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Molecular Phylogenetics and Evolution of Maternal Care in Membracine Treehoppers

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Abstract.—The treehopper subfamily Membracinae (Insecta: Hemiptera: Membracidae) comprises the majority of genera and species diversity in the New World tropics. These treehoppers exhibit a wide range of social behaviors, making them an excellent group for studying patterns of social evolution in insects. However, to date the tribal and generic relationships have remained unclear. We reconstructed the phylogeny of the Membracinae using a combined mitochondrial (COI, COII, tRNA-Leu, and 12S) and nuclear (Wg) gene data set. A total of 2608 aligned nucleotide sites were obtained for 112 species, representing 25 of 38 currently recognized genera and all four tribes. A strict consensus of five equally parsimonious trees recovered the subfamily and three of its four tribes. The majority rule consensus tree derived from the Bayesian analyses based on the GTR+I+G and mixed-models recovered many clades shared with the parsimony trees and is identical to the single best tree inferred from maximum likelihood analysis, aside from the rearrangement of one node. A comparison of mitochondrial and nuclear genes indicated that Wg provided higher consistency index (CI), data decisiveness (DD), partitioned Bremer support (PBS) than any of the mitochondrial genes analyzed. The combined mitochondrial and nuclear DNA provide strong support for the monophyly of the subfamily and three of its four tribes (Aconophorini, Hoplophorionini, and Hypsoprorini). Membracini is paraphyletic with respect to Hoplophorionini and contains two lineages, the Membracini *sensu strictu* and the newly resurrected tribe Bolbonotini. Our analyses show that there is a strong phylogenetic component to the evolution of maternal care. Given the widespread occurrence of maternal care within the subfamily, this trait is estimated to have $\leq$3 origins, two reversals, and one loss. Our results suggest that the evolution of maternal care in insects may not be as evolutionarily labile as previously thought. [Bayesian analysis; maximum likelihood; Membracidae; mitochondrial gene; nuclear gene; phylogeny; social evolution; treehoppers.]

Treehoppers in the family Membracidae (Insecta: Hemiptera) exhibit diversity in behavioral and life history traits including maternal care (subsociality), ant mutualism, host-plant specialization and plant-borne vibrational communication (Wood, 1993; Cocroft, 1996, 2001). The New World treehopper subfamily Membracinae (Fig. 1) has a mostly Neotropical distribution, with the highest generic and species diversity in the tropical regions of Central America and northern South America on the eastern slope of Andes adjacent to the edge of Amazon basin (Metcalf and Wade, 1965; Wood, 1993; McKamey, 1998). Members of the subfamily exhibit variation in social behavior ranging from solitary individuals, nymphal or adult aggregations to highly developed maternal care with parent-offspring communication (Wood, 1993; Cocroft, 1996, 1999). The variation in social behavior among closely related species within the Membracinae provides an excellent opportunity for addressing a number of important questions in insect social evolution. Does the evolution of maternal care represent a convergent adaptation to ecological conditions, reflect phylogenetic patterns, or both? How frequently has this behavior evolved, and how easily can it be lost once developed?

Treehoppers provide some of the best-studied examples of subsocial behavior in insects. Subsocial behavior in treehoppers is restricted to maternal care of eggs and nymphs. Egg-guarding is the most common form of maternal care in treehoppers and is defined as a female remaining on top of her egg mass for a period of time after oviposition (Fig. 1c, g, and h). The body of the female is used as a physical shield to protect eggs against predators or parasitoids. Most females display egg-guarding behavior that leads to body contact with egg masses (Beamer, 1930; Wood, 1974, 1976). Females of most subsocial species guard eggs without leaving the egg masses. In some treehoppers, the female extends egg-guarding behavior until offspring reach the adult stage (McKamey and Deitz, 1996). Treehoppers are phytophagous insects with piercing and sucking mouthparts used to feed on the phloem and xylem of plants. Early treehopper instars may not be able to penetrate the epidermis of host plant tissues with their stylets. Females of some subsocial species modify branches to make food resources accessible to nymphs (Wood, 1974, 1976). A series of feeding slits around the stem made by a female's ovipositor creates sites where early instars feed (McKamey and Deitz, 1996). Maternal care in treehoppers therefore represents an important behavioral and life history modification which improves offspring survival rates (Wood, 1976, 1984; Dowell and Johnson, 1986; Eberhard, 1986).

The evolutionary history of maternal care in the Membracinae is not well understood because phylogenies necessary for reconstructing the evolution of social traits are not available. We do not know whether maternal care is a derived or a primitive trait for the subfamily, or whether maternal care gives rise to solitary behavior. In the absence of a clear understanding of phylogenetic relationships, even the number of origins of maternal care is unknown. The historical origins of subsocial behavior in treehoppers can be addressed through

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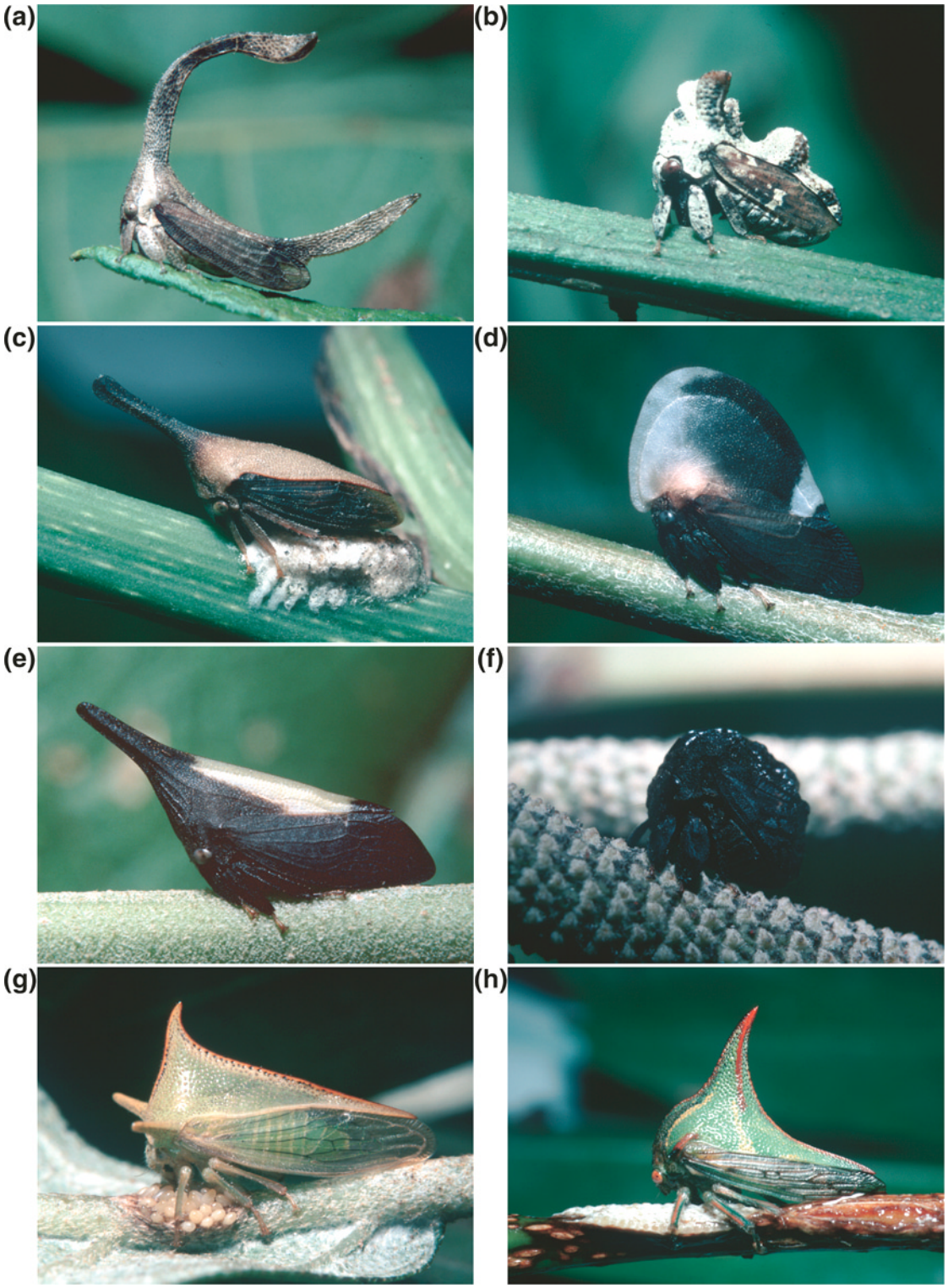

FIGURE 1. Representative genera of membracine treehoppers. (a) *Cladonota* sp. (Hypsoprorini), Peten province, Guatemala, December 1999. (b) *Notocera* sp. (Hypsoprorini), Guatemala, December 1999. (c) *Guayaquila* sp. (Aconophorini), a female sitting on eggs covered with accessory secretion, Guatemala, December 1999. (d) *Membracis foliata* (Linnaeus) (Membracini), near Gamboa, Panama, January 2000. (e) *Enchophyllum* sp. (Membracini), Podocarpus National Park, Zamora, Ecuador, January 16, 2001. (f) *Bolbonota* sp. (Membracini), San Jose de Rio Tinto, Honduras, July 25, 2001. (g) *Alchisme tridentata* (Fairmaire) (Hoplophorionini), a female sitting on eggs, Cosanga, Napo province, Ecuador, January 10, 2001. (h) *Umbonia crassicornis* (Amyot and Serville) (Hoplophorionini), a female guarding her eggs, Gamboa, Panama, January 2000. (Photos by C.-P. Lin.)

a comparative phylogenetic analysis (Felsenstein, 1985a; Coddington, 1988; Donoghue, 1989; Brooks and McLennan, 1991; Harvey and Pagel, 1991), with extensive taxon sampling including both asocial and subsocial taxa with and without maternal care. This allows the interpretation of the origins and transitions of these characters within and between treehopper lineages.

The subfamily Membracinae contains 38 genera and nearly 450 described species in five tribes: Aconophorini, Hoplophorionini, Hypsoprorini, Membracini, and Talipedini (Metcalf and Wade, 1965; Deitz, 1975; McKamey, 1998). Monophyly of the subfamily has not been well supported by previous morphological studies (Dietrich et al., 2001). Only one morphological synapomorphy was discovered for the subfamily, but this character (the reduced or absent metathoracic tibial setal row III) is also present in *Dysyncritus intectus* (Heteronotinae), suggesting either that the monophyly of Membracinae is not supported or the genus *Dysyncritus* is misplaced. Furthermore, Dietrich et al. (2001) assumed *a priori* that the Aconophorini, Hoplophorionini, and Talipedini are monophyletic based on a previous study (Dietrich and McKamey, 1995) and included only two to three genera for the remaining Membracini and Hypsoprorini in the analysis. Their analysis suggested that Hypsoprorini is a basal clade within the subfamily and Membracini is paraphyletic or polyphyletic with respect to Aconophorini and Hoplophorionini + Talipedini (Fig. 2a). Previous morphological studies (Dietrich and Deitz, 1993; Dietrich and McKamey, 1995; Dietrich et al., 2001) provided some additional resolution but did not completely resolve the tribal or generic relationships, suggesting the limited phylogenetic utility of morphological characters at higher levels within treehoppers.

Cryan et al. (2000) presented the first molecular phylogenetic analysis of Membracidae based on two nuclear genes, elongation factor-1α (EF-1α) and 28S ribosomal DNA (28S) (Fig. 2b). Their analyses recovered the monophyly of subfamily Membracinae and the monophyletic Aconophorini, Hoplophorionini, Hypsoprorini, and Membracini with moderate support. However their taxon sampling was limited (three to four genera per tribe) and probably inadequate for testing the monophyly of these tribes, some of which include up to 17 genera (e.g. Membracini). Contrary to morphological analyses (Dietrich et al., 2001; Fig. 2a), their analysis suggested that the Aconophorini and Membracini are sister clades (Fig. 2b).

Overall, monophyly of the subfamily Membracinae is suggested but not well supported by either morphology or nuclear DNA. Monophyly of the tribes Aconophorini and Hoplophorionini is supported by several morphological synapomorphies (Dietrich and Deitz, 1991; McKamey and Deitz, 1996), whereas monophyly of the Hypsoprorini, Talipedini, and Membracini is less clear (Dietrich and McKamey, 1995). Relationships among tribes and genera are not well resolved using adult morphology, nuclear DNA sequence data, or recent combined nuclear DNA and morphology (Cryan et al., in press). Resolving the tribal and generic relationships of the Membracinae is important because it is at these levels where treehoppers show behavioral variation and sufficient life history information is available for interpreting patterns of social evolution.

