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Evolution of the planthoppers (Insecta: Hemiptera: Fulgoroidea)

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Abstract

The planthopper superfamily Fulgoroidea (Insecta: Hemiptera) comprises approximately 20 described insect families, depending on which classification is followed. Multiple competing hypotheses of fulgoroid phylogeny have been published, based on either morphological character coding or DNA sequence data; however, those hypotheses disagree in several key aspects regarding the evolution of planthoppers. The current paper seeks to test these hypotheses, including the Asche (Asche, M. 1987. Preliminary thoughts on the phylogeny of Fulgoromorpha (Homoptera Auchenorrhyncha). In: Proceedings of the 6th Auchenorrhyncha Meeting, Turin, Italy, 7–11 September, 1987, pp. 47–53.) hypothesis of a trend in ovipositor structure, which may be correlated with planthopper feeding ecology. Presented here are phylogenetic reconstructions of Fulgoroidea based on analysis of DNA nucleotide sequence data from four loci (18S rDNA, 28S rDNA, Histone 3, and Wingless) sequenced from 83 exemplar taxa representing 18 planthopper families and outgroups. Data sets were analyzed separately and in various combinations under the maximum parsimony criterion, and the total combined dataset was analyzed via both maximum parsimony and partitioned Bayesian criteria; results of the combined analyses were concordant across reconstruction paradigms. Relationships recovered suggest several major planthopper lineages, including: (1) Delphacidae + Cixiidae; (2) Kinnaridae + Meenoplidae; (3) Fulgoridae + Dictyopharidae; (4) Lophopidae + Eurybrachidae (possibly + Flatidae); (5) Ricaniidae + Caliscelidae (possibly + Tropiduchidae). Results also suggest the placement of Achilixiidae outside of Cixiidae and of Tettigometridae as one of the more recently diversified lineages within Fulgoroidea. The resulting phylogeny supports Asche's (1987) hypothesis of a functional trend in ovipositor structure across families.

Keywords: Planthopper phylogeny; Planthopper evolution; Fulgoroidea

1. Introduction

Planthoppers (Insecta: Hemiptera: Fulgoroidea) are a diverse group of phytophagous insects, with more than 9000 described species worldwide (O'Brien and Wilson, 1985), that display a number of qualities making them of keen interest to biologists. A number of planthopper species are economically significant pests of major agricultural crops such as corn, wheat, rice, and barley, as well as of grapes, sugarcane, and taro (O'Brien and Wilson, 1985). Many planthoppers display bizarre morphology whose adaptive significance has never been tested, such as the fulgorids *Fulgora laternaria* (Linnaeus), the peanut-headed bug, or *Phrictus quinquepartitus* (Distant), the dragon-headed bug. Many species excrete honeydew, a mixture of partially digested phloem with products excreted via the insect's Malpighian tubules (Delabie, 2001), that serves as a carbohydrate-rich food source for a variety of other organisms including ants, bees, and wasps (Dejean et al., 2000; O'Brien, 2002), cockroaches (Roth and Naskrecki, 2001), and even geckos (Föelling et al., 2001). While adult females of many planthopper species produce wax that is associated with egg deposition (Mason et al., 1989; O'Brien and Wilson, 1985), the nymphs of planthoppers in a number of families such as Lophopidae and Flatidae, also produce waxy skeins whose function has not been formally tested.

Unfortunately, the list of fascinating aspects of planthopper biology is far more extensive than is our current

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understanding of the evolutionary diversification of this insect group. Despite the numerous publications on planthopper phylogeny (Muir, 1923, 1930; Asche, 1987; Emeljanov, 1990; Bourgoin, 1993; Chen and Yang, 1995; Bourgoin et al., 1997; Yeh et al., 1998, 2005; Yeh and Yang, 1999), two over-arching questions remain: (1) how are planthopper families related evolutionarily? and (2) how many families of planthoppers are there?

Muir (1930) proposed an informal grouping of planthopper families (Fig. 1A) based on the number of spines on the second segment of the hind tarsi. Planthopper phylogeny was not more rigorously addressed until more than 50 years later, when Asche (1987) presented an analysis of relationships among fulgoroid families (Fig. 1B), based primarily on adult morphological characters (including features of the female genitalia). Like Muir, Asche recognized three groups of families, but these assemblages were based on a functional trend that Asche observed in the modification of the ovipositor. In the most anciently diversifying lineage were placed families which possess a "piercing-sawing" type of ovipositor and in an intermediate lineage were placed families characterized by ovipositors adapted to either burying eggs or covering eggs with wax; the remaining planthopper families, placed in the most recently diversifying lineage, possess a piercing-excavating ovipositor that originates developmentally in a manner different from that of "piercing-sawing" group (Asche, 1987).

Such a trend in ovipositor function suggests that there might be an associated phylogenetic trend in host plant use across families as well. Wilson et al. (1994) used Asche's (1987) hypothesized phylogeny to examine patterns of host plant association among planthopper lineages. Those data suggested that the phylogenetically more basal planthopper families display a greater association with monocot hosts, whereas the phylogenetically more derived families have a greater association with dicot hosts. Wilson et al. (1994) interpreted this finding in light of a correlated trend in feeding location: the families Cixiidae, Achilidae, and Derbidae tend to feed in concealed locations, either underground or under bark, whereas the families Delphacidae and Meenoplidae tend to feed near the ground on their host plants, also a presumably more concealed location. The remaining families tend to feed higher on their host plants, in more exposed feeding locations. Therefore, Wilson et al. (1994) concluded that the piercing-sawing ovipositor would be adapted for oviposition in debris at the base of the host

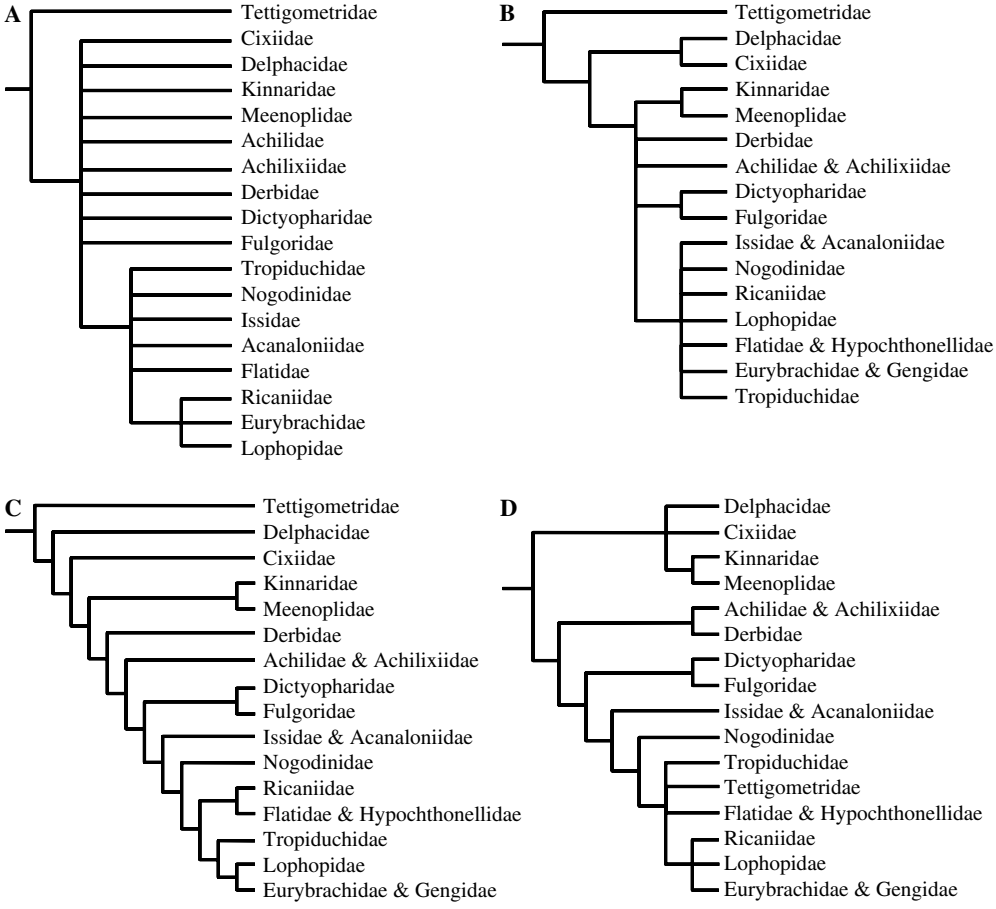

Fig. 1. Alternative hypotheses of the phylogeny of Fulgoroidea (redrawn and simplified; branch lengths are not to scale). (A) Muir (1930) recognized three general family groups based on hind tarsal spines. (B) Asche (1987) also recognized three general family groups defined on ovipositor structure. (C) Emeljanov (1990) proposed a fully resolved phylogeny based on adult and larval morphology. (D) Bourgoin (1993) analyzed features of the adult female genitalia to infer phylogeny.

plant, or for inserting eggs into the plant tissue of monocots. The spadelike ovipositor would facilitate covering the eggs, either at the base of the plant (i.e., burying in soil), or covering eggs (e.g., with wax or other materials) that are adhered to a substrate higher up on the plant, in either monocot or dicot hosts. The piercing-excavating ovipositor would be an adaptation for excavating the woody tissue of dicot hosts for oviposition.

