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Molecular Phylogeny of the Homoptera: A Paraphyletic Taxon

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Abstract. Homoptera and Heteroptera comprise a large insect assemblage, the Hemiptera. Many of the plant sap-sucking Homoptera possess unusual and complex life histories and depend on maternally inherited, intracellular bacteria to supplement their nutritionally deficient diets. Presumably in connection with their diet and lifestyles, the morphology of many Homoptera has become greatly reduced, leading to major controversies regarding the phylogenetic affiliations of homopteran superfamilies. The most fundamental question concerns whether the Homoptera as a whole are monophyletic. Recent studies based on morphology have argued that the Homoptera Sternorrhyncha (Aphidoidea, Coccoidea, Psylloidea, Aleyrodoidea) is a sister group to a group comprising the Homoptera Auchenorrhyncha (Fulgoroidea, Cicadoidea, Cercopoidea, Cicadelloidea) and the Heteroptera, making the Homoptera paraphyletic. We sequenced the 5′ 580–680 base pairs of small-subunit (18S) ribosomal DNA from a selection of Homoptera, Hemiptera, and their putative outgroups, the Thysanoptera and Psocoptera, to apply molecular characters to the problem of Homoptera phylogeny. Parsimony, distance, maximum-likelihood, and bootstrap methods were used to construct trees from sequence data and assess support for the topologies produced. Molecular data corroborate current views of relationships within the Sternorrhyncha and Auchenorrhyncha based on morphology and strongly support the hypothesis of homopteran paraphyly as stated above. In addition, it was found that Homoptera Sternorrhyncha have extra, GC-rich sequence concentrated in a variable region of the 18S rDNA, which indicates that some unique evolutionary processes are occurring in this lineage.

Key words: Homoptera — Heteroptera — Hemiptera — Paraphyletic taxon — Phylogeny, 18S rDNA

Introduction

Homoptera and Heteroptera comprise the largest nonholometabolan insect assemblage, the Hemiptera (Kristensen 1991; Carpenter 1992). The distinctive piercing-and-sucking mouthparts of Hemiptera may have played an adaptive role in their extensive evolutionary radiation (Goodchild 1966). High reproductive rates and an exceptional ability to transmit diseases make homopterans some of the worst pests known to agriculture. They attract biologists, however, by their unusual life histories, behaviors, and interspecific interactions. Members of the Sternorrhyncha (Aphidoidea, Coccoidea, Aleyrodoidea, Psylloidea), in particular, may follow complex, multiple-host life cycles incorporating parthenogenesis, polymorphism, and gall formation (Nur 1971; Hodkinson 1974; Miller and Kosztarab 1979; Byrne and Bellows 1991; Moran 1992). Members of the Auchenorrhyncha (Cicadoidea, Cicadelloidea, Cercopoidea, Fulgoroidea) are known for their auditory signaling and elaborate substrate mimicry (Ossiannilsson 1981; Nault and Rodriguez 1985). In connection with their specialized mouthparts, essentially all Hemiptera use only liquid food. Whereas heteropteran species use a variety of types of food, including vertebrate blood, invertebrate prey, and plant fluids, homopteran species are restricted to plant

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fluids, with most using the phloem or xylem saps of vascular plants (MacGavin 1993). Presumably in connection with these nutritionally deficient diets, most Homoptera harbor intracellular, apparently mutualistic bacteria that are transmitted maternally (Buchner 1965). In the case of aphids, bacterial and host phylogenies have matching topologies, indicating a long history of cospeciation following a single, ancient infection (Munson et al. 1991).

Reconstructing the phylogeny of Homoptera has proven less tractable than studying their biology, and the history of the former has been contentious. As a consequence of their feeding mode, homopterans generally have evolved sedentary lifestyles, which in turn have promoted morphological simplification (most extreme in the Sternorrhyncha). Reductions and losses of structures have made it difficult to define shared derived traits, and are themselves problematic as synapomorphies because of the possibility that they arose through convergence. While the monophylies of homopteran superfamilies are well established on the basis of morphology, almost every possible arrangement of relationships among superfamilies has been proposed (Kramer 1950; Hennig 1981). Most studies, however, portray both the Sternorrhyncha and the Auchenorrhyncha as monophyletic (Szelegiewicz 1971; Kristensen 1973; Hennig 1981; Carver et al. 1991), but some schemes impose paraphyly on the Auchenorrhyncha (Ross 1965; Goodchild 1966; Hamilton 1981). The most widely accepted hypotheses of Sternorrhyncha phylogeny recognize a monophyletic group consisting of Aphidoidea + Coccoidea and Psylloidea + Aleyrodoidea as sister groups (Theron 1958; Goodchild 1966; Schlee 1969; Hennig 1981; Carver et al. 1991). The consensus of Auchenorrhyncha relationships recognizes four major groups: Cicadelloidea, Cercopoidea, Cicadoidea, and Fulgoroidea. The Cicadelloidea + Cercopoidea are hypothesized to be a sister group to the Cicadoidea, with the Fulgoroidea presumed to be the most primitive auchenorrhynchan lineage (Evans 1946, 1963; Kramer 1950; Hennig 1981; Hamilton 1981; Carver et al. 1991).

A more fundamental question about homopteran phylogeny concerns the monophyly of the taxon as a whole. Early taxonomists (e.g., Poisson and Pesson 1951) considered Homoptera and Heteroptera to be either separate orders or equivalently ranked suborders of the order Hemiptera. Three distinct lineages, Sternorrhyncha, Auchenorrhyncha, and Heteroptera, are usually accepted (Hennig 1981; Kristensen 1973), but whether the former two constitute a monophyletic group has been debated. Features that distinguish the Homoptera from the Heteroptera are essentially primitive (Boudreaux 1979; Hennig 1981). Boudreaux (1979) and Hamilton (1981) claim that several synapomorphies justify the monophyly of Homoptera; these are: enlarged foramen magnum, large loral sutures defining the mandibular plate, characteristic folding of the hindwing, larger forewing than hindwing, reduced tarsomeres, and reduced simple sperm. Most authors, however, maintain either that it is not possible to determine the relationships between Sternorrhyncha, Auchenorrhyncha, and Heteroptera (Szelegiewicz 1971; Kristensen 1973, 1983; Scudder 1973; Hennig 1981) or that sufficient synapomorphies exist (wing, alimentary, mandibular, salivary, and embryological features) to unite the Heteroptera with the Auchenorrhyncha (Goodchild 1966; Cobben 1978; Wootten and Betts 1986; Carver et al. 1991; Kukalová-Peck 1991). Figure 1 summarizes the current consensus of Homoptera relationships based on morphology.

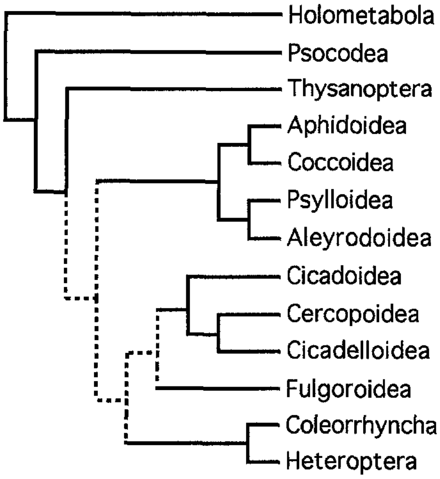

Fig. 1. Consensus of the most recent views of Hemiptera phylogeny and outgroup relationships based on morphology. *Dotted lines* represent relationships that have been debated. The Coleorrhyncha (a group confined to the Southern Hemisphere) was not included in this study.

Here we present a molecular phylogenetic analysis of partial 18S ribosomal DNA sequences from Homoptera representing all eight superfamilies, as well as representatives of one derived and three primitive heteropteran families. We also included representatives of the Thysanoptera and Psocoptera as outgroups to the Hemiptera (Ross 1955; Boudreaux 1979; Hennig 1981; Lyal 1985; Kristensen 1991) (Fig. 1). In addition, we used published sequences to test the utility of holometabolous insects (the putative sister group to the Paraneoptera [Kristensen 1991]) as more distant outgroups. The purposes of our study were to determine whether molecular characters supported currently accepted relationships within the Sternorrhyncha and Auchenorrhyncha based on morphology and whether they also supported the hypothesis of Homoptera paraphyly.

Materials and Methods

We determined approximately 580–680 base pairs from the 5′ end of the 18S rDNA molecule for a total of 27 species (Fig. 2). This region of 18S spanned the universal helices 1–18 as proposed for the secondary structure of small-subunit ribosomal RNAs and included the variable regions V1, V2, and V3 (Nelles et al. 1984).