Here we examine phylogenetic relationships of Membracinae using mitochondrial DNA and nuclear DNA sequences with extensive taxon sampling. Four mitochondrial genes, COI, tRNA-Leu, COII, and 12S were sequenced. A nuclear gene, Wingless (Wg), was added to our mitochondrial data set because it provides an independent estimation of species phylogeny. The multiple genes also cover a broad range of sequence divergences for various taxonomic levels. This is the first attempt to resolve the tribal and generic relationships of Membracinae using a combination of DNA sequences from both mitochondrial and nuclear genomes. The aims of this study are (1) to determine whether the DNA sequence from mitochondrial COI, tRNA-Leu, COII, 12S, and nuclear Wg genes provide sufficient characters to resolve tribal and generic relationships; (2) to test the monophyly of the subfamily and its inclusive tribes and genera; and (3) to interpret patterns of maternal care evolution in Membracinae using a phylogenetic framework.

Materials and Methods

Taxon Sampling

To determine whether the subfamily Membracinae is monophyletic, we included taxa from 11 of the 12 currently recognized subfamilies of Membracidae (only Centronodinae was lacking from our data set; Table 1). *Aetalion reticulatum* of Aetalionidae was used as an outgroup because the family is presumed to be a sister to Membracidae (Dietrich and Deitz, 1993). Although it was not possible to obtain specimens of all the genera in Membracinae, 25 of 38 (66%) currently recognized genera in the subfamily were used. These genera are drawn from all four recognized tribes. More than half of the genera were represented by at least three species. Seven of the 13 missing genera are monotypic and the remainder contains 3 or fewer species. We included a total of 112 OTUs in the data matrix.

Bolbonota and related genera.—The genus *Bolbonota* (Fig. 1f) represents a major lineage in the tribe Membracini and among the most frequently encountered treehoppers in the tropical lowlands of Central and South America. *Bolbonota* and related *Bolbonotodes*, *Paragara*, and *Tritropidia* are distinct from other members in the Membracini by their unique black oval shape of the pronotum, relatively small body size and short ovipositor, and only slightly notched (emarginate) sternum II (Deitz, 1975). The placement of genera related to *Bolbonota* has been problematic. *Bolbonota* and related taxa were once treated as the Bolbonotini (Goding, 1926; Metcalf and Wade, 1965) but later considered as a synonym of the Membracini (Deitz, 1975) because no distinct characters were found between the Bolbonotini and Membracini. Relationships of *Bolbonota* to other genera in the Membracinae are not clear because pronotal

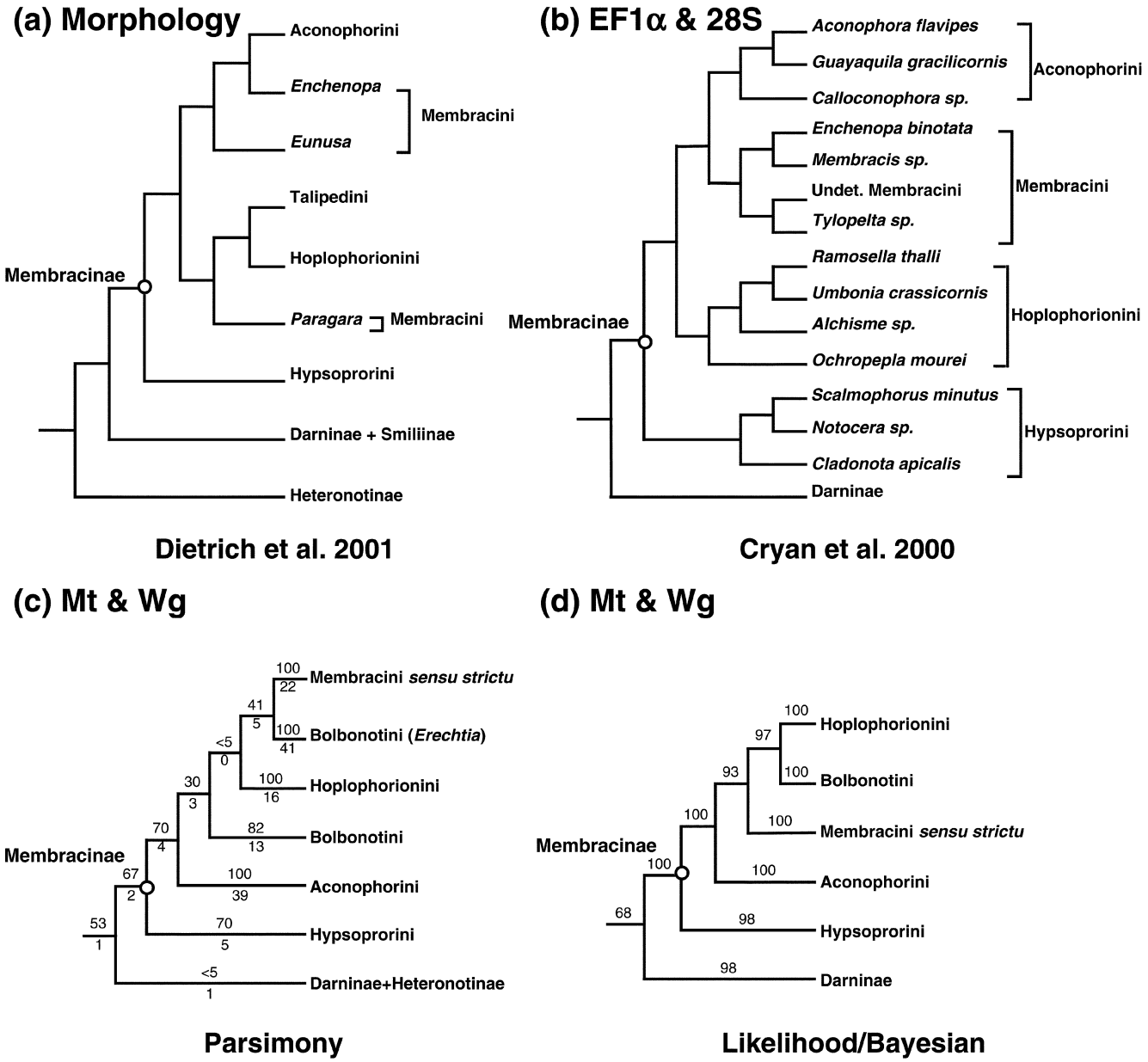

FIGURE 2. Summary of tribal relationships of the Membracinae. (a) Preferred tree of Dietrich et al., 2001 (redrawn from their Fig. 10). (b) Parsimony trees of Cryan et al., 2000 (redrawn from their Fig. 5). (c) Parsimony tree of the present study, numbers above and below branches are bootstrap and Bremer values. (d) Likelihood/Bayesian tree of the present study, numbers above branches are Bayesian posterior probabilities.

characters used to delineate them from other genera are highly reduced or absent. *Bolbonota* and related taxa were not included in the previous morphological, nuclear DNA, or combined analyses (Cryan et al., 2000, in press; Dietrich et al., 2001). In this study we included *Bolbonota* and related *Erechtia* and *Tritropidia*. Inclusion of *Bolbonota* in the analysis is important not only because it comprises a major lineage in the subfamily, but also because their morphology and unique oviposition behavior (oviposition on the plant surface rather than embedded in plant tissue) suggest that together with *Erechtia* (Membracini) and *Trinarea* (Talipedini), *Bolbonota* may represent a transitional lineage among the Aconophorini, Hoplophorioni, and Membracini.

DNA Extraction and Sequencing

Treehoppers used in this study were obtained by the author or generously provided by collaborators. Specimens were collected as adults or nymphs at various localities in Asia, North, Central, and South America (Table 1). Field-collected treehoppers were immediately preserved in 95% ethanol, followed by long-term storage at −20°C. DNA extraction was done using the abdomen or thoracic

TABLE 1. Membracine treehoppers included in the present study, with collecting information and DNA codes.

Subfamily	Tribe	Genus	Species	DNA code	Locality	Date	Collector
Aetalioninae	Aetalionini	*Aetalion*	*reticulatum* (Linnaeus)	AETA2	Gamboa, Panama	6/12/98	R. Cocroft
Biturritiinae	Biturritiini	*Gerridius*	sp.	GESP	Gamboa, Panama	3/30/98	R. Cocroft
Centrotinae	Platycentrini	*Tylocentrus*	*reticulatus* Van Duzee	K5	Rodeo, AZ	8/4/97	M. Adams
Centrotinae	Platycentrini	*Platycentrus*	sp.	PLAT	Green Valley, AZ	7/28/99	T. Wood
Centrotinae	Boocerini	*Brachybelus*	*cruralis* Stål	BRA	San Luis, Costa Rica	Jul-96	R. Cocroft
Centrotinae	Boocerini	*Campylocentrus*	sp.	CASP2	Gamboa, Panama	1/26/98	R. Cocroft
Centrotinae	Micreunini	*Leptobelus*	*gazella sauteri* Schumacher	LESA	Shenyuan, Taiwan	6/17/99	R. Hoebeke
Centrotinae	Centrocharesini	*Centrochares*	sp.	CENCHA1	Mt. Banahaw, Philippines	7/9/00	C.-P. Lin
Centrotinae	Hypsaucheniini	*Pyrgauchenia*	*brunnea* Funkhouser	PYBU	Mt. Kinabalu, Sabah	2/15/98	U. Stegmann
Centrotinae	Hypsaucheniini	*Hypsauchenia*	sp.	HYPSAU	Genting Highland, Malaysia	8/9/00	C.-P. Lin
Centrotinae	Leptocentrini	*Leptocentrus*	sp.	LEIN3	Masinequdi, India	5/2/99	R. Cocroft
Centrotinae	Tricentrini	*Tricentrus*	sp.	TRICME	Nuntou, Taiwan	11/10/98	C.-S. Lin/W.-T. Yang
Endoiastinae	Endoiastinae	*Stictodepsa*	sp.	STIECU	Mondayacu, Ecuador	1/24/01	C.-P. Lin/F.-T. Yang
Centrodontinae	Centrodontini	*Centrodontus*	*atlas* (Goding)	K6	Picacho Peak, AZ	4/18/97	C.-P. Lin
Centrodontinae	Centrodontini	*Multareoides*	*digitatus* (Van Duzee)	MUDI	Florence, AZ	7/29/99	T. Wood
Nicomiinae	Tolaniini	*Tolania*	sp.	TOSP	Gamboa, Panama	3/30/98	R. Cocroft
Oxyrhachinae	Oxyrhachini	*Oxyrhachis*	*taranda* (Fabricius)	OXTA	Masinequdi, India	8/10/99	R. Cocroft
Stegaspidinae	Microcentrini	*Microcentrus*	*caryae* (Fitch)	LMC	Watertown, NY	8/30/97	T. Wood
Stegaspidinae	Microcentrini	*Smerdalea*	*horrescens* Fowler	SMEHOR	Cerro Campana, Panama	5/2/99	G. Keller/D. Windsor
Stegaspidinae	Microcentrini	*Centruchoides*	*laticornis* Fowler	CERNA	Cerro Campana, Panama	5/2/99	G. Keller/D. Windsor
Stegaspidinae	Stegaspidini	*Stegaspis*	*fronditia* (Linnaeus)	STFR	Gamboa, Panama	2/25/98	T. Wood
Nessorhininae	Nessorhinini	*Nessorhinus*	sp.	VIRSP3	St. John, Virgin Islands	3/28/00	R. Hoebeke
Heteronotinae	Heteronotini	*Heteronotus*	*trinodosus* Butler	K3	Gamboa, Panama	12/14/94	R. Cocroft
Heteronotinae	Heteronotini	*Nassunia*	sp.	NASP2	Cerro Campana, Panama	3/19/98	R. Cocroft
Heteronotinae	Heteronotini	*Dysyncritus*	sp.	DYSYNC	Mondayacu, Ecuador	1/24/01	C.-P. Lin/F.-T. Yang
Darninae	Cymbomorphini	*Cymbomorpha*	sp.	CYMB	Gamboa, Panama	3/21/98	R. Cocroft
Darninae	Darnini	*Darnis*	*latior* Fowler	DALA	Gamboa, Panama	2/25/98	T. Wood
Darninae	Darnini	*Darnis*	*partita* Walker	DARPAR	Cerro Campana, Panama	5/2/99	G. Keller/D. Windsor
Darninae	Darnini	*Stictopelta*	sp.	STC	Guanacaste, Costa Rica	6/21/96	R. Cocroft
Darninae	Procyrtini	*Procyrta*	*pectoralis* (Fabricius)	PRPE	El Caoba, Guatemala	1/6/00	C.-P. Lin
Darninae	Hyphinoini	*Hyphinoe*	*asphaltina* (Fairmaire)	HYA	San Luis Valley, Costa Rica	1/14/98	D. Tallamy
Smilinae	Acutalini	*Acutalis*	sp.	ACUECU8	Papallacta Pass, Ecuador	1/8/01	C.-P. Lin/F.-T. Yang
Smilinae	Micrutalini	*Micrutalis*	*calva* Say	MIC1	Ulster Co., NY	6/24/98	T. Wood
Membracinae	Aconophorini	*Calloconophora*	sp.	ACCO	Rio Guanche, Panama	3/14/98	R. Cocroft
Membracinae	Aconophorini	*Aconophora*	*mexicana* Stål	ACME	Gamboa, Panama	3/12/98	R. Cocroft
Membracinae	Aconophorini	*Guayaquila*	sp.	LAC1	Guanacaste, Costa Rica	6/18/96	R. Cocroft
Membracinae	Aconophorini	*Guayaquila*	sp.	ACOCAM	Cerro Campana, Panama	5/2/99	G. Keller/D. Windsor
Membracinae	Aconophorini	*Guayaquila*	sp.	ACONOP	Darien, Panama	6/8/98	G. Keller/D. Windsor
Membracinae	Aconophorini	*Guayaquila*	*emarginata* Dietrich	ACOPAN1	Bambito, Panama	1/18/00	M. Cast/R. Cocroft/C.-P. Lin/ M. Rothschild/T. Wood
Membracinae	Aconophorini	*Guayaquila*	*pallescens* (Stål)	ACOPAN2	Volcan, Panama	1/17/00	M. Cast/R. Cocroft/C.-P. Lin/ M. Rothschild/T. Wood
Membracinae	Aconophorini	*Guayaquila*	sp.	BAC	Brazil	NA	A. Sakakibara
Membracinae	Aconophorini	*Aconophora*	nsp.	ACOGUA1	San Lucas, Guatemala	12/29/99	C.-P. Lin
Membracinae	Aconophorini	*Aconophora*	*compressa* Walker	ACOGUA2	Sacapulas, Guatemala	12/30/99	C.-P. Lin
Membracinae	Aconophorini	*Guayaquila*	sp.	PORAP	Gamboa, Panama	1/25/00	R. Cocroft

