

# Treehopper trees: phylogeny of Membracidae (Hemiptera: Cicadomorpha: Membracoidea) based on molecules and morphology

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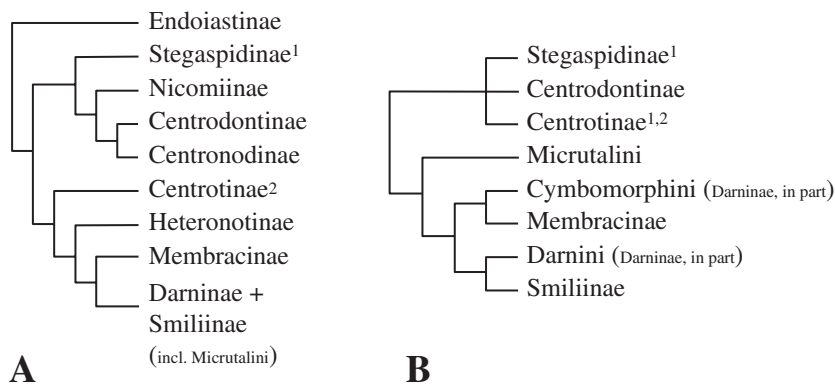
**Abstract.** Recent independent phylogenetic analyses of membracid relationships based on molecular and morphological data have identified monophyletic lineages within the family. However, the results of these studies have not fully resolved treehopper phylogeny, and relationships among some higher membracid lineages remain in doubt. Portions of three datasets (958 aligned nucleotides from elongation factor-1 $\alpha$ , 2363 aligned nucleotides from 28S ribosomal DNA, and eighty-three morphological features of adults and nymphs) introduced in recent studies were reanalysed separately and in combination with two new molecular datasets (321 aligned nucleotides from wingless and 1829 aligned nucleotides from 18S ribosomal DNA). The results of the combined data analyses, contrary to previous analyses of morphological data alone, grouped membracids into two well-supported lineages, one comprising Stegaspidae and Centrotinae, the other comprising Membracinae, Darninae and Smiliinae. The analyses recovered Centrotinae, Membracinae and Darninae as monophyletic groups, but Stegaspidae was paraphyletic with respect to Centrotinae, and Smiliinae was polyphyletic with Micrutalini placed as a sister group to the clade comprising Membracinae, Darninae and Smiliinae. These results are consistent with the following hypotheses, proposed previously based on an analysis of morphological data: (1) the posterior pronotal process was derived and lost multiple times during the evolution of Membracidae; (2) Membracidae originated in the New World and reached the Old World subsequently via dispersal; (3) maternal care evolved independently multiple times and may or may not have been preceded by the acquisition of ant mutualism.

## Introduction

The treehopper family Membracidae (Hemiptera: Cicadomorpha: Membracoidea), including nine subfamilies, forty-seven tribes, and more than 3000 described species (Deitz & Dietrich, 1993; McKamey, 1998; Wallace & Deitz, in press), has been the subject of several evolutionary studies. Nonetheless, relatively few investigations (Sakakibara, 1979; Dietrich & Deitz, 1993; Cryan *et al.*, 2000, 2003; Dietrich

*et al.*, 2001) have used quantitative phylogenetic methods to estimate relationships among membracid tribes and subfamilies. Recent phylogenetic analyses sampled either morphological data (Dietrich *et al.*, 2001; see also Fig. 1A) or DNA nucleotide sequence data (Cryan *et al.*, 2000; see also Fig. 1B), but the two kinds of data have not previously been combined to infer treehopper phylogeny. The synthesis of multiple sources of data is essential to elucidating treehopper phylogeny as some previously recognized membracid taxa (notably the subfamilies Smiliinae, Stegaspidae, Nessorhininae and Darninae) either were not well resolved or did not merit subfamily status in one or more of the phylogenetic analyses, and estimates of relationships among membracid tribes varied from study to study.

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- <sup>1</sup> paraphyletic  
<sup>2</sup> includes Nessorhinini and Oxyrhachini

Although morphological and molecular evidence separately seemed to recover many congruent relationships within Membracidae (Cryan *et al.*, 2000), there were also some notable differences between the resulting phylogenetic hypotheses. As shown in Fig. 1, molecular evidence indicated the potential paraphyly of the subfamilies Stegaspidinae and Centrotinae with respect to Centrodontinae (now considered a tribe of Centrotinae; Wallace & Deitz, in press), whereas a morphology-based analysis of Membracidae (Dietrich *et al.*, 2001) recovered Centrotinae (including Nessorhinini and Oxyrhachini, previously treated as distinct subfamilies) as a monophyletic lineage distinct from Stegaspidinae. Additionally, Smiliinae was polyphyletic, with two tribes derived from within Darninae in a morphology-based analysis, whereas molecular evidence indicated that Darninae was polyphyletic – Darnini grouped with Smiliinae, but Cymbomorphini grouped with Membracinae. Clearly, phylogenetic discordance remained.

The aim of this work was to explore further the relationships among the tribes and subfamilies of Membracidae, and specifically (1) to resolve relationships left equivocal in previous studies; and (2) to test various evolutionary hypotheses proposed as a result of morphology-based phylogenetic analyses. To those ends, we introduced two new molecular datasets (nucleotide sequence data from wingless (Wg) and 18S ribosomal DNA (18S)) and re-examined three previously published datasets: one morphological (modified from Dietrich *et al.*, 2001) and two molecular (elongation factor-1 $\alpha$  (EF-1 $\alpha$ ) and 28S ribosomal DNA (28S); Cryan *et al.*, 2000).

## Materials and methods

### Taxon sampling

Insect specimens (Table 1) were collected into 95–100% ethanol and stored at  $-80^{\circ}\text{C}$ . Sixty-six species were sampled in these analyses, including representatives of twenty-two membracid tribes in five currently recognized subfamilies. Unless otherwise stated, family group names

are used here in the sense of McKamey's (1998) catalogue, with updated nomenclature for Centrotinae based on Wallace & Deitz (in press). Because all but one of the currently recognized treehopper subfamilies are restricted to the New World, most taxa sampled are from the New World (including the Caribbean islands); six representatives of Old World Centrotinae (from Africa, Malaysia, and Australia) were also included because this subfamily is most diverse in the Old World. The five outgroup taxa belong to the membracoid families Cicadellidae (from Old World and New World localities) and Aetalionidae (from New World localities).

### Morphological data

Morphological data, consisting of eighty-three anatomical characters of adults and immatures, were obtained from Dietrich *et al.* (2001) or newly coded. Characters were uniformly weighted, and analysed as both all unordered and with character polarity following Dietrich *et al.* (2001). Character states were scored as missing values (–) in cases where the state was ambiguous or unavailable for examination.

### Nucleotide sampling and laboratory procedures

Genomic nucleic acids were extracted from thoracic flight muscle and/or abdominal tissue using DNA/RNA isolation kits (Amersham Life Sciences, Inc., Cleveland, Ohio, U.S.A.) and stored at  $-80^{\circ}\text{C}$  in either TE buffer or purified water. Intact portions of the specimens (i.e. heads, pronota, wings and legs) were given voucher numbers (Table 1) and are stored at  $-80^{\circ}\text{C}$  in 95–100% ethanol in the New York State Museum Genome Bank.

Cryan *et al.* (2000) presented a detailed description of the polymerase chain reaction (PCR) amplification and sequencing of the EF-1 $\alpha$  and 28S nucleotide sequences used here. Universal and newly designed (membracid specific; synthesized by Integrated DNA Technologies, Inc., Coralville,

**Fig. 1.** Alternative phylogenetic hypotheses of Membracidae based on (A) morphological (Dietrich *et al.*, 2001) and (B) molecular (Cryan *et al.*, 2000) evidence.

