the formerly recognized higher taxa, Emeljanov (1993) placed 22 genera (mostly Palearctic, Indo-Malayan and Afrotropical) in the subtribe Numatina, implying that the remainder of the Delphacini would be in the

'Delphacida'. As *Bostaera* was the only one of those genera included in the present analyses, the monophyly of the Numatina could not be tested. *Bostaera* was recovered deep within the Delphacini, closely allied with *Peregrinus* and *Phacalastor*, which would render the Delphacina paraphyletic. These results suggest that continued recognition of Numatina may imply the need for recognition of a series of additional tribes within the 'basal' Delphacini.

Our results divided Delphacini consistently into three major clades (plus several 'intermediate' taxa). One clade, poorly supported in all analyses, includes *Stobaera*, *Pissonotus*, *Megamelus*, *Peregrinus*, *Conomelus*, *Euides* and *Delphax,* subtended by *Bakerella* in the Bayesian and ML analyses, and *Liburniella* in the MP analyses. A second clade, well-supported in all analyses, includes *Nilaparvata*, *Muellerianella*, *Nothodelphax*, *Javesella*, *Laccocera*, *Achorotile* and *Paraliburnia*; this clade is subtended by *Liburniella* in the Bayesian and ML analyses (in MP this clade includes *Bakerella*, but that placement may be an analytical artifact). A third clade, subtended by *Eumetopina*, consists of several subclades, the composition of which is consistent among analyses, but the relationships of which are only partially resolved (probably as a result of short branch lengths). This clade includes *Caenodelphax*, *Dicranotropis*, *Kosswigianella*, *Metadelphax*, *Muirodelphax*, *Neomegamelanus*, *Prokelisia*, *Sardia*, *Sogatella*, *Tagosodes*, *Toya* and most *Delphacodes* species. The short branch lengths indicated throughout Delphacini (Fig. 2, inset phylogram) suggest that this large planthopper lineage experienced relatively rapid diversification. Resolution of relationships within Delphacini will require the addition of new data sources, such as sequence data from other genes with a higher rate of mutation.

Several previous studies (Muir, 1923; Asche, 1985; Urban & Cryan, 2007; Ceotto & Bourgoin, 2008; Ceotto *et al*., 2008) have discussed hypotheses of the origins of Delphacidae with respect to Cixiidae, but there is no clear consensus. Our results are equivocal regarding relationships between Delphacidae and Cixiidae. Exemplars of most major cixiid lineages were included in our analyses, including newly generated data and data from Ceotto *et al.* (2008). Cixiidae was recovered as a paraphyletic assemblage by MP (Figure S1) and ML (Fig. 2) analyses, but relationships were unresolved in the Bayesian topology. Nevertheless, statistical tests of all topologies failed to reject the possibility of a monophyletic Cixiidae. The ML phylogram (Fig. 2, inset) indicates short internode lengths among Cixiidae, and therefore we speculate that the early diversification events within Cixiidae + Delphacidae may have occurred very rapidly, contributing to the difficulty associated with resolving these relationships. Such a scenario suggests that future investigations may benefit from expanded data sampling of additional loci, particularly of nuclear protein coding genes.

A detailed examination of the impact of these analyses on the taxonomy and classification of Delphacidae will be examined separately, in a work that will review the standing of each of the higher taxa, including clades within Delphacini, and recommend a nomenclatural scheme consistent with these phylogenetic results, and with further analysis of morphological data. In the interim we recommend retaining a conservative nomenclature consistent with these results, and with minimal impact on current usage. In particular, we recommend following Asche (1990) with six subfamilies (Asiracinae, Vizcayinae, Plesiodelphacinae, Stenocraninae, Kelisiinae and Delphacinae), with three tribes in the Delphacinae (Tropidocephalini, Saccharosydnini, Delphacini) and provisional acceptance of Emeljanov's (1996) seven asiracine tribes (Asiracini, Eodelphacini, Idiosystatini, Neopunanini, Platysystatini, Tetrasteirini and Ugyopini) until they can be more fully evaluated. We recognize that the treatment of some taxa as subfamilies (i.e. Asche, 1985; 1990) or tribes (Emeljanov, 1996; Hamilton, 2006) is a matter of convention, and we are cognizant that Hamilton (2006) advocated nomenclatural modifications, but we advocate the retention of a consistent nomenclature until there is firm confirmation from quantitative analyses.

