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Paraphyly of Homoptera and Auchenorrhyncha inferred from 18S rDNA nucleotide sequences

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Abstract. Evolutionary affiliations of eighteen families of Hemiptera (*s.l.*) are inferred using molecular phylogenetic analysis of nucleotide (nt) sequences of 18S rDNAs. Exemplar taxa include: Archaeorrhyncha (=Fulgoromorpha): flatid, issid, dictyopharid, cixiid and delphacid; Prosorrhyncha (=Heteropterodea): Peloridiomorpha (=Coleorhyncha) — peloridiid, Heteroptera – gerrid, lygaeid and mirid; Clypeorrhyncha [=extant (monophyletic) cicadomorphs]: cicadid, cercopoids (cercopid, aphrophorid), membracid and cicadellids (deltocephaline and cicadelline); and Sternorrhyncha: psyllid, aleyrodid, diaspidid and aphid. Analysed sequences encompass a region beginning ≈550 nucleotides (nts) from the 5′-end to ≈200 nts upstream from the 3′-end of the gene [≈1150 base pairs (bp) in euhemipteran to >1400 bp in sternorrhynchan taxa]. Maximum parsimony and bootstrap analyses (PAUP) identify four principal hemipteran clades, Stenorrhyncha, Clypeorrhyncha, Archaeorrhyncha and Prosorrhyncha. These lineages are identified by synapomorphies distributed throughout the gene. Sternorrhyncha is a sister group to all other Hemiptera (i.e. Euhemiptera *sensu* Zrzavy), rendering Homoptera paraphyletic. Within Euhemiptera, clades Clypeorrhyncha, Archaeorrhyncha, Prosorrhyncha and Heteroptera are supported by one, three, two and three synapomorphic sites, respectively. There is equitable parsimonious inference for Archaeorrhyncha as the sister group to Prosorrhyncha (Neohemiptera *sensu* Sorensen *et al.*) or Clypeorrhyncha, in either case rendering Auchenorrhyncha paraphyletic. Neohemiptera is supported by one synapomorphy. Within Clypeorrhyncha, clade cicada + cercopoids is the sister group of the clade cicadellids + membracid (Membracoidea *sensu* Dietrich & Deitz). Among archaeorrhynchans, clade delphacid + cixiid is the sister group of the clade dictyopharid + flatid + issid. Within Prosorrhyncha, the peloridiid is sister to the Heteroptera. Within Heteroptera, gerrid is the sister group of the clade mirid + lygaeid (Panheteroptera *sensu* Schuh). Based on secondary structure of synonymous 18S rRNA, two synapomorphies each of Sternorrhyncha, Prosorrhyncha and Heteroptera are compensatory substitutions on stem substructures. All other synapomorphies identifying major lineages of Hemiptera are noncompensatory substitutions on either bulges or stems. Short basal internodal distances suggest radiation of hemipteran lineages at the suborder level occurred rapidly. Morphological, palaeoentomological and eco-evolutionary factors supporting the 18S rDNA-based phylogenetic tree are discussed.

Introduction

The ordinal classification and evolutionary affiliations of higher taxa in Hemiptera (true bugs) and Homoptera (planthoppers, leafhoppers, treehoppers, cicadas, spittlebugs, aphids, psyllids, scales, whiteflies, etc.) have been debated from the time Linneaus originally established these orders in 1758 on the basis of wing morphology. In 1775 Fabricius merged the orders into the Ryngota [later modified to Rhynchota (Burmeister, 1835)] based upon mouthpart structure. While in 1810 Latreille recognized Heteroptera and Homoptera as sections of his order Hemiptera (*sensu lato*). This latter hierarchical arrangement is largely accepted at present (for discussions see Henry & Froeschner, 1988).

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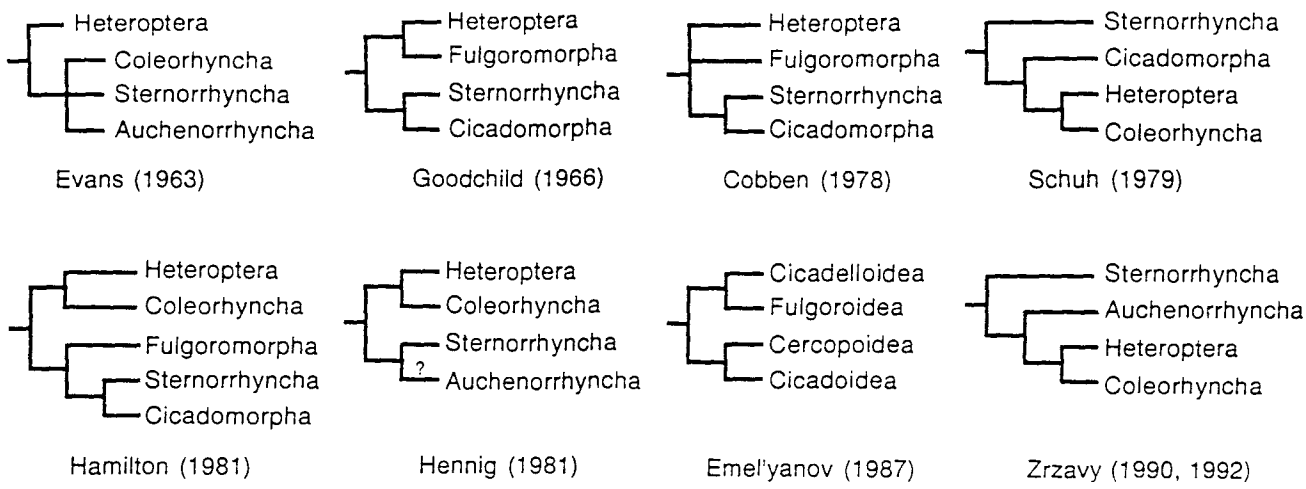

Fig. 1. Depiction of evolutionary affiliations of major lineages of Hemiptera (*s.l.*) inferred from morphologically-based studies by authors cited. [Reference to 'Schuh (1979)' refers to Schuh (1979) ex Fig. 1 in Wheeler *et al.* (1993).]

One interpretation, with the orders Hemiptera and Homoptera comprising Hemipteriforma (Metcalf, 1951), is still largely recognized in the United States.

Numerous studies using cladistic analysis of morphological characters indicate that Homoptera is not a monophyletic group (Kristensen, 1975; Hennig, 1981; Popov, 1981; Wootton & Betts, 1986; Minet & Bourgoin, 1986; Zrzavy, 1990, 1992). More recent molecular methods based on 18S rDNA nucleotide (nt) sequences (Wheeler *et al.*, 1993; Campbell *et al.*, 1994; Sorensen *et al.*, 1995) support morphologically based cladistic inferences that Sternorrhyncha is a sister-clade to all other Hemiptera (*s.l.*). Such findings, in combination with morphological evidence, support an increasingly accepted consensus to abandon the ordinal name Homoptera (e.g. Carver *et al.*, 1991).