Higher Taxon	Species	Collection Information and Preservation method	DNA extraction method	GenBank Accession
HOMOPTERA Sternorrhyncha				
Aphidoidea (Aphids)				
Aphididae	*Schlechtendalia chinensis* (Bell)	1		U20411
Adelgidae	*Adelges* sp.	Aug 1992, Stanley ID (live)	Liq. N, Phenol	U20400
Phylloxeridae	*Phylloxera notabilis* Pergande	June 1993, Gonzales TX (live) 2	Phenol	U20398
Coccoidea (Scales)				
Pseudococcidae	*Pseudococcus longispinus* (Targioni-Tozzetti)	1	/	U20399
	Dysmicoccus neobrevipes (Beardsley)	1	/	U20429
Dactylopiidae	*Dactylopius confusus* (Cockerell)	July 1990, Tucson AZ (-70°) 3	Liq. N, Phenol	U20402
Psylloidea (Psyllids)				
Triozidae	*Paratrioza cockerelli* (Sulc) 4	May 1992, Tucson AZ (-70°)	Liq. N, Phenol	U20416
Psyllidae	*Pachypsylla venusta* (Osten-Sacken) 4	April 1993, Tucson AZ (live)	Liq. N, Phenol	U20409
Aleyrodoidea (Whiteflies)				
Aleyrodidae	*Bemisia tabaci* (Gennadius)	1	/	U20401
	Aleurodicus dugesii 5	March 1994, Guatemala (EtOH)	Abbreviated	U20412
HOMOPTERA Auchenorrhyncha				
Cicadelloidea (Hoppers)				
Membracidae	*Philya ferruginosa* (Goding) 6	April 1993, Continental AZ (-70°)	Liq. N, Phenol	U20405
	Platycotis vittata (F.) 6	April 1993, Catalina Mts AZ (-70°)	Liq. N, Phenol	U20410
Cicadellidae	*Erythroneura variabilis* Beamer	Oct 1989, Tucson AZ, from culture (-70°)	Liq. N, Phenol	U20403
Cercopoidea (Froghoppers)				
Cercopidae	*Clastoptera arizonana* Doering	Aug 1993, Continental AZ (-70°)	Phenol	U20395
Cicadoidea (Cicadas)				
Cicadidae	*Magicicada cassini* (Fisher) 7	June 1991, Cumberland Co. OH (EtOH)	Liq. N, Phenol	U20404
Cicadidae	*Cacama dissimilis* (Distant) 8	May 1993, Tucson AZ (-70°)	Liq. N, Phenol	U20413
Fulgoroidea (Planthoppers)				
Delphacidae	*Prokelisia dolus* Wilson 9	June 1993, Slaughter Beach, DE (-70°)	Phenol	U20406
Flatidae	*Ormenis saucia* Van Duzee	May 1993, Tucson AZ (-70°)	Liq. N, Phenol	U20397
Fulgoridae	*Poblicia fuliginosa* (Olivier)	April 1993, Continental AZ (-70°)	Phenol	U20417
HETEROPTERA				
Gerridae	*Aquarius remigis* (Say) 10	May 1993, Continental AZ (-70°)	Liq. N, Phenol	U20394
Belostomatidae	*Abedus herberti* Hidalgo 10	May 1993, Continental AZ (-70°)	Liq. N, Phenol	U20393
Gelastocoridae	*Gelastocoris oculatus* (F.) 10	May 1993, Continental AZ (-70°)	Phenol	U20430
Rhopalidae	*Boisea trivittatus* (Say)	Dec 1992, Pocatello ID (live)	Liq. N, Phenol	U20408
THYSANOPTERA (Thrips)				
Phlaeothripidae	*Hoplothrips karnyi* (Hood)	11	/	U20415
	Elaphrothrips tuberculatus (Hood)	11	/	U20396
Aeolothripidae	*Aeolothrips albicinctus* Haliday	11	/	U20407
PSOCOPTERA (Booklice)				
Ectopsocidae 4	(species unidentified)	Oct 1993, Portal AZ (-70°, then EtOH)	Abbreviated	U20414

1 DNA from P. Baumann
2 Collected by W. Ree
3 Collected by R. Ziegler
4 ID by M.-M. Yang
5 Collected by D. Frohlich
6 Collected, ID by R. Cocroft
7 Collected by C. Simon
8 Collected by J. M. Porter; ID by C. Olsen
9 Collected by R. Denno
10 ID by R. Smith
11 DNA from B. Crespi

Fig. 2. Insect species sequenced in this study. Classification is according to Carver et al. (1991).

DNA was extracted from either live, 95% ethanol-preserved, or frozen (–70°C) insects. For most homopterans, multiple individuals were combined for extraction, but single individuals of the Cicadidae and one species of Aleyrodidae, all Hemiptera, and the ectopsocid were extracted. Most material was either ground in liquid nitrogen and added to extraction buffer (10 mM tris-HCl, pH 8.0; 10 mM EDTA; 50 mM NaCl; 0.5% SDS) or ground in extraction buffer with a small pestle in a 1.5-ml Eppendorf tube. Proteinase K was added and homogenates were incubated at 60–65°C for 1–3 h. Homogenates were extracted with phenol/chloroform and chloroform and the DNA was precipitated with sodium acetate and 100% ethanol. RNA was removed with heat-treated RNase A. Two templates (*Aleurodicus dugesii* and the ectopsocid) were prepared using an abbreviated procedure (Gawel and Bartlett 1993) in which single insects were ground in a lysis buffer and heated, and the homogenate was used directly for PCR.

Sequencing templates were amplified with the polymerase chain reaction (PCR) using standard conditions (Saiki et al. 1988). Double-stranded PCR products were sequenced directly by cycle sequencing, as implemented with the dsDNA Cycle Sequencing System (GIBCO BRL Life Technologies, Inc.) or by an automated sequencer (Applied

Biosystems, Inc.). Templates were sequenced in both directions. The amplification primers were 2880 (5′ CTGGTTGATCCTGCCAGTAG3′; binding at sites 4–23 on *Drosophila melanogaster* [Tautz et al. 1988]) and B-(5′CCGCGGCTGCTGGCACCAGA 3′; binding at 565–584), which were also used for sequencing in addition to two internal sequencing primers, A (5′ AGGGTTCGATTCCGGAGAGG 3′: binding at 374–393) and A-(5′ CAGGCTCCCTCTCCGGAATC 3′; binding at 381–400).

Sequences were aligned with the PILEUP program of the University of Wisconsin Genetics Computer Group (1991) software package. Regions of alignment ambiguity (all in the variable regions V1, V2, and V3) were omitted manually. Sequences were deposited in GenBank (accession numbers are listed in Fig. 2).

Phylogenetic analyses were performed with the computer programs PAUP 3.1.1 (Swofford 1993) and PHYLIP 3.5 (Felsenstein 1993); subsequent tree manipulations were performed with MacClade 3.0 (Maddison and Maddison 1992). The PTP test for cladistic structure (Archie 1989; Faith and Cranston 1991) was implemented with PHYLIP. Shortest trees, as determined by the criterion of maximum parsimony (Felsenstein 1982), were found with PAUP using the heuristic option (random-addition sequence, tree bisection-reconnection branch-swapping, 10 replicates) with all substitutions weighted equally. The most probable tree, as determined by maximum likelihood (Felsenstein 1981), was found using the DNAML program of PHYLIP, with a transition/transversion ration of 2.1579. The transition/transversion ratio was determined using the State Changes & Stasis option of MacClade, based on the most-parsimonious tree including only the Cicadelloidea and Cercopoidea. Transition and transversion rates were estimated using a set of closely related taxa because of the higher probability that more recent divergences would reflect the true rates: over time, transitions mask transversions and thus obscure the true ratio (C. Simon, personal communication).

Relative support for the branching events found in parsimony searches was evaluated by bootstrap methods (Felsenstein 1985). Bootstrap parsimony analyses were performed with PAUP. In addition, bootstrapping of a distance-based method, neighbor-joining (Saitou and Nei 1987), was performed with the SEQBOOT and NEIGHBOR program of PHYLIP. Distance matrices were generated using the maximum-likelihood option of the DNADIST program and the transition/transversion ratio of 2.1579. All analyses were performed both including and excluding the Aleyrodoidea because of the potential for their unusually long common branch (see below) to cause misleading results (Felsenstein 1978).

Maximum-likelihood methods also were used to compare specific tree topologies. Eight different topologies, which reflected the alternative placements of the Aleyrodoidea and Heteroptera found in heuristic parsimony searches, and/or constrained the Homoptera to be monophyletic, were evaluated. The test of Kishino and Hasegawa (1989) to detect significant differences between each tree and the one with the highest likelihood was performed with the User Tree option of DNAML and the transition/transversion ratio of 2.1579.