**Table 1.** Taxa sampled. Classification follows McKamey (1998) and Dietrich *et al.* (2001). Voucher numbers refer to material currently in the New York State Museum Genome Bank. GenBank accession numbers are given for wingless (Wg) and 18S nucleotide sequences; for elongation factor-1 $\alpha$  and 28S accession numbers, refer to Cryan *et al.* (2000).

Taxon	Voucher number	Geographical source	GenBank accession no. (Wg–18S)
Centrodontinae			
Centrodontini			
<i>Centrodontus atlas paucivenosus</i> Cook	97-02-19-36	Arizona, U.S.A.	AY498481–AY498415
Centrotinae			
Boocerini			
<i>Brachybelus cruralis</i> Stål	97-02-19-71	San Luis, Costa Rica	AY498477–AY498411
<i>Campylocentrus</i> sp.	95-05-12-52	La Selva, Costa Rica	AY498487–AY498421
<i>Ischnocentrus niger</i> Stål	96-09-16-63	La Selva, Costa Rica	AY498501–AY498435
Centrotini			
<i>Anchon</i> sp.	95-02-01-35	Morogoro, Tanzania	AY498509–AY498443
Gargarini			
<i>Gargara</i> sp.	Lin GAR-1	Taipei, Taiwan	AY498497–AY498431
Hypsauchenini			
<i>Pyrgauchenia</i> sp.	Stegmann Pyr2	Sabah, Malaysia	AY498521–AY498455
Nessorhini			
<i>Callicentrus</i> sp.	97-02-19-45	Hispaniola	AY498478–AY498412
<i>Nessorhinus</i> sp.	CRB	Tortola, British Virgin Is.	AY498511–AY498445
Platycentrini			
<i>Platycentrus acuticornis</i> Stål	97-02-19-95	Mexico	AY498519–AY498453
<i>Tylocentrus reticulatus</i> Van Duzee	Lin K5	Arizona, U.S.A.	AY498530–AY498464
Terentiini			
<i>Eufairmairia fraterna</i> Distant	CHD M20	Australia	AY498494–AY498428
<i>Ceraon vitta</i> (Walker)	Lin LCV-1	Australia	AY498502–AY498436
<i>Sextius virescens</i> (Fairmaire)	Lin LSV-1	Australia	AY498524–AY498458
Darninae			
Cymbomorhini			
<i>Cymbomorpha</i> sp. 1	95-02-01-49	Mérida, Venezuela	AY498490–AY498424
Darnini			
<i>Darnis latior</i> Fowler	Lin DALA	Gamboa, Panama	AY498492–AY498426
<i>Stictopelta</i> sp.	Lin LSCT-1	New Mexico, U.S.A.	AY498523–AY498457
Membracinae			
Aconophorini			
<i>Aconophora flavipes</i> (Germar)	95-02-01-43	Mérida, Venezuela	AY498471–AY498405
<i>Calloconophora</i> sp.	95-02-01-28	Mérida, Venezuela	AY498488–AY498422
<i>Guayaquila gracilicornis</i> (Stål)	95-05-12-40	La Selva, Costa Rica	AY498499–AY498433
Hoplophorionini			
<i>Alchisme</i> sp.	96-09-16-68	Monteverde, Costa Rica	AY498474–AY498408
<i>Ochropepla mourei</i> (Sakakibara)	96-09-16-37	Caracas, Venezuela	AY498517–AY498451
<i>Umbonia crassicornis</i> (Amy. & Serv.)	96-09-16-57	Lara, Venezuela	AY498534–AY498468
Hypsoprini			
<i>Cladonota apicalis</i> (Stål)	96-09-16-54	Caracas, Venezuela	AY498479–AY498413
<i>Notocera</i> sp.	96-09-16-79	Manuel Antonio, Costa Rica	AY498512–AY498446
<i>Scalmophorus minutus</i> Ball	97-02-19-63	Dominica	AY498526–AY498460
Membracini			
<i>Enchenopa binotata</i> (Say)	95-02-01-66	Maryland, U.S.A.	AY498493–AY498427
<i>Membracis</i> sp.	97-02-19-42	Cayo, Belize	AY498510–AY498444
<i>Tylopelta</i> sp.	97-02-19-17	Playa Tamarindo, Costa Rica	AY498533–AY498467
Undet. Membracini (nymph)	97-02-19-86	La Selva, Costa Rica	AY498531–AY498465
Smiliinae			
Acutalini			
<i>Acutalis tartarea</i> (Say)	95-02-01-69	Maryland, U.S.A.	AY498476–AY498410
Amastrini			
<i>Harmonides</i> sp.	95-02-01-46	Mérida, Venezuela	AY498500–AY498434
Ceresini			
<i>Cyphonia clavata</i> (Fabricius)	96-09-16-59	Caracas, Venezuela	AY498482–AY498416
<i>Spissistilus festinus</i> (Say)	95-05-12-58	North Carolina, U.S.A.	AY498525–AY498459

**Table 1.** Continued.

Taxon	Voucher number	Geographical source	GenBank accession no. (Wg–18S)
<i>Stictocephala brevitylus</i> (Van Duzee)	95-02-01-64	Maryland, U.S.A.	AY498522–AY498456
<i>Stictocephala taurina</i> (Fitch)	95-02-01-71	Maryland, U.S.A.	AY498527–AY498461
<i>Vestistilus variabilis</i> (Fowler)	95-05-12-27	Madraselva, Costa Rica	AY498535–AY498469
<b>Micrutalini</b>			
<i>Micrutalis calva</i> (Say)	95-02-01-68	Maryland, U.S.A.	AY498506–AY498440
<b>Polyglyptini</b>			
<i>Ennya</i> sp.	95-02-01-45	Mérida, Venezuela	AY498495–AY498429
<i>Metheisa lucillodes</i> Fowler	96-09-16-42	Caracas, Venezuela	AY498508–AY498442
<i>Polyglypta</i> sp.	96-09-16-37	Caracas, Venezuela	AY498520–AY498454
<b>Smiliini</b>			
<i>Antianthe</i> sp.	95-02-01-39	Mérida, Venezuela	AY498475–AY498409
<i>Carynota mera</i> (Say)	95-02-01-65	Maryland, U.S.A.	AY498486–AY498420
<i>Cyrtolobus arcuatus</i> (Emmons)	95-02-01-16	Maryland, U.S.A.	AY498480–AY498414
<i>Cyrtolobus fenestratus</i> (Fitch)	95-02-01-62	Maryland, U.S.A.	AY498483–AY498417
<i>Cyrtolobus fuliginosus</i> (Emmons)	95-02-01-11	Maryland, U.S.A.	AY498484–AY498418
<i>Cyrtolobus maculifrontis</i> (Emmons)	95-02-01-63	Maryland, U.S.A.	AY498485–AY498419
<i>Cyrtolobus tuberosus</i> (Fairmaire)	95-05-12-21	North Carolina, U.S.A.	AY498489–AY498423
<i>Glossonotus acuminatus</i> (Fabricius)	95-02-01-20	Maryland, U.S.A.	AY498496–AY498430
<i>Ophiderma definita</i> Woodruff	95-02-01-60	Maryland, U.S.A.	AY498513–AY498447
<i>Ophiderma evelyna</i> Woodruff	95-02-01-10	Maryland, U.S.A.	AY498514–AY498448
<i>Ophiderma flavicephala</i> Goding	95-05-12-18	North Carolina, U.S.A.	AY498515–AY498449
<i>Ophiderma grisea</i> Woodruff	95-02-01-17	Maryland, U.S.A.	AY498516–AY498450
<i>Ophiderma pubescens</i> (Emmons)	95-02-01-67	Maryland, U.S.A.	AY498518–AY498452
<i>Telamona monticola</i> (Fabricius)	95-02-01-18	Maryland, U.S.A.	AY498529–AY498463
<i>Telamona unicolor</i> Fitch	95-02-01-19	Maryland, U.S.A.	AY498532–AY498466
<i>Xantholobus muticus</i> (Fabricius)	95-05-12-13	North Carolina, U.S.A.	AY498536–AY498470
<b>Stegaspinae</b>			
<b>Microcentrini</b>			
<i>Microcentrus caryae</i> (Fitch)	95-02-01-75	North Carolina, U.S.A.	AY498507–AY498441
<b>Stegaspidini</b>			
<i>Lycoderes</i> sp.	96-09-16-34	Maracay, Venezuela	AY498505–AY498439
<b>Unplaced</b>			
<i>Antillotolania microcentroides</i> Cryan & Bartlett	CRB Steg	Tortola, British Virgin Is.	AY498528–AY498462
<i>Deiroideres inermis</i> Ramos	CRB Din	Tortola, British Virgin Is.	AY498491–AY498425
<b>Aetalionidae</b>			
<i>Gerridius fowleri</i> (Haviland)	97-02-19-46	Guyana	AY498498–AY498432
<i>Lophyraspis</i> sp.	95-05-12-96	Sirena, Costa Rica	AY498504–AY498438
<b>Cicadellidae</b>			
<i>Flexamia areolata</i> (Ball)	CHD LH38	Virginia, U.S.A.	AY498503–AY498437
<i>Putoniessa rivularis</i> (Walker)	CHD AH3	New South Wales, Australia	AY498472–AY498406
<i>Paracephaleus brunneus</i> (Waterhouse)	CHD AH6	New South Wales, Australia	AY498473–AY498407