Membracinae	Aconophorini	*Calloconophora*	sp.	BRACON7	Uberlandia, Brazil	8/12/00	K. Tilmon/T. Wood
Membracinae	Aconophorini	*Guayaquila*	*pallescens* (Stål)	GUAGUA7	Jocotenango, Guatemala	12/25/99	C.-P. Lin
Membracinae	Aconophorini	*Guayaquila*	*pallescens* (Stål)	GUAGUA8	El Rancho, Guatemala	1/2/00	C.-P. Lin
Membracinae	Aconophorini	*Aconophora*	*robusta* Dietrich	CALPAN3	Boquete, Panama	1/14/00	M. Cast/R. Cocroft/C.-P. Lin/ M. Rothschild/T. Wood
Membracinae	Aconophorini	*Aconophora*	*marginata* Walker	CALCAL	Panajachel, Guatemala	12/26/99	C.-P. Lin
Membracinae	Aconophorini	*Calloconophora*	*caliginosa* (Walker)	CALGUA3	Cutarina, Guatemala	12/28/99	C.-P. Lin
Membracinae	Aconophorini	*Aconophora*	*marginata* Walker	CALGUA4	Chicua, Guatemala	12/29/99	C.-P. Lin
Membracinae	Aconophorini	*Aconophora*	*laminata* Fairmaire	CALGUA6	Jocotenango, Guatemala	12/25/99	C.-P. Lin
Membracinae	Hoplophorionini	*Alchisme*	*grossa* (Fairmaire)	ALCGRO	Cosanga, Ecuador	1/9/01	C.-P. Lin/F.-T. Yang
Membracinae	Hoplophorionini	*Alchisme*	*virescens* (Fairmaire)	ALCVIR	Cosanga, Ecuador	3/24/00	D. Penrose
Membracinae	Hoplophorionini	*Alchisme*	sp.	Als	Monteverde, Costa Rica	7/14/95	T. Johnson
Membracinae	Hoplophorionini	*Alchisme*	*apicalis* (Walker)	ALCAPI	Volcan, Panama	1/17/00	M. Cast/R. Cocroft/C.-P. Lin/ M. Rothschild/T. Wood
Membracinae	Hoplophorionini	*Metcalfiella*	*monogramma* (Germar)	METMON	Jocotenango, Guatemala	12/25/99	C.-P. Lin
Membracinae	Hoplophorionini	*Ramosella*	*thalli* McKamey & Deitz	RAM	Constanza, Dominican Republic	1/3/98	S. McKamey
Membracinae	Hoplophorionini	*Platycotis*	*minax* Goding	PLMI	Santa Paula Co., CA	8/18/99	R. Dowell
Membracinae	Hoplophorionini	*Platycotis*	sp.	PLAVOL1	Bambito, Panama	1/19/00	M. Cast/R. Cocroft/C.-P. Lin/ M. Rothschild/T. Wood
Membracinae	Hoplophorionini	*Platycotis*	*tuberculata* (Fairmaire)	PLATUB	Jocotenango, Guatemala	12/25/99	C.-P. Lin
Membracinae	Hoplophorionini	*Umbonia*	*ataliba* Fairmaire	UMA	Puntarenas, Costa Rica	8/16/96	R. Cocroft
Membracinae	Hoplophorionini	*Umbonia*	*reducta* Walker	Ure	La Selva, Costa Rica	4/20/94	S. McKamey
Membracinae	Hoplophorionini	*Umbonia*	*spinosa* (Fabricius)	UMBOSP	Gamboa, Panama	2/4/95	R. Cocroft
Membracinae	Hoplophorionini	*Umbonia*	*crassicornis*	UC0601	Lab colony	NA	R. Cocroft
Membracinae	Hoplophorionini	*Umbonia*	*crassicornis*	UMCRP	Gamboa, Panama	3/1/98	T. Wood
Membracinae	Hoplophorionini	*Ochropepla*	sp.	OCV	Edo. Miranda, Venezuela	1/3/98	S. McKamey
Membracinae	Hoplophorionini	*Ochropepla*	*inaequalis* Fowler	OCHINE	Chacte, Guatemala	1/7/00	C.-P. Lin
Membracinae	Hoplophorionini	*Potnia*	*gladiator* (Walker)	POGL	Gamboa, Panama	2/25/98	T. Wood
Membracinae	Hoplophorionini	*Potnia*	sp.	POSP1	Cerro Campana, Panama	2/27/98	T. Wood
Membracinae	Hoplophorionini	*Stalotypa*	*fairmairii* (Guerin-Meneville)	STAL	Soroa, Cuba	6/19/01	C.-P. Lin
Membracinae	Hypsoprorini	*Hypsoprora*	sp.	HYSP2	Pl Llano, Panama	12/9/98	R. Cocroft
Membracinae	Hypsoprorini	*Philya*	sp.	PHIL2	Riverside, CA	7/20/99	R. Dowell
Membracinae	Hypsoprorini	*Cladonota*	*biclavata* (Westwood)	SPGU	Gamboa, Panama	2/25/98	T. Wood
Membracinae	Hypsoprorini	*Cladonota*	sp.	SPSP2	Chirique Grande, Panama	3/5/98	T. Wood
Membracinae	Hypsoprorini	*Notocera*	sp.	NOSP2	Gamboa, Panama	2/25/98	T. Wood
Membracinae	Hypsoprorini	*Notocera*	sp.	NOSP4	Fortuna, Panama	3/4/98	T. Wood
Membracinae	Hypsoprorini	*Notocera*	sp.	NOSP6	Guanacaste, Costa Rica	5/7/96	R. Cocroft
Membracinae	Hypsoprorini	*Notocera*	sp.	NOSP3	Chirique Grande, Panama	3/5/98	T. Wood
Membracinae	Membracini	*Bolbonota*	*insignis* Fowler	BOIN	Chirique Grande, Panama	3/5/98	R. Cocroft
Membracinae	Membracini	*Bolbonota*	sp.	BOSP2	Coclecito, Panama	1/29/98	R. Cocroft
Membracinae	Membracini	*Bolbonota*	sp.	BOV	Caracas, Venezuela	1/2/98	S. McKamey
Membracinae	Membracini	*Bolbonota*	sp.	BOSP1	Gamboa, Panama	2/25/98	T. Wood
Membracinae	Membracini	*Erechtia*	sp.	BER	Brazil	NA	A. Sakakibara
Membracinae	Membracini	*Erechtia*	sp.	TRINSP	El Valle, Panama	1/24/99	R. Cocroft
Membracinae	Membracini	*Erechtia*	sp.	TRINPAN	Volcan, Panama	1/17/00	M. Cast/R. Cocroft/C.-P. Lin/ M. Rothschild/T. Wood
Membracinae	Membracini	*Campylenchia*	*latipes* (Say)	LCL	Newark, DE	8/30/95	T. Wood

(Continued on next page)

TABLE 1. (CONTINUED)

Subfamily	Tribe	Genus	Species	DNA code	Locality	Date	Collector
Membracinae	Membracini	*Campylenchia*	sp.	ENCV2	San Antonio, Venezuela	7/26/97	S. McKamey
Membracinae	Membracini	*Campylchchia*	sp.	CAMP1	Fairview, UT	8/5/99	T. Wood
Membracinae	Membracini	*Tylopelta*	sp.	TYLON	Fortuna, Panama	3/6/98	R. Cocroft
Membracinae	Membracini	*Enchenopa*	sp.	ENBO1	Boquete, Panama	3/6/98	T. Wood
Membracinae	Membracini	*Enchenopa*	sp.	ENCA	Panama City, Panama	2/7/98	R. Cocroft
Membracinae	Membracini	*Enchenopa*	*binotata* (Say)	K8	Newark, DE	8/25/96	C.-P. Lin
Membracinae	Membracini	*Enchophyllum*	sp.	EEN	Gamboa, Panama	12/29/94	R. Cocroft
Membracinae	Membracini	*Enchophyllum*	sp.	ENPHCA	Cerro Campana, Panama	5/2/99	G. Keller/D. Windsor
Membracinae	Membracini	*Enchenopa*	*ignidorsum* Walker	ENCHIG	Cerro Campana, Panama	5/2/99	G. Keller/D. Windsor
Membracinae	Membracini	*Enchophyllum*	sp.	ENP2	San Luis Valley, Costa Rica	1/14/98	D. Tallamy
Membracinae	Membracini	*Membracis*	*tectigera* Olivier	MEMTE	Maracas Valley, Trinidad	10/13/99	C. Chaboo
Membracinae	Membracini	*Membracis*	*tectigera* Olivier	BRMEMTEC	Manaus, Brazil	8/1/00	K. Tilmon/T. Wood
Membracinae	Membracini	*Membracis*	*orteguazaensis* Richter	BRMEMORT	Rio preta da Eva, Brazil	8/9/00	K. Tilmon/T. Wood
Membracinae	Membracini	*Membracis*	*caquetaensis* Richter	MEMCA	Igritus, Peru	8/1/99	J. Castner
Membracinae	Membracini	*Membracis*	*dorsata* Fabricius	MEDO	Gamboa, Panama	2/25/98	T. Wood
Membracinae	Membracini	*Membracis*	*tectigera* Olivier	MEMELEP	Gamboa, Panama	1/21/00	M. Cast/R. Cocroft/C.-P. Lin/ M. Rothschild/T. Wood
Membracinae	Membracini	*Membracis*	*trimaculata* Fairmaire	MEMTRI	Miraflores, Panama	3/5/00	R. Cocroft
Membracinae	Membracini	*Membracis*	*flava* Richter	MEFL	Chirique Grande, Panama	3/5/98	T. Wood
Membracinae	Membracini	*Membracis*	*mexicana*	MEMMEXG1	Panajachel, Guatemala	12/26/00	C.-P. Lin
Membracinae	Membracini	*Membracis*	*mexicana*	MEMMEXCO	Tole, Panama	1/12/00	M. Cast/R. Cocroft/C.-P. Lin/ M. Rothschild/T. Wood
Membracinae	Membracini	*Membracis*	*foliata* (Linnaeus)	MEMFO	Igritus, Peru	8/1/99	J. Castner
Membracinae	Membracini	*Kronides*	*incumbens* (Germar)	KROIN	Brazil	NA	A. Sakakibara
Membracinae	Membracini	*Enchenopa*	sp.	ENCHDS	Darien, Panama	6/8/98	G. Keller/D. Windsor
Membracinae	Membracini	*Leioscyta*	sp.	LEBE	Cerro Campana, Panama	2/27/98	T. Wood
Membracinae	Membracini	*Leioscyta*	*nitida* Fowler	LEINIT	Volcan, Panama	1/19/00	M. Cast/R. Cocroft/C.-P. Lin/ M. Rothschild/T. Wood
Membracinae	Membracini	*Tritropidia*	sp.	BRTRIT	Manaus, Brazil	8/2/00	K. Tilmon/T. Wood

TABLE 2. Primers used to amplify and sequence various gene regions. Position information refers to the 5′ end of primer sequence in *Drosophila yakuba* (Clay and Wolstenholme, 1985). The standardized primer names (Simon et al., 1994) are in parentheses.