Numerous post-Darwinian assessments of hemipteran genealogy at suborder and infraorder levels have been proposed (Fig. 1). Within the last half century various interpretations of fossils, placement of ocelli, morphological features of the head, genitalia, antennae and wings, and structure of the alimentary canal, have resulted in contradictory and non-congruent phylogenies. For example, Heteroptera was treated as the sister group of all other Hemiptera (*s.l.*), with Coleorhyncha and Sternorrhyncha as, each sister groups of Auchenorrhyncha (Cicadomorpha + Fulgoromorpha) (Evans, 1963). Goodchild (1966), using morphology and histology of the alimentary canal, placed the clade Heteroptera + Fulgoromorpha in a sister position to clade Sternorrhyncha + Cicadomorpha, with the position of Coleorhyncha unclear. Using cladistic analysis of mouthparts, Cobben (1978) placed both Heteroptera and Fulgoromorpha in a sister position to clade Sternorrhyncha + Cicadomorpha; Coleorhyncha were uninterpreted. Hennig (1981) placed the clade Heteroptera + Coleorhyncha (Heteropteroidea) (see Schlee, 1969) as the sister group of clade Sternorrhyncha + Auchenorrhyncha. However, he was not able to prove the monophyly of fulgoromorphs and cicadomorphs. Hamilton (1981), using mouthparts and features of the head, placed the clade Heteroptera + Coleorhyncha (Heteropteroidea *sensu* Schlee) in a sister position to clade Fulgoromorpha + (Sternorrhyncha + Cicadomorpha). Emely'yanov (1987) generally placed fulgoromorphs with cicadomorphs (i.e. Auchenorrhyncha) with the preferred arrangement being the clade Cicadelloidea + Fulgoroidea as the sister group of the clade Cercopoidea + Cicadoidea

Some morphological analyses showed paraphyly of Homoptera, with Sternorrhyncha the sister group of all other hemipterans (Fig. 1). In a different interpretation of a similar cladistic dataset as used by Cobben (1978), Schuh (1979) surmised that Sternorrhyncha may have a sister-group relationship to all other Hemiptera and that Cicadomorpha were a sister group of Heteropteroidea *sensu* Schlee. Eco-evolutionary history and antennal morphology of various groups of Hemiptera were construed to indicate that Sternorrhyncha was the sister group of the remaining hemipterans (Euhemiptera), with Auchenorrhyncha the sister group of Heteropterodea (=Heteropteroidea) (Zrzavy, 1990, 1992).

Certain studies have proposed different interpretations of genealogical affiliations of taxonomic groups within some of the major lineages of Hemiptera (*s.l.*). The palaeoentomological record did not necessarily support a monophyletic Auchenorrhyncha. Fossil evidence did not provide a clear picture of the evolution of fulgoromorphs, but suggested that they arose independently of a polyphyletic Cicadomorpha at the end of the early Permian (Shcherbakov, 1984). Recent anatomical interpretations of the tentorium and structures associated with reproductive organs have been invoked as probable synapomorphies for a monophyletic affiliation of fulgoromorphs and heteropterans (Bourgoin, 1985, 1986, 1988, 1993). Based on fossil evidence, Popov & Shcherbakov (1991) argued that Coleorhyncha and Heteroptera do not have an immediate common ancestor and descended independently from separate lineages of auchenorrhynchan ingruids. Cladistic analyses of morphological characters indicated that cercopoids are sisters to clade membracids + cicadellids (Membracoidea) (Dietrich & Deitz, 1993). Shcherbakov (1988) further interpreted that modern (extant) cicadomorphs and heteropterans are descendants of a common prosboloidean ancestor.

In several recent molecular studies based on nucleotide (nt)

sequences of the nuclear gene encoding 18S ribosomal RNA (18S rDNA) of exemplary hemipteran taxa. Sternorrhyncha was placed as the sister group of all other Hemiptera (Wheeler *et al.*, 1993; Campbell *et al.*, 1994; Sorensen *et al.*, 1995). Furthermore, molecular phylogenetic evidence (Campbell *et al.*, 1994) verified morphologically based evidence (Evans, 1963; Schuh, 1979; Hennig,'1981) that Sternorrhyncha (Psylloidea, Aleyrodoidea, Coccoidea and Aphidoidea) is monophyletic. Key molecular synapomorphies of Sternorrhyncha were discovered to be large expansion regions in 18S rDNAs synonymous with variable helices of 18S rRNAs (Kwon *et al.* 1991; Campbell *et al.*, 1993, 1994). Such considerably expanded regions are absent in variable helices of all other hemipterans.

Nucleotide sequences of conserved genes ($<2 \times 10^{-10}$ base substitutions/nt position · year) can be used to generate sizable character sets to infer ancient phylogenetic affiliations (e.g. Woese, 1987; Jorgensen & Cluster, 1988; Sogin, 1989; Hillis & Dixon, 1991; Turbeville *et al.*, 1991, 1992; Wainright *et al.*, 1993; Smothers *et al.*, 1994). Nuclear 18S rDNA has been found suitable to estimate ancient phylogenies of various groups of holometabolous insects (Wheeler, 1989; Carmean *et al.*, 1992; Martin & Pashley, 1992; Weller *et al.*, 1992; Whiting & Wheeler, 1994) and insects preserved in amber (DeSalle *et al.*, 1992; Cano *et al.*, 1993). Wheeler *et al.* (1993), using two relatively conserved fragments (≈680 nt) of 18S rDNA in concert with a morphological cladistic analysis, concluded that cicadomorphs (one cicada and two cicadellids in their analysis) comprised a sister clade to Heteropterodea *sensu* Zrzavy. The phylogenetic affiliation of fulgoromorphs was not evaluated in their study. Sorensen *et al.* (1995) used parsimony analysis of full-length sequences (>1950 nt) of 18S rDNA of euhemipterans, three cicadomorphs (a membracid, a cicada and an aphrophorid), a fulgoromorph (a delphacid) and heteropteran (a mirid). In this analysis, cicadomorphs were also placed in a sister position to Heteroptera. However, fulgoromorphs were placed in a clade with Heteroptera (and presumably Coleorhyncha). This finding rendered Auchenorrhyncha paraphyletic. To deal with taxonomic recognition of new monophyletic groups, Sorensen *et al.* (1995) established four suborders of Hemiptera (*s.l.*) and conformed their names using a -rrhyncha suffix. The suborder names of Sorensen *et al.* (1995) are as follows: Sternorrhyncha retained; Clypeorrhyncha ('shield-nose') for extant, monophyletic cicadomorphs [fossil interpretation of Cicadomorpha *sensu* Shcherbakov is polyphyletic]; Archaeorrhyncha ('ancient-nose') to include the extant fulgoromorphs; and Prosorrhyncha ('front' or 'forward nose'), as a replacement for Heteropterodea, to include clade Peloridiomorpha *sensu* Zrzavy (=Coleorhyncha) + Heteroptera. The clade incorporating fulgoromorphs + Heteropterodea was named Neohemiptera.

In the current study a broader assemblage of exemplary euhemipteran taxa is analysed to test more rigorously the support for phylogenetic arrangements deduced by earlier molecular studies (Wheeler *et al.*, 1993; Campbell *et al.*, 1994; Sorensen *et al.*, 1995). The expanded taxonomic dataset includes: a cicada, two cercopoids (a cercopid and an aphrophorid), a membracid, two cicadellids (a deltocephaline and a cicadelline), five archaeorrhynchans (a delphacid, a cixiid, a dictyopharid, an issid and a flatid), four prosorrhynchans (a peloridiid, and three heteropterans, a gerrid, a mirid and a lygaeid), and four stenorrhynchans (an aphid, whitefly, a psyllid and a scale insect). Phylogenetic inferences are based upon most parsimonious trees from datasets which include all nt sites and datasets that exercised step-wise exclusion (attenuation) of homoplasious sites or regions manifesting higher molecular clock tempos (Van de Peer *et al.*, 1993). The expanded number of taxa and attenuation of homoplasy enabled a rigorous assessment of genealogical polarity, synapomorphic sites, and level of nodal support for inferred topologies (Felsenstein, 1985; Li & Gouy, 1991).

Methods

Protocols used in this study were performed in the following order: collection of insects, isolation of genomic DNA, amplification of 18S rDNA by polymerase chain reaction (PCR) (Saiki *et al.*, 1988), cloning PCR-reaction products, selection of positive 18S rDNA-containing clones, nt sequencing, alignment of nt sequences, attenuation of variable regions and sites of respective datasets, and phylogenetic analysis using parsimony (PAUP) (Swofford, 1993).