While it is an intriguing hypothesis that planthopper diversification could reflect an evolutionary progression in feeding location, results of additional phylogenetic studies have yielded mixed results with respect to this hypothesis. Emeljanov (1990) used adult and nymphal characters to propose a phylogeny of Fulgoroidea that is consistent with the Asche (1987) hypothesis with respect to the general ordering of relationships among families. Emeljanov (1990) further specified a relative order of diversification among families, thereby proposing a fully resolved phylogeny for the superfamily (Fig. 1C) hypothesizing several new sister-group relationships beyond those determined by Asche (1987, Fig. 1B).

Bourgoin (1993), however, presented a phylogenetic hypothesis based on morphology of the adult female genitalia that is not concordant with the Asche (1987) hypothesis of three groups of functional ovipositor types. Rather, Bourgoin (1993) proposed two monophyletic lineages within Fulgoroidea (Fig. 1D), reproduced from Bourgoin et al. (1997); in one lineage are placed four families characterized by orthopteroid-like female genitalia, which was interpreted to be the ancestral state for fulgoroids. The remaining families are characterized by a derived condition of the female genitalia.

Chen and Yang's (1995) phylogenetic hypothesis of Fulgoroidea, based on analysis of larval metatarsi, suggested three family groups similar in identity to those of Asche (1987) and Muir (1930). However, relationships (Fig. 2A) among the lineages suggested a polarity reversal relative to the other morphology-based hypotheses. Thus, despite numerous attempts to reconstruct phylogenetic relationships within Fulgoroidea based on morphology, little is generally agreed upon.

The monophyly of Fulgoroidea is well supported based on morphology (Asche, 1987; Bourgoin et al., 1997; Emeljanov, 1990), with synapomorphies including shape of the middle and hind coxae, bristle-like antennae beneath the compound eye, basal antennal placoids, and the "fulgoromorphan face" (Asche, 1987; Bourgoin et al., 1997; Yeh et al., 1998). Recent studies using molecular evidence further support the monophyly of Fulgoroidea (Bourgoin et al., 1997; Yeh et al., 2005). However, the monophyly of some of the approximately 20 currently recognized planthopper families remains in doubt. Emeljanov (1999) questioned the monophyly of the planthopper family Issidae; based on features of the ovipositor, he proposed the recognition of the brachypterous issid subfamily Caliscelinae as a distinct family. A reduction in number of families was favored by Liang (2001), who considered Achilixiidae, with the two included subfamilies Achilixiinae and Bebaiotinae, as members of Cixiidae based on common features of the antennal sensilla. Several additional groups are given family level names (e.g., Gengidae, Hypochthonellidae, Acanaloniidae), yet are placed within larger families in some published phylogenetic hypotheses and classification schemes (Fig. 1).

A number of studies have applied molecular data to some of the questions surrounding planthopper phylogeny. Bourgoin et al. (1997) analyzed 18S ribosomal DNA nucleotide sequence data generated from exemplars of seven fulgoroid families (Fig. 2B) in order to test the placement of the family Tettigometridae (see Bourgoin's (1993) placement in Fig. 1D, relative to Figs. 1A–C). Similarly, Yeh et al. (1998) analyzed partial regions of the mitochondrial genes 16S rDNA and Cytochrome b from exemplars of seven families in order to determine whether the issid subfamily Caliscelinae should be split out of Issidae and recognized as its own family (Fig. 2C). Although those studies achieved their intended objectives (i.e., testing the relative placements of the Tettigometridae and Caliscelidae), their limited taxonomic sampling failed to provide comprehensive insight into relationships across Fulgoroidea.

Most recently, two molecular phylogenetic studies were conducted that included greater taxonomic representation. Yeh and Yang (1999) analyzed a partial region (~720 bp) of 28S rDNA for exemplars of 13 fulgoroid families (Fig. 2D) and Yeh et al. (2005) analyzed a partial region (~600 bp) of 16S rDNA for exemplars of 15 fulgoroid families (Fig. 2E). Results of both studies agreed on several issues: (1) placement of Delphacidae, Cixiidae, and Meenoplidae among the most anciently diversifying lineages within the superfamily, (2) placement of the Tettigometridae among more recently diversifying lineages, (3) placement of Dictyopharidae and Fulgoridae as sister taxa, and (4) paraphyly of Issidae. Unfortunately, these studies were methodologically limited in that they each included a relatively small amount of sequence data from only a single gene per analysis, they employed only a distance-based method (i.e., neighbor-joining) of phylogenetic reconstruction, and outgroup choice was suboptimal. Despite these limitations, however, the results of these studies clearly illustrate the power of the molecular approach for providing much needed insight into planthopper diversification. However, there have been no comprehensive studies (with regard to inclusion of multiple genetic loci or thorough taxonomic sampling) performed to date that test existing hypotheses of planthopper evolution.

Therefore, the current study represents a more rigorous test of existing morphology based hypotheses by increasing the breadth of taxonomic sampling, the number of molecular markers used for analysis, and the analytical methodologies beyond any previous investigation of fulgoroid phylogeny. Phylogenetic relationships within Fulgoroidea

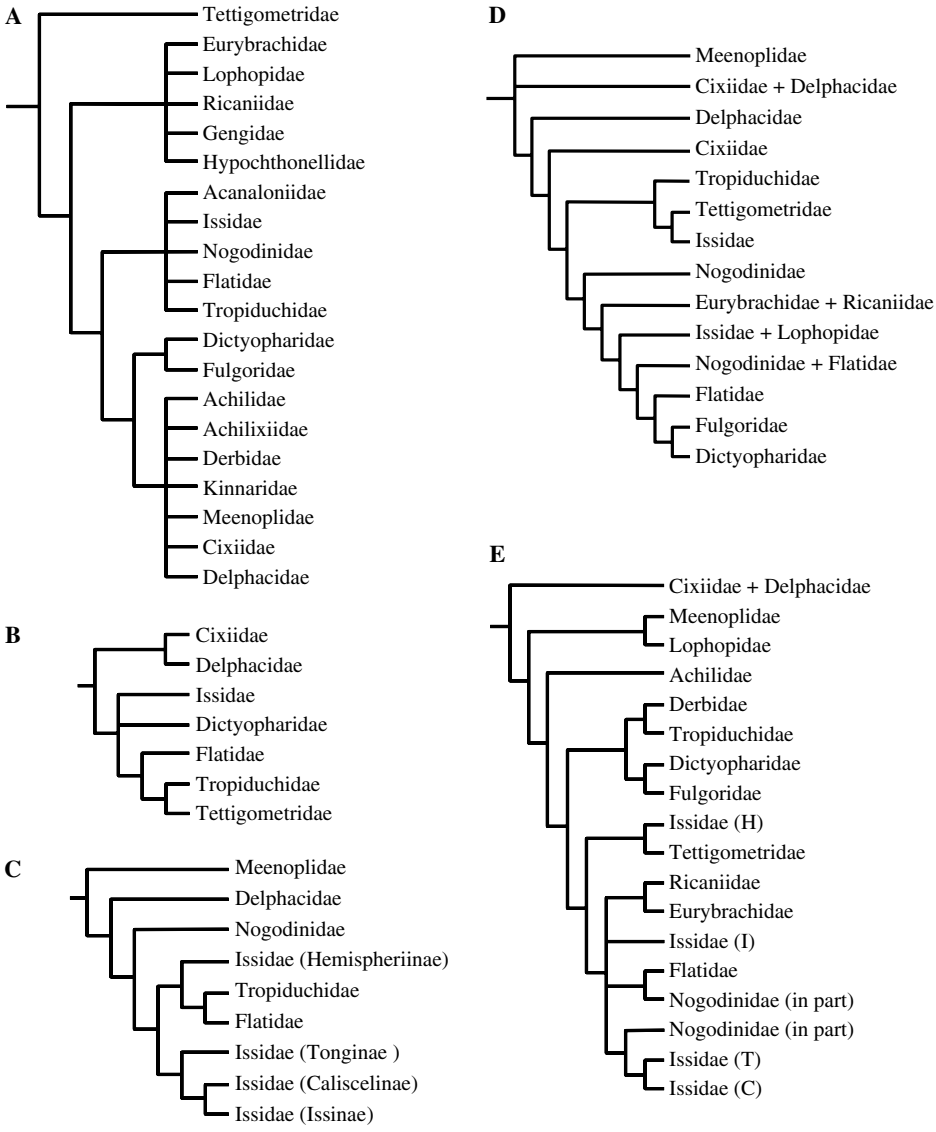

Fig. 2. Alternative hypotheses of the phylogeny of Fulgoroidea (redrawn and simplified; branch lengths are not to scale). (A) Chen and Yang (1995), based on larval metatarsi. (B) Bourgoin et al. (1997), based on partial 18S rDNA sequences. (C) Yeh et al. (1998) analyzed partial 16S rDNA sequences of the "Tropiduchidae group". (D) Yeh and Yang (1999) reconstructed planthopper phylogeny based on analysis of partial 28S rDNA sequences. (E) Yeh et al. (2005) analyzed partial 16S rDNA sequences.

are here reconstructed based on nucleotide sequences from four nuclear genes (18S rDNA, 28S rDNA, Histone subunit 3, and Wingless) for exemplars of 18 of the 20 fulgoroid families. Outgroup taxa were selected from within Hemiptera (s.l.), as well as from lineages in other paraneopteran orders to insure the correct polarity of the reconstructed phylogenies. In so doing, the current study seeks to answer the following questions: (1) How planthopper families are related evolutionarily by testing the morphology-based hypotheses of Muir (1930), Asche (1987), Emeljanov (1990), Bourgoin (1993), and Chen and Yang (1995) and (2) How many planthopper families should be recognized? Specifically, the sampling of this study allows detailed investigation of the placement of achilixiids with respect to Cixiidae, and Acanaloniidae and Caliscelidae with respect to Issidae.