Gene	Position and Reference		Sequence
COI			
Ron (C1-J-1751)	1729	Simon et al., 1994	5′ GGATCACCTGATATAGCATTYCC 3′
Dick (C1-J-2441)	2410	Simon et al., 1994	5′ CCAACAGGAATTAAAATTTTTTAGATGATTAGC 3′
Rick (C1-J-2441)	2410	Simon et al., 1994	5′ CCAACAGGAATTAAAGTTTTTTAGATG 3′
Calvin (C1-N-2725)	2725	Lin and Wood, 2002	5′ GGRAARAAWGTTAARTTWACTCC 3′
COII			
Barb1 (C2-N-3661)	3684	Simon et al., 1994	5′ CCACAAATTTCTGAACATTGACCA 3′
12S			
12Sbi (SR-J-14233)	14214	Simon et al., 1994	5′ AAGAGCGACGGGCGATGTGT 3′
12Sai (SR-N-14588)	15179	Simon et al., 1994	5′ AAACTAGGATTAGATACCCTATTAT 3′
Wingless			
Wg1a	Brower and DeSalle, 1998		5′ GARTGYAARTGYCAYGGYATGTCTGG 3′
Wg2a	Brower and DeSalle, 1998		5′ ACTXCGCARCACCARTGGAATGTRCA 3′

muscles of a single individual following protocols outlined in Danforth (1999) with the remainder of the specimen preserved as a voucher in 95% ethanol at −20°C. Oligonucleotide primers used to amplify and sequence various gene regions are listed in Table 2. The cycling profile of polymerase chain reaction (PCR) amplifications began with one cycle of DNA denaturation at 94°C for 2 min followed by 35 cycles of sequence amplification (DNA denaturation at 94°C for 30 s, primer annealing at 48°C to 53°C for 30 s, and sequence extension at 72°C for 1 to $1^1/_2$ min). The primers Ron-Calvin produced a PCR product of nearly 1000 bp in the COI. A combination of Dick/Rick and Barb1 primers produced a PCR product of approximately 1200 bp across COI, tRNA-Leu, and COII. The 12Sbi and 12Sai primer produced a PCR product of about 350 bp in the 5′ end of 12S gene. The combination of Wg1a and Wg2a primer produced a PCR product of nearly 400 bp in the wnt-1 wingless paralog (Jockusch and Ober, 2000). The PCR amplifications of COI, COII, and 12S gene in general yielded a single visible band on agarose gel.

Amplification of the Wg gene fragment sometimes yielded two bands (350 bp and 400 bp in length). The 350-bp band may represent another paralogue of the Wg gene family. PCR products were gel-purified in low-melting-point agarose gels (FMC, Rockland, ME) overnight at 4°C. PCR products were recovered from the gel slices using Wizard PCR Preps DNA purification kit (Promega, Madison, WI) and sequenced from both directions on an ABI 377 automated sequencer (Applied Biosystems, Foster City, CA) in the Evolutionary Genetics Core Facility (EGCF) at Cornell University, the DNA Sequencing Facility of the BioResource Center at Cornell University or the DNA Sequencing Facility in the University of Delaware. The Ron-Calvin and Dick-Barb fragments have 300-bp and 200-bp overlap, respectively, from two strands. These two fragments were checked against each other for sequencing error in a region of 200-bp overlap. A region of 300 bp in the 5′ end of Ron-Calvin and 400 bp in the 3′ end of Dick-Barb was sequenced from one direction. For 12S and Wg fragments, sequences have complete overlap from two strands. Sequences that were not clear were resequenced or new PCR products were generated for sequencing. Sequences of various DNA fragments were edited and assembled using EDITSEQ (DNASTAR, Madison, WI) and SEQUENCHER version 4.1 (GeneCodes Corp., Ann Arbor, MI). DNA sequences used in this study can be obtained from GENBANK (accession AY513370-AY513481, AY593499-AY593722).

Sequence Alignment

The protein-coding sequences in the COI, COII, and Wg genes were initially aligned using MEGALIGN (DNASTAR, Madison, WI) and this initial alignment was later refined manually. Mitochondrial sequences of *Drosophila yakuba* (Clary and Wolstenholme, 1985) were used as a reference to determine the reading frame of COI and COII sequences and to facilitate manual alignment of insertions or deletions of amino acids. The assignment of codon positions was confirmed by translating nucleotide sequences into amino acid sequences using MacClade version 4.0 (Maddison and Maddison, 2000) with reference to a mitochondrial genetic code of *Drosophila* for COI and COII and a universal genetic code for Wg. The alignment of the tRNA-Leu gene was done manually with reference to tRNA secondary folding structure in *Apis mellifera* (Crozier and Crozier, 1993). The 12S ribosomal gene sequences were initially aligned using MEGALIGN and later adjusted by eye. The secondary structure model of the third domain of 12S rRNA for periodical cicada (Hemiptera) (Hickson et al., 1996) was used as a reference. Stem and loop regions could be identified and the sequences within these regions were subsequently aligned against closely related taxa. Inferred insertion and deletion gaps were coded as fifth characters for phylogenetic analyses. Missing characters from short sequences at the ends of the alignment were trimmed and excluded from the analyses. The alignments from each gene were combined in MacClade to form the complete matrix (TreeBase accession M1727). Treating gaps as fifth characters, especially for long gaps, is known to have potential problems including nonindependence of adjacent gap characters, inflated synapomorphies, and overweighting of characters (Baldwin et al., 1995; Kjer, 1995; Giribet and Wheeler, 1999, Lutzoni et al., 2000).

Although gaps and indels can introduce complications to phylogenetic analysis, most gap lengths in the tRNA-Leu and 12S alignment are relatively short (from 1 to 6 bp).

Parsimony Analyses

Maximum parsimony.—Equally weighted parsimony analyses were done using PAUP* (version 4.0b10, Swofford, 1998). Heuristic tree searches were performed on gene partitions and combined data using 1000 random sequence additions and TBR branch swapping. The parsimony ratchet procedure (Nixon, 1999) was performed to search tree space more effectively in the combined analysis. The ratchet procedure was run 20 times using 200 replicates each and repeated with varying percentages of weighted characters using batch files implemented in Pauprat (Sikes and Lewis, 2000). Phylogenetic analyses were also carried out using the neighbor-joining method on log determinant distances (LogDet, Lockhart et al., 1994), with invariable sites excluded to assess the effect of nucleotide compositional bias (see results) on phylogenetic reconstruction.

Branch support.—Nonparametric bootstrap (Felsenstein, 1985b) values were calculated on gene partitions and combined data using 1000 replicates and 100 random taxon additions to evaluate branch support. Separate bootstrap analysis was not conducted on the tRNA-Leu gene alone because of the limited number of characters (75 sites). To assess the relative contribution of data partitions to the total support of combined analysis, the Bremer support (BS; Bremer, 1988) of combined data and partitioned Bremer support (PBS; Baker and DeSalle, 1997) of each partition were calculated using a command file of constraint trees generated in TreeRot (version 2; Sorenson, 1999) with 100 heuristic searches (but see DeBry, 2001, for limitations of the Bremer support in parsimony analysis). To measure the information content (hierarchical structure) of each data partition and how strongly the data partition preferred the most parsimonious trees over other trees, data decisiveness (DD; Goloboff, 1991) values were calculated for each gene and the combined data. The mean length of all possible trees used in calculating DD values was estimated using 100,000 random trees generated by PAUP*.

Likelihood Analyses

Model selection.—For maximum likelihood analysis, trees obtained from equal weights parsimony analysis were used to compare the goodness of fit of 20 models of sequence evolution with increasing complexity. The four basic models are: (1) Jukes-Cantor (JC; 1969); (2) Kimura two-parameter (K2P; 1980); (3) Hasegawa-Kishino-Yano (HKY; 1985); and (4) the General Time Reversible model (GTR; 1994a). Within each model there are five methods of accommodating for rate heterogeneity among sites: (1) no rate variation; (2) gamma distribution (G; Yang, 1994b); (3) proportion of invariable sites (I); (4) I+G; and (5) site-specific rates (SSR). For SSR, characters were partitioned into 11 rate categories according to the functional properties of a site: three codon positions for each of the three protein-coding genes and all sites in tRNA-Leu and 12S gene. To determine an appropriate model of sequence evolution, the significance of the difference in the likelihood scores of the models was evaluated using the likelihood ratio test (LRT) statistic (LRT: $-2\ln\Lambda$, where Λ is equal to the difference between the likelihood under the null and the alternative [more complex] model). When the models compared are nested, the distribution of LRT statistic is expected to be approximately a χ^2 distribution with degrees of freedom equal to the difference in the number of free parameters between the two models being compared (Huelsenbeck and Crandall, 1997; Goldman, 1993).

Maximum likelihood.—Once the likelihood scores were calculated and LRT statistics of comparing nested models were done, the best-fitting model was used to find the maximum likelihood topology. To search for a maximum likelihood tree, the equal weights parsimony trees were used as starting trees and heuristic searches were performed using increasingly exhaustive branch swapping methods in the following order: nearest neighbor interchange (NNI), subtree pruning and regrafting (SPR), second round of SPR, tree bisection and reconnection (TBR), and a second round of TBR. At each iteration, the maximum likelihood parameters were reestimated from the trees that were obtained from the previous round of branch swapping. Given the size of the data set it was impractical to calculate bootstrap values based on the likelihood analysis. Instead, we relied on posterior probabilities derived from the Bayesian analyses based on the same set of models (see below).

Rates of Nucleotide Substitutions

The likelihood models GTR+I+G and GTR+SSR were used to estimate the rate of nucleotide substitution among genes, codon positions, and transformations among nucleotide bases. Equal weights parsimony trees obtained from combined analyses of all genes were used as tree topologies in parameter estimation of proportion of invariant site (*Pinv*), gamma distribution (α), and transformation rate matrices (r) under the GTR+I+G model. The transition/transversion (TS/TV) ratios were estimated under the HKY+I+G model. The relative rate of nucleotide substitution in each data partition was evaluated by searching for the maximum likelihood tree using the GTR+SSR model.

Bayesian Analyses

We performed Bayesian analyses using MrBayes 2.0 and 3.0 (Huelsenbeck and Ronquist, 2001). The results from the LRTs indicated that the model GTR+I+G best fit the sequence data given the parsimony topology. This model was used for subsequent Bayesian analyses. Alternatively, we analyzed the data using a mixed-model approach in MrBayes 3.0. Characters were partitioned into four categories: 1st and 2nd positions in COI and COII, 3rd positions in COI and COII, tRNA-Leu and 12S, and Wg. The appropriate model for each data partition was

chosen separately in Modeltest 3.0 (Posada and Crandell, 1998) (TVM+I+G for 1st and 2nd positions in COI and COII, tRNA+12S; TrN+G for 3rd positions in COI and COII; TrN+I+G for Wg). The Bayesian analyses were then performed with the best model in each character partition. We ran the analysis for 2.0×10^6 generations. Trees were sampled at intervals of 100 generations for a total of 20,000 trees. Stability of the process was achieved when ln likelihood values approached equilibrium, as determined by plotting the ln likelihood scores of the sampled trees against generation time. All trees sampled before reaching stability are discarded as "burn in" (Huelsenbeck and Ronquist, 2001). After discarding burn-in samples, the remaining trees were used to generate a 50% majority rule consensus tree with the percentage of trees recovering the node representing the node's posterior probability. Analyses were run independently five times to compare for convergence determined by similar ln likelihood value at each run. The resulting trees from each gene partition were compared to detect potential areas of incongruence as indicated by conflicting nodes with posterior probability values $\geq$95%.

Character Optimization and Tests for Phylogenetic Correlation

Patterns of maternal care evolution were examined by mapping egg-guarding behavior onto the phylogenetic tree. Character coding of the behavioral trait was based on the literature (Haviland, 1925; Wood, 1974, 1976, 1978, 1984, 1993; Hinton, 1977; Dietrich and Dietz, 1991; McKamey and Deitz, 1996) and personal observations (summarized in Lin, 2003). Character transformations were optimized under the parsimony criterion using MacClade 4.0 (Maddison and Maddison, 2000). Character evolution was assessed using the Trace Character Option with the default of most parsimonious state shown at each node setting. Randomization tests were employed to test whether or not a pattern of discrete behavioral trait is correlated with the phylogeny. Two randomization tests were done. First, 10,000 randomized trees were generated and the number of transitions of the character was obtained on each tree using MacClade. Second, the tree topology was held constant and the distribution of character states was randomized among taxa with 1000 replicates using the "shuffle" utility in MacClade. The distributions of the frequencies of the number of steps (i.e., the tree length) resulting from both randomized trees and character states were used to test whether the observed number of steps was significantly less than expected (i.e., more correlated with phylogeny) under a random model. The resulting P values indicated the probability of incorrectly rejecting the null hypothesis that the observed patterns of character association arose by chance.

We used maximum likelihood method to estimate the degree of confidence in ancestral character state reconstructions (Pagel, 1994, 1999; Schluter et al., 1997). Maximum likelihood estimates of rates of evolution between gains and losses of egg-guarding on the tree were calculated using Discrete 4.0 (Pagel, 1999). Differences in the rate of gain and loss were tested statistically by comparing the likelihood score of the data under a model with independent rates of gain and loss (two-rate model) with the likelihood score under a model in which rates of gains and losses are constrained to be equal (one-rate model). The significance of the difference was evaluated using the LRT statistic (χ^2 distribution with one degree of freedom, corresponding to a likelihood ratio of 7.4). Tree branch lengths were initially set to be equal and later set to the values derived from the Bayesian analysis to evaluate the sensitivity of rate estimates to the assumption of branch lengths. We used an one-rate model as recommended by Schluter et al. (1997) and Mooers and Schluter (1999) for reconstruction of ancestral character states and calculated their "local" likelihood estimates on the tree in Discrete (Pagel, 1999). The degree of support for a particular character state was evaluated using the LRT statistic.