Insects

Live insects were collected and stored at –70°C until extraction of genomic DNA (exceptions where noted). 18S rDNA nucleotide sequences of exemplary taxa included: HEMIPTERA: ARCHAEORRHYNCHA: Flatidae: *Siphanta acuta* (Walker) (GenBank acc. no. U06476), on *Acacia* sp., Berkeley, CA, 27.ix.1993. Issidae: *Hysteropterum severini* Caldwell & DeLong (GenBank acc. no. U15214), on milk thistle, Sacramento, CA, 20.v.1994. Dictyopharidae: *Scolops fumida* Uhler (GenBank acc. no. U15216), on ground in walnut orchard, Yuba City, CA, 8.vi.1994. Cixiidae: *Oliaruus hesperinus* Van Duzee (GenBank acc. no U15215), on walnut, Yuba City, CA 8.vi. 1994. Delphacidae: *Prokelista marginata* (Van Duzee) (GenBank acc. no. U09207), on *Spartina foliosa* Trin., Richmond, CA, 28.ix.1993. PROSORRHYNCHA: Peloridiidae: *Hemiodoecus leai* China [partial 18S rDNA sequence (Wheeler *et al.*, 1993)]. Heteroptera: Gerridae: *Aquarius remigis* Say (GenBank acc. no. U15691), from fresh-water stream, Anguin, CA, 12.iii.1994. Lygaeidae: *Oncopeltus fasciatus* (Dallas) (GenBank acc. no U15188), on milkweed, Sutter Buttes, Sutter Co., CA, 16.vi.1994. Miridae: *Lygus hesperus* Knight (GenBank acc. no. U06476), on alfalfa, Davis, CA, 20.ix.1993. CLYPEORRHYCHA: Cicadidae: *Okanagana utahensis* Davis (GenBank acc. no. U06478), on *Artemisia tridentata* Nuttall, Milford, CA, vii.1993. Cercopoidea: Cercopidae (Tomaspinae): *Prosapia plagiata* (Distant) (Gen Bank acc. no. U16264), Finca Sulza, Chiriqui Prov, Panama, 28.v.1994. Aphrophoridae: *Philaenus* (sp.) *spumarius* L. (identification based on a nymph) (GenBank acc. no. U06480), on geranium, Pinole, CA, 29.vi 1993. Membracidae: *Spissistilus festinus* (Say) (GenBank acc. no. U06477), on alfalfa, Davis, CA, 20.ix.1993. Cicadellidae: Deltocephalinae: *Euscelidius variegatus* Kirschbaum (GenBank acc. no. U15148), from a colony at Univ. of California, Berkeley, CA 26.i.1994, Cicadellinae: *Graphocephala atropunctata* (Signoret) (GenBank acc. no. U15213), from a colony at Univ. of California, Berkeley,

CA, 26.i.1994. STERNORRHYNCHA: Psyllidae: *Trioza eugeniae* Froggatt (GenBank acc. no. U06482), on *Eugenia* sp., Albany, CA, 7.iv.1993. Aleyrodidae: *Pealius kelloggii* (Bemis) (GenBank acc. no. U06479), on *Prunus lyoni* (Eastwood), Sacramento, CA, 4.v.1993. Diaspididae: *Aonidiella aurantii* (Maskell) (GenBank acc. no U06475), on *Laurus nobilis* L. Sacramento, CA, 14.vii. 1993. Aphididae: *Acyrthosiphon pisum* (Harris) [EMBL acc. no. X62623 (Kwon *et al.*, 1992)]. COLEOPTERA: Tenebrionidae: *Tenebrio molitor* L. [EMBL acc. no. X07801 (Hendriks *et al.*, 1988a)]. Voucher specimens are available from R.J.G. or J.T.S., at CDFA, Sacramento, CA.

Isolation of genomic DNA and PCR amplification

Total genomic DNA was purified by homogenizing whole insects or thoracic muscle of larger insects (e.g. cicada, lygaeid, etc.) using a pestle in microcentrifuge tubes to which was added 200 µl buffer [10 mM Tris (pH 8.0), 2.5 mM $MgCl_2$, 50 mM KCl], 200 µl phenol, and 20 µl 10% SDS. Phases were separated by centrifugation, and DNA was ethanol-precipitated and resuspended in 20 µM TE-buffer [10 µM Tris (pH 8.0), 1 mM EDTA]. All buffers, microcentrifuge tubes, and pestles were sterilized before use.

PCR was conducted in 25 µl reactions using components of the GeneAmp® Kit (Perkin Elmer Cetus, Norwalk, CT, U.S.A.) and contained 1 µl DNA template (≈100 ng), 2.5 µl PCR buffer, 0.5 µl each dNTP, 2 µl (50 nM) each respective forward and reverse primer, 0.125 µl *Taq* DNA polymerase (0.625 Units), and 15.375 µl water. Initially, amplification of full 18S rDNAs of all taxa by PCR was attempted. This, as well as sequencing, was successful in some taxa (i.e. psyllid, whitefly, scale insect, membracid, aphrophorid, cicada, flatid, delphacid and mirid – full-length 18S rDNA sequences deposited with GenBank), however, not successful for others. A matrix of forward and reverse PCR primers was tested until a suitable section comprising >1000 bases of the 18S rDNA of all taxa was amplified by PCR. This section comprised a central portion of the gene encompassing a region ≈550 nt downstream from the 5′-end to ≈200 nt upstream from the 3′-end. This portion was amplified using the following primers: forward primer, 5′-GCC·GCG·GTA·ATT·CCA·GCT-3′ (corresponding to base position nos. 587–604 of *T.molitor*); reverse primer, 5′-CGG·TGT·GTA·CAA·AGG·GCA·GGG-3′ (corresponding to reverse complement of base position nos. 1752–1772 of *T.molitor*). The PCR cycling programme was 30 s at 95°C followed by 39 cycles of 1 min at 95°C, 2 min at 50°C and 4 min at 74°C, and another 7 min at 74°C after the last cycle.

Cloning and sequencing

PCR products were cloned using plasmids and competent cells supplied in the TA Cloning™ System (Invitrogen, La Jolla, CA) and following procedures in the instruction manual. Cloning enabled separation of PCR-amplified 18S rDNAs of target taxa from other potentially contaminating genomes. Clones of potentially contaminating 18S rDNAs could be distinguished from target 18S rDNAs according to size (see Appendix) restriction digestion with *Eco* RI (Sambrook *et al.*, 1989), or ultimately by nt sequencing and examination of synapomorphic nucleotides (see Table 1). Inadvertent inclusion of nt sequences from contaminants has proved to be a problem in some molecular phylogenetic studies of insects in which cloning was not performed (e.g. see Derr *et al.*, 1992; Pashley & Ke, 1992). Accidental incorporation of contaminating nt sequences into a phylogenetic data matrix for studies of distantly related taxa [divergence > 100 million years ago (Mya)] can occur when short fragments of DNA (<600 bp), deficient in synapomorphic sites, are used. Cloned 18S rDNAs of a number of nonhemipteran contaminants [e.g. fungi, plant, nematodes, microarthropods (mites, parasitic wasps)] were detected in our study by performing similarity searches on sequences deposited with GenBank.

Top and bottom strands of 18S rDNAs were completely sequenced by the dideoxynucleotide-termination method (Sanger *et al.*, 1977), with a total of twelve internal primers, using components and protocols supplied with the Sequenase® ver 2.0 DNA Sequencing Kit and [α^{33}P]dATP (Amersham, Arlington Heights, IL). Stock cultures of 18S rDNA clones are maintained by BCC and JDS-C at USDA, Albany, CA.