2. Materials and methods

2.1. Taxon sampling

Insect specimens (Table 1) were collected into 95–100% ethanol and are stored at −80 °C in the New York State Museum Genome Bank, a frozen tissue collection (NYSM, Albany, NY). The 71 ingroup specimens included in this analysis represent 18 fulgoroid families, as well as the putative family Caliscelidae (exemplars of Gengidae and Hypochthonellidae were not available for this analysis); ingroup taxa were selected in an attempt to maximize the sampled phyletic and biogeographic diversity of each family (Table 2), to the extent possible with available specimens. Nine non-fulgoroid taxa from within Hemiptera were included as outgroups (Table 1); because relationships

Table 1
Taxa sampled

Taxon	Voucher Code[a]	Geographical source	GenBank Accession Number			
			18S	28S	H3	Wg
Acanaloniidae						
Acanalonia depressa	02-06-17-04	St. John[b]	DQ532503	DQ532582	DQ532660	DQ532729
Acanalonia bivitatta	02-06-17-78	USA (NY)	DQ532504	DQ532583	DQ532661	DQ532730
Acanalonia vivida	02-06-20-20	St. John[b]	DQ532505	DQ532584	DQ532662	DQ532731
Philatis sp.	03-01-09-75	Ecuador	DQ532546	DQ532626	—	DQ532760
Achilidae						
Catonia sp.	02-06-20-02	St. John[b]	DQ532506	DQ532585	DQ532663	DQ532732
Amblycratus sp.	04-12-17-52	French Guiana	DQ532507	DQ532586	DQ532664	DQ532733
Spino sp.	04-12-28-64	Costa Rica	DQ532508	DQ532587	DQ532665	DQ532734
Achilixiidae						
Bebaiotes sp.	03-01-09-49	Ecuador	DQ532509	DQ532588	DQ532666	DQ532735
Caliscelidae						
Bruchomorpha sp.	02-06-17-70	USA (MD)	DQ532544	DQ532624	DQ532697	—
Aphelonema sp.	02-06-20-23	USA (DE)	DQ532545	DQ532625	DQ532698	DQ532759
Fitchiella sp.	04-05-15-70	USA (AZ)	DQ532549	DQ532629	DQ532701	DQ532763
Cixiidae						
Pintalia alta Osborn	02-06-17-11	St. John[b]	AY744804	DQ532589	AY744876	—
Oliarus slossonae	02-06-17-01	St. John[b]	DQ532510	DQ532590	—	—
Bothriocera eborea	02-06-17-03	St. John[b]	DQ532511	DQ532591	DQ532667	—
Oecleus sp.	02-06-17-52	USA (UT)	DQ532512	DQ532592	DQ532668	—
Delphacidae						
Megamelus distinctus	01-07-24-09	USA (NC)	DQ532513	DQ532593	—	DQ532736
Nothodelphax gillettei (VD)	01-07-24-15	USA (UT)	DQ532514	DQ532594	DQ532669	—
Opiconsiva sp.	04-12-09-67	Australia	DQ532515	DQ532595	—	—
Harmalia ostorius	04-12-09-69	Australia	DQ532516	DQ532596	DQ532670	—
Derbidae						
Cedusa obscura	02-06-17-06	USA (NY)	DQ532517	DQ532597	DQ532671	DQ532737
Cedusa sp.	02-06-17-67	USA (MD)	DQ532518	DQ532598	DQ532672	DQ532738
Omalicna sp.	04-12-17-14	USA (FL)	DQ532519	DQ532599	DQ532673	DQ532739
Derbe sp.	04-12-17-47	Fr. Guiana	DQ532520	DQ532600	DQ532674	DQ532740
Rhotana sp.	04-12-28-38	Australia	DQ532521	DQ532601	—	DQ532741
Dictyopharidae						
Scolops sulcipes	02-06-17-07	USA (NY)	DQ532522	DQ532602	DQ532675	DQ532742
Rhynchomitra microrhina	02-06-20-22	USA (NC)	DQ532523	DQ532603	DQ532676	—
Lappida sp.	03-07-21-58	Costa Rica	DQ532524	DQ532604	DQ532677	—
Hyalodictyon sp.	04-12-17-57	Fr. Guiana	DQ532525	DQ532605	DQ532678	DQ532743
Dictyophara sp.	04-12-28-51	India	DQ532526	DQ532606	DQ532679	DQ532744
Rhaphiophora sp.	04-12-28-79	Ghana	DQ532527	DQ532607	DQ532680	DQ532745
Eurybrachidae						
Platybrachys sp.	03-07-21-81	Australia	DQ532528	DQ532608	DQ532681	—
Platybrachys group *sicca*	04-05-15-10	Australia	DQ532529	DQ532609	DQ532682	DQ532746
Platybrachys group *sicca*	04-12-28-10	Australia	DQ532530	DQ532610	DQ532683	DQ532747
Olonia sp.	04-12-28-11	Australia	DQ532531	DQ532611	DQ532684	DQ532748
Platybrachys group *sicca*	04-12-28-50	Australia	DQ532532	DQ532612	DQ532685	DQ532749
Flatidae						
Petrusa epilepsis	02-06-20-05	St. John[b]	DQ532533	DQ532613	DQ532686	DQ532750
Melormenis basalis	02-06-17-14	St. John[b]	DQ532534	DQ532614	DQ532687	—
Pseudoflatoides sp.	02-06-17-54	St. John[b]	DQ532535	DQ532615	DQ532688	DQ532751
Ormenis saucia	04-12-17-34	USA (AZ)	DQ532536	DQ532616	DQ532689	DQ532752
Massila sp.	04-12-28-40	Australia	DQ532537	DQ532617	DQ532690	DQ532753
Siphanta sp.	04-12-28-62	Malaysia	DQ532538	DQ532618	DQ532691	DQ532754
Fulgoridae						
Amerzanna sp.	04-05-15-42	Peru	DQ532539	DQ532619	DQ532692	DQ532755
Rentinus dilatatus	04-05-15-27	Australia	DQ532540	DQ532620	DQ532693	DQ532756
Penthicodes pulchella	04-12-15-34	India	DQ532541	DQ532621	DQ532694	DQ532757
Scaralis semillimpida	04-12-17-78	Fr. Guiana	DQ532542	DQ532622	DQ532695	DQ532758

Table 1 (*continued*)

Taxon	Voucher Code[a]	Geographical source	GenBank Accession Number			
			18S	28S	H3	Wg
Issidae						
Thionia argo	02-06-17-10	St. John[b]	DQ532543	DQ532623	DQ532696	—
Kinnaridae						
Oeclidius sp.	03-07-21-72	USA (CA)	DQ532551	DQ532631	DQ532703	DQ532765
Quilessa maculata	04-11-29-07	Dominica	DQ532552	DQ532632	DQ532704	DQ532766
Lophopidae						
Lophops sp.1	03-01-09-53	PNG[c]	DQ532553	DQ532633	DQ532705	DQ532767
Onycta sp.	03-01-09-55	PNG[c]	DQ532554	DQ532634	DQ532706	DQ532768
Lophops sp.2	03-01-09-60	PNG[c]	DQ532555	DQ532635	DQ532707	DQ532769
Makota sp.	03-01-09-64	PNG[c]	DQ532556	DQ532636	DQ532708	DQ532770
Meenoplidae						
Nisia sp.	03-01-09-65	PNG[c]	DQ532557	DQ532637	DQ532709	DQ532771
Nogodinidae						
Biolleyana costalis	02-06-17-42	Costa Rica	DQ532558	DQ532638	DQ532710	DQ532772
Bladina sp.	03-01-09-78	Belize	DQ532560	DQ532640	DQ532712	DQ532774
Danepteryx sp.	04-12-17-23	USA (CA)	DQ532547	DQ532627	DQ532699	DQ532761
Dictyssa sp.	04-12-17-39	USA (CA)	DQ532548	DQ532628	DQ532700	DQ532762
Paradetya sp.	03-01-09-59	PNG[c]	DQ532559	DQ532639	DQ532711	DQ532773
Undet. Lipocalliini	04-12-28-24	Australia	DQ532550	DQ532630	DQ532702	DQ532764
Ricaniidae						
Pochazia guttifera	02-06-17-34	PNG[c]	DQ532561	DQ532641	DQ532713	DQ532775
Euricania sp.	02-06-17-25	PNG[c]	DQ532562	DQ532642	DQ532714	DQ532776
Privesa sp.	02-06-17-29	PNG[c]	DQ532563	DQ532643	DQ532715	DQ532777
Scolypopa sp.	02-06-17-36	PNG[c]	DQ532564	DQ532644	DQ532716	—
Aprivesa sp.	04-12-28-27	Australia	DQ532565	DQ532645	DQ532717	DQ532778
Tettigometridae						
Euphyonarthex phyllostoma			DQ532566	DQ532646	DQ532718	—
Hilda undata	04-12-28-72	Ghana	DQ532567	DQ532647	DQ532719	DQ532779
Hilda sp.	04-12-28-76	Ghana	DQ532568	DQ532648	DQ532720	DQ532780
Tropiduchidae						
Tangia viridis (Walker)	02-06-17-09	St. John[b]	DQ532569	DQ532649	DQ532721	DQ532781
Tangiopsis sp.	03-01-09-46	Ecuador	DQ532570	DQ532650	DQ532722	DQ532782
Chasmocephalus pluvialis	04-11-29-09	Dominica	DQ532571	DQ532651	DQ532723	DQ532783
Neotangia caribea	04-11-29-10	Dominica	DQ532572	DQ532652	DQ532724	DQ532784
Outgroups						
Cercopoidea: Aphrophoridae						
Neophilaenus linneatus Linn.	01-07-15-03	USA (VT)	DQ532499	DQ532579	DQ532658	—
Cicadoidea: Tettigarctidae						
Tettigarcta crinata Distant	CS 97-3	Australia	DQ532500	DQ532580	AY744874	—
Membracoidea: Membracidae						
Ophiderma definata Woodr.	NCSU 95-02-01-60	USA (MD)	DQ532502	DQ532581	DQ532659	AY498513
Flexamia areolata (Ball)	CHD LH38	USA (Virginia)	DQ532501	AY744845	AY744883	AY498503
Heteroptera						
Undetermined Berytidae	01-08-15-25	St. John[b]	DQ532493	DQ532573	DQ532653	DQ532725
Undetermined Pentatomidae	01-08-15-26	St. John[b]	DQ532494	DQ532574	DQ532654	DQ532726
Undetermined Miridae	01-08-15-43	USA (MD)	DQ532495	DQ532575	DQ532655	DQ532727
Undetermined Belostomatidae	02-12-10-15	Belize	DQ532496	DQ532576	—	—
Undetermined Psyllidae	03-12-09-39	Australia	DQ532498	DQ532578	DQ532657	DQ532728
Mantodea						
Undetermined Mantidae	04-10-28-81	Peru	DQ532497	DQ532577	DQ532656	—
Plecoptera						
Malenka californica			AY338724	AY338680	AY338642	—
Cerconychia sp.			AY338725	AY338682	AY338643	—