Iowa, U.S.A.) oligonucleotide primers (Table 2) were used to amplify and sequence the nuclear developmental gene Wg and 18S via standard PCR using *Taq* DNA polymerase (PE Applied Biosystems, Foster City, California, U.S.A.). Approximately 321 bp of Wg were amplified and sequenced using the primer pairs 1A–7B or 1.5A–7.5B (Table 2). Nearly the entire 18S gene ( $\approx$ 1850 bp) was sequenced in three contiguous regions using the primer pairs 1F-b3.9, a0.7-bi and a2.0-9R (Table 2). All PCR reactions included negative controls to detect possible contamination. Double-stranded PCR amplification products were visualized on 1–2% agarose gels, purified using GeneClean III DNA Purification Kits (Bio 101, Vista, California, U.S.A.), and directly sequenced with D-Rhodamine Terminator Cycle Sequencing

Ready Reaction with AmpliTaqFS<sup>®</sup> DNA polymerase (PE Applied Biosystems). Sequences were fractionated by polyacrylamide gel electrophoresis on a Prism<sup>™</sup>377 automated DNA sequencer (PE Applied Biosystems).

#### Sequence alignment and phylogenetic analyses

Sequence confirmation was accomplished by comparison of complementary DNA strands. Editing nucleotide sequences, contig assembly, consensus sequence calculation and manual alignment of consensus sequences were performed using the software program SEQUENCHER<sup>™</sup> 4.0.5 (Gene Codes Corporation, Ann Arbor, Michigan, U.S.A.)

**Table 2.** Oligonucleotide primer sequences. Primers used for the polymerase chain reaction amplification and sequencing of wingless (Wg) and 18S ribosomal DNA from Membracidae and outgroups; primers used for amplification and sequencing elongation factor-1 $\alpha$  and 28S ribosomal DNA were listed by Cryan *et al.* (2000).

Primer	Sequence (5'–3')	Reference
Wg 1A	GARTGYAARTGYCAYGGYATGTCTGG	Cryan <i>et al.</i> (2001)
Wg 7B	ACCAGTGGGAATGTGCACGCGC	Cryan <i>et al.</i> (2001)
Wg 1.5A	G TSAARACBTGYTGGATGCG	Membracidae specific (designed by JRC)
Wg 7.5B	GTCCTGTAMCCVCGVCCACAACACAT	Membracidae specific (designed by JRC)
18S 1F	TACCTGGTTGATCCTGCCAGTAG	Conserved (within Insecta) primer
18S b3.9	TGCTTTRAGCACTCTAA	Whiting (2002)
18S a0.7	ATTAAAGTTGTTGCGGTT	Whiting (2002)
18S bi	GAGTCTCGTTCGTTATCGGA	Whiting (2002)
18S a2.0	ATGGTTGCAAAGCTGAAAC	Whiting (2002)
18S 9R	GATCCTTCCGAGGTTACCTAC	Whiting (2002)

for PC. Complete nucleotide sequences (including regions of ambiguous alignments) are available in GenBank under the accession numbers listed in Table 1 (note that EF-1 $\alpha$  and 28S accession numbers are listed in Cryan *et al.*, 2000), and the concatenated alignment (including all molecular and morphological characters) is available on TreeBASE and at the following website: <http://www.nysm.nysed.gov/lceg>. Alignments of EF-1 $\alpha$  and Wg contained no alignment ambiguities due to the functional codon constraints imposed on these genes. Alignments of 18S and 28S sequences were mostly unambiguous, but did identify a few short, length-variable regions of alignment where assessment of homology was ambiguous. Therefore, a few ambiguously aligned regions of each gene were excluded from the analysis (Table 3). In total, twenty-two nucleotide positions were excluded from throughout the 18S alignment where single taxa had short length-variable insertions (i.e. not corresponding to defined regions of the locus); 447 nucleotide positions were excluded from the 28S alignment, corresponding to hypervariable portions of the divergent domains D2, D3, D6–7a and D9–10.

Phylogenetic analyses using the maximum parsimony criterion were performed using the software programs PAUP\* 4.0b10 (Swofford, 1998) and NONA (Goloboff, 1998).

Heuristic tree searches were performed using 1000 random addition replications with the tree bisection and reconnection option; gaps were treated as missing data.

Incongruence length difference tests (ILD; Farris *et al.*, 1995, as implemented by the partition homogeneity test in PAUP\*) were used to assess character incongruence and combinability (but see below for a synopsis of the controversy regarding the ILD test). This test putatively determines whether the original data partitions are significantly less homogenous than random partitions of identical size constructed from the set of combined data, as measured by  $I_{MF}$  (the incongruence index of Mickevich & Farris, 1981). Significance indicates heterogeneity in the distribution of phylogenetic information among the original data partitions, suggesting incongruence among the datasets.

Support for individual nodes on the strict consensus topology resulting from total combined data analyses was assessed by calculation of partitioned Bremer support values (Baker & DeSalle, 1997; computed using the computer program TREEROT.V2 (Sorenson, 1999) in conjunction with PAUP\* 4.0b10) and nonparametric bootstrap analysis (1000 replicates). Alternative phylogenies were compared using the Templeton (Wilcoxon signed-ranks; Templeton, 1983), winning-sites (sign) and Kishino–Hasegawa (Kishino

**Table 3.** Descriptive statistics for separate and combined data partitions.

Data partition	EF-1 $\alpha$	Wg	18S	28S	Molecular <sup>a</sup>	Morphology
Alignment length (bp)	958	321	1853	2810	5942	83
Data partition length (bp)	958	321	1829	2363	5471	83
No. variable sites/% data partition	410/42.8	172/53.6	362/19.8	728/30.8	1672/30.6	76/91.6
No. informative sites <sup>b</sup> /% data partition	319/32.4	121/37.7	141/7.7	371/15.7	952/17.4	68/82.0
%A	0.27	0.22	0.24	0.20	0.22	–
%C	0.24	0.28	0.28	0.30	0.31	–
%G	0.25	0.31	0.23	0.28	0.26	–
%T	0.24	0.19	0.25	0.22	0.21	–
% pairwise distance (uncorrected)	1–19%	1–30%	1–7%	1–26%	1–33%	–

EF-1 $\alpha$  = elongation factor-1 $\alpha$ ; Wg = wingless.