RESULTS

Sequence Alignment

An alignment of 2608 nucleotide sites (including gaps) was obtained for 112 species. This alignment consisted of 1236 sites from the 3′ end of COI, the complete tRNA-Leu (75 sites), 517 sites from the 5′ end of COII, 407 sites from the 5′ end of 12S, and 373 sites from Wg. Thirteen stem and nine loop regions were identified as secondary structures in the 12S alignment. A region of five sites in the 3′ end of COI gene (position 1232 to 1236) was inferred to be noncoding. Three indels of one amino acid (three nucleotide sites) were detected in the alignment of COI. A region of four amino acid indels (ranging from three to nine nucleotides, positions 1693 to 1704) was detected in the alignment of COII. For the Wg gene, a region of 13 amino acids indels (ranging from 6 to 27 nucleotides, positions 2334 to 2372) was detected in most of the taxa and one amino acid deletion (positions 2382 to 2384) was detected in *Metcalfiella monogramma*. All inferred indels in protein coding genes retained the reading frame of the gene.

Base Composition

The base composition varied greatly among genes and codon positions. Overall nucleotide frequencies for mitochondrial genes were biased toward A+T (73.1%) (Table 3), consistent with other insect taxa (Simon et al., 1994). The base composition of the nuclear Wg is G+C rich (39.5% A+T). Chi-square tests of base frequency stationarity indicate the third positions in three protein-coding genes show significant among-taxa variation in composition ($P < 0.001$), whereas the remaining character partitions were not significant. The overall significant nucleotide compositional variation among taxa ($P < 0.001$) is due to these third position sites. Substantial nucleotide compositional bias could potentially affect the phylogenetic results, we analyzed the data set using LogDet to assess the effect of compositional bias

TABLE 3. Nucleotide composition of COI, COII, tRNA-Leu, 12S, and Wg sequences. The *P*-value is the probability of rejecting the null hypothesis of homogeneity of base composition among taxa.

Character partitions	Base frequency (%)					*P*-value
	A	C	G	T	A+T	
COI	31.2	15.5	14.4	39	70.2	<0.001
COI nt1	31.3	14.4	22.9	31.3	62.6	1
COI nt2	19.1	22.9	15.1	43	62.1	1
COI nt3	43.1	9.4	5.1	42.5	85.6	<0.001
tRNA-Leu	40.2	8.9	11.9	39.1	73.3	1
COII	37.1	14.2	10	38.7	75.8	<0.001
COII nt1	38	14.8	18	29.2	67.2	0.999
COII nt2	27.9	19.4	8.1	44.7	72.6	1
COII nt3	45.4	8.4	3.9	42.3	87.7	<0.001
12S	33.2	7.3	13.9	45.6	73.1	0.999
Mitochondrial	33.2	13.8	13.2	39.9	73.1	<0.001
Wg	22.1	28.1	32.4	17.4	39.5	1
Wg nt1	26.3	25.2	32.9	15.6	41.9	1
Wg nt2	31.5	14.4	29.5	24.6	56.1	1
Wg nt3	8.4	44.9	34.8	11.9	20.3	<0.001
Overall	31.6	15.8	15.9	36.7	68.3	<0.001

on the resulting phylogeny. The topology of neighbor-joining tree resulted from the LogDet analysis is largely in agreement with that of the parsimony and likelihood/Bayesian analyses (see below).

Parsimony Analyses

Maximum parsimony.—None of the individual gene trees (not shown) recovers a monophyletic Membracinae. No tree topologies are identical to one another or to that of combined data. Even though the tree topologies are different, no strongly supported nodes (bootstrap value >75%) are in conflict with nodes of the tree based on combined genes, except one node of the COII and five nodes of the 12S tree. Although alternative explanations, including nuclear copy of mitochondrial genes and contamination, cannot be excluded, the differences in these topologies are likely due to the stochastic noise from a small number of informative sites rather than conflict of phylogenetic signals in individual gene trees. The number of nodes with bootstrap value >50% increased as various gene combinations were added, indicating an increased resolving power of combined data.

A combined analysis of all the data using equal weights parsimony found five equally parsimonious trees (Fig. 3). Relationships in general were well resolved. This tree recovered the subfamily Membracinae and three of its four tribes with moderate to strong bootstrap and Bremer support. Most of the generic and species relationships are resolved and supported by high bootstrap and Bremer support values except among the Hypsoprorini. Relationships among outgroup subfamilies are reasonably well resolved but with weak branch support. Statistics of the strict consensus of the parsimony trees for five gene partitions and combined data are presented in Table 4. Despite the relatively high resolution (four parsimony trees and 107 resolved nodes), the COI gene is the most homoplasious (CI = 0.121, RI = 0.419) and least decisive (DD = 0.32). Wg alone does not provide much structure (>1000 parsimony trees and 62 resolved nodes), but together with tRNA-Leu, these two genes are the least homoplasious (CI = 0.255/0.255, RI = 0.607/0.617) and most decisive (DD = 0.56/0.55).

Partitioned Bremer support.—Partitioned Bremer support (PBS) values were used to examine the signal of individual gene partitions within the context of combined data (Table 4). PBS values were divided by the minimum possible number of steps for each partition to control for the difference in the size of each data partition. All gene partitions provide support for the parsimony topology of combined analysis except COII (negative score of summed PBS, Table 4). The standardized PBS value is highest in Wg, followed by tRNA-Leu, 12S, and COI. The value of nuclear Wg is nearly twice of that of remaining mitochondrial gene partitions. The relatively high PBS, data decisiveness, and less homoplasy of Wg indicate that this gene shows the greatest phylogenetic utility for higher level relationships in treehoppers.

Likelihood Analyses

Model selection.—As we expected, ln likelihood scores increase as additional parameters are added to the model. Applying the GTR model of variable rates of six nucleotide transformations and nonequal base frequencies greatly improved the likelihood scores among the four basic models. Accounting for rate heterogeneity using a gamma distribution (G) significantly improves the likelihood scores while the proportion of invariant site (I) and the site-specific rate (SSR) model provide little improvement on the scores. All LRT statistics are

TABLE 4. Summary of tree statistics for separate and combined parsimony analyses. CI = consistency index; RI = retention index; DD = data decisiveness; PBS = partitioned Bremer support. Resolved nodes = the number of nodes resolved in a strict consensus of parsimony trees in the data partition. Congruent nodes = the number of the resolved nodes that also appear in the topology of the combined analysis (Fig. 3). PBS values were summed across all the nodes on each of the data partitions and standardized by the minimum possible number of steps.

Partition	Characters				Trees					Resolved nodes	Congruent nodes	Summed PBS	PBS/min. steps
	Total	Constant	Varied	Informative	Number	Length	CI	RI	DD				
COI	1236	430	806	726	4	13974	0.121	0.419	0.32	107	59	992	0.59
tRNA-Leu	75	17	58	42	>1000	423	0.255	0.617	0.55	18	4	87	0.81
COII	517	109	408	371	72	6383	0.139	0.45	0.36	96	53	−39	−0.04
12S	407	62	345	293	57	4124	0.182	0.455	0.38	101	45	586	0.78
Wg	373	131	242	170	>1000	1724	0.255	0.607	0.56	62	39	599	1.36
Combined	2608	749	1859	1602	5	27211	0.142	0.437	0.35	107	—	2225	0.57

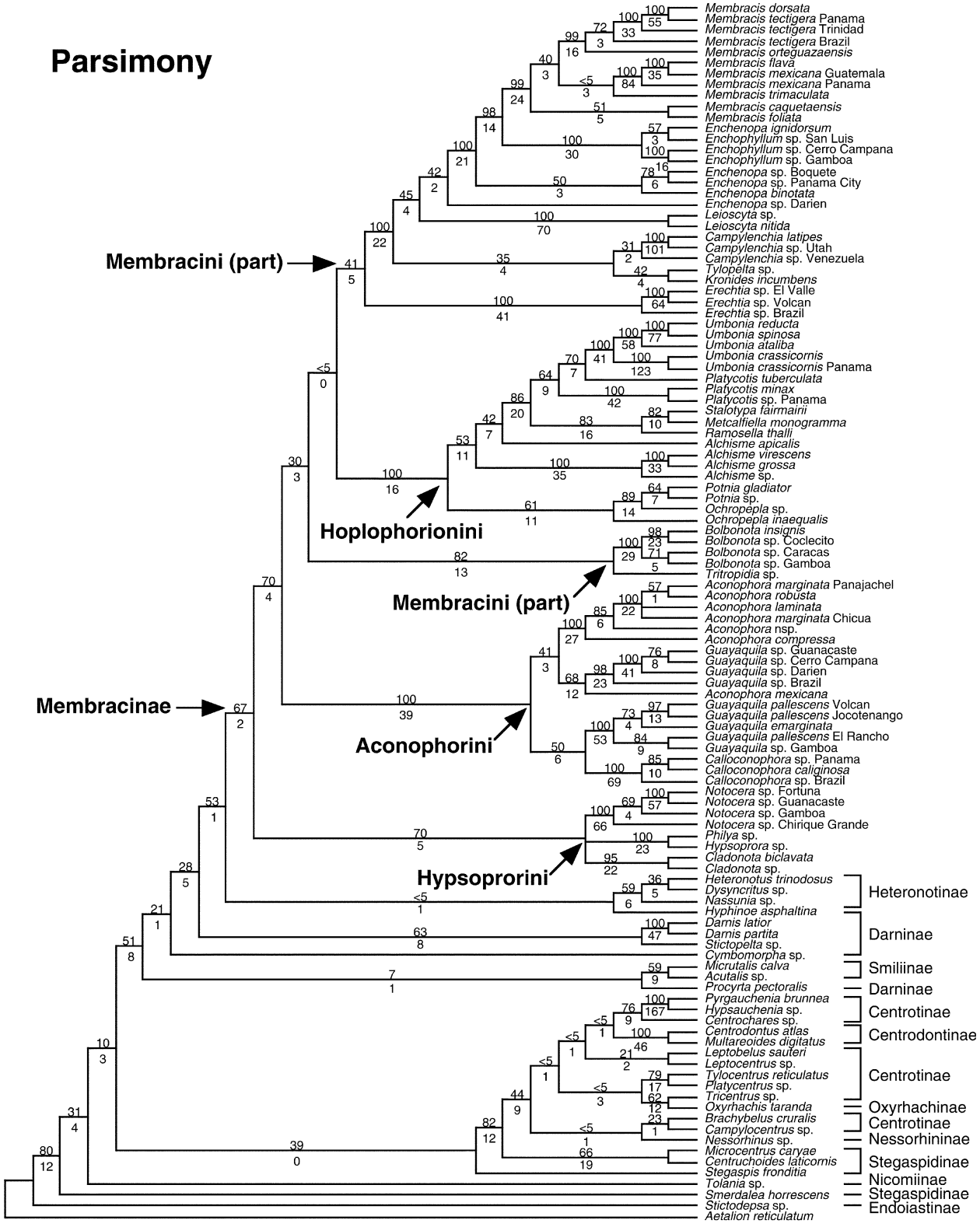

FIGURE 3. Strict consensus of five equally parsimony trees (tree length = 27211, CI = 0.142, RI = 0.437) based on combined analysis of equally weighted characters. Bootstrap and Bremer support values are shown above and below branches, respectively.

TABLE 5. Substitution model parameters estimated for various genes and character partitions using the GTR+I+G model on the equal weights parsimony topology. Transitions/Transversions (TS/TV) ratios are estimated using the HKY+I+G model.