Results

Sequence Alignment and Variation

Alignment of our sequence data yielded a matrix of 729 characters (Fig. 3), of which 544 were unambiguously aligned and 125 were potentially informative. Sternorrhyncha had in common sequences that were on average 10% longer, and more variable in length, than those of Auchenorrhyncha, Heteroptera, and outgroups. The majority of additional sequence in the Sternorrhyncha was concentrated in the V2 variable region, specifically in the eucaryote-specific helices E9-1 and E9-2 (as numbered in Nelles et al. [1984], but also known as E10-1 and E10-2 [Hendricks et al. 1988]), which together were 11–38% more GC-rich than conserved regions.

Pairwise distance estimates between taxa in the final 544-character matrix were between 0.2% and 20% (Fig. 4). Distances within orders and suborders averaged 2.3% for Auchenorrhyncha, 4.4% for Sternorrhyncha (excluding Aleyrodoidea), and 8.2% for Heteroptera. A high substitution rate was apparent in the Aleyrodoidea, whose distances to outgroup taxa were 60% greater than were those for other Sternorrhyncha to outgroups. Distances between the ectopsocid and ingroup taxa were no greater (in fact, they were 1–4% lower) than distances between two Thysanoptera to ingroup taxa, implying that variable sites in the ectopsocid were close to saturated (Moritz et al. 1987). The same pattern was found for distances between Hemiptera and holometabolous insects (representatives of Coleoptera, Mecoptera, and Hymenoptera), which were 2–3% lower than distances between the ectopsocid and Hemiptera (not shown). Because of this saturation of sites over the time since their earlier divergence, holometabolous taxa were uninformative as more distant outgroups and were omitted from our analyses.

Phylogenetic Analyses

Initial heuristic parsimony searches including all taxa gave an implausible result with respect to the ectopsocid and the Aleyrodoidea (Fig. 5), confirming our suspicions raised by the distance matrix. Most-parsimonious trees could not be rooted such that the Hemiptera were monophyletic; instead, the ectopsocid moved into the Hemiptera, attracting the long aleyrodoid branch, to become the sister group of the Auchenorrhyncha + Heteroptera. The association of the ectopsocid with Aleyrodoidea, and the position of their common branch, was supported with less than 50% bootstrap confidence (Fig. 5). Because these and other analyses suggested that the ectopsocid was too distant an outgroup to be informative, it was omitted in further searches. Both with and without the ectopsocid included, the PTP test indicated that the data set had significant phylogenetic structure ($P < 0.01$).

Heuristic parsimony searches excluding the ectopsocid supported the hypothesis that the Homoptera are paraphyletic (Fig. 6). The Auchenorrhyncha and Heteroptera formed a monophyletic group that was sister to the Sternorrhyncha. Constraining the Homoptera to be monophyletic added five steps to the most-parsimonious trees. The Auchenorrhyncha were not monophyletic in most-parsimonious trees; however, it cost only one extra step to make the Heteroptera a sister group to a monophyletic Auchenorrhyncha. Within the Auchenorrhyncha, Cicadelloidea were monophyletic and a sister group to the Cercopoidea, with the Cicadoidea taking a basal

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[                           10        20        30        40        50        60        70]
[                            .         .         .         .         .         .         .]
                    CTGGTTGATCCTGCCAGTAG 2880->                                V1 ->    XX
Ectopsocidae        CTGGTTGATCCTGCCAGTAGTCATATGCTTGTCTCAAAGATTAAGCCATGCATGTCTCAGTGCAAGCC--   [68]
Elaphrothrips       .......................................................G.A..........--   [68]
Hoplothrips         .......................................................G.A..........--   [68]
Aeolothrips         .........................................................A..........--   [68]
Aleurodicus         .............................C..........C...........................--   [68]
Bemisia             .............................C..........C....G......................--   [68]
Pachypsylla         ....................................................................GT   [70]
Paratrioza          ....................................................................GT   [70]
Schlechtendalia     ....................................................................--   [68]
Adelges             ..............................................................T.....--   [68]
Phylloxera          ..............................................................T.....--   [68]
Dactylopius         ....................................................................GT   [70]
Dysmicoccus         ....................................................................GT   [70]
Pseudococcus        ....................NNNNNNNNNNNNNNNNNNNNNNNNNNNN....................GT   [70]
Ormenis             ....................................................A........A......--   [68]
Poblicia            ....................................................A........A......--   [68]
Prokelisia          ....................................................A........A......--   [68]
Magicicada          ....................................................A........A..T...--   [68]
Cacama              ....................................................A....A...A..T...--   [68]
Philya              ....................................................A....A...AT.....--   [68]
Platycotis          ....................................................A....A...AT.....--   [68]
Erythroneura        ....................................................A........A......--   [68]
Clastoptera         ....................................................A........A.G....--   [68]
Gelastocoris        .......................................C............A........A......--   [68]
Abedus              .......................................C............A........A......--   [68]
Aquarius            .......................................C............A............A.T--   [68]
Boisea              .......................................C............A........A.G...T--   [68]

[                           80        90       100       110       120       130       140]
[                            .         .         .         .         .         .         .]
                    XXXXXXXXXXXX         <-V1                   V2->               XXXXXXX
Ectopsocidae        ----------ATATTAAGGTGAAACCGCGAAAGGCTCATTAAATCAGTTATGGTTCATTGGATG-GTAC-   [126]
Elaphrothrips       ----------GA.C.....C.....................C.......G......C.....CT-....C   [127]
Hoplothrips         ----------GA.......C.....................C.......G......C.....CC-....C   [127]
Aeolothrips         ----------.A.............................T...............A.A...C-....-   [126]
Aleurodicus         ---------CG...C.G...............A................G.C.....CA....C-..G.C   [128]
Bemisia             ----------GA.C.......G...........................G.C.....CA....C-..G.C   [127]
Pachypsylla         ACGCGCTCGCGCG.CT........................................C..A...C-....C   [139]
Paratrioza          ACGCGTTCGCGCG.CT........................................C......C-....C   [139]
Schlechtendalia     ----------GC.....................................G......C..A...C-....A   [127]
Adelges             ----------GC.....................................G......C..A...C-....C   [127]
Phylloxera          ----------GC....C................................G......C..A...C-....C   [127]
Dactylopius         ----------CCCG..C.....................G.................C..A...T-....C   [129]
Dysmicoccus         ----------CC.G..C.......................................C..A...C-....C   [129]
Pseudococcus        ----------C..G..C.......................................C..A...C-....C   [129]
Ormenis             ----------.A.C..............A...........................C......C-....A   [127]
Poblicia            ----------GA.C..............A...........................C......C-....A   [127]
Prokelisia          ----------.C................A...........................C......C-....A   [127]
Magicicada          ----------GC.C..............A...........................C......C-T..-A   [126]
Cacama              ----------GC.C..............A...........................C......C-T..-A   [126]
Philya              ----------.A.......C........A...........................C......C-....A   [127]
Platycotis          ----------.A................A...........................C......C-....A   [127]
Erythroneura        ----------.C................A...........................C......C-....A   [127]
Clastoptera         ----------GA................A...........................C..A...C-....A   [127]
Gelastocoris        ----------GA................A...T................T.......C.A.TGCT.A..A   [128]
Abedus              ----------GC.C..............A..C.................T.A.....C.T..GC-.ATAA   [127]
Aquarius            ----------.A.....A..............A........................G.T..GC--A---   [123]
Boisea              ----------GAC....A..........C.........G....C.G..C..A...T.CC....--A..GC   [126]
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Fig. 3. Data matrix of aligned, partial 18S sequences. Unknown nucleotides are indicated by *N;* gaps are marked by *hyphens; periods* indicate sequence identical to the first taxon. Primer sequences are shown above the alignment at their respective binding sites. Regions omitted from the analysis are indicated by *x*'s in *bold.* Beginnings and endings of variable regions are indicated by *arrows.*

position in the clade. The Sternorrhyncha were monophyletic; consistent with morphology, the Aphidoidea and Coccoidea were sister groups. Psylloidea and Aleyrodoidea were not sister taxa, however, but branched independently from the common lineage, with Psylloidea basal in the clade. Constraining the Psylloidea and Aleyrodoidea to be monophyletic added two steps to the tree.