Alignment of nucleotides and phylogenetic analysis

Nucleotide sequences were aligned using the 'DNA Alignment' subprogram of Gene Works™ v. 2.3.1 based on least cost maximum similarity (IntelliGenetics, Mountain View, CA). Because of different sizes of internal expansion regions among taxa between clades, the maximum diagonal offset was adjusted prior to alignment. The offset for alignment of all archaeorrhynchans was set at 10, except 35 for the delphacid, 60 for the gerrid, 45 for the remaining heteropterans and clypeorrhynchans, and 85 for the beetle. For alignment of sternorrhynchans, taxa having the largest and most variable expansion regions, the offset was adjusted as follows: psyllid 130, scale insect 150, and aphid and whitefly 200. The available 18S rDNA nucleotide sequence (≈550 nt) of a peloridiid, *H.leai* (Wheeler *et al.*, 1993) was aligned manually. The alignment of 18S rDNAs of all taxa is shown in the Appendix and served as the 'full' dataset.

In a second analysis, the phylogenetic dataset was attenuated by extracting variable regions and sites to reduce homoplasy and establish a dataset of sites having superior similarity of base substitution rates. Such attenuation can facilitate resolution of ancient phylogenies inferred from 18S rDNA sequences (Van de Peer *et al.*, 1993; Dixon & Hillis, 1993; Smothers *et al.*, 1994). Such sites are generally located in conserved regions that are responsible for maintaining secondary structure in synonymous rRNA necessary for a translationally active ribosome (Aagaard & Douthwaite, 1994; Kooi *et al.*, 1993). Phylogenetic inferences resulting from an analysis confined to sites in these conserved regions facilitate resolution of basal nodes (Vawter & Brown, 1993; Dixon & Hillis, 1993).

The largest aggregation of sites having discernibly high base-substitution rates was in expansion regions synonymous with variable helices E21 and 41 of 18S rRNAs (Kwon *et al.*, 1991) (Fig. 2). Large portions of nt sequences (some taxa >200 nt) in these regions could not be aligned coherently (viz <70% similarity; Hillis & Huelsenbeck, 1992) between taxa above the

superfamilial level, especially among sternorrhynchans (Appendix). Additional, smaller variable regions (2–20 bp) and sites were interspersed throughout the 18S rDNAs. These variable regions were typically indicated by telltale deletions/insertions (indels) in the nt alignment (Van de Peer *et al.*, 1993) and corresponded to some of twenty-four variable helices reported by De Rijk *et al.* (1992). Removal of ambiguously aligned sequences in datasets of distantly related taxa is justified by the fact that their homology within the secondary structure of synonymous rRNAs is unknown (Sogin & Elwood, 1986; Vawter & Brown, 1993). We adopted a practical and objective approach for extracting variable sites in the dataset by removing all nts on either side of an indel region until a site was reached where the same nt occurred in all taxa. Using this technique, the remaining aligned nucleotide sites were designated the 'attenuated' dataset.

In both the full and attenuated dataset analyses, a coleopteran served as an outgroup. Justification for using a beetle sequence was based upon fossil evidence (Hennig, 1981; Kukalova-Peck, 1991) and a molecularly based phylogenetic analysis (Carmean *et al.*, 1992) which showed the beetles comprise a basal lineage of Holometabola and, as endopterygotans, are in a sister clade to Hemipterodia. Both these neopteran lineages are well represented by Permian (≈250 Mya) fossils (Hennig, 1981; Labandeira & Sepkoski, 1993). Permian hemipterodians include ancestral thrips, psocids, cicadomorphs and psyllids (Morgan, 1984; Popov & Shcherbakov, 1991; Labandeira & Sepkoski, 1993). The available sequence of a psocid (Wheeler *et al.*, 1993) possessed too few nucleotides to serve as a suitable outgroup. Attempts to amplify the complete section of 18S rDNA used in this study of a psocid by PCR were unsuccessful.

To better resolve affiliations of basal lineages within the euhemipteran clade, an analysis was performed on a dataset consisting of sites lacking homoplasy between sternorrhynchan and euthemipteran lineages (in all trees to this point, a sternorrhynchan clade was sister to a euhemipteran clade). Outgroup polarization (Hennig, 1966) was used to anchor the tree in this analysis, and was achieved by first selecting symplesiomorphic sites between psyllid and beetle from informative sites of the attenuated dataset. Then, all homoplasious sites between sternorrhynchan and euhemipteran lineages were discarded. Remaining sites were analysed as the polarized dataset. A psyllid was chosen to represent the ancestral lineage of Sternorrhyncha based on molecular, physiological, feeding, reproductive and fossil criteria. First, all molecular analyses to this point indicated that sternorrhynchans had a sister relationship to Euhemiptera and that psyllids were the basal lineage of Sternorrhyncha (Campbell *et al.*, 1994; Sorensen *et al.*, 1995). Second, psyllids are deficient in a number of derived characteristics associated with other sternorrhynchans. Unlike other stenorrhynchans, psyllids retain all four Malpighian tubules, lack a well-developed sternorrhynchan-type filter chamber (embryologically and anatomically distinct from that found in clypeorrhynchans) (Goodchild, 1966), are not parthenogenetic (Evans, 1963), are not phloem specialists (Backus, 1988; Ullman & McLean, 1988), and have significantly shorter expansion regions in their 18S rDNA (Fig. 2) [large expansions in 18S rDNAs are generally construed to represent a derived condition within a common lineage (Kwon *et al.*, 1991)]. Moreover, psyllids are well represented by Permian fossils (250 Mya) (Hennig, 1981; Morgan, 1984). The earliest fossils of aphids and scales appear in the Triassic (≈220 Mya) (Heie, 1987; Shcherbakov & Wegierek, 1991) and whiteflies in the Cretaceous (≈135 Mya) (Schlee, 1970).

Phylogeny was analysed using algorithms provided in PAUP v. 3.1.1 for the Macintosh computer (Swofford, 1993). Only informative sites were included in the data matrix from each dataset. Because of the large number of taxa, an exhaustive search for shortest trees of the datasets was not feasible. Also, the large size of the data matrix in the full dataset rendered a branch-and-bound analysis unachievable on our computer (Mac IIci). To infer a topology of the full dataset, a bootstrap strict-consensus tree was determined using the heuristic option (1000 replicates). Gaps and deletions were scored as missing data. The attenuated dataset was analysed by 1000 bootstrap replicates using the branch-and-bound option. The inferred phylogeny using the polarized dataset was obtained by finding the shortest (most parsimonious) tree using a branch-and-bound search. This was accompanied by construction of 50% majority-rule consensus trees based on all trees one and two steps longer than the shortest tree. Analyses of all datasets included nonweighting and weighting of transitions versus transversions 1:10.