		Substitutions						Rate parameters	
		Transitions		Transversions					
Partitions	TS/TV	C ↔ T	A ↔ G	A ↔ T	A ↔ C	C ↔ G	G ↔ T	*Pinv*	*α*
COI	1.766	19.465	6.987	4.325	1.37	2.752	1	0.33	0.733
COI nt1	1.749	15.555	3.971	3.553	2.173	0.212	1	0.406	0.902
COI nt2	1.331	4.893	6.1	2.245	1.555	5.559	1	0.545	0.612
COI nt3	14.986	6.016	5.433	0.032	0.014	0.241	1	0	0.901
COII	1.931	10.841	7.76	2.434	0.98	3.223	1	0.187	0.63
COII nt1	1.546	9.336	5.241	3.121	1.257	0.715	1	0.228	0.809
COII nt2	1.335	2.778	4.725	0.929	0.638	4.588	1	0.291	0.646
COII nt3	41.905	29.387	27.352	0.023	0.51	0.015	1	0	0.704
Wg	2.014	3.974	2.712	1.298	0.971	0.508	1	0.321	0.564
Wg nt1	1.182	3.759	4.695	3.933	3.165	0.508	1	0.172	0.358
Wg nt2	0.995	16.346	5.442	5.117	13.092	3.315	1	0.343	0.788
Wg nt3	2.269	3.223	4.973	2.873	0.954	0.18	1	0.03	2.131
All nt1	1.551	12.912	4.839	4.23	2.147	0.585	1	0.343	0.732
All nt2	1.258	5.631	5.255	2.251	1.918	4.77	1	0.47	0.572
All nt3	2.107	24.662	13.755	5.38	0.817	9,836	1	0.006	1.172
tRNA-Leu	1.382	583.358	556.253	285.992	314.191	455.183	1	0	0.222
12S	0.701	2.687	4.238	2.334	0.399	0.082	1	0.168	0.168
Combined	1.331	11.246	5.939	4.23	1.267	2.799	1	0.285	0.285

significant ($P < 0.01$) for nested models and the best GTR+I+G model was chosen for subsequent likelihood and Bayesian analyses. The selection of GTR+I+G model is appropriate because there is considerable heterogeneity in base frequencies and in nucleotide transformations among genes and codon partitions. Accounting for rate heterogeneity using a gamma distribution (G) and invariant sites (I) model is preferred over a site-specific rate (SSR) model. The SSR model, which assumes rate homogeneity within each rate class, is likely to underestimate the number of multiple substitutions within rate classes where among-site rate variation is extreme (Buckley et al., 2001). Applying substitution models separately for each data partitions can overcome this problem (see below, mixed-model Bayesian analyses).

Maximum likelihood.—One tree resulted from the likelihood analysis of the combined character matrix using GTR+I+G model after branch swapping with little improvement (1.2%) of −ln likelihood score from 105152.41 to 103846.26, indicating that the parsimony tree topologies are close to the likelihood tree after branch swapping. The estimated model parameters from the likelihood tree are within the 95% confidence intervals obtained from the Bayesian analysis, suggesting the convergence of the two analyses. Table 5 summarizes the parameter estimates obtained from the GTR+I+G analysis. The likelihood tree topology (not shown) is very similar to that of parsimony trees and Bayesian trees (see Fig. 5 for similar tree topology). It recovers many of the same tribal and generic relationships with robust bootstrap support. The tribe Membracini is paraphyletic with respect to Hoplophorionini, and contains two main lineages. The large lineage consists of *Campylenchia*, *Enchenopa*, *Enchophyllum*, *Kronides*, *Leioscyta*, *Membracis*, and *Tylopelta*. We refer to this group as the Membracini *sensu strictu*. The other lineage contains *Bolbonota*, *Erechtia*, and *Tritropidia*, and we refer to this group as the Bolbonotini of older classifications (Goding, 1926; Metcalf and Wade, 1965). The major difference between the topology of the parsimony and likelihood tree is the placement of the Bolbonotini relative to the Hoplophorionini and Membracini *sensu strictu* (Fig. 2c and d). The likelihood tree places the Bolbonotini sister to the Hoplophorionini, whereas in the parsimony trees the Bolbonotini is paraphyletic (*Bolbonota* + *Tritropidia* basal to the Hoplophorionini and *Erechtia* sister to the Membracini *sensu strictu*).

Relative rates among genes.—Likelihood parameter estimation reveals that the nucleotide substitution process is highly heterogeneous among various data partitions (Fig. 4). Nucleotide substitution rates of various sites estimated under the GTR+SSR model shows the substitution rates are the highest at the third position of COI

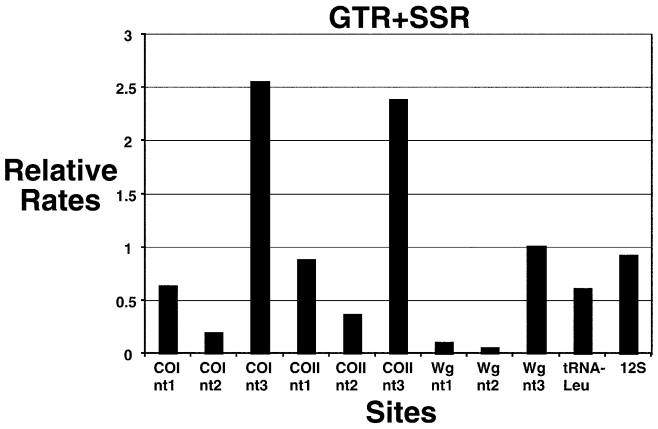

FIGURE 4. Site-specific rate estimation based on the GTR+SSR model and equal weights parsimony tree topology of the combined data (estimated relative rate: A ↔ C, 1.455; A ↔ G, 5.118; A ↔ T, 4.064; C ↔ G, 1.825; C ↔ T, 8.539; G ↔ T, 1).

and COII; intermediate at the first position of COI, COII, the third position of Wg, tRNA-Leu, and 12S; and the lowest at the first and second position of Wg. The first codon position of COII and the third position of Wg and 12S have similar overall rates of nucleotide substitutions. The third codon position of COI with the greatest rate of substitution (2.56) is about 40 times faster than the lowest rate in the second position of Wg (0.06).

Bayesian Analyses

A posterior probability distribution containing 20,000 sampled trees was obtained in the Bayesian analysis for 2×10^6 generations based on the GTR+I+G model. Five independent Bayesian analyses converged on similar likelihood scores and reached stability no later than 5×10^5 generations. The first 10,000 trees of each run were discarded and a majority rule consensus tree was constructed using the pooled 50,000 trees from five analyses. The Bayesian tree is well resolved and contains a majority (80%) of ingroup (Membracinae) nodes with posterior probability >95%. The tree topologies of the Bayesian and likelihood analysis are identical except in one node, the placement of *Campylenchia* + *Kronides* + *Tylopelta* relative to *Enchenopa* sp. Darien + *Leioscyta*. Applying mixed-model Bayesian analyses resulted in an improved likelihood score (likelihood values increased from to −103895 to −100607). This tree (Fig. 5) is well supported and similar to the analyses based on GTR+I+G model with the exception of one node within the Hypsoprorini. Using trees based on GTR+I+G model (not shown) does not change the phylogenetic conclusions or the interpretation of character evolution.

Ancestral States and Character Evolution

The minimum number of transitions of egg-guarding behavior on the phylogeny was eight (4 within the Membracinae) under parsimony. Using 1000 random tree topologies for these 112 taxa, we estimated that on average 37.2 ± 2.9 transitions in egg-guarding behavior with a minimum of 29 steps. Our observed number (eight) is significantly less ($P < 0.001$) than the null distribution obtained from 1000 random trees. Similar result was obtained by holding the tree topology constant and randomizing distribution of this character among taxa 1000 times. The number of transitions for 1000 randomized egg-guarding distributions averaged 37.2 ± 2.6, with a minimum of 29 steps. Randomization tests of correlation between egg guarding and phylogeny were all significant ($P < 0.001$), suggesting that the evolutionary transitions (gains and losses) of this trait is phylogenetically conservative and this trait is highly correlated with phylogeny. Figure 6 shows that the presence of egg guarding within the Membracinae is restricted to three lineages, Aconophorini, Hoplophorionini + Bolbonotini, and *Leioscyta*. Egg-guarding appears to be a derived (apomorphic) trait for the Membracinae and the common ancestor of the subfamily appears to have had no egg-guarding. There is one possible loss of egg-guarding in *Bolbonota* of the Bolbonotini. However, the character optimization of two ancestral nodes within the subfamily (Fig. 6, node 1 and 2) cannot be unambiguously resolved under parsimony. The interpretation of egg guarding with two origins (Fig. 6, node 1 and *Leioscyta*) and one reversal (Fig. 6, Membracini *sensu strictu*) is equally parsimonious with that of three origins (Fig. 6, node 3, Aconophorini, and *Leioscyta*).

Under the assumption of equal branch lengths, maximum likelihood method estimated the rate of gains to be higher (1.8 to 1; $P = 0.49$) than the rate of losses. Rate estimates using models with Bayesian branch lengths substantially increased the relative difference of rates between gains and losses (3600 to 1; $P < 0.02$). The overall higher rate of gains than that of the losses corroborates the result from the parsimony character mapping and suggests that the egg-guarding in membracines is easily gained than lost. Maximum likelihood reconstruction of ancestral states assuming equal branch lengths was similar to the parsimony reconstruction (data not shown). The result strongly supports ($P < 0.01$) all ancestral states reconstructed by parsimony with the exception of two internal nodes being moderately supported (node 3 and Membracini *s. s.* in Fig. 6; likelihood ratio = 6.3). When branch lengths were set to the values derived from Bayesian analyses, maximum likelihood significantly favored ancestral states of parsimony reconstruction with the exception of four internal nodes having moderate support (Fig. 7, node 3, 4, 5, and Bolbonotini; likelihood ratio ranging from 1.1 to 6.6). Similar to parsimony reconstruction, two nodes deep in the tree were ambiguous with no support using maximum likelihood (Fig. 7, node 1 and 2). Overall, maximum likelihood reconstructed the same ancestral states found in the parsimony analysis and revealed moderate degree of uncertainty in ancestral estimates of four internal nodes.

DISCUSSION

Phylogenetic Utility of the Genes

Analyses of combined mitochondrial COI, tRNA-Leu, COII, 12S, and nuclear Wg genes provided overall reasonably good resolution, with moderate to strong support for the tribal and generic relationships within the Membracinae. In contrast, the analyses of separated gene partitions rarely recovered higher taxonomic groups (tribes or subfamilies), suggesting the limited phylogenetic utility of individual genes for the tribal relationships of Membracinae. The short length of individual gene fragments (small number of informative sites) may be the limiting factor for their performance as suggested by the low number of nodes with bootstrap >50 %. For insect molecular systematics, nuclear protein-coding genes have been shown to exhibit less homoplasy and greater support than do mitochondrial genes at higher taxonomic levels or deeper divergences (e.g., Reed and Sperling, 1999; Baker et al., 2001; Johnson et al., 2002; Leys et al., 2002; Morris et al., 2002; Danforth et al., 2003; but see Monteiro and Pierce, 2001; Kjer et al., 2001). Whereas mitochondrial genes in general are more useful for resolving closely related taxa that have diverged recently

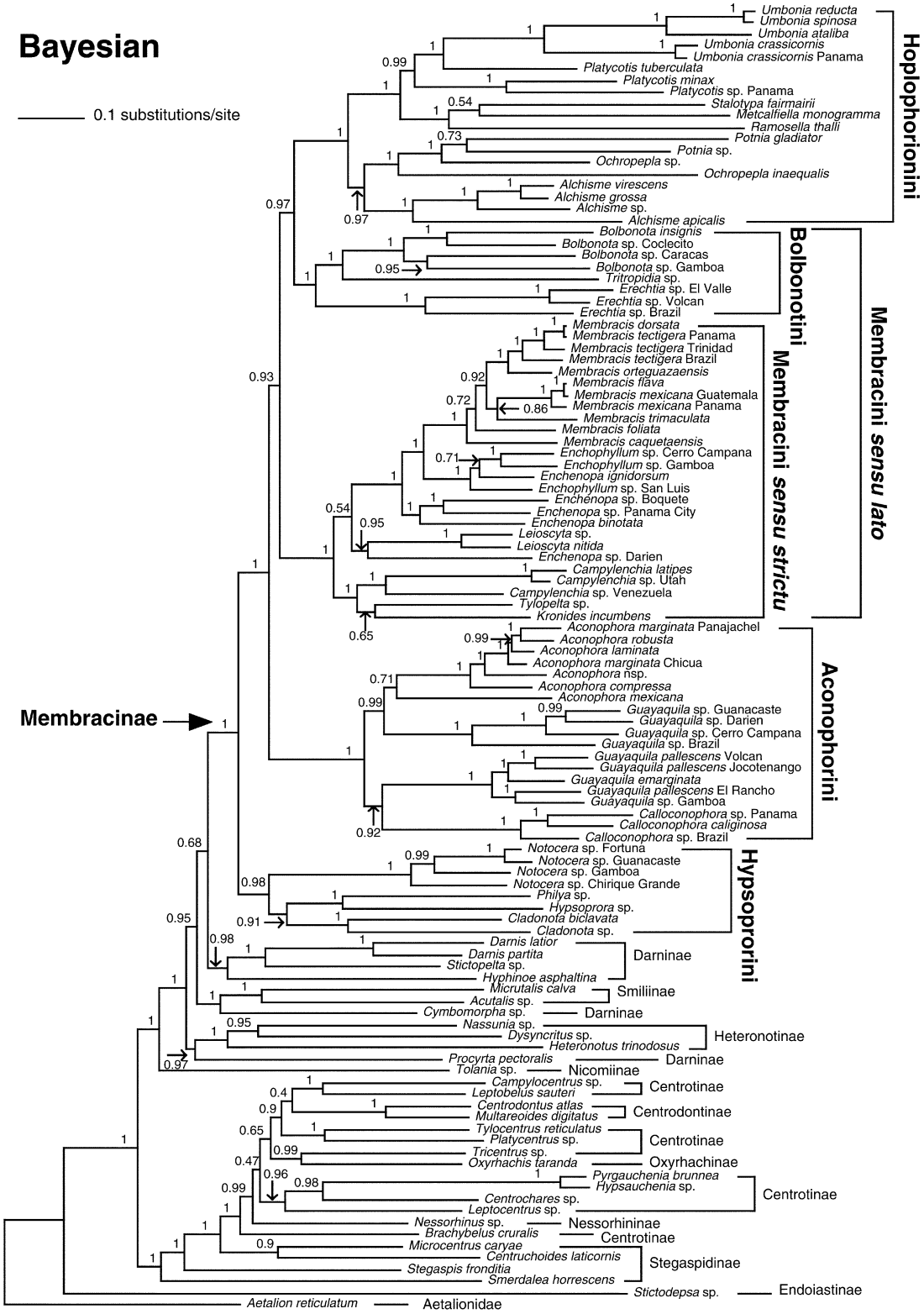

FIGURE 5. The 50% majority rule consensus tree from the Bayesian analyses of combined data based on mixed models (TreeBase accession M1727). Numbers above the branches are posterior probability values of the nodes (e.g., 1 = 100%, 0.95 = 95%, etc.). Branch lengths are optimized using estimated mean parameter values and drawn proportional to character changes as indicated by the scale bars. This tree topology is identical to that of likelihood and Bayesian analyses based on the GTR+I+G model except for one node (see text for discussion).