<sup>a</sup>Combined molecular dataset, comprising EF-1 $\alpha$  + Wg + 18S + 28S.

<sup>b</sup>Under parsimony criteria.

& Hasegawa, 1989) tests as implemented in PAUP\* 4.0b10 (under the maximum parsimony criterion), to determine whether the topological differences could be explained by random error.

## Results

Nucleotide sequence editing and alignment resulted in a Wg data partition of 321 bp, and an 18S data partition of 1829 bp. When added to the data presented by Cryan *et al.* (2000; 958 bp of EF-1 $\alpha$  and 2363 bp of 28S), a resulting combined molecular dataset of  $\approx$ 5.5 kb was obtained for sixty-six taxa; additionally, the morphological data partition comprised eighty-three characters (Table 3 lists descriptive statistics for each data partition). As expected, informative nucleotide sites in the protein coding genes largely occupied the third codon positions; the following distributions of informative characters were observed: EF-1 $\alpha$ , nt1 = 30 (9.4%), nt2 = 8 (2.5%), nt3 = 281 (88.1%); Wg, nt1 = 17 (14.0%), nt2 = 10 (8.3%), nt3 = 94 (77.7%).

### Phylogenetic analyses

The results of ILD tests among data partitions are listed in Table 4; the hypothesis of congruence was rejected ( $\alpha = 0.05$ ) only for the comparison between 28S and morphology. As the indicated incongruence is not symmetric (i.e. 28S  $\neq$  morph, but 28S  $\approx$  Wg and morph  $\approx$  Wg, etc.), and in the interest of exploring the combined evidence approach, we included all molecular data partitions in the combined molecular analysis and all data partitions in a final combined data analysis.

Statistical and topological results from the analyses of the individual molecular data partitions, of the individual morphological data partition, and of various combinations of data partitions are summarized in Tables 5 and 6. Unweighted parsimony analysis of the combined molecular dataset (including all molecular data partitions) resulted in twelve equally parsimonious topologies, the strict consensus

**Table 4.** Results from incongruence length difference tests among data partitions. Values of  $\alpha < 0.05$  indicate sufficient evidence to reject the hypothesis of congruence.

Data partition	EF-1 $\alpha$	Wg	18S	28S	Molecular <sup>a</sup>	Morphology <sup>b</sup>
EF-1 $\alpha$	–	1.000	1.000	0.505	–	0.121
Wg	–	–	1.000	1.000	–	1.000
18S	–	–	–	1.000	–	1.000
28S	–	–	–	–	–	0.001*
Molecular <sup>a</sup>	–	–	–	–	–	1.000

EF-1 $\alpha$  = elongation factor-1 $\alpha$ ; Wg = wingless.

<sup>a</sup>Combined molecular dataset, comprising EF-1 $\alpha$  + Wg + 18S + 28S.

<sup>b</sup>Characters unordered.

\*Statistically significant value.

of which is shown in Fig. 2(A). This is compared with the results of a separate analysis of morphology (with characters unordered), shown in Fig. 2(B) (relationships recovered when morphological character polarity follows Dietrich *et al.*, 2001, not shown, are similar but less resolved).

Unweighted parsimony analysis of the total combined dataset (including all molecular and morphological data partitions) resulted in twenty-four shortest length topologies, the strict consensus of which is shown in Fig. 3 (corresponding nodal support values are listed in Table 7). The combined dataset was analysed twice, once with all data unordered and once with polarity of the morphological data following Dietrich *et al.* (2001). The sole topological difference between the two analyses was the placement of *Acutalis tartarea* (representing Acutalini), either basal to Ceresini (data unordered) or in a trifurcation with Ceresini and the remaining Smiliinae (morphology ordered); this difference is shown as a broken line in Fig. 3.

Our combined data analyses are largely congruent with previously published analyses of molecular and morphological data, but also differ from previous morphology-based estimates (Dietrich & McKamey, 1995; Dietrich *et al.*, 2001) in several important respects. Like the previous morphology-based analysis (Dietrich *et al.*, 2001), the combined data analyses supported the monophyly of Membracinae and indicated that Nessorhinini, treated as a separate subfamily by Deitz (1975), arose from within Centrotinae. Also, like the previous analysis, the combined data analyses found little or no support for the monophyly of Smiliinae and Stegaspidinae. The combined data analyses recovered as monophyletic all but two of the tribes for which more than one exemplar was included: Nessorhinini, Platycentrini, Terentiini (*sensu* Wallace & Deitz, in press), Hypsoprornini, Hoplophorionini, Aconophorini, Membracini, Darnini, Ceresini and Polyglyptini. Contrary to the previous morphology-based estimate, Smiliini (*sensu lato*, sampled more extensively in the current analyses) was paraphyletic with respect to Polyglyptini on some of the most parsimonious topologies. In contrast to the recent morphology-based analysis of Centrotinae by Wallace & Deitz (in press), Boocerini was polyphyletic, with *Ischnocentrus* placed in a basal trifurcation (Fig. 3, node 6) and *Brachybelus* and *Campylocentrus* grouped together as sister to Centrodontini + Platycentrini. Also, in the previous morphology-based analyses (Dietrich & McKamey, 1995; Dietrich *et al.*, 2001), Membracini was paraphyletic with respect to Aconophorini, Talipedini and Hoplophorionini, and Polyglyptini was paraphyletic with respect to Tragopini. Exemplars of Talipedini and Tragopini were not available for the molecular analysis.

Unlike the previous morphology-based analysis (Dietrich *et al.*, 2001), in which separate stegaspidine and centrotine clades were placed as a paraphyletic grade, giving rise to the 'higher' membracid subfamilies (Heteronotinae (unavailable for the molecular analysis), Membracinae, Darninae and Smiliinae), the combined data analyses recovered two major membracid lineages with strong branch support (Fig. 3, Table 7): one comprising Stegaspidinae +

**Table 5.** The results of separate and combined data partition analyses.

Data partition	No. trees	Length	Consistency index	Retention index
EF-1 $\alpha$	12	2361	0.28	0.58
Wg	40	899	0.32	0.61
18S	>10 000	768	0.57	0.62
28S	2400	1911	0.52	0.54
Molecular <sup>a</sup>	12	6135	0.39	0.56
Morphology <sup>b</sup>	1836	223	0.44	0.83
Morphology <sup>c</sup>	>10 000	252	0.39	0.83
Combined <sup>d</sup>	24	6398	0.39	0.57
Combined <sup>e</sup>	24	6428	0.39	0.58

EF-1 $\alpha$  = elongation factor-1 $\alpha$ ; Wg = wingless.

<sup>a</sup>Combined molecular dataset, comprising EF-1 $\alpha$  + Wg + 18S + 28S.

<sup>b</sup>Characters unordered.

<sup>c</sup>Character polarity follows Dietrich *et al.* (2001).

<sup>d</sup>Combined analysis of molecular (EF-1 $\alpha$ , Wg, 18S, 28S) and morphological data (unordered).

<sup>e</sup>Combined analysis of molecular (EF-1 $\alpha$ , Wg, 18S, 28S) and morphological data (polarity follows Dietrich *et al.*, 2001).

Centrodontinae + Centrotinae (Fig. 3, node 2) and the other including the 'higher' treehopper subfamilies (Fig. 3, node 17). Also in contrast with the previous morphology-based analysis, the combined data analysis indicated (with 72% bootstrap support) that Centrodontini arose from within

Centrotinae; Dietrich *et al.*'s (2001) morphology-based analysis placed Centrodontini within a clade comprising the genera *Antillotolania* and *Deiroideres*, Centronodinae, and Nicomiinae (exemplars of the latter two groups were not available for the molecular analysis).