### *Host–plant associations*

Based on more than 500 host–plant records and Asche's (1985, 1990) phylogenetic scheme, Wilson *et al.* (1994) proposed a hypothesis of the role host–plant shifts played in the diversification of Delphacidae. Our results support the major assertion of the Wilson *et al*. (1994) hypothesis: the rapid diversification of Delphacini is associated with a shift to feeding on Poaceae and particularly C3 grasses within Poaceae. Early (basal) nodes of Delphacini (e.g. *Bakerella*, *Stobaera*, *Pissonotus*, *Megamelus* and *Conomelus*) are associated primarily with sedge, rush or dicot hosts, whereas subsequent clades are mainly grass feeders, with *Euides* on *Phragmites* and *Arundo* (C3 grasses), *Delphax* on *Phragmites*, and *Peregrinus* on *Zea* (a C4 grass). Intermediately derived Delphacini (the 'second clade' discussed above) are largely C3 grass feeders, whereas shifts to C4 grass feeding appear to have occurred among the most recently derived lineages of Delphacini (especially the 'third clade' discussed above), and may be partly responsible for the diversification of the advanced Delphacini. An example of this trend is seen in the New World clade consisting basally of *Delphacodes detecta* and *Delphacodes penedetecta*, followed by *Tumidagena*, *Neomegamelanus* and *Prokelisia*, which are all primarily *Spartina* feeders (a C4 grass). This *Spartina*-feeding clade was uniformly supported in all our analyses. In plants, C4 plants have a competitive advantage over C3 plants under nitrogen or carbon dioxide limitation, drought or high temperatures, allowing C4 plants greater dominance potential in warmer or more xeric clines. For delphacids, feeding on C4 plants would presumably be an adaptation for dispersal into tropical or xeric climates.

Results of this study suggest no phylogenetic constraints governing the pestiferous tendency of certain species of Delphacidae, their association with specific crops or plant virus type (i.e. Rhabdovirus, Fijivirus and Tenuivirus) transmitted by some pest species. For example, the virus MRDV is vectored by taxa that are distantly related within Delphacini (*Javesella pellucida*, *Dicranotropis hamata* and *Metadelphax propinqua*), whereas the viruses EWSMV (transmitted by *J. pellucida*

and *Javesella dubia*) and PGSV (transmitted by *D. hamata* and *Megadelphax sordidulus*) are each vectored by closely related delphacid species. A broader phylogenetic perspective of vectors is gained when one considers additional delphacid species not represented in the taxonomic sample of this study (Table S9), as some viruses are vectored by congeneric (and presumably closely related) species. For example, Fiji disease virus (FDV) is transmitted by three *Perkinsiella* species and rice hoja blanca virus (RHBV) is transmitted by two *Sogatella* species. However, maize sterile stunt virus (MSSV) is transmitted by *Peregrinus maidis* and two *Sogatella* species. The position of *P. maidis* and the *Sogatella* species sampled here suggests that the vectors of MSSV are quite distantly related within the family.

Mechanisms of plant virus transmission by Hemiptera have been grouped by degree of viral persistence within the insect vector (Nault, 1987; Hogenhout *et al*., 2008), with four defined categories ranging from less to more persistent (nonpersistent stylet-borne, semi-persistent foregut-borne, persistent circulative and persistent propagative; Hogenhout *et al*., 2008). All plant viruses vectored by delphacids belong to the latter, most persistent category, with the virus often propagated transovarially, and thereby transmitted vertically from parent to offspring (Hogenhout *et al*., 2008). Notably, plant viruses transmitted by Delphacidae are not transmitted by any other auchenorrhynchous insect species, and within the planthopper superfamily Fulgoroidea, Delphacidae are the only family known to serve as vectors of plant viruses. Therefore, it seems likely that there is some evolutionary association between delphacids and certain viral plant pathogens. Because our current knowledge of viral vectors is primarily limited to economically important crop systems, the extent and nature of these evolutionary associations is only speculative at the moment.

Taken together, evidently host relationships have played a key role in the evolutionary history of Delphacidae. These results suggest that Delphacidae did not strictly coevolve with their host plants, but rather experienced a pattern of repeated host shifts, with each shift to a novel host leading to a radiation of delphacid species. Because these shifts appear not to track the phylogenetic relationships among the host plants (or, if so, then only in a broad sense), the host shifts were more likely driven by ecological opportunity. Host–plant associated diversification within Delphacidae and associations with pathogenic plant viruses may be mediated by coevolutionary relationships with endosymbiotic bacteria and fungi (Müller, 1940, 1949, 1962; Ermisch, 1960; Buchner, 1965). Future research that integrates these multiple dimensions promises not only to unveil intriguing aspects of delphacid evolution, but may also provide much needed insight relevant to the control of present and potential delphacid pest species.

## Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: DOI: 10.1111/j.1365-3113.2010.00539.x

**Figure S1.** Strict consensus topology.

**Table S1.** Taxa sampled.

**Table S2.** Morphological characters and character states.

**Table S3.** Morphological data matrix.

**Table S4.** Oligonucleotide primer sequences.

**Table S5.** Host–plant associations.

**Table S6.** Descriptive statistics for data partitions.

**Table S7.** Nodal support for Figure S1.

**Table S8.** Results of SH tests of Delphacidae classification.

**Table S9.** Delphacid species known to transmit viral plant pathogens.

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