Many of the relationships found in heuristic searches were well supported in the bootstrap parsimony analysis (Fig. 7). The major result of homopteran paraphyly—namely, that Auchenorrhyncha and Heteroptera form a monophyletic group—was supported with 95% bootstrap confidence. The relative lack of potentially informative characters in the Auchenorrhyncha (evident from the distance matrix) led to low bootstrap support for relationships among the major auchenorrhynchan clades and the Heteroptera. Nevertheless, auchenorrhynchan taxa were assigned to their respective superfamilies generally with high bootstrap support. The monophyly of the Sternorrhyncha was supported with high bootstrap values, as

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[                         150       160       170       180       190       200       210]
[                          .         .         .         .         .         .         .]
                  xxxxxxxxxxxxxxxx                                                     x
Ectopsocidae      -A-----CAGTTACT-TGGATAACTGTGGTAATTCTAGAGCTAATACATGCAAACTGAGTCCCGACCAT-   [188]
Elaphrothrips     -------........-..........C..A..A...G.........T....CG..A............G-   [188]
Hoplothrips       -------........-..........C..A..A...G..............CG..A............G-   [188]
Aeolothrips       ------T.......A-..........C..A...A..G..............C...A...........GG-   [188]
Aleurodicus       A.----A...-...A-...................................CG..A........G.G.C-   [191]
Bemisia           AC----A.CC-...A-...................................CG.TA........G.A.C-   [190]
Pachypsylla       AC----T---.....-...................................CT..A....T......GCC   [201]
Paratrioza        AT----T........-...................................CG..A....T......GCC   [204]
Schlechtendalia   CG------.......-...................................CG..A....T......G.C   [190]
Adelges           C.------.......-...................................CG..A....T......GGC   [190]
Phylloxera        C.------.......-...................................CG..A....T......GGC   [190]
Dactylopius       C.CATTTGT......-...................................CG..A....T.......GC   [198]
Dysmicoccus       C.C------......-...................................CG..A....T.......AC   [192]
Pseudococcus      C.C------......-...................................CG..A....T.......AC   [192]
Ormenis           -.----T.CA.....-.......................................A............G-   [190]
Poblicia          -.----T.CA.....-.......................................A...........G.-   [190]
Prokelisia        -.----T.CA.....-.......................................A............G-   [190]
Magicicada        -.-----........-.......................................A-...........G-   [187]
Cacama            -.-----........-....C..................................A-...........G-   [187]
Philya            -.----T.CA.....-.......................................A..A.........G-   [190]
Platycotis        -.----A.CA.....-.......................................A..A.........G-   [190]
Erythroneura      -.----T.CA.....-.......................................A..A.........G-   [190]
Clastoptera       -.----T.CA.....-.......................................A............G-   [190]
Gelastocoris      T.----TTT.-.-G.-....C................................-.AA...........G-   [189]
Abedus            C.----T.C...-T.-.............C.......................-.CA...........G-   [189]
Aquarius          ------T.CACCT..G.................C...................T.A-.......T...G-   [185]
Boisea            C.---CT..C-----G.......................................A-.....TCT...GC   [187]

[                         220       230       240       250       260       270       280]
[                          .         .         .         .         .         .         .]
                  xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx
Ectopsocidae      ---------------------------TCATTT-------------GGCAGGGATGCTTTTATTAGATCA   [218]
Elaphrothrips     ---------------------------AG.--.-------------..G.....C...............   [216]
Hoplothrips       ---------------------------AG.--.-------------..G.....C...............   [216]
Aeolothrips       ---------------------------.G.--C-------------..A.....C...............   [216]
Aleurodicus       ----------------------GCCCCGGGCA.CAC----GCCCGG........C...............   [235]
Bemisia           ----------------------TCCCCGGG---CTC----GCCCGG........C...............   [231]
Pachypsylla       TCAT--CAG------------------------------------C..G...A.C...............   [233]
Paratrioza        TCGT----G------------------------------------C..G...A.C...............   [234]
Schlechtendalia   GCGG--CGG---------CGCCTCCG--GGCG.CGT----GC--GC..G...A.C...............   [241]
Adelges           GCGC--GTC---------CGCTCACG--.G-G.CGT----GCCGTC..A...A.C...............   [242]
Phylloxera        -CGG--CGG---------C-CTCCGG--GTCG.CGC----GT---C..A...A.C...............   [238]
Dactylopius       --GC--CGGGT-GTATTAGGCTTCG--CGCCGACGCC-TGGTGTTC..A...A.C...C...........   [260]
Dysmicoccus       TGTA--CGGATGGGCGGCGGCTTCGGTCGTCG.CGCCGTACGGTTC..A...A.C...............   [260]
Pseudococcus      TGGTGACAGATGTGCGTCGGCCTCGGTCGTCG.CGCTGTACGGTTC..A...A.C...............   [262]
Ormenis           ---------------------------AA.--.-------------..A.....C...............   [218]
Poblicia          ---------------------------AA.--.-------------..A.....C...............   [218]
Prokelisia        ---------------------------AA.--.-------------..A....GC...............   [218]
Magicicada        ---------------------------AG.--.-------------..A.....C...............   [215]
Cacama            ---------------------------AG.--.-------------..G.....C...............   [215]
Philya            ---------------------------AG.--.-------------..A.....C...............   [218]
Platycotis        ---------------------------AG.--.-------------..A.....C...............   [218]
Erythroneura      ---------------------------AG.--.-------------..G.....C...............   [218]
Clastoptera       ---------------------------AG.--.-------------..A.....C............C..   [218]
Gelastocoris      ---------------------------.G.--.-------------..A.A...C...............   [217]
Abedus            ---------------------------.A.--.-------------..A.A...C...............   [217]
Aquarius          ---------------------------.G.--.-------------..T.A...C...............   [213]
Boisea            ----------------------------G..---------------..AG....C.........G.....   [214]
```

Fig. 3. Continued.

were most of the relationships among superfamilies, with the exception of the Aleyrodoidea.

Bootstrap analyses with the distance method recovered relationships and support similar to the parsimony methods, with some exceptions (Fig. 8). The neighbor-joining method placed Aleyrodidae at the base of the sternorrhynchan lineage, with moderate bootstrap support. Moderate support was also found for the same relationships of Cicadelloidea, Cercopoidea, and Cicadoidea identified in heuristic parsimony searches. Homopteran paraphyly was well supported, but, again, the monophyly of the Auchenorrhyncha was unresolved.

Trees recovered in maximum-likelihood searches were very similar to those found using parsimony methods. In particular, under maximum likelihood, the Homoptera were found to be paraphyletic. Auchenorrhyncha were paraphyletic and Sternorrhyncha were monophyletic, as with parsimony. Of the eight user-defined topologies tested, the tree positioning Aleyrodoidea as the sister group to Aphidoidea + Coccoidea and placing Heteroptera as the sister group to the Cicadelloidea, Cercopoidea, and Cicadoidea had the highest log likelihood (–2,666.41), but it was not significantly different from any other topology. The two topologies that

```
[                          290       300       310       320       330       340       350]
[                            .         .         .         .         .         .         .]
                           XXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXXX
Ectopsocidae        AAACCAA----TC--AG--------------ACCTTTT------G------------------------G   [238]
Elaphrothrips       .......-A----TCTT--------------C..C.-------C.-------------------------   [235]
Hoplothrips         .......-AAA..CCC.--------------C..C.-------C.------------------------.   [240]
Aeolothrips         .......----..GGTC--------------G.---..------C------------------------.   [235]
Aleurodicus         .....G.CACCGGGGG.CTCCGCTCGCCGGGGT.CCCCG-GT---------------------------C   [277]
Bemisia             .....G.CACCG.GGGCCTCC--------GGGT.CGCGGTTT---------------------------C   [266]
Pachypsylla         .......TCGCG.GCG.TGTCGCACGCTTCACG.G.CGCGTCCC.CCGCGAACACATACGGTGCCGCGC.   [303]
Paratrioza          .....C.TCG-GTACG.--------GCTT----.GGCCCATACC.AC---AACA----------CTCG-.   [277]
Schlechtendalia     .....GGCCCG..GCG.G-------CGC-GCCT.GCGCGCG-CCC------------------------C   [278]
Adelges             .....GGCCCG..GCG.T-------CGCGGST..GRCCGCG-GCC------------------------C   [280]
Phylloxera          .....GGCC----------------CGA-TCCT.G-----------------------------------   [256]
Dactylopius         .....GGCCAAA.AGT.TTTGCTAACGCGTTTT.GAACGCGTTC.------------------------C   [306]
Dysmicoccus         .....GGCTAAA.GGC.GTT----ACGC---TT.G--CGGGTTTC------------------------C   [297]
Pseudococcus        .....GGCCAGA.GGT.GTT------TCGCGTT.GCGCGCGTCTC-----------------------CA   [303]
Ormenis             .......----..GAC.--------------T..GC..TCCTTC.------------------------.   [246]
Poblicia            .......----..GAC.--------------T..G.C.GCTTTC.------------------------.   [246]
Prokelisia          .......----..GAC.--------------T.AG..GCTT-----------------------------   [242]
Magicicada          .....G.----..GGC.--------------T..GGC.-----CC------------------------.   [238]
Cacama              .....G.----..GGC.--------------T..GGC.-----CC------------------------.   [238]
Philya              .......----..GGG.---------------.T---------C--------------------------   [234]
Platycotis          .......----..GGG.---------------.T---------T--------------------------   [234]
Erythroneura        .......----..GGG.---------------.T---------CC------------------------.   [236]
Clastoptera         .......----..GGT.---------------.TCGG.TCGTCC.------------------------.   [245]
Gelastocoris        .......----..-GGT--------------..GG--.T-----T------------------------C   [237]
Abedus              ...T...----..GGG.--------------T.GGACAA-----.------------------------C   [240]
Aquarius            .......----..G--.--------------...GGC.-CGTCC.------------------------C   [238]
Boisea              ......T----..---------------------------------------------------------   [223]