Results

Alignment of necleotide sequences

Alignment of nt sequences of the partial 18S rDNA region of all taxa analysed is shown in the Appendix. This partial 18S rDNA was composed of both conserved (core) regions and variable (expansion) regions homologous with variable helices in the secondary structure of synonymous 18S rRNA. Variable helices are interspersed throughout all 18S rRNAs examined to date (Neefs & De Wachter, 1990; De Rijk *et al.*, 1992; Van de Peet *et al.*, 1993). These regions possess differing base-substitution rates and, consequently, 18S rDNAs do not possess a homogenous molecular clock (Jorgensen & Cluster, 1988; Hillis & Dixon, 1991; Van de Peer *et al.*, 1993; Vawter & Brown, 1993). Variable regions are generally characterized by deleted sites in nucleotide alignments of disparate taxa (Van de Peer *et al.*, 1993). The two major variable regions encountered in our partial 18S rDNA sequences are synonymous with helices E21 and 41 of eukaryotic 18S rRNAs (Hendriks *et al.*, 1988b; Kwon *et al.*, 1991). The number of nucleotide sites comprising the E21 and 41 variable regions in our taxa varied considerably. The most significant difference in length of these helices occurs between sternorrhynchan and other insect taxa examined. These regions are significantly expanded in sternorrhynchans (Fig. 2). The length of E21 ranged from ≈400 nt in the psyllid to ≈600 nt in the whitefly; E21 in the scale insect and aphid were ≈500 nt, each. In comparison, E21 regions in all other taxa were ≈240 to ≈300 nt. The 18S rDNA region synonymous with helix 41 showed even a greater disparity between sternorrhynchan and other insect taxa. The 41 region of all sternorrhynchans was >240 nt, except for the psyllid, which had ≈160 nt. Contrastingly, all euhemipterans possessed ≈40 to ≈50 nt and the beetle 91 nt. These expansion regions in sternorrhynchan taxa are a reliable molecular synapomorphy of the clade. These expansions generate significantly longer primary structures of synonymous 18S rRNAs of sternorrhynchans compared to other insects studied to date. For example, lengths of full 18S rDNAs are ≈1900 nt in most insects, including

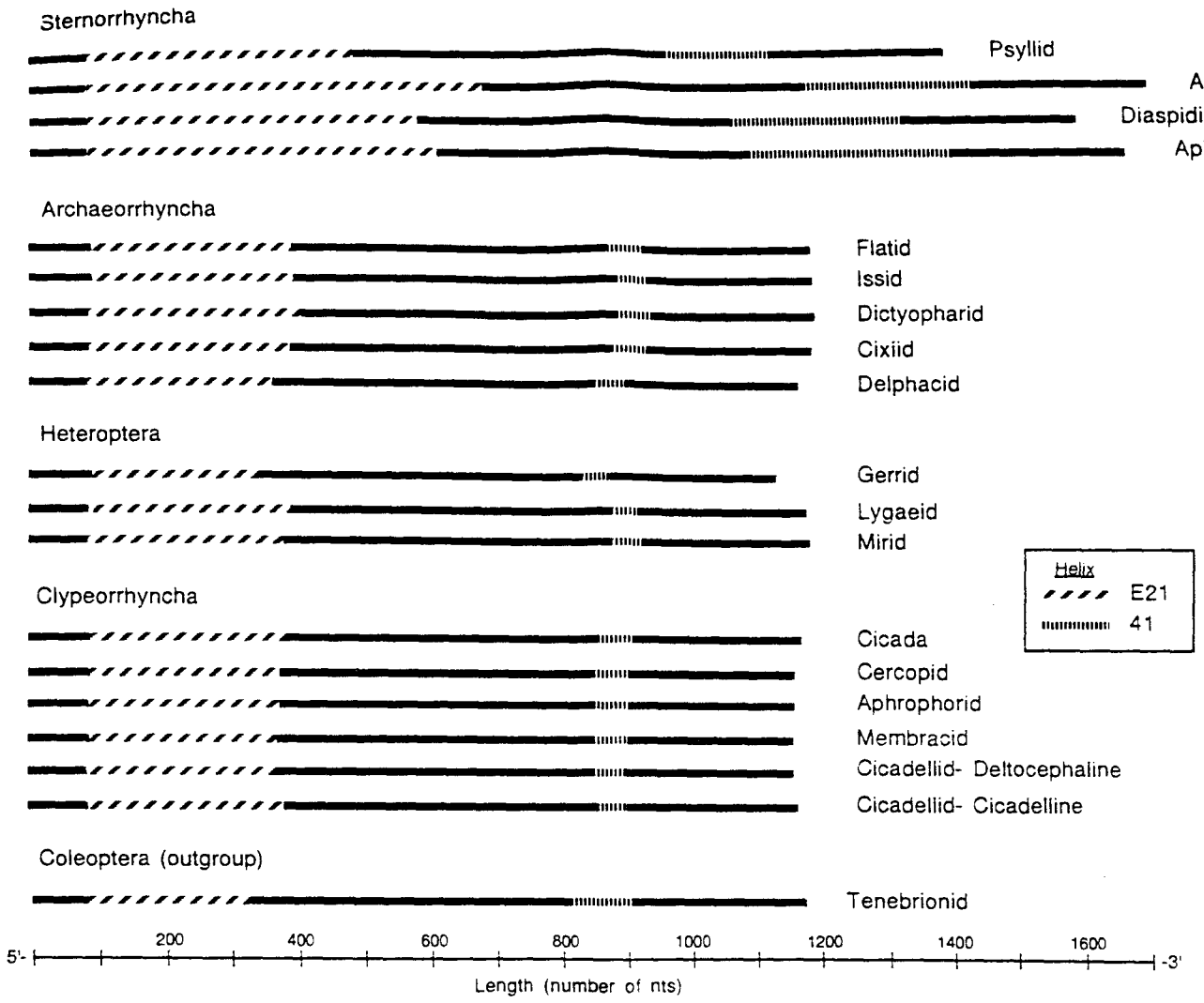

Fig. 2. Comparative lengths of 18S rDNAs used as the phylogenetic dataset. Exemplary insect taxa are grouped according to four major hemipteran lineages (Archaeorrhyncha, Heteroptera, Clypeorrhyncha and Sternorrhyncha) and the outgroup (Coleoptera). Solid black sections correspond to conserved regions of the gene wherein nucleotides (nts) can be unambiguously aligned between taxa. Hatched sections show variable regions of the gene that correspond to helices E21 (diagonal hatches) and 41 (vertical hatches) in the secondary structure of synonymous rRNAs proposed by Kwon *et al.* (1991). Scale indicates relative number of nucleotides (nts) from the 5'-end.

euhemipterans, compared with the full length of ≈250 nt of a whitefly (Campbell *et al.*, 1994) and an aphid (Kwon *et al.*, 1991). E21 and 41 helices of a strepsipteran, *Xenos vesparum* Rossius (EMBL acc. no. X77784, deposited by Chalwatzis *et al.*, January 1994), have also been found to be extremely expanded. However, strepsipteran expansions are extraordinarily A-T rich possessing poly A or poly T repeats. These expansions are readily distinguishable from expansions characteristic of sternorrhynchan E21 and 41 helices.

Within some hemipteran groups, lengths of E21 and 41 expansions varied (Fig. 2). Lengths of both E21 and 41 differed among sternorrhynchan taxa, with psyllid having the shortest and whitefly the longest expansions. Among the heteropterans, the E21 region of the gerrid was ≈40 nt shorter than the two panheteropteran taxa. Respective lengths of these expansions were similar among archaeorrhynchans with the exception of E21 in the delphacid, which was ≈25–35 nt shorter. Length variation of the E21 expansion among clypeorrhynchans was negligible (10 or fewer nt).

Phylogenetic analyses

The inferred genealogical proximities of hemipteran lineages according to analysis of the full dataset (all nucleotide sites) is represented in Fig. 3A. The full dataset consisted of 2058 sites. This tree shows a sternorrhynchan clade sister to a clade including all other hemipterans (Euhemiptera) having bootstrap support of 95% and 73%, respectively. This arrangement is in concordence with the morphological and molecular based phylogeny of Wheeler *et al.* (1993). Only 19% of reconstructed partitions supported Homoptera. Other strongly supported nodes (>90%) include individual clades for archaeorrhynchans (99%), prosorrhynchans (100%), heteropterans (100%) and clypeorrhynchans (95%) within the euhemipteran clade.