Taxa included in 18S, 28S, Histone 3 (H3), and Wingless (Wg) nucleotide sequence datasets.

[a] Voucher material preserved in the New York State Museum Genome Bank (in 95% ethanol at −80 °C).

[b] US Virgin Islands.

[c] Papua New Guinea.

Table 2
Diversity of taxon sampling

Family	Subfamilies sampled	Tribes sampled	Biogeographic regions sampled[b]
Acanaloniidae	1 of 1	1 of 1	NEA, NEO
Achilidae[c]	1 of 2	1 of 7	NEO
Achilixiidae	1 of 1	1 of 1	NEO
Caliscelidae[d]	1 of 1	1 of 3	NEA
Cixiidae[e]	2 of 3	4 of 17	NEA, NEO
Delphacidae[f]	1 of 7	1 of 9	NEA, AUS
Derbidae	1 of 2	3 of 6	NEA, NEO, AUS
Dictyopharidae	1 of 2	1 of 4	NEA, NEO, AFR, IND
Eurybrachidae	1 of 2	1 of 7	AUS
Flatidae	2 of 2	4 of 9	NEA, NEO, AUS, IND
Fulgoridae	4 of 5	4 of 13	NEO, AUS, IND
Issidae[g]	1 of 4	1 of 6	NEO
Kinnaridae	1 of 1	1 of 1	NEA, NEO
Lophopidae	1 of 2	2 of 6	AUS
Meenoplidae	1 of 2	1 of 1	AUS
Nogodinidae[h,i]	1 of 1	4 of 7	NEA, NEO, AUS
Ricaniidae	1 of 1	1 of 1	AUS
Tettigometridae	2 of 3	2 of 3	AFR
Tropiduchidae	1 of 2	2 of 9	NEO

Phyletic and geographic diversity represented by taxa included in these analyses. Taxonomic classification follows Metcalf catalogues[a] unless otherwise indicated.

[a] Metcalf (1932, 1945, 1946, 1947, 1954a,b, 1955a,b, 1956, 1957, 1958).

[b] Biogeographic region abbreviations: AFR = Afrotropical region (= Ethiopian) AUS = Australian region (= Australasian) IND = Indomalayan region (= Oriental) NEA = Nearctic region NEO = Neotropical region PAL = Palearctic region.

[c] Fennah (1950).

[d] Emeljanov (1999).

[e] Emeljanov (2002).

[f] Asche (1985).

[g] Metcalf (1958) with Caliscelinae removed as per Emeljanov (1999).

[h] Fennah (1978).

[i] Fennah (1984).

among the major hemipteran lineages remain controversial, three additional paraneopteran exemplars were also included (Table 1) to ensure accurate character polarity.

2.2. Molecular markers

The nuclear genes 18S rDNA and 28S rDNA, encoding the small and large ribosomal subunits, respectively, were chosen for use in the current study because the levels of variation at these loci have provided phylogenetically useful information for family level divergences in studies of other auchenorrhynchan insects (Cryan et al., 2000, 2004; Dietrich et al., 2001; Cryan, 2005). Similarly, the nuclear protein coding genes H3 and Wg were chosen for use in the current study because they are not highly variable, due to selective constraint, making alignment less problematic. These markers also have been shown to be phylogenetically informative in studies of insects with comparable levels of divergence (Colgan et al., 1998; Ogden and Whiting, 2003; Cryan et al., 2004; Cryan, 2005). Furthermore, combining ribosomal and protein coding genes is an approach recommended by Danforth et al. (2005), based on their analyses comparing the phylogenetic utility and performance of these two types of genes across 12 datasets. Mitochondrial markers were not included in these analyses; the higher rate of nucleotide substitution evident in mtDNA made saturation a confounding issue with regard to reconstructing phylogenetic relationships at or above the tribal level in Fulgoroidea (Urban and Cryan, unpublished).

2.3. DNA extraction and PCR amplification

For all specimens, DNA was extracted from thoracic flight muscle tissue using FastDNA Extraction Kits (Qbiogene Inc., Carlsbad, CA, USA) or Qiagen DNEasy Kits (Qiagen, Inc., Valencia, CA, USA). Polymerase chain reaction (PCR) was conducted in 25 μl and 50 μl reactions, using either AmpliTaq DNA polymerase (PE Applied Biosystems, Foster City, CA, USA) or ISIS DNA polymerase (Qbiogene). The PCR cycling program was 30 s. (AmpliTaq) or 2 min. (ISIS) at 94 °C, 30–35 cycles of 1 min at 42–54 °C and 1 min at 72 °C, followed by 7 min at 72 °C. All PCR reactions included negative controls to detect any possible contamination.

Oligonucleotide primers used in PCR reactions are listed in Table 3, and were synthesized at Wadsworth Laboratories (NY Department of Health, Albany, NY). Amplified DNA was visualized using 1–2% agarose gel electrophoresis with ethidium-bromide staining, and was purified using GeneClean purifying kits (BIO 101, Vista, CA, USA). Sequences were obtained from complementary strands using d-Rhodamine terminator cycle sequencing on ABI

Table 3
Oligonucleotide primer sequences

Primer	Sequence (5′ → 3′)	Primer source
18S Fulfwd1	GGATAACTGTGGTAATTCTAG	New, designed by JMU/JRC
18S Fulrev2	ACACAGATCCAACTACGAG	New, designed by JMU/JRC
18S Fulfwd2	CTCGTAGTTGGATCTGTGT	New, designed by JMU/JRC
18S Fulrev3	TCAAATTAAGCCGCAGGC	New, designed by JMU/JRC
18S Fulfwd3	GCCTGCGGCTTAATTTGA	New, designed by JMU/JRC
18S Fulrev4	CTACGGAAACCTTGTTACG	New, designed by JMU/JRC
18S 1F	TACCTGGTTGATCCTGCCAGTAG	Cryan et al. (2000)
18S DelR1	AATTTGTTCAAAGTAAACGTGCCGG	Cryan et al. (2000)
18S a0.7	ATTAAAGTTGTTGCGGTT	Cryan et al. (2000)
18S bi	GAGTCTCGTTCGTTATCGGA	Cryan et al. (2000)
18S a2.0	ATGGTTGCAAAGCTGAAAC	Cryan et al. (2000)
18S 9R	GATCCTTCCGCAGGTTCACCTAC	Cryan et al. (2000)
28S EE	CCGCTAAGGAGTGTGTAA	Cryan et al. (2000)
28S MM	GAAGTTACGGATCTARTTTG	Cryan et al. (2000)
28S Lalt	CCTCGGACCTTGAAAATCC	Cryan et al. (2000)
28S Galt	TGTCTCCTTACAGTGCCAGA	Cryan et al. (2000)
28S V	GTAGCCAAATGCCTCGTCA	Cryan et al. (2000)
28S X	CACAATGATAGGAAGAGCC	Cryan et al. (2000)
H3AF	ATGGCTCGTACCAAGCAGACVGC	Colgan et al. (1998)
H3AR	ATATCCTTRGGCATRATRGTGAC	Colgan et al. (1998)
HexAF	ATGGCTCGTACCAAGCAGACGGC	Colgan et al. (1998)
HexAR	ATATCCTTGGGCATGATGGTGAC	Colgan et al. (1998)
H3BF	ATGGCTCGTACCAAGCAGAC	Colgan et al. (1998)
H3BR	ATRTCCTTGGGCATGATTGTTAC	Colgan et al. (1998)
Wg 1A	GARTGYAARTGYCAYGGYATGTCTGG	Cryan et al. (2001)
Wg DelR1	GTCCTGTARCCRCGKCCACAACACAT	New, designed by JRC

Primers used for the PCR amplification of 18S, 28S, H3, and Wg from Fulgoroidea and outgroups.