[                          360       370       380       390       400       410       420]
[                            .         .         .         .         .         .         .]
                    XXXXXXXXXXXXXXXXXXXXXXXXX                                      XXXXXXX
Ectopsocidae        GTCTGTTGC-------------TCTTGGTGACTCTGAATAACTTTGGGCTGATCGCA-TGGTC--TC---   [289]
Elaphrothrips       ---..GGAAA--ACG-----AC..AA....................T..C.......-C..C.--CTC--   [290]
Hoplothrips         .GTG.GGAAA--ACG------G.TAA....................T..C.......-C..C.--C.C--   [297]
Aeolothrips         .CGA--------TCGTGC--AG...................................-C..C.--..C--   [290]
Aleurodicus         TC.AA.C..ACCCCGTGTGCACGT.G.AC..T..C........CC.-..C.......CC..C.CT..CCG   [346]
Bemisia             TC.A---..ACTTCGCGTGCACA..G.AC..T..C........CC.-..C.......TC..A.CC..GCG   [332]
Pachypsylla         TC.GCGC..GGTCCGTAACAAA---.....................T..........-C....--ATCAA   [367]
Paratrioza          TC.GT.C..GGGCCG-ACCAAG---.....................T..........-C....---TCA-   [338]
Schlechtendalia     .AT---C..GGCCCGCGCAAAGA.C...................C.A..........-C....--..C--   [340]
Adelges             .AT---C..GGCCCGCACAAAGA.C...................C.A..........-C....--CTG--   [342]
Phylloxera          .AT---C..GGCCCGCACAAAGA.C...................C.A..........-C....--CTG--   [318]
Dactylopius         .CTGTAA..GCCCCGCGCAAAGA....AC............T..C.A..........-C....--.TG--   [371]
Dysmicoccus         ...GTAA..GGCCCGCGCAAAGAT....................C.A..........-C....--CTC--   [362]
Pseudococcus        T..GTAA..GGCCCGCGCAAAGA.....................C.A..........-C....--CTC--   [368]
Ormenis             .C.G.CG..G--CCGTAC---TC......................T...A.......-C....--.TC--   [306]
Poblicia            .C.GACG..G--CCGTAC---TC......................T...A.......-C....--..C--   [306]
Prokelisia          .C.AAC...G--CCGTAC---TC......................C...A.......-C....--..A--   [302]
Magicicada          .C.---G..G--CCGTCT---C.T............G........TA..........-C....--A.A--   [295]
Cacama              .C.G.-G..G--CCGTCT---C.T............G........CA..C.......-C....--A.A--   [297]
Philya              .C.---------CCGCAC---T.......................AA..........-C....--CTT--   [287]
Platycotis          .C.---------CCGCAC---T.......................AA..........-C....--..C--   [287]
Erythroneura        .C.---------TCGCAC---TA.....................ACA..........-C....--CTA--   [289]
Clastoptera         .C.---------CAGATA---CA.....................CTA..........-C....--..C--   [298]
Gelastocoris        .C.----.TA--CCGTTT-GAC.TC..A..............C-AAA..G.......-C....--AAC--   [294]
Abedus              .C.----..T--CCGTTT-GAC.--..A..............C--A.T.G.......-C....--..A--   [294]
Aquarius            ...----------CGTCT-TTGC....A.......TGG....A--T.C.AC......-C....--CTC--   [290]
Boisea              .C...G.T.G--CCGGGCGTCTG....A.......AG.....A--CCA.A.......-C....--.TA--   [284]
```

Fig. 3. Continued.

constrained the Homoptera to be monophyletic, however, had the lowest log likelihoods (–2,686.96 and –2,687.59), and of these two, the tree that also constrained the Psylloidea and Aleyrodoidea to be sister groups had the lower value.

Discussion

Using parsimony, distance, and maximum-likelihood methods, our molecular data strongly support the hypothesis that Homoptera are paraphyletic: Heteroptera and Auchenorrhyncha share a more recent common ancestor than do Sternorrhyncha and Auchenorrhyncha. The fossil record is also consistent with this conclusion in that Heteroptera appear later than the first Homoptera. Fossils assignable to homopterous forms can be dated to the lower Permian, while hemipteran representatives do not emerge until the upper Permian (Wootton 1981; Carpenter 1992; Kukalová-Peck 1991; Labandiera and Sepkoski 1993), suggesting that Heteroptera were descended from an existing homopteran lineage. Our data, however, could not distinguish among the possible alternative origins of the Heteroptera with respect to the Auchenor-

```
[                            430       440       450       460       470       480       490]
[                            .         .         .         .         .         .         .]
                                             <-V2
Ectopsocidae        GTACCGGCGACGCATCTTTCAAATGTCTGACTTATCAACTTTCGATGGTAGGCTC-TGCGCCTATTACGG   [358]
Elaphrothrips       .CG..........G...............C.........................-........CC.T..   [359]
Hoplothrips         .CG..........G...............C.........................-........CC.T..   [366]
Aeolothrips         ..G..........................C..........G...........T..-........CC.T..   [359]
Aleurodicus         .GC.G...........C............C...........G...C...C.AG.A-GAG.....CCGT..   [415]
Bemisia             ..C.G...........C............C...............C...C..G.A-.TG.....CCGT..   [401]
Pachypsylla         ....A.....T..................C..........G...........T.A-........CC.T..   [436]
Paratrioza          ..G.T.....T..................C..........G...........T.A-........CC.T..   [407]
Schlechtendalia     .............................C..........G....C......T..-CAT.....CCGT..   [409]
Adelges             .............................C..........G....C......T..-CAT.....CCGT..   [411]
Phylloxera          .............................C..........G....C......T..-CAT.....CCGT..   [387]
Dactylopius         ............T................C..........G....C......T..-GAT.....CCGT..   [440]
Dysmicoccus         ............T................C..........G....C......T..-GA......CCGT..   [431]
Pseudococcus        ............T................C..........G....C......T..-GA......CCGT..   [437]
Ormenis             ................C............C..........G...........T..-........CC.T..   [375]
Poblicia            .A..............C............C..........G...........T..-........CC.T..   [375]
Prokelisia          ................C............C..........G...........T..-........CC.T..   [371]
Magicicada          ........................................G..............-........CC.T..   [364]
Cacama              ........................................G..............-........CC.T..   [366]
Philya              ........................................G..........AT..T........CC.T..   [357]
Platycotis          ........................................G..........AT..-........CC.T..   [356]
Erythroneura        ........................................G..........AT..-........CC.T..   [358]
Clastoptera         ........................................G..........AT..-........CC.T..   [367]
Gelastocoris        ...........AA............C..............G..........AT..-.A..T...CC.T..   [363]
Abedus              ..G........C.............C..............G..........AT..-.A..T...CC.T..   [363]
Aquarius            ...........T.G...........A..............G..........AT.T-.AT.T...CC.T..   [359]
Boisea              .......T...AT...C....................G..G..........A...-..T.T...CC.T..   [353]