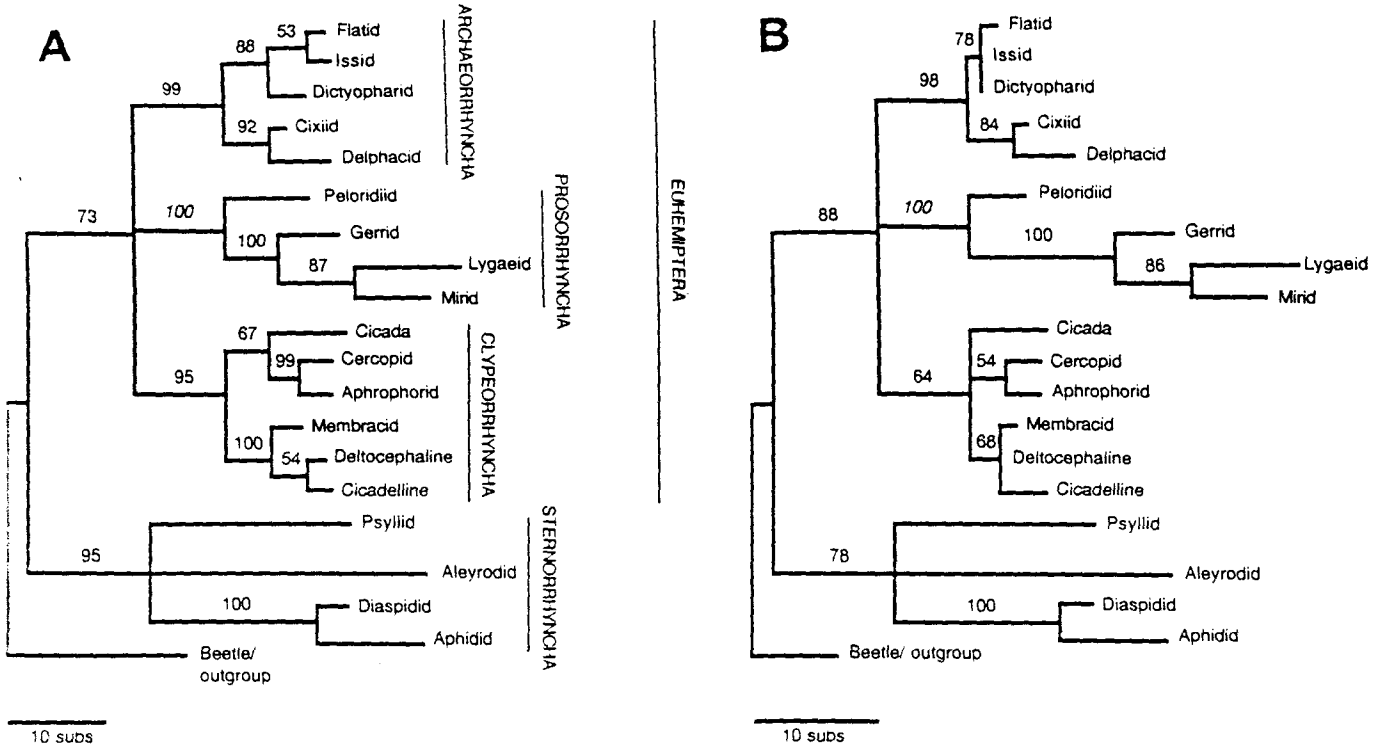

Fig. 3. Most parsimonious phylogenetic trees based on heuristic and branch and bound analysis (1000 replicates), respectively, of informative sites of full (A) and attenuated (B) datasets. Numbers above branches represent percentages of tree reconstructions supporting the corresponding node (bootstrap support). Support for prosorrhynchan node (italics) based on 50% majority rule consensus. Scale at bottom of each tree represents branch lengths equivalent to ten substitutions. Tree A: length = 823 steps; consistency index = 0.568; homoplasy index = 0.432; rescaled consistency index = 0.304; number of informative sites = 275. Tree B: length = 455 steps; consistency index = 0.654; homoplasy index = 0.346; rescaled consistency index = 0.351; number of informative sites = 75.

A number of distal affiliations are depicted within each of the major lineages. Among archaeorrhynchans, there is a separate clade (92%) of cixiid + delphacid, sister to a clade (88%) that includes the flatid, issid and dictyopharid. This arrangement of archaeorrhynchans supports that proposed by Asche (1988), Yang & Fang (1993) and Bourgoin (1993) based on morphological interpretations. The polarity of archaeorrhynchan lineages in our study (e.g. Fig. 3A) agrees with those proposed by Emel'yanov (1990) with the exception that his morphologically based study inferred delphacids basal and paraphyletic to cixiids. Among clypeorrhynchans, the membracoid clade (membracid + cicadellids) is strongly supported (100%) and agrees with morphologically based inferences by Dietrich & Deitz (1993). The membracoid clade is the sister group of a weakly supported (67%) clade of cicada as the sister group of a strongly supported (99%) cercopoid clade. Emel'yanov (1987) also inferred a cicadoid + cercopoid clade as the sister group of a cicadelloid clade among his three preferred trees (out of fifteen) based on ninety pairs of characters. However, fulgoromorphs were variously placed in each of these three trees. A significant synapomorphic trait that may support a cercopoid + cicada clade entails anatomical structures of the alimentary canal and specializations associated with the excretion of urine (Boulard, 1991). Among heteropterans, the gerrid is placed in a sister group relation to a panheteropteran clade, an arrangement in agreement with prior morphological (Schuh, 1979) and 18S rDNA (Wheeler *et al.*, 1993) inferences. There was also strong support (100%) for an Aphidiformes clade (aphids + scales) among sternorrhynchans (Hennig, 1981).

The full dataset analysis did not resolve the basal genealogy of the three major euhemipteran lineages. The archaeorrhynchan, clypeorrhynchan, and prosorrhynchan lineages were depicted as emanating from a trichotomous node (Fig. 3A). Only 13% of tree reconstructions supported Auchenorrhyncha, whereas, 32% and 33% of tree reconstruction suggested a Clypeorrhyncha + Prosorrhyncha or an Archaeorrhyncha + Prosorrhyncha clade, respectively.

Tree topology derived from analysis of the attenuated dataset (ambiguously aligned sites extracted) (Fig. 3B) agreed with topology inferred from analysis of the full dataset (Fig. 3A). The consistency index of the attenuated dataset was improved by ≈15%, but remained low. The bootstrap strict consensus tree showed a sternorrhynchan clade as the sister group of a euhemipteran clade. The bootstrap support for a sternorrhynchan clade (78%) was lower and the euhemipteran clade (88%) higher than calculated using the full dataset. Homoptera was inferred by only 2% of the trees. The affiliations of the three euhemipteran lineages continued to be represented as a trichotomy. An auchenorrhynchan clade was supported by only 17% and a Clypeorrhyncha + Prosorrhyncha or a Archaeorrhyncha + Prosorrhyncha clade were each supported by 32% of bootstrap trees. The inferred existence of archaeorrhynchan (98%), prosorrhynchan (100%) and heteropteran (100%) clades were