**Table 6.** Relative support for currently recognized groups. Taxa follow current classification *sensu* McKamey (1998) and Dietrich *et al.* (2001). Scores to the left of the dash are bootstrap values<sup>a</sup> for each group, based on unconstrained analysis; scores to the right of the dash are the number of additional steps (above the most parsimonious tree length for that data partition) necessary to recover each group, with no other relationships constrained. Numbers in parentheses are the most parsimonious tree length for that data partition from unconstrained analysis.

Taxon	EF-1 $\alpha$ (2361)	Wg (899)	18S (768)	28S (1911)	Molecular <sup>b</sup> (6135)	Morphology <sup>c</sup> (223)	Combined <sup>d</sup> (6398)
Centrotinae	<50–18	<50–10	<50–6	<50–14	<50–17	<50–0	<50–10
Boocerini	<50–1	<50–0	<50–3	<50–3	<50–0	69–0	<50–0
Nessorhinini	74–0	<50–3	<50–0	<50–0	84–0	54–0	94–0
Platycentrini	98–0	73–0	<50–5	<50–6	100–0	77–0	100–0
Darninae	<50–7	<50–2	<50–2	<50–3	<50–0	<50–0	53–0
Darnini	97–0	<50–2	<50–0	<50–0	100–0	75–0	99–0
Membracinae	<50–1	<50–7	<50–1	<50–6	79–0	<50–2	95–0
Aconophorini	100–0	<50–0	<50–3	98–0	100–0	70–0	100–0
Hoplophorionini	79–0	<50–5	<50–6	<50–0	90–0	97–0	100–0
Hypsoprurini	67–0	<50–2	<50–2	<50–6	77–0	<50–1	87–0
Membracini	81–0	<50–13	<50–4	<50–2	<50–0	<50–0	68–0
Smiliinae	<50–19	<50–10	<50–3	<50–15	<50–24	<50–0	<50–23
Ceresini	85–0	61–0	<50–0	<50–0	90–0	93–0	97–0
Polyglyptini	<50–1	<50–2	<50–4	<50–7	<50–3	95–0	56–0
Smiliini	<50–1	<50–0	<50–2	<50–6	<50–3	<50–0	<50–0
Telamonini <sup>e</sup>	99–0	75–0	<50–1	65–0	100–0	54–0	100–0
Stegaspidae	<50–12	<50–11	<50–9	<50–15	<50–16	<50–1	<50–15
Microcentrini + unplaced <sup>f</sup>	84–0	<50–7	<50–5	<50–14	62–0	<50–3	54–0

EF-1 $\alpha$  = elongation factor-1 $\alpha$ ; Wg = wingless.

<sup>a</sup>EF-1 $\alpha$ , molecular and combined data partition bootstrap values calculated with full heuristic searches (100 replications); Wg, 18S, 28S, and morphology values calculated with fast stepwise-addition searches (10 000 replications).

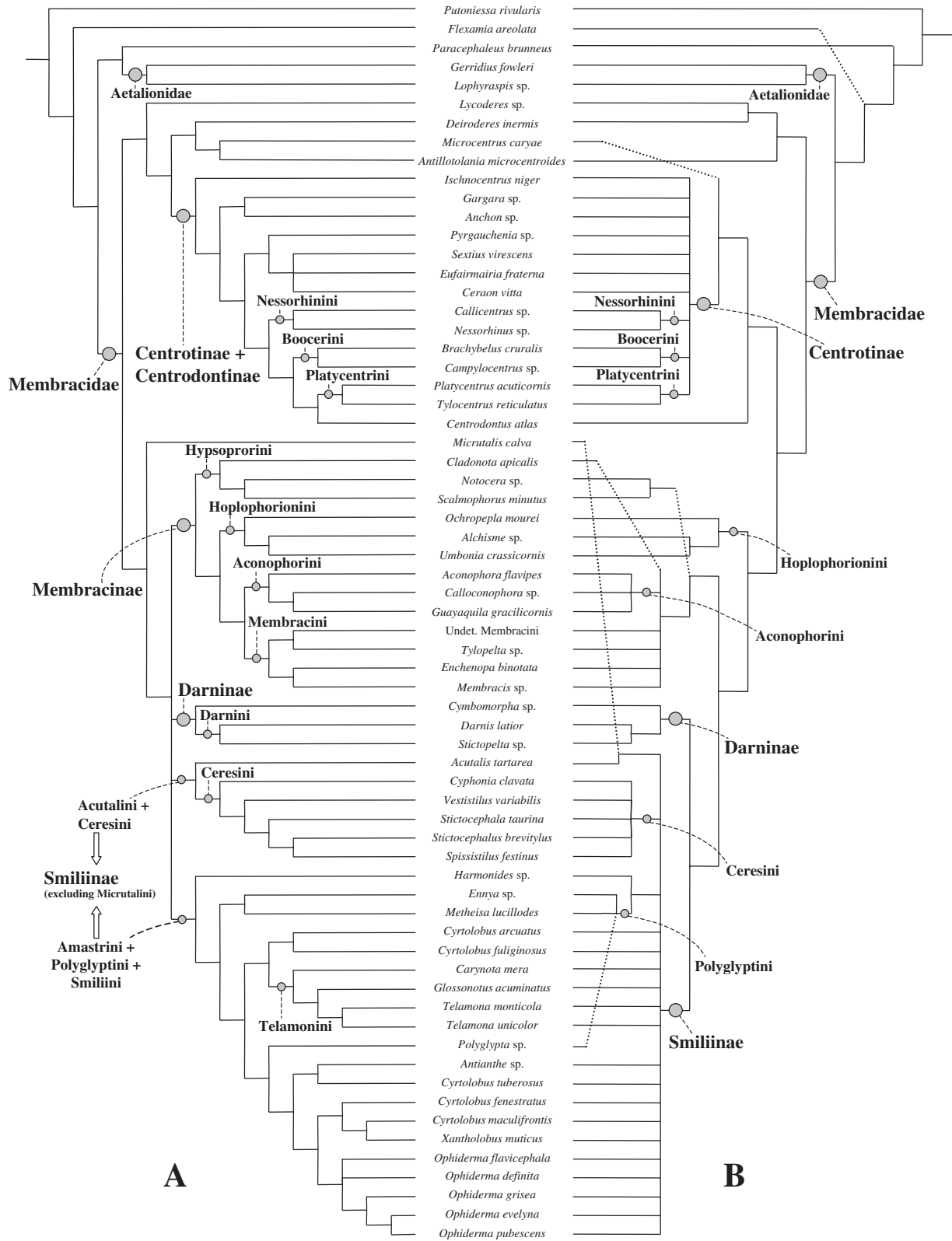
<sup>b</sup>Combined analysis of molecular (EF-1 $\alpha$ , Wg, 18S, 28S) data.

<sup>c</sup>Characters unordered.