Prism 3100/3700 DNA sequencers at Wadsworth Laboratories.

2.4. Sequence alignment and phylogenetic analysis

All chromatography data were visually inspected, edited, and assembled in contiguous sequences using Sequencher 4.0.5 for Windows (GeneCodes Corp., Ann Arbor, MI, USA). Multiple sequence alignment was performed manually using Sequencher. Highly variable regions of 18S and 28S that differed in base composition and sequence length across the sampled fulgoroid taxa were excluded from phylogenetic analysis due to the extreme ambiguity involved in any possible alignment. Excluded from the 18S alignment were four non-contiguous regions with a combined length of 310 bp; excluded from the 28S alignment were six non-contiguous regions with a combined length of 710 bp. Exclusion of these highly variable regions of 18S and 28S is a practice consistent with previous phylogenetic analyses of insect taxa using these markers (e.g., Sanderson and Shaffer, 2002; Cryan et al., 2004). Within the aligned regions, gaps in 18S and 28S sequences were coded as missing data.

Multiple sequence alignment of the nuclear protein coding gene H3 was unambiguous, and included no gaps. Codon position was determined by comparison of amplified H3 sequences with sequences available on GenBank for which amino acid identity and reading frame had been determined (Ogden and Whiting, 2003). Likewise, multiple sequence alignment of Wg was unambiguous, but did include one gap that did not interrupt or shift the reading frame (as determined using Sequencher) for any of the taxa showing an insert in this region. All missing data were coded as gaps.

Two phylogenetic reconstruction paradigms were used to analyze the data generated during this investigation. Analyses of the individual and combined data partitions were conducted under the criterion of maximum parsimony using PAUP*4.0b10 (Swofford, 2001). Heuristic tree searches were conducted using 1000 replications of random additions with the tree bisection and reconnection option (TBR). However, due to excessive computational time required to complete heuristic parsimony searches of the individual 18S and 28S partitions, the nchuck and chuckscore options were employed as implemented by PAUP* such that no more than 500 trees of score 1750 were saved in each of the 1000 replications of the heuristic search of the 18S partition (nchuck = 500, chuckscore = 1750), and no more than 500 trees of score 2750 were saved for the 28S partition (nchuck = 500, chuckscore = 2750). The time required to perform the heuristic search of the combined 18S + 28S data partitions was also extensive, and therefore only 250 replications of random sequence addition with TBR were conducted, as compared to the 1000 replications performed in all other searches. Bootstrap analyses were performed using 1000 standard replicates to provide an estimate of support for individual nodes in parsimony-based phylogenies resulting from the combined data partitions. Bremer and partitioned Bremer support values were

computed for phylogenies resulting from combined data partitions using TreeRot (version 2; Sorenson, 1999) in conjunction with PAUP*.

Partitioned Bayesian analysis was conducted for the combined 18S + 28S + H3 + Wg dataset using MrBayes 3 (Ronquist and Huelsenbeck, 2003). Five data partitions were specified in this analysis (18S, 28S, H3 + Wg codon position 1, H3 + Wg codon position 2, and H3 + Wg codon position 3). Modeltest 3.5 (Posada and Crandall, 1998) was used to evaluate alternative molecular evolution models; results indicated that the GTR + I + G model was most appropriate, and so that model was used in subsequent analyses. Parameters under this model were unlinked for the five partitions. Partitioned Bayesian analysis was run for 25 million generations, with four chains (one cold and three heated), flat priors, and trees sampled at intervals of 1000 generations for a total of 25,000 sampled trees. Log-likelihood scores were plotted to determine the number of sampled trees to be discarded as "burn-in" (Huelsenbeck and Ronquist, 2001). The first 5000 sampled trees were discarded, and the remainder used to construct a 50% majority rule consensus tree.

To compare each of the five previous morphology based hypotheses (Muir, 1930; Asche, 1987; Emeljanov, 1990; Bourgoin, 1993; Chen and Yang, 1995) with results from the current study, heuristic searches (using 1000 replications with random sequence additions and TBR) were conducted on the combined 18S + 28S + H3 + Wg dataset imposing the constraints of each hypothesis under the criterion of parsimony. The resulting topology of each search was compared to the topology obtained in the current study (i.e., the unconstrained topology) using the Templeton (Wilcoxon signed-ranks; Templeton, 1983), winning-sites (sign), and Kishino–Hasegawa (Kishino and Hasegawa, 1989) tests under parsimony using PAUP*. Where the constrained heuristic searches yielded multiple equally parsimonious trees, multiple trees were tested in the topology comparisons. Because the unconstrained tree was compared to multiple topologies, a Bonferroni correction was employed to control for the possibility of inflated Type I error (Cohen, 2001). Therefore, for each of the five comparisons, an $\alpha \leqslant 0.01$ was the criterion for significant difference (i.e., not due to random error) between the two topologies in question.

3. Results

Approximately 2 kb of 18S rDNA were amplified in three contiguous, overlapping sections, each of which was approximately 600–700 bp in length. For several taxa, however, the combination of primers that successfully amplified the first and second section of 18S failed to overlap, resulting in a gap of approximately 50bp in this region for some taxa. Approximately 2.2 kb of 28S rDNA were amplified in three contiguous, overlapping sections, each of which was approximately 700 bp in length. Approximately 350 bp of H3, and 340 bp of Wg, were each amplified in one section.

After the ambiguously aligned regions of each data partition were excluded (as described above), the retained data partitions consisted of 1900 bp from 18S, 1697 bp from 28S, 357 bp from H3, and 345 bp from Wg, yielding a combined molecular dataset of approximately 4.3 kb for 83 taxa (71 ingroup and 12 outgroup taxa). The number of characters, constant sites, variable sites, and parsimony informative sites for the individual and combined data partitions are summarized in Table 4. Mean base frequencies for 18S, 28S, each codon position of H3 and Wg, respectively, and for the combined data partitions, are shown in Table 5. Sequence divergences (Table 6) were computed between fulgoroid taxa and outgroups, between fulgoroid taxa across families, and between fulgoroid taxa within families, for 18S, 28S, and each codon position of H3 and Wg. Descriptive statistics for the results of maximum parsimony analyses conducted on individual data partitions and on combinations of the four data partitions are listed in Table 7.

Analysis of each individual data partition often resulted in unresolved topologies. As indicated in Table 7, data from two, three, and all four partitions were combined, analyzed,

Table 4
Descriptive statistics for separate and combined data partitions

Gene partition	Characters			
	Total	Constant	Varied	Informative
18S	1900	1235	665	380
28S	1697	893	804	602
H3	357	212	145	136
Wg	345	140	205	187
18S + 28S + H3 + Wg	4299	2480	1819	1305

Table 5
Base frequencies for separate and combined data partitions

Gene partition	Base frequencies				*P*-value
	A	C	G	T	
18S	24.4	23.9	27.9	23.8	1
28S	23.4	24.1	31.4	21.1	1
H3 codon 1	28.8	28.1	31.5	11.6	1
H3 codon 2	28.8	26.4	21.1	23.7	1
H3 codon 3	13.2	37.7	33.7	15.4	0.00
Wg codon 1	29.3	21.7	29.0	20.0	1
Wg codon 2	30.6	18.6	28.2	22.6	1
Wg codon 3	25.9	21.2	21.3	31.6	0.00
Overall	24.3	24.3	29.3	22.1	0.99

Table 6
Uncorrected pairwise distances among taxa for each data partition

Gene partition	Minimum	Maximum
18S	0.0000	0.1689
28S	0.0000	0.3339
H3 codon position 1	0.0000	0.1441
H3 codon position 2	0.0000	0.0200
H3 codon position 3	0.0000	0.7218
Wg codon position 1	0.0000	0.3787
Wg codon position 2	0.0000	0.2449
Wg codon position 3	0.0087	0.8419

Table 7
Descriptive statistics for parsimony analyses of separate and combined data partitions

Data partition	Trees			
	Number	Length	CI	RI
18S	178,000	1854	0.4256	0.5838
28S	7913	2827	0.4133	0.5978
H3	12	1869	0.1435	0.4414
Wg	4	1648	0.2192	0.5413
18S + 28S	1810	4624	0.4040	0.5726
H3 + Wg	12	3461	0.1808	0.4724
18S + 28S + H3	63	6949	0.3118	0.5001
18S + 28S + Wg	97	5895	0.3525	0.5509
18S + 28S + H3 + Wg (no H3 codon 3)	36	6848	0.3361	0.5380
18S + 28S + H3 + Wg (no Wg codon 3)	4	7284	0.3148	0.4999
18S + 28S + H3 + Wg (no H3/Wg codon 3)	290	5379	0.3892	0.5574
18S + 28S + H3 + Wg	1	8752	0.2874	0.4965

and the topologies evaluated; across all analyses, resolution increased as more data were included. Because the third codon positions of H3 and Wg exhibited somewhat high genetic divergences (Table 6), analyses were also run excluding the third codon position from either or both of these partitions. Exclusion of the third codon position of H3 resulted in failure to recover a monophyletic Fulgoroidea. Similarly, exclusion of the third codon position of Wg also resulted in failure to recover a monophyletic Flatidae or Acanaloniidae (one of the four acanaloniid taxa was placed sister to Delphacidae); analogous results were obtained when the third codon positions of both H3 and Wg were excluded. Analysis of the complete dataset under the criterion of parsimony yielded a single most parsimonious topology (length = 8752, CI = 0.2874, RI = 0.4965). Bootstrap, Bremer, and partitioned Bremer support values for each node of this topology are listed in Table 8. The position of groups of taxa across that topology (depicted in Fig. 3) and across the topology of the 50% majority rule consensus tree obtained from the Bayesian analysis (depicted in Fig. 4, with posterior probability values listed at each node) is treated in the following Discussion section.