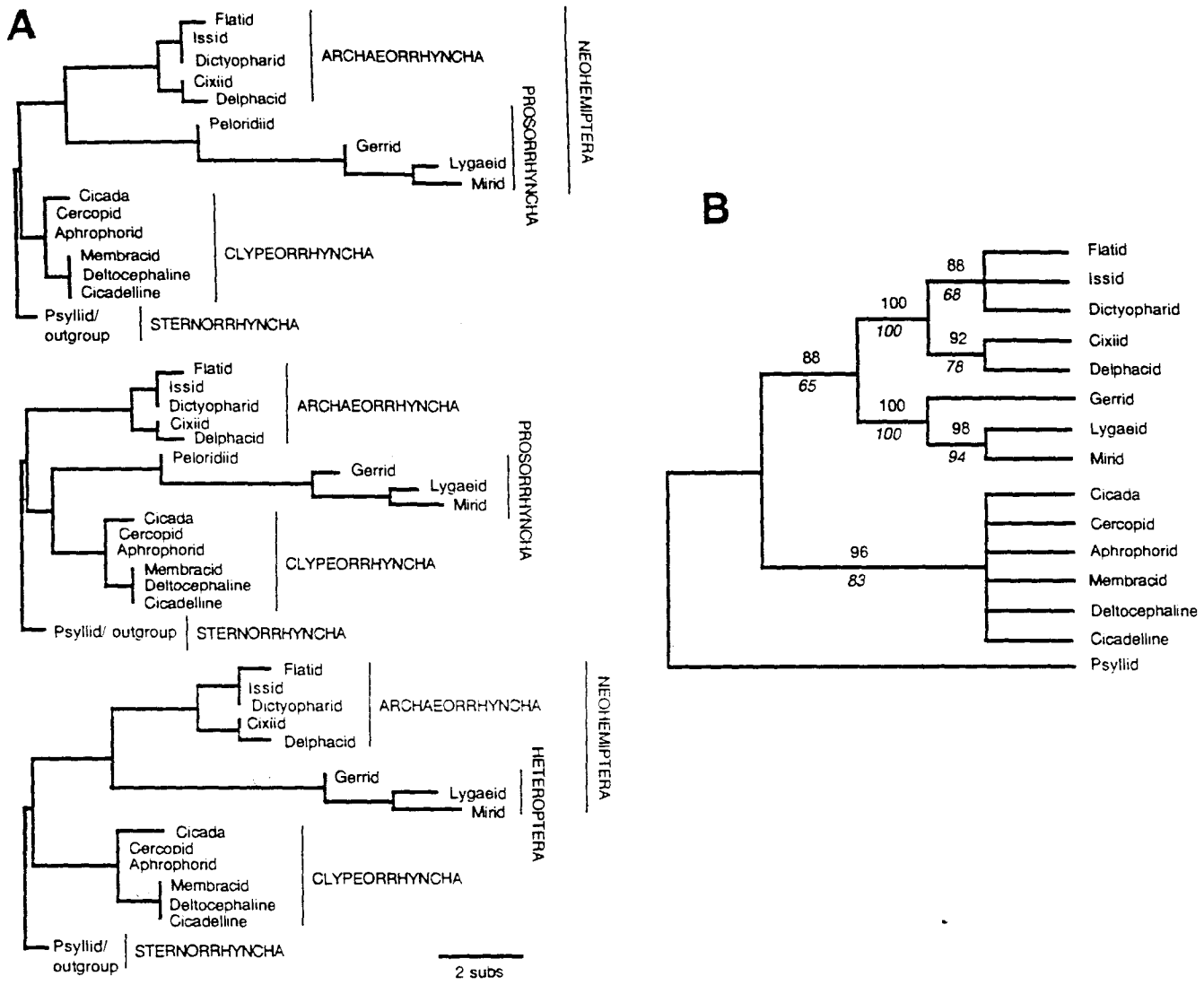

Fig. 4. Most parsimonious phylogenetic trees based on analysis of polarized dataset. (A) Top two trees generated from a branch and bound search including the peloridiid (tree lengths = 35; consistency indices = 0.812; homoplasy index = 0.188; rescaled consistency index = 0.740; number of informative sites = 24). Bottom tree generated excluding the peloridiid (tree length = 34; consistency index = 0.824; homoplasy index = 0.176; rescaled consistency index = 0.751). Scale equivalent to branch length of two substitutions. (B) 50% majority rule consensus tree of 49 and 767 trees and two steps longer, respectively, than shortest tree generated excluding the peloridiid. Percentages of tree reconstructions supporting corresponding node for 35- (roman) and 36- (italics) step trees appear above and below branches, respectively.

strongly supported, whereas that for a clypeorrhynchan clade (64%) was reduced compared to the full dataset analysis (Figs 3A and 3B). Within the clypeorrhynchan clade, cicada + cercopoid collapsed forming a trichotomy with the membracoid clade. Thus, attenuation resulted in forfeiture of phylogenetic information pertaining to distal genealogy. The inferred sister relationship of two archaeorrhynchan clades (cixiid + delphacid) + (dictyopharid + issid + flatid) was again well supported (Fig. 3B). Also, as in the full dataset analysis, the gerrid was placed in a sister position to a reasonably supported panheteropteran clade.

Removal of all ambiguously aligned sites from the full dataset resulted in minor reduction of homoplasy. However, despite extracting these sites, a large number of homoplasious sites shared between sternorrhynchan and euhemipteran lineages remained. 'Hennigean' approach of outgroup polarization (Hennig, 1966) achieved a significant reduction of this homoplasy. Use of only sites symplesiomorphic between psyllid and beetle and eliminating sites exhibiting homoplasy between sternorrhynchan and euhemipteran lineages improved the consistency index of the polarized dataset ≈45% over the full dataset.

Analysis of the polarized dataset was performed including and excluding the peloridiid to examine effects of unavailable sites (e.g. the neohemipteran synapomorphy, Table 1; Appendix) of this taxon on tree topology. One most parsimonious tree was generated excluding the peloridiid and two were generated including the peloridiid (Fig. 4A). In the two trees having the peloridiid, each tree supported distinct clypeorrhynchan, archaeorrhynchan and prosorrhynchan clades. Prosorrhyncha was inferred as sister to Archaeorrhyncha (viz Neohemiptera

Table 1. Location of synapomorphic sites that identify lineages of Hemiptera in substructures of synonymous rRNAs [based on secondary structure of 18S rRNA of *Acyrthosiphon pisum* (Harris) (Kwon *et al.*, 1991)].

Site [1]	Position[2]	Substitution[3]	Substructure[4]	Type[5]
Sternorrhyncha				
1349	1711	G→T**	Stem (40)	Noncomp.
1965	2207	T→C*	Stem (45)	Comp. (2228)
1986	2228	A→G*	Stem (45)	Comp. (2207)
Euhemiptera				
n/a	56	T→A*	Stem	Noncomp.
n/a	90	G→A*	Stem	Noncomp.
Clypeorrhyncha				
1840	2183	C→T*	Stem (43)	Noncomp.
Neohemiptera				
1353	1715	G→A*	Bulge (40)	Noncomp.
Archaeorrhyncha				
600	1073	A→T**	Stem (E21-7)	Noncomp.
1039	1418	A→T**	Stem (26)	Noncomp.
1103	1475	A→G*	Bulge (29)	Noncomp.
Prosorrhyncha				
1234	1606	C→T*	Stem (35)	Comp. (1643)
1271	1643	G→A*	Stem (35)	Comp. (1606)
Heteroptera				
64	696	A→T**	Stem (20)	Noncomp.
634	1107	C→T*	Stem (E21-8)	Noncomp.
934[6]	1315	G→A*	Bulge (24)	Noncomp.
1233[6]	1605	C→T*	Stem (35)	Comp. (1650)
1278[6]	1650	G→A*	Stem (35)	Comp. (1605)
1354	1716	C→T*	Stem (40)	Comp. (2033)
1788	2033	G→A*	Stem (40)	Comp. (1716)
1962	2204	G→A*	Bulge (45)	Noncomp.