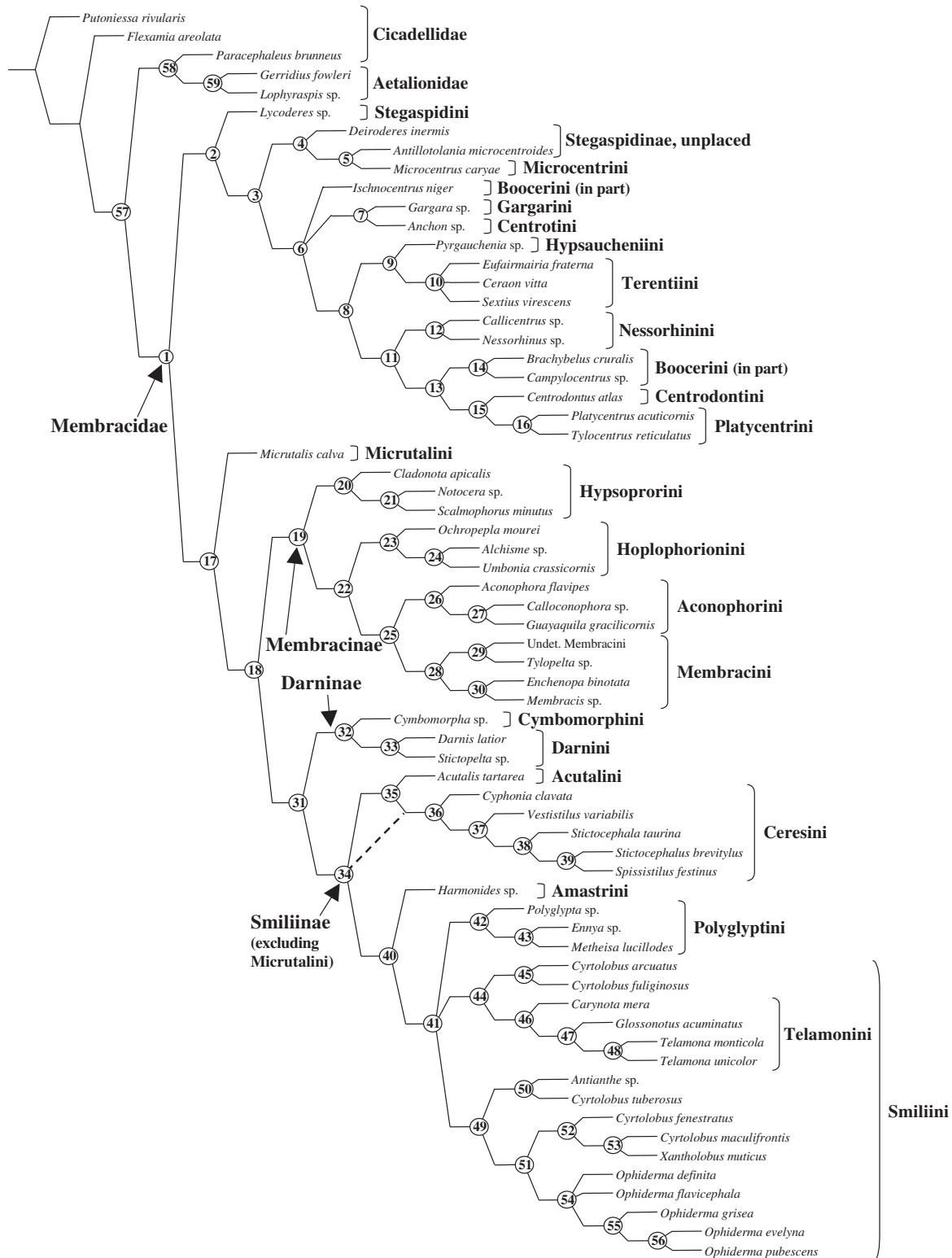
<sup>d</sup>Combined analysis of molecular (EF-1 $\alpha$ , Wg, 18S, 28S) and morphological data (characters unordered).

<sup>e</sup>Telamonini Goding (1892) is currently considered a synonym of Smiliini (Deitz, 1975; McKamey, 1998).

<sup>f</sup>Unplaced = the genera *Deiroideres* and *Antillotolania* (represented here by one species each).



**Fig. 2.** A comparison between the results of the combined molecular data analysis and the separate analysis of morphological data. A, Combined molecular data (including elongation factor-1 $\alpha$ , wingless, 18S and 28S): strict consensus of twelve shortest length trees (6135 steps, consistency index = 0.39, retention index = 0.56); B, morphology (characters unordered): strict consensus of 1836 shortest length trees (223 steps, consistency index = 0.44, retention index = 0.83).



**Fig. 3.** Strict consensus of twenty-four shortest length trees (6398 steps, consistency index = 0.39, retention index = 0.57) resulting from a combined evidence parsimony analysis (including elongation factor-1 $\alpha$ , wingless, 18S, 28S and unordered morphology data partitions). Nodes are circled and numbered; 'node numbers' are referred to in the text. Total and partitioned Bremer support and bootstrap values for each node are listed in Table 7. The broken line indicates the only topological difference when the combined datasets were analysed with the polarity of morphological characters following Dietrich *et al.* (2001).

**Table 7.** Nodal support for Fig. 3. The columns list bootstrap, Bremer, and partitioned Bremer support (the contribution of a specified gene to the total Bremer support at the indicated node) as calculated for the combined data partition phylogeny (Fig. 3). Bootstrap support values result from 1000 bootstrap analysis replicates.

Node no.	Bootstrap support	Bremer support	Partitioned Bremer					Node no.	Bootstrap support	Bremer support	Partitioned Bremer				
			EF-1 $\alpha$	Wg	18S	28S	Morphology				EF-1 $\alpha$	Wg	18S	28S	Morphology
1	99	11	1.0	0.5	-2.0	7.0	4.5	31	<50	3	-2.8	0.4	-0.6	4.3	1.7
2	98	12	8.0	-2.5	1.0	3.0	2.5	32	53	8	-4.0	7.0	0.0	2.0	3.0
3	99	15	8.5	1.5	7.0	0.5	-2.5	33	100	24	10.0	-1.0	2.5	9.0	3.5
4	60	5	2.6	-1.1	1.2	3.0	-0.7	34	<50	3	-2.7	0.7	-1.7	4.5	2.2
5	54	3	-5.0	-0.5	5.5	4.0	-1.0	35	<50	2	-1.0	1.0	2.0	1.0	-1.0
6	72	3	-10.0	0.5	6.0	7.0	-0.5	36	98	9	4.0	5.0	0.0	-3.0	3.0
7	<50	3	-10.0	0.5	6.0	7.0	-0.5	37	100	38	18.0	5.0	6.0	9.0	0.0
8	<50	3	-10.0	0.5	6.0	7.0	-0.5	38	70	2	2.0	0.0	-2.0	2.0	0.0
9	<50	3	1.5	-0.5	-0.5	2.5	0.0	39	100	9	8.0	1.0	0.0	0.0	0.0
10	81	3	1.0	0.5	0.0	1.5	0.0	40	97	12	7.7	5.0	0.3	-0.8	-0.2
11	<50	2	-1.0	2.5	0.0	1.0	-0.5	41	86	4	1.0	5.0	-2.0	-3.5	3.5
12	98	10	-5.0	0.5	6.0	7.0	1.5	42	65	2	0.0	1.0	-2.0	-2.5	5.5
13	<50	2	3.0	0.5	-1.0	0.0	-0.5	43	100	27	-1.7	0.8	5.2	17.2	5.5
14	<50	4	1.7	0.8	-0.3	0.0	1.8	44	80	5	-7.0	-1.0	-1.0	13.5	0.5
15	59	5	2.0	2.5	1.0	2.0	-2.5	45	85	3	-2.0	0.0	3.0	2.5	-0.5
16	100	31	16.2	7.2	-1.4	5.5	3.5	46	100	23	10.0	4.0	5.0	1.5	2.5
17	99	14	6.0	0.0	-1.0	7.0	2.0	47	64	2	0.0	0.0	0.0	2.0	0.0
18	93	17	6.0	4.0	1.0	4.5	1.5	48	99	8	3.0	0.0	0.0	5.0	0.0
19	97	10	5.5	-0.5	0.0	3.5	1.5	49	51	2	-1.0	0.0	0.0	3.5	-0.5
20	87	9	4.0	2.0	-1.0	2.0	2.0	50	71	2	1.0	1.0	-1.0	1.0	0.0
21	76	4	-1.0	2.0	-5.0	5.5	2.5	51	<50	1	1.0	-1.0	1.0	0.5	-0.5
22	85	9	12.6	-1.0	2.7	-1.8	-3.5	52	<50	1	1.4	-0.2	0.2	-0.3	-0.1
23	100	19	6.0	-1.0	-4.0	4.0	14.0	53	84	4	3.1	2.0	0.0	-1.0	-0.1
24	75	4	1.0	1.0	1.0	0.0	1.0	54	100	10	6.2	1.7	0.3	1.8	-0.2
25	63	6	5.0	-2.0	-2.0	2.0	3.0	55	72	1	1.0	0.0	0.0	0.0	0.0
26	100	34	8.0	3.0	3.0	13.0	7.0	56	70	1	1.0	0.0	0.0	0.0	0.0
27	82	6	1.0	4.0	0.0	1.0	0.0	57	100	24	15.0	3.5	-1.0	8.0	-1.5
28	68	6	4.7	-1.7	-1.0	1.7	2.3	58	67	4	9.0	2.0	-5.0	0.0	-2.0
29	58	4	3.0	0.0	0.0	1.0	0.0	59	100	73	23.0	15.5	27.0	0.0	7.5
30	100	16	5.0	6.0	3.0	3.0	-1.0	Total		580	174.7	87.6	67.4	181.6	68.7
								Percentage		100	30.1	15.1	11.6	31.3	11.9