As shown in Table 8, partitioned Bremer support values summed across each data partition indicate an overall negative value for the influence of H3. However, exclusion of this data partition yielded topologies that were problematic (e.g., resulted in a non-monophyletic Fulgoroidea) and more poorly resolved. Similar results were obtained when any other data partition or third codon positions were excluded. These results suggest that phylogenetic signal is increased with the addition of more data, as has been found in other phylogenetic studies (Wenzel and Siddell, 1999; Cryan, 2005). With respect to the higher homoplasy levels expected to occur in the third codon positions of H3 and Wg, our results are consistent with those of Wenzel and Siddell (1999), who showed using simulated data that phylogenetic signal can combine in an additive manner across multiple homoplasious data partitions. Similarly, a recent study by Vogler et al. (2005) using empirical data from tiger beetles showed that third codon positions provided phylogenetic signal, and results were not improved when these positions were excluded or downweighted. Therefore, the single most parsimonious tree resulting from the combined analysis of the complete dataset (Fig. 3) was used to evaluate existing morphology based hypotheses of planthopper evolution.

4. Discussion

4.1. Tree topologies

Parsimony analysis of the combined dataset yielded a single shortest-length tree (Fig. 3), which is similar in several key aspects to the topology of the 50% consensus tree reconstructed using partitioned Bayesian analysis (Fig. 4). Under both reconstruction methods, the Cixiidae and Delphacidae are placed in a clade sister to the remaining planthopper families. Under parsimony, the Delphacidae are placed as arising from within the Cixiidae, which was a possibility suggested by Asche (1985), although bootstrap and Bremer support values are low for this placement; the Bayesian analysis recovers Cixiidae and Delphacidae as monophyletic sister taxa. To date, morphological data have been unable to resolve the relationship between these two families, due in part to the extensive plesiomorphy observed in cixiid characters (Asche, 1985, 1987); indeed, Muir (1923) proposed that the Delphacidae arose from within the Cixiidae. The taxonomic sampling for this investigation was not designed to address this question, and while the obtained results cannot rule out the possibility that the Delphacidae arose from within Cixiidae, it is likely that accurate resolution of this issue requires more extensive taxonomic sampling.

In both the parsimony and Bayesian topologies, Kinnaridae + Meenoplidae were placed as the next most anciently diversifying lineage in Fulgoroidea. Placement of these two families as sister taxa is consistent with previous hypotheses (Asche, 1987; Emeljanov, 1990; Bourgoin, 1993), and is supported by the morphological synapomorphy of wax producing plates on abdominal segments VI–VIII (Asche, 1987). Additionally, nymphs of both families occur exclusively underground (O'Brien, 2002).

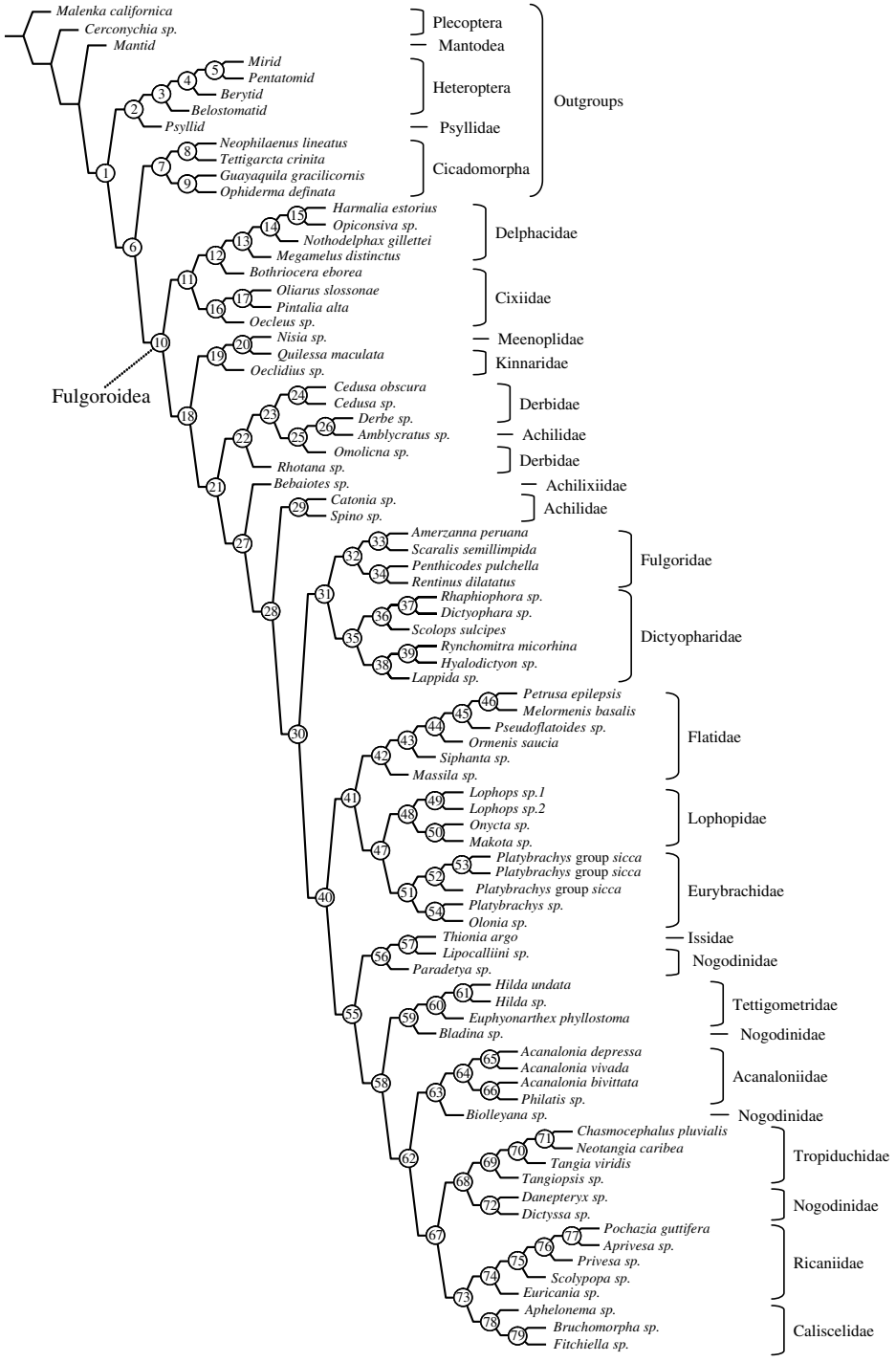

Fig. 3. Single most parsimonious topology (length = 8752; CI = 0.2874; RI = 0.4965) resulting from unconstrained and unweighted parsimony analysis of the combined data partitions 18S + 28S + H3 + Wg. Nodes are numbered (numbers in node circles); nodal support, including bootstrap, Bremer, and partitioned Bremer support values, are listed in Table 8.