[                            500       510       520       530       540       550       560]
[                            .         .         .         .         .         .         .]
                                           AGGGTTCGATTCCGGAGAGG A ->
                                            <- A- CTAAGGCCTCTCCCTCGGAC
Ectopsocidae        TT-GTAACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGGTACCACATCCA   [427]
Elaphrothrips       ..-.........G.............................................C...........   [428]
Hoplothrips         ..-.........G.............................................C...........   [435]
Aeolothrips         ..-.......................................................C...........   [428]
Aleurodicus         ..-.CG...........................C........................C...........   [484]
Bemisia             ..-.CG....................................................C...........   [470]
Pachypsylla         .G-.........G.....G..T...........C........................C...........   [505]
Paratrioza          .G-.........G.....G..T...........C........................C...........   [476]
Schlechtendalia     .G-.......................................................C...........   [478]
Adelges             .G-.......................................................C...........   [480]
Phylloxera          .G-.......................................................C...........   [456]
Dactylopius         .G-.......................................................C...........   [509]
Dysmicoccus         .G-.......................................................C...........   [500]
Pseudococcus        .G-.......................................................C...........   [506]
Ormenis             ..-.......................................................C...........   [444]
Poblicia            ..-.......................................................C...........   [444]
Prokelisia          ..-.......................................................C...........   [440]
Magicicada          ..-..T....................................................C...........   [433]
Cacama              ..-.......................................................C...........   [435]
Philya              ..G.......................................................C...........   [427]
Platycotis          ..G.......................................................C...........   [426]
Erythroneura        ..-.......................................................C...........   [427]
Clastoptera         ..-.......................................................C...........   [436]
Gelastocoris        .A-A....................................................A.C...........   [432]
Abedus              ..-.........G...........................................T.C...........   [432]
Aquarius            ..-..................................................C..T.C...........   [428]
Boisea              CG-C.C......G.............................................C...........   [422]
```

Fig. 3. Continued.

rhyncha. Equally, or nearly equally, parsimonious solutions placed the Heteroptera either as the sister group to the Fulgoroidea, to the Cicadelloidea + Cicadoidea, or to the Auchenorrhyncha as a whole. Bootstrap analysis did not support any one of these hypotheses with greater than 50% confidence, and maximum likelihood methods could not distinguish among the alternative hypotheses. The comparative lack of informative characters in the Auchenorrhyncha probably led to this ambiguity. Evidence from morphology is equivocal on this question. On the one hand, features of the alimentary canal, and perhaps some wing characters, seem to unite the Fulgoroidea and Heteroptera (Goodchild 1966; Wootton and Betts, 1986). On the other hand, the complex acoustical system, bristlelike antennal flagella, and possibly jumping ability and some wing characters may all be synapomorphies of the Auchenorrhyncha (Kristensen 1973; Hennig 1981; Carver et al. 1991). From a molecular standpoint, additional sequence data will be necessary to resolve this aspect of hemipteran phylogeny.

In a recent phylogenetic study of the Heteroptera, Wheeler et al. (1993) also found some support for para-

```
[                           570       580       590       600       610       620       630]
[                            .         .         .         .         .         .         .]
                                                                          V3->
Ectopsocidae        AGGAAGGCAGCAGGCGCGCAAATTACCCACTCCCAGCAC-GGGGAGGTAGTGACGAAAAATAACGATGCA   [496]
Elaphrothrips       ..................................G..T.-..................G...........   [497]
Hoplothrips         ..................................G..T.-..................G...........   [504]
Aeolothrips         ..................................G..T.-..................G........A.G   [497]
Aleurodicus         ..................................G....-...............G......C......G   [553]
Bemisia             ..................................G....-...............G......C......G   [539]
Pachypsylla         ...............A.....................C.A..................T........A.G   [575]
Paratrioza          ...............A.....................T.A..................T........A.G   [546]
Schlechtendalia     ..................................G.A..-...........................A.G   [547]
Adelges             ..................................G.T..-...........................A.G   [549]
Phylloxera          ..................................G.T..-.............................G   [525]
Dactylopius         .....................................T.-.............................G   [578]
Dysmicoccus         .....................................T.-...........................A.G   [569]
Pseudococcus        .....................................T.-...........................A.G   [575]
Ormenis             ..................................G....-...........................A.G   [513]
Poblicia            ..................................G....-...........................A.G   [513]
Prokelisia          ..................................G....-...........................A.G   [509]
Magicicada          ..................................G....-...........................A.G   [502]
Cacama              ..................................G....-...........................A.G   [504]
Philya              ..................................G....-...........................A.G   [496]
Platycotis          ..................................G....-...........................A.G   [495]
Erythroneura        ..................................G....-...........................A.G   [496]
Clastoptera         ..................................G....-...........................A.G   [505]
Gelastocoris        ...............A..................G....-..............A............A.G   [501]
Abedus              ...............A..................G....-..............A............A.G   [501]
Aquarius            ...............A..................G....-..............A............A.G   [497]
Boisea              ...............A..................G....-..............A............A.G   [491]

[                           640       650       660       670       680       690       700]
[                            .         .         .         .         .         .         .]
                                                                           <-V3
                                                                      XXXXXX
Ectopsocidae        GAACTCATTCCGAGGC-TCTGCAATCGGAATGAGAACACTCTAAATCATTT----AACGAGTATCCATTG   [561]
Elaphrothrips       .G....T-.AT.....-C...T...........CT...T.T......TC..----..G.......A....   [561]
Hoplothrips         .G....T-.TT.....-C...T...........CT...T.T......TC..----..G.......A....   [568]
Aeolothrips         .G.....-........-C.C.T...........CT.....T......C...----..G.......A..GA   [561]
Aleurodicus         .G.....-A.......-C.C.TG...........CG.C.CG....CA.GGC----G.....G.C......   [617]
Bemisia             .G.....-A.......-C.C.TG...........CG.C.CG....CA.GGC----G.....G.C......   [603]
Pachypsylla         .G.....-........-CTC.T............T.....T....A.G...----......G........   [639]
Paratrioza          .G.....-........-CTC.T............T.....T....A.G...----......G........   [610]
Schlechtendalia     .G.....-........-C.C.T..................T....A.C...---A......G...A....   [612]
Adelges             .G.....-........-C.C.T..................T....A.C...---A......G...A....   [614]
Phylloxera          .G.....-........-C.C.T............T.....T....A.C..G---A......G...A....   [590]
Dactylopius         .G....T-.AT.....T..C.T............T.....T....A.C..GGTCAC.....G...A....   [647]
Dysmicoccus         .G.....-.T......-C.C.T............T.....T....A.C..GGTCACG....G...A....   [637]
Pseudococcus        .G.....-.T......-C.C.T............T.....T....A.C..GGTCAC.....G...A....   [643]
Ormenis             .G.....-........-C.C.T............T.T..........C..A----T.........A....   [577]
Poblicia            .G.....-........-C.C.T............T.T..........C..A----T.........A....   [577]
Prokelisia          .G.....-........-C.C.T............T.T..........C..G----T..A......A....   [573]
Magicicada          .G.....-........-C.C.T............T.....T.....AC...----........C.A....   [566]
Cacama              .G.....-........-C.C.T............T.....T.....AC...----........C.A....   [568]
Philya              .G.....-........-C.C.T............T.....T......C...----........C.A....   [560]
Platycotis          .G.....-........-C.C.T............T.....T......C...----........C.A....   [559]
Erythroneura        .G.....-........-C.C.T............T.....T......C...----........C.A....   [560]
Clastoptera         .G.....-........-C.C.T............T.....T......C...----........C.A....   [569]
Gelastocoris        .G.....-........-C.C.T............T.....T......C...----...A..G........   [565]
Abedus              .G.....-........-C.C.T.............................----...A..G.C.T....   [565]
Aquarius            .G.....-........-C.C.T............T.....T......C...----......G........   [561]
Boisea              .G.....-........-C.C.T.........................C...----......G...T....   [555]
```

Fig. 3. Continued.

phyly of the Homoptera using sequence from several regions of 18S rDNA. Although that study focused on Heteroptera, one sternorrhynchan species and three auchenorrhynchan species were included. We experimented with adding the non-overlapping, variable sites (47 total) from the Wheeler et al. study of the corresponding groups in our data set (Psocoptera, Aphididae, Cicadellidae, Cicadidae, Gerridae, Belostomatidae, and Gelastocoridae). The addition of these characters did not change our results. The sequence data presented by Wheeler et al. included a single nonhomoplasious site and two homoplasious sites (i.e., showing synapomorphies for the node in question, but having homoplasy elsewhere on the tree) that supported Auchenorrhyncha + Heteroptera as a monophyletic sister group to the Sternorrhyncha. (But all three characters were mapped onto that node as nonhomoplasious sites in the cladogram presented.) Our data support this relationship with two nonhomoplasious and seven homoplasious sites. The region that we sequenced overlaps with one of their fragments (the "A" fragment). However, of the set of sites that supports Auchenorrhyncha + Heteroptera (corre-