EF-1 $\alpha$  = elongation factor-1 $\alpha$ ; Wg = wingless.

## Discussion

### Combining data

Whether disparate kinds of data should be combined for phylogenetic analysis is a topic of continued debate (de Queiroz *et al.*, 1996; Huelsenbeck & Bull, 1996; Huelsenbeck *et al.*, 1996; Lutzoni, 1997; Wenzel & Siddall, 1999). Cunningham (1997) concluded that the 'ILD' test (also called the partition homogeneity test; Mickevich & Farris, 1981; Farris *et al.*, 1995; Johnson & Soltis, 1998), which assesses the amount of extra homoplasy resulting from the combination of data partitions, was the most useful among tests of data partition concordance. Other studies concluded that the ILD test had limited utility as an assessment of topological congruence, data partition homogeneity and data partition combinability (Dolphin *et al.*, 2000; Barker &

Lutzoni, 2002). Meanwhile, an ever-increasing number of studies incorporate some combination of morphological, molecular, behavioural and ecological data for phylogenetic reconstruction; proponents of the so-called 'total evidence' approach advocate the consideration and combination of all possible data when reconstructing phylogenies, often despite homogeneity test results indicating discordant phylogenetic signals among combined data types (Poe, 1996; Soltis *et al.*, 1998; Wiens, 1998). Nevertheless, we agree with Wiens (1998) that, if the results of a combined data analysis are taken as the best estimate of phylogeny, cases where the separately analysed datasets are strongly discordant should be viewed as questionable in the combined data analysis until additional evidence is found to support one topology over another. Wenzel & Siddall (1999) provided a sound theoretical justification of data partition combination, even in cases where the level of homoplasy in one or

more data partitions is relatively high, in that concordant phylogenetic signals within data partitions are additive, whereas the noise (homoplasy) is averaged. Our analyses support this view, as the combined data topology (Fig. 3) is better resolved, with stronger nodal support, than any of the topologies resulting from the analysis of individual data partitions.

#### *Treehopper evolution*

Estimates of treehopper relationships incorporating both molecular and morphological data reveal an emerging consensus of the pattern of higher-level diversification of membracid clades. The most striking result of the present analysis is the grouping of treehoppers into two well-supported sister clades. One of these lineages, comprising the currently recognized subfamilies Stegaspidinae (*sensu* Cryan *et al.*, 2003), and Centrotinae (*sensu* Wallace & Deitz, in press, including the tribe Centrodontini), is approximately equivalent to the concept of Centrotinae (*sensu lato*) espoused by Funkhouser (1951) and Strümpel (1972). This lineage includes those membracids having the scutellum exposed, traditionally regarded as the 'primitive' treehoppers, but also includes two tribes (Centrodontini, Nessorhinini) which have the pronotum completely concealing the scutellum and two genera (*Antillotolania* and *Deiroideres*) which have apparently lost the posterior pronotal process secondarily. The second major lineage comprises the 'higher' treehopper subfamilies Darninae, Membracinae and Smiliinae, all species of which have the pronotum completely concealing the scutellum (and often parts of the resting forewing as well). Previous morphology-based analyses had found, albeit with weak branch support, that these 'higher' treehoppers arose from within a paraphyletic grade comprising the treehoppers with the pronotum not completely concealing the scutellum.

Interestingly, our results support the hypothesis, based on analyses of morphological data, that the acquisition of a fully developed posterior pronotal process in Membracidae did not follow a single stepwise progression from absent, to partially concealing the scutellum, to completely concealing the scutellum. Instead, the evolution of this structure exhibited considerable homoplasy, with treehoppers lacking the posterior process (e.g. *Antillotolania* and *Deiroideres*) derived from ancestors having such a process, and treehoppers with the scutellum fully concealed (Nessorhinini, Centrodontini and all members of Fig. 3, node 17) arising independently multiple times. The presence of a posterior pronotal process is a unique feature found only in this lineage of Cicadomorpha, and therefore it has traditionally been used to diagnose the family Membracidae.

Despite the presence of a possible morphological synapomorphy in the forewing venation uniting Stegaspidinae (Dietrich & Deitz, 1993), the separate and combined data analyses presented here suggest that this subfamily is paraphyletic. The analyses placed *Lycoderes* (Stegaspidini; reviewed by Cryan & Deitz, 1999a, 1999b, 2000) as sister to

a clade comprising the remaining exemplars of Stegaspidinae and Centrotinae, and recovered a clade comprising *Microcentrus* (Microcentrini; monographed in Cryan *et al.*, 2003), *Antillotolania*, and *Deiroideres* (Stegaspidinae, unplaced to tribe; Cryan *et al.*, 2003) as sister to Centrotinae. The morphology-based analyses of Dietrich *et al.* (2001) did not consistently recover Stegaspidinae as a monophyletic group, placing the included representatives in a weakly supported clade also comprising Centrodontini, Nicomiinae and Centronodinae (exemplars of the latter two subfamilies were not available for inclusion in the DNA datasets). The results of Templeton (Wilcoxon signed-ranks), winning-sites (sign), and Kishino–Hasegawa tests comparing Fig. 3 with a topology constrained to the results of Dietrich *et al.* (2001; but including only taxa represented in Fig. 3) indicated a statistically significant difference between the trees at  $P < 0.05$ .

Analyses of combined molecular and morphological data placed *Ischnocentrus* in a basal position within the Centrotinae lineage (Fig. 3, node 6), whereas *Brachybelus* and *Campylocentrus* (exemplars of Boocerini) were placed as a more derived lineage within Centrotinae (Fig. 3, node 14). *Ischnocentrus* was previously classified in the centrotine tribe Abelini, but Wallace & Deitz (in press) presented evidence to support the monophyly of Abelini and Boocerini (with Boocerini as the senior synonym). Although the placement of these groups in the current analyses is contradictory, nodal support for the internal nodes of the Centrotinae lineage is relatively weak, and therefore these results do not strongly contradict the results of Wallace & Deitz.

In agreement with the morphology-based analysis of Dietrich *et al.* (2001), the combined analysis recovered a well-supported clade (Fig. 3, node 40) comprising the members of Smiliinae which have hindwing vein A unbranched, but did not support the broader traditional concept of this subfamily which includes the tribes Micrutralini, Acutalini and Ceresini (McKamey, 1998). The previous morphology-based analysis grouped Micrutralini and Acutalini with the subfamily Darninae. In our combined analysis, Micrutralini was consistently placed as sister to Membracinae + Darninae + Smiliinae (Fig. 3, nodes 17–18) with strong bootstrap (>90%) and Bremer (>10) support (when this relationship was compared with a topology constraining the traditional definition of Smiliinae by Templeton, winning-sites, and Kishino–Hasegawa tests, the results indicated a statistically significant difference at  $P < 0.05$ ). Thus, analyses of the combined molecular (Fig. 2A) and combined molecular and morphological data (Fig. 3) agree with the finding of Dietrich *et al.* (2001) that the confluent R and M veins of the forewing, a character traditionally used to diagnose Smiliinae, arose independently at least twice: once in Micrutralini and again in Smiliinae (Fig. 3, node 34).