The next set of lineages recovered by both parsimony and Bayesian analyses (Figs. 3 and 4, respectively) include the families Achilidae, Derbidae, and Achilixiidae, although the relationships within and among these groups differed across the reconstructed topologies. The Achilidae show paraphyletic placement in both the parsimony and Bayesian topologies, with one achilid placed as arising from within the Derbidae. Bourgoin (1993) proposed a sister relationship between Achilidae and Derbidae based on characters of female genitalia. Of the Achilidae, Achilixiidae, Kinnaridae, and Meenoplidae, Fennah (1950) wrote that "While these four families form a natural group, it is remarkable how some of their lines of development exactly parallel those found in Derbidae". Convergence between achilids and derbids include laying simple eggs, and concealment of nymphs under bark or within dead wood

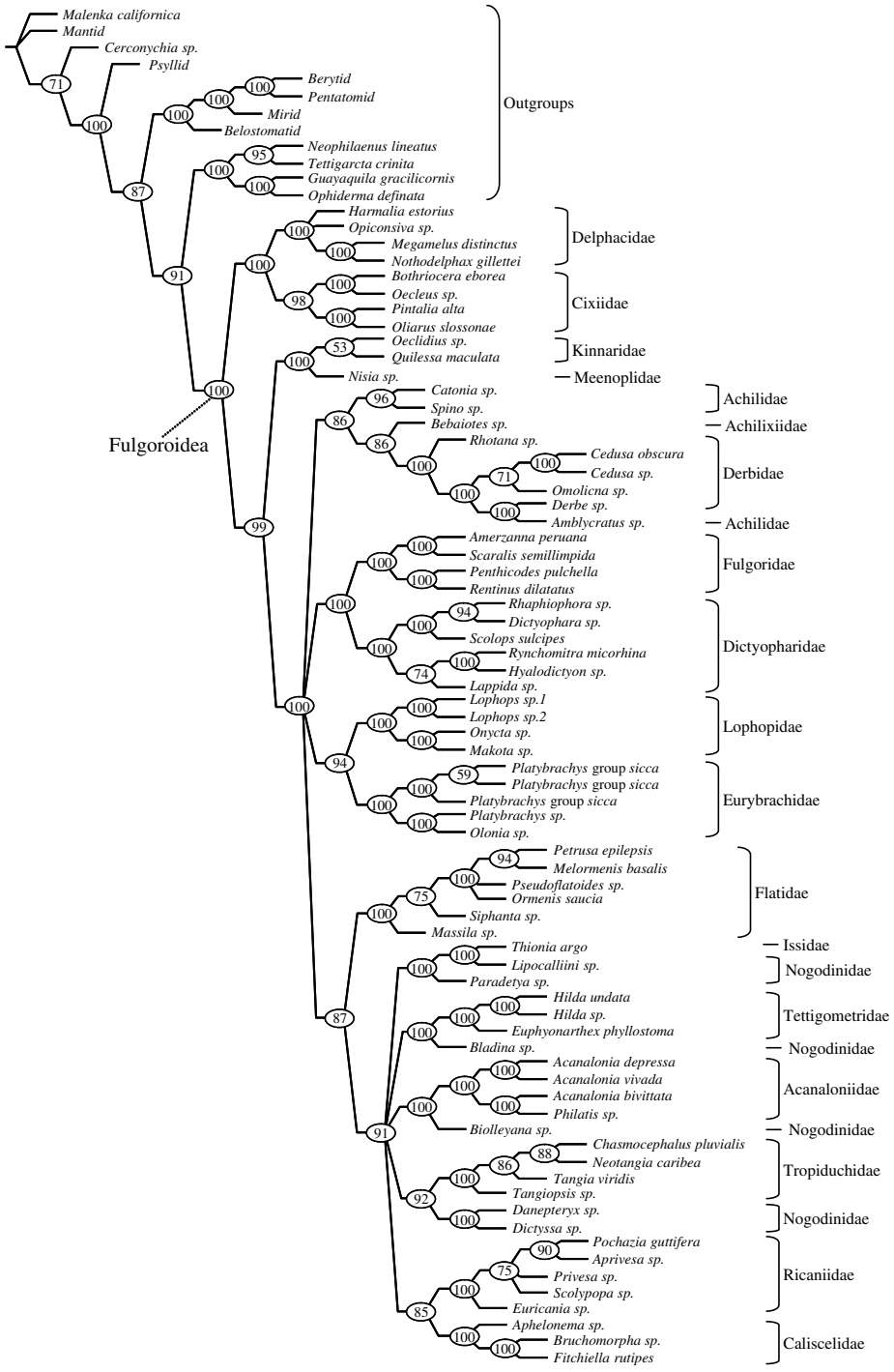

Fig. 4. Bayesian 50% consensus tree based on partitioned analysis of combined data 18S + 28S + H3 + Wg. Numbers in node ovals are posterior probability values.

(Fennah, 1950). Furthermore, neither the monophyly of Achilidae nor of Derbidae have yet been tested (Szwedo, 2004). These molecular data clearly suggest a close association between these families; however, more detailed investigation is needed to test the monophyly of Achilidae and its affinity to Derbidae.

The families Dictyopharidae and Fulgoridae were consistently recovered as sister taxa with strong nodal support, in concordance with numerous hypotheses that unite these lineages based on unique features of the male genitalia (Muir, 1923, 1930; Asche, 1987; Emeljanov, 1990; Bourgoin, 1993). Although many genera of Dictyopharidae and Fulgoridae possess elongate head processes, this characteristic is not a synapomorphy uniting these two families (in fact, six other planthopper families have at least one genus with a head process; O'Brien, 2002). Eurybrachidae and Lophopidae were also placed as sister taxa by both reconstruction methodologies, consistent

Table 8
Nodal support for Fig. 3

Node #	Bootstrap support (%)	Bremer support	Partitioned Bremer			
			18S	28S	H3	Wg
1	54	9	40	7	−17	−21
2	52	9	16	18	1	−26
3	86	6	43	−3	−16	−18
4	86	6	43	−3	−16	−18
5	<50	3	2	−6	1	6
6	<50	4	1	3	0	0
7	57	12	4	1	3	4
8	52	2	−2	2	2	0
9	100	30	13	21	−4	0
10	62	9	17	−4	−7	3
11	56	19	7	6	6	0
12	<50	3	0	−2	5	0
13	100	59	36	−9	8	24
14	81	1	1	0	0	0
15	62	1	0.5	0.5	0	0
16	<50	3	−1	−3	7	0
17	87	5	3	−5	7	0
18	<50	3	2	1	3	−3
19	86	6	11	−3	−5	3
20	67	2	1	1	3	−3
21	<50	5	4	−5	−8	14
22	55	11	5	9	2	−5
23	<50	6	5	4	0	−3
24	100	138	58	55	7	18
25	<50	11	4	6	2	−1
26	100	27	3	37	−15	2
27	<50	7	−4	2.5	2.5	6
28	<50	5	−1	−5	−1	12
29	51	5	2	−5	−5	13
30	<50	5	2	5	2	−4
31	89	10	3	−2	1	8
32	<50	11	2	3	3	3
33	97	8	6	0	1	1
34	82	5	1	0	2	2
35	68	7	3	1	3	0
36	84	6	10	−1	−4	1
37	59	1	2	−1	0	0
38	52	1	0	0	1	0
39	99	11	5	7	−1	0
40	<50	6	8	1	−8	5
41	<50	1	0	2	−1	0
42	<50	3	0	4	−1	0
43	<50	4	1	2	−3	4
44	85	11	1	0	4	6
45	<50	4	1	2	−3	4
46	69	4	0	0	4	0
47	78	5	2	1	−2	4
48	<50	6	1	0	4	1
49	100	30	5	8	8	9
50	79	5	0	2	1	2
51	99	14	3	9	1	1
52	100	17	4	5	5	3
53	79	1	0	0	1	0
54	90	5	−1	2	4	0
55	<50	5	5	−3	−4	7
56	100	88	1	87	1	−1
57	91	7	0	8	−2	1
58	<50	2	1	−2	2	1
59	<50	5	10	−7	−9	11
60	100	86	47	41	6	−8
61	100	27	0	27	0	0
62	<50	2	1	−2	2	1
63	51	9	−0.5	−0.5	9.5	0.5
64	<50	10	8	−13	5	10
65	100	13	7	0	2	4
66	86	10	3	1	2	4
67	<50	2	1	−1	2	0
68	<50	4	1.5	−3	2.5	3
69	100	24	13	−3	−9	23
70	<50	2	1	−1	2	0
71	<50	1	1	−1	−2	3
72	100	13	1.5	2.5	1	8
73	<50	4	0	2	−3	5
74	100	34	1	9	2	22
75	63	1	−1	2	−2	2
76	<50	1	0	2	−3	2
77	<50	1	0	2	−3	2
78	100	38	7	27	4	0
79	98	6	−1.5	4.5	3.5	−0.5
Total		983	479.5	349.5	−3	157
Percentage		100	48.8	35.5	−0.3	16.0

Columns list bootstrap, Bremer, and partitioned Bremer support (the contribution of specified gene to the total Bremer support at the indicated node) as calculated for nodes on the single most parsimonious topology resulting from unconstrained analysis of 18S + 28S + H3 + Wg (Fig. 3). Bootstrap support values result from 1000 bootstrap analysis replicates.

with Emeljanov's (1990) hypothesis, based on putatively synapomorphic head, wing, and genitalic characters.

The monophyly of the family Flatidae was supported in each analysis, although the placement of Flatidae differed across analyses. The parsimony analysis recovered Flatidae as the sister lineage to Eurybrachidae + Lophopidae (Fig. 3), whereas Flatidae was placed as a more recently diverged lineage in the Bayesian analysis (Fig. 4). Placement of Flatidae sister to Eurybrachidae + Lophopidae is a novel placement, as no morphological evidence or previous hypothesis supporting this relationship could be found in the existing literature. Emeljanov (1990) noted that flatids, eurybrachids, and lophopids have all lost the posterior tentorial arms; however, this reduced condition is also observed in Ricaniidae and Tropiduchidae (Emeljanov, 1990). The more general placement of Flatidae in the parsimony analysis (i.e., in a clade with the families Lophopidae, Eurybrachidae, Issidae (including Acanaloniidae and Caliscelidae), Nogodinidae, Tropiduchidae, and Ricaniidae) is consistent with Asche's (1987) morphological hypothesis in that these families all share a similar piercing-excavating ovipositor.