```
[                                     710         720        ]
[                                      .           .         ]
                                <- B- AGACCACGGTCGTCGGCGCC
Ectopsocidae             GAGGACAAGTCTGGTGCCAGCAGCCGCGG    [590]
Elaphrothrips            ....G........................    [590]
Hoplothrips              ....G........................    [597]
Aeolothrips              ....G........................    [590]
Aleurodicus              ....G........................    [646]
Bemisia                  ....G........................    [632]
Pachypsylla              ....G........................    [668]
Paratrioza               ....G........................    [639]
Schlechtendalia          ....G........................    [641]
Adelges                  ....G........................    [643]
Phylloxera               ....G........................    [619]
Dactylopius              ....G........................    [676]
Dysmicoccus              ....G........................    [666]
Pseudococcus             ....G........................    [672]
Ormenis                  ....G........................    [606]
Poblicia                 ....G........................    [606]
Prokelisia               ....G........................    [602]
Magicicada               ....G........................    [595]
Cacama                   ....G........................    [597]
Philya                   ....G........................    [589]
Platycotis               ....G........................    [588]
Erythroneura             ....G........................    [589]
Clastoptera              ....G........................    [598]
Gelastocoris             ....G........................    [594]
Abedus                   ....G........................    [594]
Aquarius                 ....G........................    [590]
Boisea                   ....G........................    [584]
```

Fig. 3. Continued.

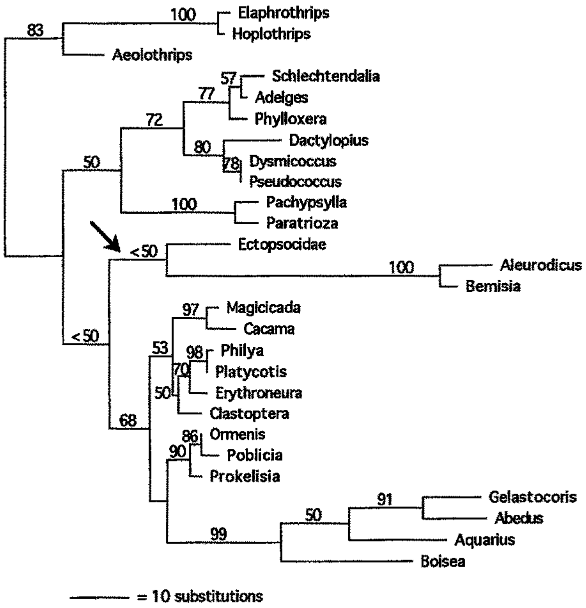

Fig. 5. One representative tree from heuristic searches including all taxa. Sixteen most-parsimonious trees were found (length = 308, CI = 0.604, RI = 0.742, uninformative characters excluded). Branch lengths are proportional to the maximum number of steps. *Bootstrap percentages* (from 500 replicates) are shown above the branches. The *arrow* indicates the misleading placement of the ectopsocid and Aleyrodoidea lineages, probably due to saturation of variable sites in the ectopsocid and to the unusually long common branch of the Aleyrodoidea.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1 Ectopsocidae		0.1030	0.0986	0.0836	0.1480	0.1390	0.0940	0.0959	0.0850	0.0914	0.0958	0.1051	0.0919	0.0951	0.0625	0.0645
2 Elaphrothrips	51		0.0056	0.0664	0.1661	0.1567	0.1116	0.1050	0.1047	0.1089	0.1089	0.1110	0.1067	0.1109	0.0876	0.0920
3 Hoplothrips	49	3		0.0623	0.1612	0.1565	0.1071	0.1006	0.1003	0.1044	0.1044	0.1111	0.1023	0.1062	0.0875	0.0919
4 Aeolothrips	42	34	32		0.1545	0.1476	0.0797	0.0796	0.0710	0.0708	0.0749	0.0920	0.0729	0.0750	0.0546	0.0567
5 Aleurodicus	71	78	76	73		0.0305	0.1482	0.1439	0.1240	0.1286	0.1286	0.1406	0.1331	0.1342	0.1400	0.1419
6 Bemisia	67	74	74	70	16		0.1482	0.1438	0.1195	0.1241	0.1263	0.1387	0.1312	0.1296	0.1265	0.1310
7 Pachypsylla	47	55	53	40	71	71		0.0112	0.0603	0.0665	0.0705	0.0833	0.0645	0.0661	0.0751	0.0751
8 Paratrioza	48	52	50	40	69	69	6		0.0623	0.0684	0.0725	0.0832	0.0644	0.0660	0.0771	0.0771
9 Schlechtendalia	43	52	50	36	60	58	31	32		0.0094	0.0170	0.0444	0.0286	0.0281	0.0623	0.0603
10 Adelges	45	53	51	35	61	59	33	34	4		0.0113	0.0443	0.0305	0.0301	0.0622	0.0644
11 Phylloxera	46	52	50	36	60	59	34	35	7	3		0.0403	0.0304	0.0280	0.0663	0.0684
12 Dactylopius	52	55	55	46	67	66	42	42	23	22	19		0.0265	0.0260	0.0811	0.0876
13 Dysmicoccus	46	53	51	37	64	63	33	33	15	15	14	14		0.0020	0.0624	0.0666
14 Pseudococcus	45	52	50	36	61	59	32	32	14	14	13	13	1		0.0639	0.0683
15 Ormenis	32	44	44	28	67	61	38	39	32	31	32	41	32	31		0.0056
16 Poblicia	33	46	46	29	68	63	38	39	31	32	33	44	34	33	3	
17 Prokelisia	33	47	45	29	68	64	39	40	33	32	33	43	34	33	4	7
18 Magicicada	35	47	47	33	67	61	42	42	33	32	33	42	33	32	13	16
19 Cacama	37	44	44	34	68	62	43	43	34	35	36	45	36	35	16	19
20 Philya	35	45	43	29	69	67	40	41	33	30	31	43	34	33	14	17
21 Platycotis	34	46	44	28	68	66	39	40	32	29	30	42	33	32	13	16
22 Erythroneura	33	46	44	30	66	64	37	38	29	30	31	41	32	31	13	16
23 Clastoptera	35	49	47	29	67	65	38	41	29	28	29	39	30	29	13	16
24 Gelastocoris	54	69	67	49	77	77	54	57	47	46	47	55	48	46	36	39
25 Abedus	53	67	67	52	77	74	60	60	54	53	56	64	57	55	37	40
26 Aquarius	57	72	70	53	79	80	59	61	53	53	54	62	57	55	42	45
27 Boisea	66	84	82	68	89	87	70	71	64	63	66	68	67	65	49	52

	17	18	19	20	21	22	23	24	25	26	27
1 Ectopsocidae	0.0645	0.0688	0.0729	0.0684	0.0664	0.0645	0.0684	0.1093	0.1070	0.1170	0.1365
2 Elaphrothrips	0.0939	0.0944	0.0879	0.0895	0.0915	0.0919	0.0981	0.1441	0.1405	0.1529	0.1801
3 Hoplothrips	0.0896	0.0943	0.0878	0.0852	0.0872	0.0876	0.0937	0.1394	0.1403	0.1481	0.1751
4 Aeolothrips	0.0566	0.0650	0.0669	0.0564	0.0544	0.0587	0.0566	0.0993	0.1064	0.1090	0.1424
5 Aleurodicus	0.1423	0.1405	0.1428	0.1445	0.1423	0.1380	0.1400	0.1648	0.1654	0.1713	0.1951
6 Bemisia	0.1332	0.1269	0.1292	0.1400	0.1377	0.1334	0.1355	0.1647	0.1583	0.1736	0.1902
7 Pachypsylla	0.0772	0.0839	0.0860	0.0792	0.0772	0.0732	0.0751	0.1099	0.1237	0.1227	0.1462
8 Paratrioza	0.0791	0.0838	0.0859	0.0812	0.0791	0.0751	0.0812	0.1162	0.1235	0.1269	0.1483
9 Schlechtendalia	0.0643	0.0647	0.0667	0.0643	0.0623	0.0564	0.0563	0.0943	0.1099	0.1087	0.1319
10 Adelges	0.0642	0.0646	0.0707	0.0602	0.0582	0.0603	0.0562	0.0942	0.1097	0.1107	0.1317
11 Phylloxera	0.0683	0.0687	0.0748	0.0642	0.0622	0.0643	0.0602	0.0984	0.1187	0.1151	0.1410