No previous phylogenetic analysis based on morphological or molecular data has recovered a monophyletic subfamily Darninae (Sakakibara, 1979; Cryan *et al.*, 2000; Dietrich *et al.*, 2001). Whereas Dietrich *et al.* (2001) found support for darnine monophyly only when Micrutralini and Acutalini were included, our combined analyses grouped

the three exemplars of Darninae together in a clade (Fig. 3, node 32) with moderate statistical support (Table 7). The addition of molecular data for representatives of the remaining tribes of Darnini (Hemikypthini, Hyphinoini, Procyrtini) is necessary to test rigorously the phylogenetic status of this subfamily.

#### *Origin and geographical distribution of Membracidae*

The results of our combined analyses are consistent with the hypothesis, based on analyses of morphological data (Dietrich & Deitz, 1993; Dietrich *et al.*, 2001; Wallace & Deitz, in press) and fossil evidence (Schlee, 1990; Shcherbakov, 1992; Dietrich & Deitz, 1993), that Membracidae arose in the New World and reached the Old World subsequently via dispersal. All members of the 'higher membracid' clade (Fig. 3, node 17) and the most basal members of its sister group (Fig. 3, node 2: *Lycoderes*, *Deiroideres*, *Antillotolanina* and *Microcentrus*) are restricted to the New World. The six Old World centrotine species sampled here never grouped as a monophyletic clade in our analyses (the results of Templeton, winning-sites, and Kishino–Hasegawa tests comparing these results with a topology which constrains the monophyly of the Old World Centrotinae indicate a statistically significant difference at  $P < 0.05$ ). Rather, these taxa were placed into two clades with weak support: African species (Fig. 3, node 7) and Malaysian + Australian species (Fig. 3, node 9). *Gargara* and *Anchon*, represented here by African species, also include Indomalayan and Palearctic species (McKamey, 1998). Our results are equivocal regarding the biogeographical origin of Centrotinae. Although support for some previously recognized centrotine tribes was strong, relationships among these tribes were poorly resolved, with most internal nodes following node 6 (Fig. 3) receiving less than 50% bootstrap support. The morphology-based analysis of Wallace & Deitz (in press) indicated that Centrotinae arose in the New World and dispersed into the Old World two times. Their analysis, which included representatives of all twenty-four tribes of Centrotinae and numerous additional morphological characters diagnostic for these taxa, placed Centrodontini as sister to the remaining members of this subfamily. Topological differences between our results and those of Wallace & Deitz (in press) are probably due, at least in part, to the limited sample of centrotine taxa available for DNA sequencing.

#### *Sociality and mutualism in Membracidae*

Treehopper life histories range between the extremes of exclusively solitary to highly developed subsocial behaviour, including presociality (various degrees of maternal care and aggregation behaviour) and mutualistic interactions with social Hymenoptera (ants, bees, and wasps; Wood, 1984, 1993). Although sociality was traditionally thought to be bound by 'Dollo's Law', whereby social

behaviour proceeds along an irreversible evolutionary course (from primitive asociality to presociality to sociality to eusociality), Wcislo & Danforth (1997) reviewed cases from social bee lineages which document apparent reversals from eusociality to sociality. The results of a previous phylogenetic analysis of treehoppers suggest that gregarious and presocial behaviours have also been gained and lost multiple times (McKamey, 1994; Dietrich *et al.*, 2001).

Wood (1984) regarded presociality in treehoppers as an apomorphic trait derived from ant mutualism, arguing that maternal care behaviour evolved as a result of selective pressure to increase the concentration of the honeydew secretion provided by treehoppers to tending ants, thereby making the protective services provided by ants more constant and reliable. Tallamy & Schaefer (1997) disagreed, regarding presociality as plesiomorphic, with mutualism acquired subsequent to the evolution of maternal care as a means of transferring some of the costs of maternal behaviour to mutualistic social hymenopterans.

Our results suggest that either hypothesis may be correct, depending on which treehopper lineage is considered. Within Membracinae (Fig. 3, node 19), all species of Hoplophorionini and Aconophorini observed exhibit highly developed maternal care behaviour (Wood, 1984; McKamey, 1994). Females guard their eggs and remain with nymphal aggregations throughout their development, often aggressively confronting predators with kicks of the legs and/or buzzing wings. In Hoplophorionini (Fig. 3, node 23), ant attendance has only been recorded in one species (*Potnia granadensis* (Fairmaire); Loye, 1992), and ant mutualism in Aconophorini (Fig. 3, node 26) is limited to some species of *Guayaquila* Goding. Many species of Membracini (Fig. 3, node 28) are ant attended and some exhibit maternal care (Wood, 1984; McKamey, 1994), but in Hypsoprini (Fig. 3, node 20), ant mutualism only appears to occur consistently in the genus *Notocera* Amyot & Serville (Wood, 1984). In light of our phylogenetic results, this suggests that within Membracinae, parental care arose in the common ancestor of Hoplophorionini, Aconophorini and Membracini. Given that ant mutualism occurs, at most, in one genus each of Hoplophorionini and Aconophorini, as well as in the sister group to this lineage (Hypsoprini), it seems possible that the common ancestor of Hoplophorionini + Aconophorini + Membracini was not ant mutualistic. Thus, for Membracinae, our phylogeny is consistent with the hypothesis that ant mutualism arose subsequent to (and possibly as a consequence of) the origin of maternal care (Tallamy & Schaefer, 1997).

In the Darninae + Smiliinae lineage (Fig. 3, node 31), ant attendance has not been reported in Darninae (node 32) and occurs sporadically in Ceresini (node 36) and Telamonini (node 46). Amastrini and Polyglyptini usually occur in ant-attended aggregations but only Polyglyptini are known to exhibit maternal care. *Antianthe* (Smiliini) is also ant mutualistic and females guard eggs and nymphs. Placement of Amastrini (represented here by one species of *Harmonides*) as sister to the clade comprising Polyglyptini + Smiliini (Fig. 3, node 41) suggests that the acquisition of

ant mutualism preceded the evolution of maternal care in this lineage, consistent with Wood's (1984) hypothesis.

#### Concluding remarks

Despite strong nodal support in the current study for resolution of relationships among the major membracid lineages, the results presented here should be interpreted with caution due to the absence of some subfamilies (Endoiastinae, Nicomiinae, Centronodinae and Heteronotinae) from our molecular datasets. The morphology-based analysis consistently placed Endoiastinae as sister to the remaining treehoppers and Heteronotinae as sister to a clade comprising Membracinae, Darninae and Smiliinae (Dietrich & Deitz, 1993; Dietrich *et al.*, 2001), but the phylogenetic positions of Centronodinae and Nicomiinae remained poorly resolved. The analysis of Dietrich *et al.* (2001) placed Centronodinae as sister to Centrodontini with weak branch support, and these two groups together were sister to Nicomiinae, also with weak support. Subsequently, more detailed morphology-based phylogenetic analyses (Wallace & Deitz, in press; Albertson & Dietrich, unpublished) indicated that Centrodontini belong to Centrotinae, in agreement with the present combined data analysis. Given the strong potential of molecular data for resolving the status and relationships of treehopper subfamilies, efforts are needed to obtain exemplars of the remaining membracid subfamilies, particularly Centronodinae and Nicomiinae suitable for DNA sequencing.

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