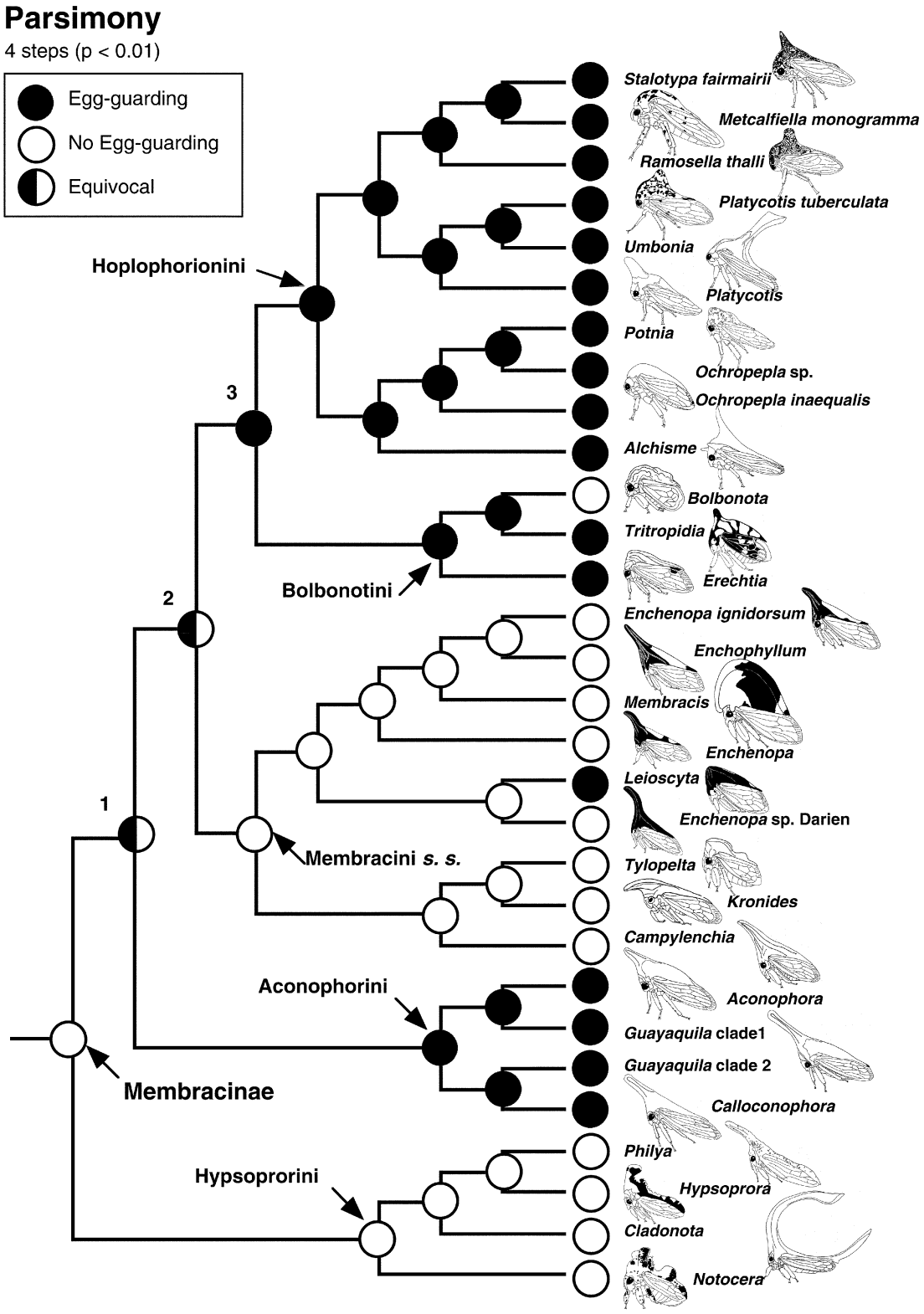

FIGURE 6. Parsimony reconstructions of ancestral states of egg-guarding in the Membracinae. Characters are optimized on the species tree derived from the Bayesian analysis of mixed models (Fig. 5). All branch lengths are equal. The ancestral state of the Membracinae is estimated to have no egg-guarding using outgroups. The tree topology is summarized to show the generic relationships of the Membracinae (relationships of outgroup subfamilies not shown), with tips representing genera or species (in cases of nonmonophyletic genera).

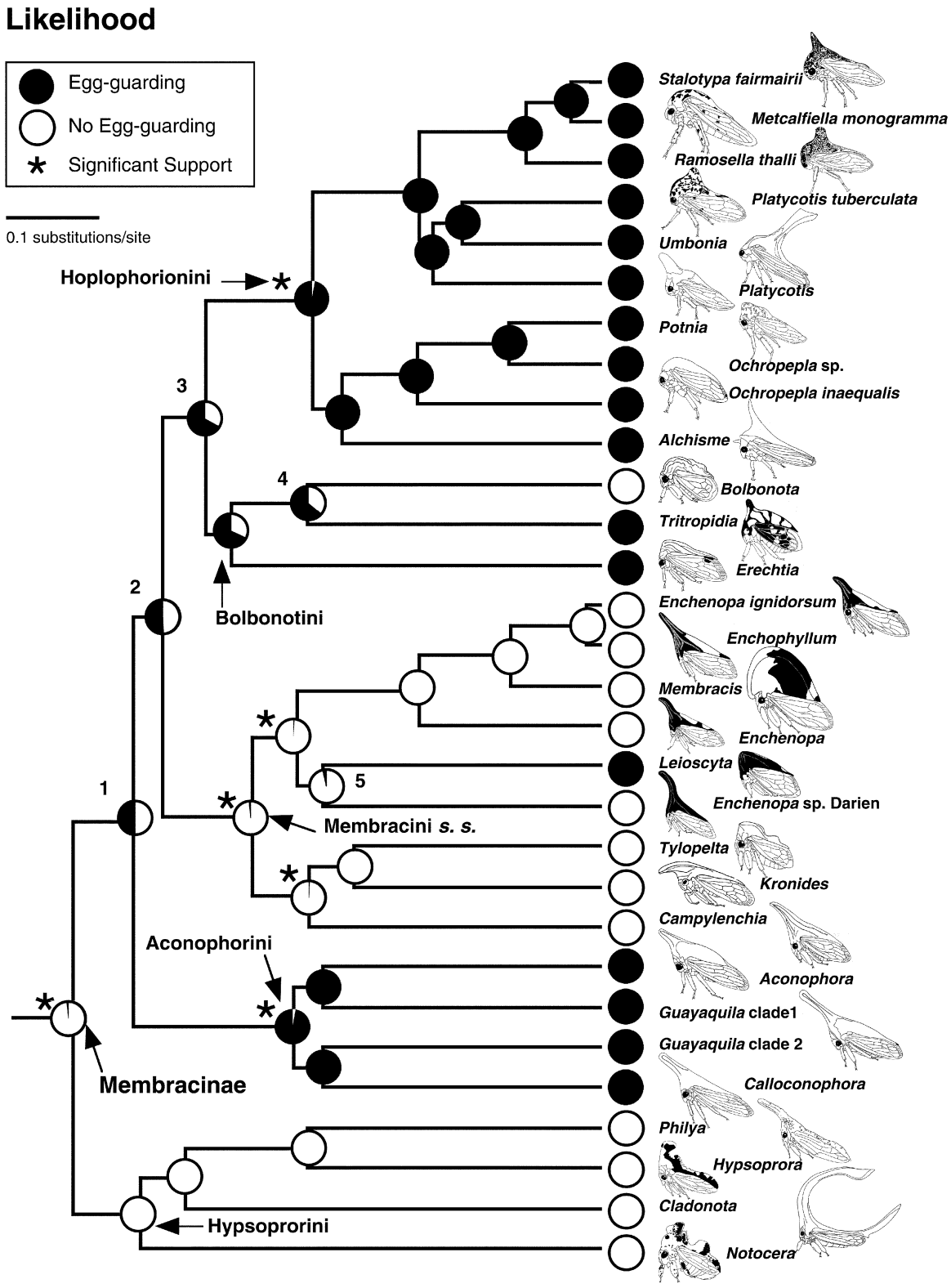

FIGURE 7. Ancestral states of egg-guarding in the Membracinae, reconstructed by maximum likelihood based on one-rate model (forcing rates of gains and losses to be equal; rate = 0.36) and the Bayesian tree. Pie diagrams indicate the relative degree of support for alternative character states, with a likelihood ratio of 7.4:1 or greater considered significant. Branch lengths were estimated based on the Bayesian analysis of mixed models (Fig. 5).

(reviewed in Simon et al., 1994; Avise, 2000; Caterino et al., 2000). Our analysis of mitochondrial and nuclear genes in Membracinae supports the notion that the nuclear Wg gene shows higher consistency index (CI), data decisiveness (DD), and partitioned Bremer support (PBS) than any of the mitochondrial genes analyzed. The nuclear Wg gene also shows more homogeneous patterns of among-site rate variation (higher values of α) and more symmetrical transformation rate matrices than mitochondrial genes. These characteristics together may explain the overall poor performance of mitochondrial genes for resolving higher level relationships when compared to nuclear genes in the same analysis (Lin and Danforth, 2004).

Congruence of Phylogenetic Methods

The concordance in tree topology resulting from different phylogeny reconstruction methods is reassuring to support for a particular clade. Our results based on parsimony, maximum likelihood, and Bayesian analyses are largely congruent. No conflicting nodes with strong support (bootstrap support, BP > 75%; posterior probability, $Pr > 95\%$) were observed between trees from the parsimony bootstrapping and Bayesian analysis. Bayesian analysis recovers many clades shared with that of parsimony and has an identical tree topology to the likelihood tree except for the rearrangement of one node. In addition, the Bayesian analysis frequently (at least 8 nodes within Membracinae) provides significant posterior probability ($Pr > 95\%$) for clades with weak support in parsimony analyses (BP < 75%). The Bayesian tree (Fig. 5) is used as the working phylogenetic hypothesis for the subfamily.

Membracinae Phylogeny and Taxonomic Implications

Our results provide the best estimate of the phylogenetic relationships among the tribes and genera within the predominantly subsocial lineages of the Membracinae. No previous phylogenetic studies (Dietrich and Deitz, 1993; Dietrich and McKamey, 1995; Cryan et al., 2000, in press; Dietrich et al., 2001) have included as many species and genera. However, we are still far from a complete understanding of Membracinae phylogeny because several small subsocial and asocial genera such as *Stirpis* (Hoplophorionini) and *Jibarita* (Hypsoprorini) are not available for the analysis. Nevertheless, our results recover many traditionally recognized taxa and in addition provide a few novel relationships among tribes and genera that have important implications for further taxonomic and phylogenetic studies of membracine treehoppers.

Monophyly of subfamily.—Maximum parsimony, maximum likelihood, and Bayesian methods all support the monophyly of the subfamily Membracinae (BP/BS/$Pr = 67\%/2/100\%$). This result is consistent with morphology (Dietrich et al., 2001), nuclear EF-1α, and 28S genes (Cryan et al., 2000), and mitochondrial genes with extensive taxon sampling across subfamilies and tribes of the Membracidae (Wood et al., in preparation). Despite the relatively less resolved relationships among outgroup subfamilies (BP < 75%, $Pr < 95\%$), our analysis suggest that the Darninae, Heteronotinae, and Smiliinae are paraphyletic lineages and basal to the Membracinae (BP/BS/$Pr = 51\%/8/100\%$), with a lineage of the Darninae consisting of *Darnis*, *Hyphinoe* and *Stictopelta* being sister to the Membracinae (Fig. 5). The subfamily Heteronotinae is monophyletic (BP/BS/$Pr = 59\%/6/100\%$) whereas the Darninae is paraphyletic with respect to the Heteronotinae and Smiliinae suggesting taxonomic revision of this subfamily is needed. This overall basal position of these three subfamilies with respect to the Membracinae is in concordance with morphological and nuclear DNA analyses. However, complete resolution of the relationships among these three subfamilies is beyond the scope of this study and requires future research.