12 Dactylopius	0.0853	0.0837	0.0900	0.0853	0.0832	0.0812	0.0770	0.1121	0.1330	0.1294	0.1414
13 Dysmicoccus	0.0664	0.0648	0.0709	0.0664	0.0644	0.0625	0.0584	0.0965	0.1168	0.1176	0.1391
14 Pseudococcus	0.0681	0.0664	0.0729	0.0681	0.0660	0.0639	0.0596	0.0977	0.1192	0.1200	0.1428
15 Ormenis	0.0075	0.0247	0.0305	0.0265	0.0246	0.0246	0.0246	0.0710	0.0734	0.0841	0.0987
16 Poblicia	0.0131	0.0306	0.0364	0.0324	0.0305	0.0304	0.0304	0.0773	0.0799	0.0907	0.1054
17 Prokelisia		0.0324	0.0344	0.0285	0.0265	0.0246	0.0284	0.0689	0.0755	0.0884	0.0987
18 Magicicada	17		0.0113	0.0247	0.0228	0.0227	0.0227	0.0734	0.0801	0.0908	0.1053
19 Cacama	18	6		0.0266	0.0246	0.0227	0.0304	0.0735	0.0843	0.0951	0.1099
20 Philya	15	13	14		0.0019	0.0113	0.0169	0.0648	0.0755	0.0886	0.1076
21 Platycotis	14	12	13	1		0.0094	0.0150	0.0628	0.0735	0.0865	0.1054
22 Erythroneura	13	12	12	6	5		0.0131	0.0607	0.0734	0.0821	0.1008
23 Clastoptera	15	12	16	9	8	7		0.0608	0.0735	0.0821	0.1008
24 Gelastocoris	35	37	37	33	32	31	31		0.0467	0.0676	0.1011
25 Abedus	38	40	42	38	37	37	37	24		0.0775	0.1037
26 Aquarius	44	45	47	44	43	41	41	34	39		0.1037
27 Boisea	49	52	54	53	52	50	50	50	51	51	

Fig. 4. Pairwise distances between species included in this study. *Below the diagonal* are absolute substitution differences calculated with the SHOWDIST command in PAUP. *Above the diagonal* are maximum-likelihood estimates calculated with the DNADIST program in PHYLIP, with the transition/transversion ratio of 2.1579.

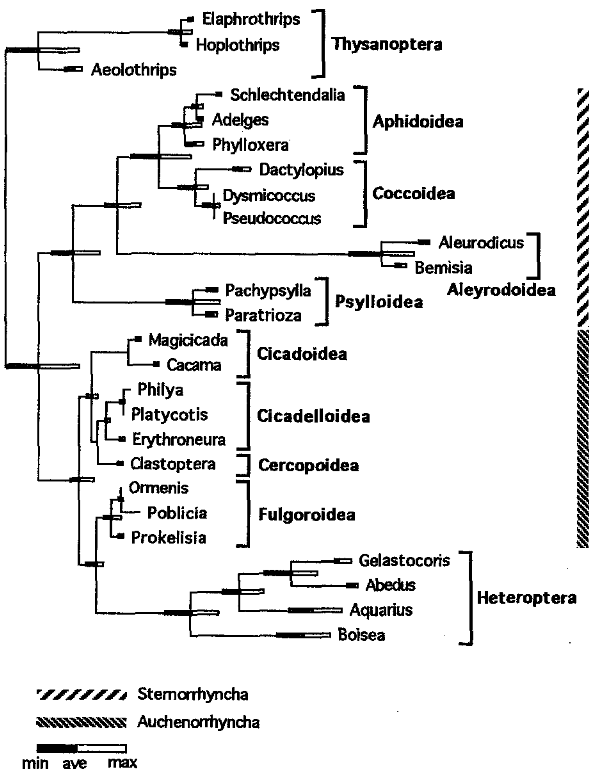

Fig. 6. Results of heuristic searches with the ectopsocid omitted, showing minimum, average, and maximum branch lengths under all equally parsimonious reconstructions. Topology shown is one of 36 most-parsimonious trees (length = 294, CI = 0.63, RI = 0.76, uninformative characters excluded). Trees differed with respect to the position of the Aleyrodoidea, relationships within the Aphidoidea and Heteroptera, and position of the Heteroptera with respect to the major auchenorrhynchan clades. When Aleyrodoidea were excluded, 16 most-parsimonious trees were recovered (length = 227, CI = 0.634, RI = 0.779), which were a subset of the topologies found with Aleyrodoidea included.

sponding to a conclusion of homopteran paraphyly), only a single homoplasious site falls within the overlapping region. Thus, the additional (nonoverlapping) sequence of Wheeler et al. adds the support of two sites to our conclusion of homopteran paraphyly.

Within the Sternorrhyncha, the lack of a sister-group relationship between Aleyrodoidea and Psylloidea was a departure from morphological evidence. Defined by a number of putative synapomorphies (Schlee 1969; Szelegiewicz 1971; Hennig 1981; Carver et al. 1991), the sister relationship of Aleyrodoidea and Psylloidea has never been questioned. In our study, the unusually high substitution rate in the aleyrodoid lineage was problematic for phylogenetic reconstruction: all methods agreed that Aleyrodoidea and Psylloidea were not sister taxa, but all disagreed on the exact placement of Aleyrodoidea. Because the neighbor-joining method tends to be more accurate under varying substitution rates than other methods (Nei 1991), the position of Aleyrodoidea as the most primitive sternorrhynchan lineage could be treated as a working hypothesis to be tested in future work.

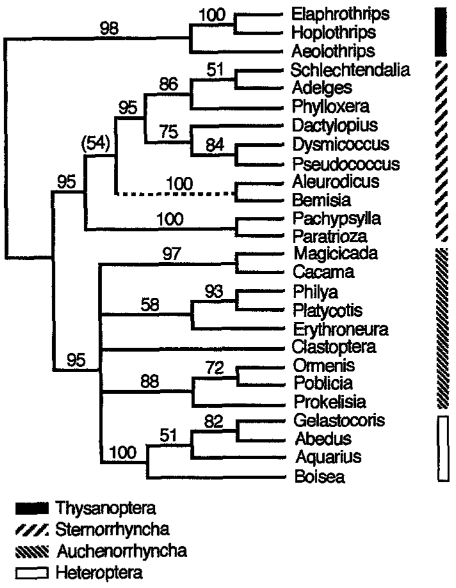

Fig. 7. Bootstrap consensus tree from parsimony searches (500 replicates). *Numbers* above the branches are bootstrap percentages resulting when Aleyrodoidea were omitted from the analyses. *Dotted line* shows the position of Aleyrodoidea when they were included; *value in parentheses* indicates support for the placement of Aleyrodoidea. (Relationships of the remaining taxa were the same with and without Aleyrodoidea included.)

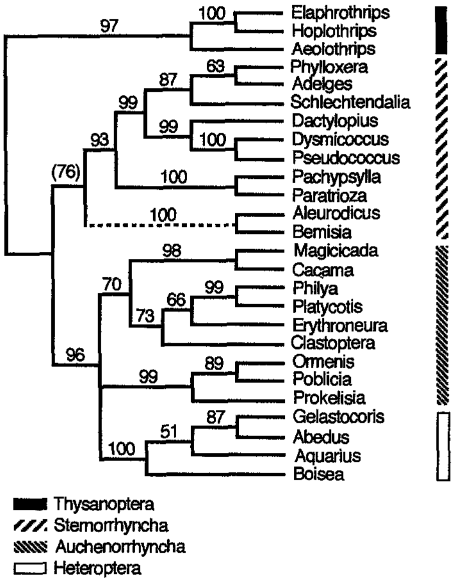

Fig. 8. Bootstrap consensus tree from the distance method (neighbor-joining, 500 replicates). *Numbers* above the branches are bootstrap percentages resulting when Aleyrodoidea were omitted from the analyses. *Dotted line* shows the position of Aleyrodoidea when they were included; *value in parentheses* indicates support for the placement of Aleyrodoidea. (Relationships of the remaining taxa were similar with and without Aleyrodoidea included.)

The 18S rDNA gene harbors phylogenetic information for diverse taxa, on a wide evolutionary scale (Hillis and Dixon 1991), despite growing evidence that the gene is evolving quite differently in different lineages (e.g., Sogin et al. 1986; Vossbrinck et al. 1987). The 18S genes of the Sternorrhyncha sequenced in this study clearly have experienced unique evolutionary events compared to Auchenorrhyncha, Hemiptera, and outgroup taxa. The extra sequence found in sternorrhynchans is consistent with the discovery that the 18S rDNA of the pea aphid (*Acyrthosiphon pisum*) is almost 500 base pairs longer than that of most other insects (Kwon et al. 1991). In the pea aphid, the extra, GC-rich sequence is found mainly in three variable regions, including the V2. We have discovered this phenomenon of extra sequence (as well as length and sequence variation and high GC content) in other variable regions of the entire 18S rDNA of Psylloidea, Coccoidea, and other Aphidoidea, but not in Auchenorrhyncha or Heteroptera (von Dohlen et al., in preparation). At least one other insect group, the Neuroptera, has a large insertion in a variable region, although in that case the insertion is AT-rich (Carmean et al. 1992). Such variable regions may contain phylogenetic information for more recent divergences, and they might also provide clues to previously unknown ribosomal functions.

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