The family Tettigometridae, represented in these analyses by three species, was recovered among the more recently diversifying lineages of Fulgoroidea (Figs. 3 and 4). This placement approximates that of Bourgoin (1993), rather than the placement of Tettigometridae as sister to the remaining planthopper families as proposed by other morphology-based hypotheses (Muir, 1930; Asche, 1987; Emeljanov, 1990; Chen and Yang, 1995). In both the parsimony and Bayesian topologies, a nogodinid in the genus *Bladina* was placed sister to the tettigometrid clade. This

placement may be an artifact of the difficulties associated with discerning relationships among the nogodinid taxa included in the current study. Interestingly, in analyses in which either the third codon position of Wg alone was excluded, or when both the third codon positions of both Wg and H3 were excluded, Tettigometridae was placed sister to the clade containing the Derbidae. Therefore, even with data from four genes, placement of this family was sensitive to a very small subset of characters. Additional data is clearly needed to more thoroughly resolve placement of tettigometrids among other planthopper lineages.

A number of planthopper families recovered as monophyletic were supported with high Bayesian posterior probabilities, but relatively low bootstrap values (i.e., Fulgoridae, Dictyopharidae, Flatidae, Lophopidae, Acanaloniidae), whereas the monophyly of some families was supported with both high posterior probabilities and bootstrap values (i.e., Delphacidae, Eurybrachidae, Tettigometridae, Tropiduchidae, Caliscelidae, Ricaniidae). While the validity of bootstrap support values has been called into question (e.g., Hillis and Bull, 1993), it could also be the case that limited taxonomic or biogeographic sampling (see Table 2) could have contributed to the higher bootstrap support values, particularly for the families Delphacidae or Tropiduchidae, which have greater taxonomic diversity (relative to other planthopper families) and occur in several biogeographic regions. Interestingly, however, sampling of the family Achilidae was also taxonomically and biogeographically limited, and these taxa showed paraphyletic placement across all analyses.

4.2. Testing of family-level status

As previously described, one of the objectives of the current study was to investigate the appropriateness of recognizing some lineages as their own family within Fulgoroidea (i.e., achilixiids with respect to Cixiidae, and of Acanaloniidae and Caliscelidae with respect to Issidae). The single representative of the family Achilixiidae included in this study was placed near Achilidae in both the parsimony and Bayesian topologies, although the paraphyletic placement of the achilid taxa makes exact placement of Achilixiidae (i.e., with respect to achilids and derbids) somewhat problematic. However, it is clear that these results do not support Liang's (2001) placement of the Achilixiidae as arising from within Cixiidae; rather, the placement of Achilixiidae near Achilidae is consistent with previous morphology-based hypotheses (Asche, 1987; Emeljanov, 1990; Bourgoin, 1993; Chen and Yang, 1995).

Both parsimony and Bayesian analyses indicated the polyphyly of lineages that had been traditionally placed within Issidae (caliscelids and acanaloniids). Caliscelidae was recovered in both analyses (Figs. 3 and 4) as the monophyletic sister lineage to Ricaniidae. In addition, Acanaloniidae was also recovered as a monophyletic lineage in both analyses, and placed apart from Issidae. Fennah (1954) placed acanaloniids and caliscelids as subfamilies within Issidae, whereas Emeljanov (1999) proposed placement of Caliscelidae outside of Issidae. Emeljanov (1999) also proposed an affinity of Acanaloniidae with Nogodinidae, as well as with two subfamilies of Issidae, the Tonginae and the Trienopinae (this relationship was also noted earlier by Fennah (1954)). Although taxa from these issid subfamilies were not available for inclusion in the current study, one specimen from the Nogodinidae was placed sister to the Acanaloniidae clade.

The monophyly of Nogodinidae was not supported in these analyses. Two of the included nogodinid taxa (sampled from the genera *Danepteryx* and *Dictyssa*, both from the southwestern US), both formerly included within Issidae, were recovered as sister taxa in the current study. However, these taxa were not placed near the other nogodinids, nor near the issid exemplar (Issinae) included in the analyses. Overall, these results support the placement of Caliscelidae and Acanaloniidae as separate lineages from Issidae and highlight the need for additional data and increased taxonomic representation in order to elucidate these relationships.

4.3. Testing of morphology based hypotheses

The single, most parsimonious topology obtained through parsimony analysis of the combined data partitions (Fig. 3) was used to test five previous morphology-based hypotheses of Fulgoroidea phylogeny (Muir, 1930; Asche, 1987; Emeljanov, 1990; Bourgoin, 1993; Chen and Yang, 1995). Constraining the molecular data to Muir's (1930) hypothesis did not result in a statistically less parsimonious tree at the 0.01 significance level (Templeton $p = 0.0344$; winning sites $p = 0.0228$; K–H $p = 0.0272$). Similarly, a significant difference was not obtained when comparison was made constraining the molecular data to Asche's (1987) hypothesis (Templeton $p = 0.0273$; winning sites $p = 0.0645$; K–H $p = 0.0336$). However, constraining data to Emeljanov's (1990) hypothesis (Templeton $p < 0.0001$; winning sites $p < 0.0001$; K–H $p < 0.0001$), Bourgoin's (1993) hypothesis (Templeton $p < 0.0001$; winning sites $p < 0.0001$; K–H $p < 0.0001$), and Chen and Yang's (1995) hypothesis (Templeton $p = 0.0006$; winning sites $p = 0.0005$; K–H $p = 0.0007$) all resulted in significantly less parsimonious trees. While Muir's (1930) and Asche's (1987) hypotheses do not define well-resolved relationships among planthopper families, failure to find significant differences under these hypotheses cannot be attributable only to this lack of resolution, as relationships among planthopper families are similarly unresolved under the Chen and Yang (1995) hypothesis which was statistically rejected.

Clearly, results obtained in the current study are in agreement with several aspects of all previous morphology-based hypotheses. For example, Emeljanov's (1990) proposed placement of Eurybrachidae in a clade with Lophopi561dae was supported, as was Bourgoin's (1993) placement of

Tettigometridae among more recently diversifying planthopper lineages. Conversely, the topology supported by these molecular data differs with previous hypotheses in several key aspects. For example, the current study did not support Emeljanov's (1990) hypothesis that placed Delphacidae as a sister group to Cixiidae + remaining families (except Tettigometridae); rather, the current study supported a Delphacidae + Cixiidae clade. Emeljanov's (1990) proposed Ricaniidae + Flatidae clade was also not supported; the current study placed Caliscelidae sister to Ricaniidae. The current study did not support Bourgoin's (1993) proposal of two monophyletic clades of planthoppers ((Cixiidae, Delphacidae, Kinnaridae, Meenoplidae) and (remaining families)) based on female genitalia. Chen and Yang's (1995) proposal of the clade (Eurybrachidae, Ricaniidae, Lophopidae) as sister to the remaining families (except Tettigometridae) was also not supported.

While results of the topology tests fail to reject Muir's (1930) and Asche's (1987) hypotheses, the reconstructed topology (Fig. 3) is not perfectly congruent either of these hypotheses. With the exception of the placement of Tettigometridae, however, results obtained under parsimony are consistent with Asche (1987). Results of the partitioned Bayesian analysis (Fig. 4) differ only in that they lack resolution concerning the placement of Eurybrachidae + Lophopidae. That is, in the Bayesian topology, placement of this clade could lie near the most derived group of families (as Asche predicted) or within the intermediate group.

4.4. Conclusions

Therefore, results of the current study support Asche's (1987) hypothesis of a phylogenetic trend in ovipositor function across planthopper families. The only exception to the trend in feeding location is the family Tettigometridae. Wilson et al. (1994) report that tettigometrid nymphs feed in concealment among plant roots. Placement of the Tettigometridae among the more cladistically derived families suggests an independent origin of concealed feeding. However, the subterranean feeding habit reported by Wilson et al. (1994) is based on records for fewer than 10 species. For two of the tettigometrid species included in this study, however, the authors observed both nymphs and adults above ground on host plant foliage (unpublished). Therefore, additional records are needed to more clearly discern tettigometrid patterns of host plant use. Perhaps of greater significance is that tettigometrids are unique among the planthoppers in that nymphs are often gregarious and exhibit mutualistic relationships with ants (O'Brien, 2002). Hence, the bucking of the phylogenetic trend in their feeding ecology may be related to these unusual behaviors.

Additionally, representatives of two planthopper families, Gengidae and Hypochthonellidae, were unavailable for inclusion in the current study. These two families are both from Africa and are known from only a limited number of specimens (Wilson et al., 1994; O'Brien, 2002). Although these families are not speciose, their inclusion in future work could provide important insights into fulgoroid evolution, particularly with respect to feeding location, as the Hypochthonellidae are known only to feed underground (Wilson et al., 1994).

That the findings of the current study provide evidence supporting Asche's (1987) functional ovipositor types, and that these adaptations are consistent with a trend in feeding location, open interesting avenues for future research on planthopper diversification. For example, exposed feeding could be associated with increased predation among the more recently diversifying families. Such predation could select for morphological or behavioral traits to reduce predation pressure, such as increased size, crypsis, etc., that might occur at a greater frequency among these more recently diversifying families. Undoubtedly, greater insight into the evolution of these fascinating insects can be achieved by additional studies that not only employ greater taxonomic representation, but also combine data from additional molecular markers with morphological data.

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