Monophyly of tribes.—Our results are largely concordant with the morphological study and higher classifications of the Membracinae by Deitz (1975) and McKamey (1998). Three of their four recognized tribes are recovered but we found that the Hoplophorionini arises from within the Membracini ($Pr = 97\%$) rendering the Membracini paraphyletic (Fig. 5). Within the Membracinae, Aconophorini and Hoplophorionini are each monophyletic with strong support (BP/BS/$Pr = 100\%/39/100\%$ and $100\%/16/100\%$). The Hypsoprorini is monophyletic (BP/BS/$Pr = 70\%/5/98\%$) as suggested by two morphological synapomorphies (Dietrich and McKamey, 1995). Our analysis and morphological studies (Dietrich and McKamey, 1995) both suggest that the Membracini is not monophyletic. But our analysis indicates the tribe contains only two clades rather than nine, as suggested by morphology. The Membracini consists of two major lineages, the Bolbonotini ($Pr = 100\%$) and Membracini *sensu strictu* (BP/BS/$Pr = 100\%/22/100\%$) (Fig. 5). This result is concordant with the earlier taxonomic study (Goding, 1926) and the recognition of the unique character combinations (short ovipositor and slightly notched sternum II) that distinguish *Bolbonota*, *Paragara*, and *Tritropidia* from all other membracine genera (Deitz, 1975). The phylogenetic placement of the Bolbonotini suggested here implies this lineage and closely related genera such as *Bolbonotodes*, *Eunusa*, and *Paragara* may form a clade. Here we resurrect the tribe Bolbonotini containing *Bolbonota*, *Bolbonotodes*, *Erechtia*, *Paragara*, and *Tritropidia*. However, the monophyly of the Bolbonotini should be tested with more extensive taxon sampling especially for genera not included in the present study.

Tribal relationships.—The Hypsoprorini is a basal lineage within the subfamily (BP/BS/$Pr = 70\%/4/100\%$) (Fig. 5). Contrary to previous phylogenetic studies, our analysis places the Aconophorini sister to the Hoplophorionini + Membracini *sensu lato* (BP/BS/$Pr = 30\%/3/93\%$). The Aconophorini has been regarded as either derived from within the Membracini (Dietrich and McKamey, 1995; Dietrich et al., 2001, Fig. 2a) or as sister to the Membracini (Cryan et al., 2000, Fig. 2b). This discordance likely resulted from the limited taxon sampling in the previous studies, in which tree topologies are less stable and likely to change with the addition of major

lineages such as the Bolbonotini. The resurrected Bolbonotini is sister to the Hoplophorionini ($Pr = 97\%$) and represents a morphologically and behaviorally transitional lineage between the Membracini and Hoplophorionini. This phylogenetic hypothesis is novel and largely congruent with the shared reduction in body size in the basal lineages, *Ochropepla* and *Potnia* of the Hoplophorionini and in the Bolbonotini.

Evolution of Maternal Care in Membracine Treehoppers

The evolution of subsocial behavior or parental care in insects has received much attention (Wilson, 1971; Eickwort, 1981; Tallamy and Wood, 1986; Tallamy and Schaefer, 1997). Ecological factors such as physically stressful environments, rich but ephemeral resources, and predation have been hypothesized to promote subsociality including maternal care (Wilson, 1971). However, few attempts have been made to analyze in detail the phylogenetic history of maternal care in insects (but see Tallamy and Schaefer, 1997). Our results with regard to the evolution of maternal care in membracine treehoppers are clear. Given the widespread occurrence of maternal care, we infer equal to or fewer than three origins, two reversals, and only one possible loss. The ancestral state of the Membracinae is estimated to have no maternal care based on both parsimony and maximum likelihood reconstruction.

Examination of the cladograms in detail suggests that the ancestors of membracine treehoppers are likely to resemble the species in the basal Hypsoprorini and closely related Darninae that are solitary or in aggregations of few individuals sporadically attended by honeydew-harvest ants. Like other honeydew-producing insects, treehoppers are frequently tended by ants and they benefit from this mutual interaction through increased survival (Wood, 1977; McEvoy, 1979; Fritz, 1982). Maternal care has evolved later in the evolutionary history of membracine treehoppers and occurs in the more apical lineages. Within the Membracinae, maternal care is a derived (apomorphic) trait and the occurrence of maternal care is mainly restricted to two lineages, Aconophorini and Bolbonotini + Hoplophorionini. Maternal care first appeared in the more basal Aconophorini. The aconophorine female guards their eggs and frequently associated with mutualist ants. The next basal clade, Membracini *sensu strictu*, contains treehoppers without maternal care but in which species form adult and nymphal aggregations frequently attended by ants. Nevertheless, within this clade maternal care has evolved at least once in the genus *Leioscyta*.

Moving away from the base of the cladogram, maternal care has evolved again in the more apical lineage, Bolbonotini + Hoplophorionini. The Bolbonotini contains a mixture of subsocial and gregarious species without maternal care. Within this clade, there is a possible loss of maternal care in *Bolbonota*. However, this interpretation should be treated with moderate degree of uncertainty as suggested by maximum likelihood estimates of ancestral states in the Bolbonotini. The loss of maternal care in this lineage is interesting because it may have been associated with the development of ant mutualism. *Bolbonota* occurs mainly in tropical lowland forests where ants are most diverse and abundant. Protection against predators with ant mutualism may have evolutionarily substituted the ecological role of maternal care in this lineage (Lin, 2003). However, members within the same lineage, the hoplophorionine female does not interact with ants but extends egg-guarding behavior to actively defend offspring until they reach the adult stage. The evolution of highly developed maternal care with nymphal guarding and aggressive antipredator behavior in the Hoplophorionini may have been associated with moving into higher elevations where ants are less diverse and abundant (Wood, 1984). Most hoplophorionine treehoppers occur in montane and submontane forests at higher elevations and latitudes (McKamey and Deitz, 1996). At higher elevations with less abundance of ants may have placed more selection pressure on these females to remain with offspring and develop behavior to protect them (Wood, 1984).

In summary, the reconstructed pattern of maternal care evolution in Membracinae deviates significantly from a random distribution of maternal care on the tree, suggesting that there is a strong phylogenetic/historical component to the evolution of maternal care. The phylogenetic conservatism of maternal care evolution suggests that this trait is highly heritable among membracine treehopper lineages and/or closely related subsocial taxa with similar life history and ecology may have been shaped by environment in a similar way. Overall, the results of this study confirm the notion that maternal care arose independently many times throughout various insect lineages and that it rarely reverses to no maternal care (Wilson, 1971; Eickwort, 1981; Tallamy and Wood, 1986).

Our findings provide new insights on the evolution of maternal care and further suggest that the evolution of subsocial behavior in insects in general may not be as evolutionarily labile as previous thought. The evolution of maternal care in insects, especially among hemipterans (true bugs), was considered to be evolutionarily labile because it requires no adaptive change in morphology and only slightly modification in behavior (reviewed in Tallamy and Schaefer, 1997). Females of egg-guarding treehoppers simply need to remain on or near their eggs for some time following oviposition. Therefore, the maternal care behavior of insects should have been gained and lost frequently throughout its evolutionary history. However, our phylogenetic results indicate that maternal care in membracine treehoppers is not evolutionarily labile but rather difficult to evolve (i.e., few origins) and relatively difficult to lose once evolved (i.e., low numbers of reversals and losses). This makes sense in light of different life histories between treehoppers with and without maternal care. Compared to solitary or gregarious treehoppers, the evolution of maternal care requires the addition or modification of several life history and ecological attributes in addition to change in egg-guarding behavior.

First, females of many subsocial treehoppers have semelparous reproduction and need to deposit their entire clutch into a short period (usually within 24 h or 2 to 3 days) and a small space (a plant stem) to be able to guard their offspring (Wood, 1984, 1993). Secondly, after oviposition, females need to stay and guard their eggs for a prolonged period of time (days or months) until they hatch or reach the adult stage. Moreover, in some subsocial treehoppers such as species in the Hoplophorionini, females need to develop aggressive behaviors including wing-fanning and leg-kicking to defend their offspring in response to the approach of arthropod predators (Wood, 1974, 1976; McKamey and Deitz, 1996). Finally, subsocial treehoppers need to develop communication systems using pheromones (Nault et al., 1974) and/or plant-borne vibrational signals (Cocroft, 1999, 2001) among siblings and between females and offspring to maintain the integrity of nymphal aggregations and to evoke antipredator response of females. Ant mutualism is also important in determining the duration of egg-guarding behavior in some subsocial treehoppers such as *Entylia*, *Publilia*, and *Guayaquila* (Wood, 1977; McEvoy, 1979; Zink, 2002). Ant mutualism may have historically correlated with the evolutionary development of maternal care in treehoppers (Lin, 2003). Overall, like other life history traits, maternal care in the form of egg guarding should be considered as a complex life history syndrome involving many correlated behavioral, reproductive, and ecological characteristics. In addition to phylogenetic conservatism, the necessity of evolutionary development of associated behavioral, life history, and ecological features may be what explains the limited number of origins and reversals of maternal care in these treehoppers.

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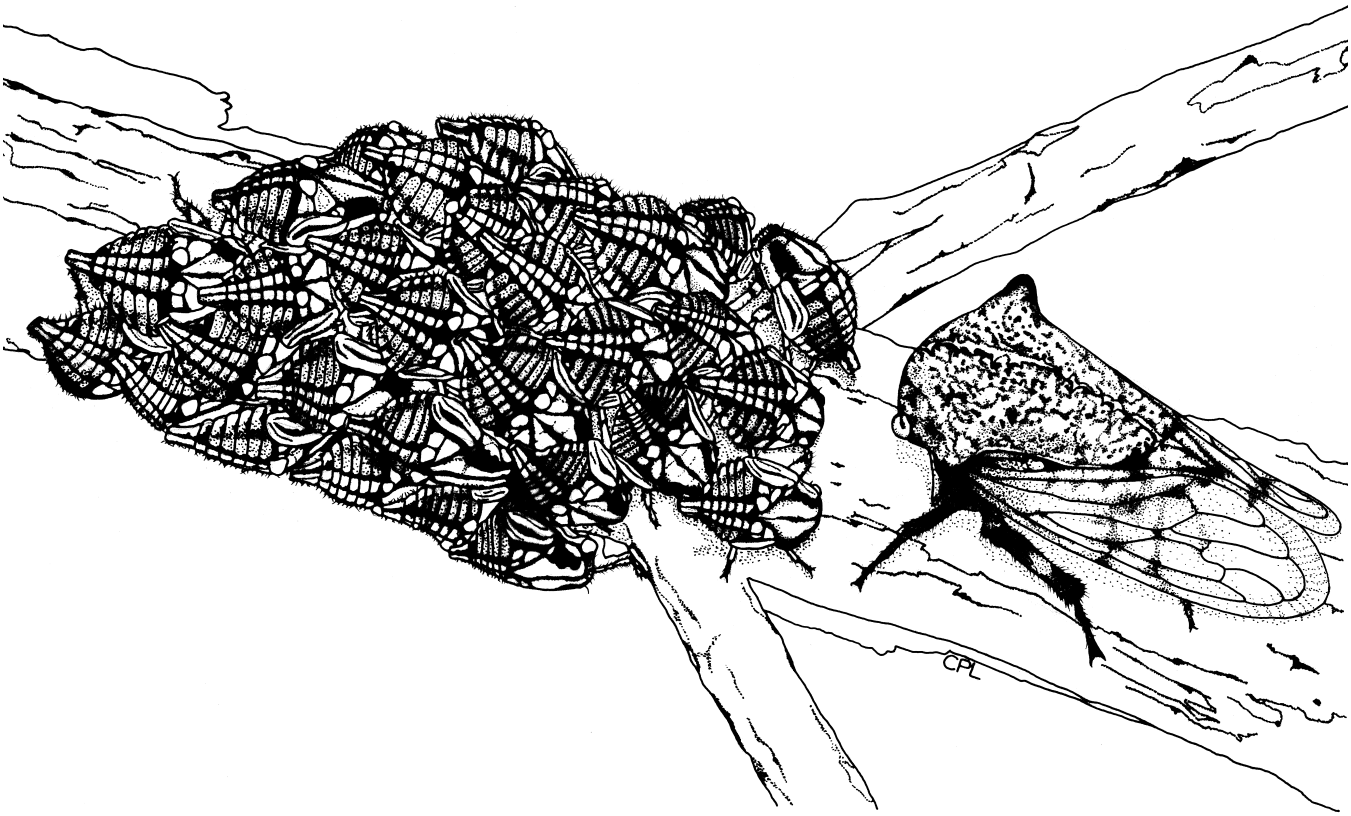

A female treehopper guarding an aggregation of her offspring (*Metcalfiella nigrihumera* McKamey). This hoplophorionine treehopper is endemic to the Andes of Ecuador and Peru. Drawing by Chung-Ping Lin.