[1] Site no. according to aligned nucleotide sequences of full dataset (Appendix).
[2] Homologous position of site within nucleotide sequence of synonymous 18S rRNA of *A.pisum*.
[3] Transitions = *; Transversions = **.
[4] Substructures as defined by Vawter & Brown (1993). Helix location in parentheses.
[5] Position of compensatory substitution (comp.) to maintain Watson-Crick pairing in parentheses.
[6] Prosorrhyncha and Heteroptera synapormorphic sites inclusive in Wheeler *et al.* (1993) dataset.

sensu Sorensen *et al.*) in one tree, and sister to Clypeorrhyncha in the other. Trees one or two steps longer than these most parsimonious trees showed practically no support for Auchenorrhyncha. Exclusion of the peloridiid from the dataset (for analysis of taxa where character states of all sites were known) yielded a singular most parsimonious tree inferring Neohemiptera (Fig. 4A). An assessment of all trees one step (forty-nine trees) and two steps (767 trees) longer, strongly supported the major clades of the most parsimonious tree (Fig. 4B). The neohemipteran clade was supported by 88% and 65% of the one- and two-step longer trees, respectively, whereas a clypeorrhynchan + heteropteran clade was supported by 10% of one- and 29% of two-step longer trees. Auchenorrhyncha was represented in none of the one-step longer trees and only one of 767 two-step longer trees. The archaeorrhynchan, clypeorrhynchan and heteropteran clades are each strongly supported by both one- and two-step longer trees. The sister group relationships of (cixiid + delphacid) and (dictyopharid + issid + flatid) clades within Archaeorrhyncha is inferred by the polarized dataset, consistent with the two longer datasets. Also, Gerridae as the sister group of the panheteropterans is strongly supported. However, the clypeorrhynchan clade collapsed to a sextachotomy after removal of faster clock-rate sites implying that superfamily-level lineages within Clypeorrhyncha diverged rapidly.

Synapomorphic sites

A number of synapomorphic sites within conserved regions of the gene identify the various hemipteran and euhemipteran lineages inferred by parsimony analysis (Table 1). These synapomorphic sites were additionally verified using large 18S rDNA sequence datasets of Holometabola (Carmean *et al.*, 1992), of Hemiptera (Wheeler *et al.*, 1993), and other insect taxa deposited with GenBank. There were over sixty-five taxa examined to confirm these synapomorphic sites. Two sites identifying Euhemiptera are located near the 5'-end of the gene (based on full-length 18S rDNA sequences). The three euhemipteran synapomorphic sites designated by Wheeler *et al.* (1993) in their

dataset are homoplasious when our wider range of taxa are incorporated. Location of remaining synapomorphic sites are designated in the Appendix. Base-position, type of substitution, and location in type of substructure of these sites within the secondary structure of synonymous 18S rRNA are listed in Table 1. Three synapomorphic sites identify Sternorrhyncha. Within Euhemiptera, one, one, three, two and three site(s) identify Clypeorrhyncha, Neohemiptera, Archaeorrhyncha, Prosorrhyncha and Heteroptera, respectively. The character state of the peloridiid at the neohemipteran synapomorphy was unavailable. Some of five additional sites that identify Heteroptera may also identify Prosorrhyncha in view of the fact that they are located beyond of the available peloridiid sequence. No synapomorphic sites identified Auchenorrhyncha.

Of twenty synapomorphic sites encompassed by the full dataset, four were transversions (Table 1). However, weighting of transitions and transversions 1:10 did not affect topologies or level of nodal support in any of the trees. Moreover, a transition/transversion bias is absent in 18S rDNAs (Vawter & Brown, 1993). Synapomorphies were also evaluated as to the type of substructure in which they were located. Base substitutions that occur within stem substructures are likely to be under selective influence. As such, compensatory substitutions are likely to maintain Watson-Crick pairing for a translationally functional ribosome (Aagaard & Douthwaite, 1994; Wheeler & Honeycut, 1988). Those substitutions not occurring on stems or which are not compensatory reflect a more random evolutionary event and, therefore, are considered to have greater phylogenetic weight (Dixon & Hillis, 1993; Vawter & Brown, 1993; Wheeler & Honeycutt, 1988). The neohemipteran identifier is situated in a bulge that lacks Watson-Crick pairing and cannot be a compensatory substitution, by definition. One of each of the synapomorphies identifying Clypeorrhyncha and Archaeorrhyncha are also situated in bulges. One sternorrhynchan, two euhemipteran, one clypeorrhynchan and two archaeorrhynchan synapomorphies were situated in stems with no evidence for compensatory substitution. This finding supports the contention that substitutions that occur in stem substructures are not necessarily compensatory (Dixon & Hillis, 1993). Two prosorrhynchan, four heteropteran and two sternorrhynchan synapomorphies were compensatory substitutions on helices, 35, 40 and 45 of the synonymous rRNA model (Table 1). Weighting of these eight compensatory sites as 50% of all other sites did not change inferred phylogenies.

Discussion

Our phylogenetic analysis of 18S rDNA sequences indicates that Homoptera is paraphyletic because Sternorrhyncha is the sister group of a clade that includes Clypeorrhyncha, Archaeorrhyncha and Prosorrhyncha (Euhemiptera *sensu* Zrzavy). This arrangement supports a number of recent inferences based on either morphological or molecular criteria concerning phylogenetic affiliations of major hemipteran lineages (Schuh, 1979; Zrzavy, 1990; Carver *et al.*, 1991; Wheeler *et al.*, 1993; Campbell *et al.*, 1994; Sorensen *et al.*, 1995).

Additionally, there is strong bootstrap support for four major hemipteran clades: Sternorrhyncha, Archaeorrhyncha, Prosorrhyncha and Clypeorrhyncha. Evidence of other phylogenetic affiliations within each of these clades concurs with certain morphologically-based inferences; within Clypeorrhyncha, Membracoidea (Dietrich & Deitz, 1993); within Heteroptera, Panheteroptera (Schuh, 1979; Wheeler *et al.*, 1993); within Sternorrhyncha, Aphidiformes (Hennig, 1981); and two separate clades within Archaeorrhyncha (Asche, 1988; Bourgoin, 1993). Possibly of greatest importance for adherents of traditional hemipteran systematics is the negligible support for the homopteran suborder Auchenorrhyncha.

Phylogenetic analysis of larger datasets of 18S rDNA sequences in our study did not clearly resolve the basal framework of Euhemiptera. Analysis of datasets that included all nucleotide sites (full) or all sites with ambiguously aligned sites removed (attenuated) showed that euhemipteran lineages formed a trichotomous node (Figs 3A and 3B). Inability to resolve clearly a phylogenetic framework for Euhemiptera using the larger datasets was in part due to a vast number of homoplasious sites shared between sternorrhynchan and euhemipteran taxa. This homoplasy may largely result from unequal evolutionary rates of the two lineages (Campbell *et al.*, 1994). Sternorrhynchans are generally smaller, have faster generation times and more generations/year than euhemipterans. These factors can contribute to different overall molecular clock rates in more rapidly evolving regions of rDNAs (Chao & Carr, 1993; Martin & Palumbi, 1993; Wu & Li, 1985). To overcome this problem a 'Hennigean' phylogenetic analysis of Euhemiptera using only nonhomoplasious sites between ingroup and outgroup lineages (viz between Euhemiptera and Sternorrhyncha) was employed. The ancestral nucleotide state was considered to be where psyllid and beetle were symplesiomorphic. The results of this analysis infer that Prosorrhyncha is either a sister group of Clypeorrhyncha or Archaeorrhyncha (Figs 4A and 4B). The latter arrangement is congruent with a prior phylogenetic study of a fewer taxa using full-length 18S rDNA sequences (Sorensen *et al.*, 1995). In that study the Archaeorrhyncha + Heteroptera clade was named Neohemiptera.

In addition to parsimonious support, our results also show a G to A transitional synapomorphic site identifying Neohemiptera. This site is not homoplasious with any outgroup taxon (sternorrhynchans and beetle) (site no. 1353; Appendix; Table 1). Despite the addition of eight phylogenetically disparate euhemipteran taxa to the number examined previously (Sorensen *et al.*, 1995), this neohemipteran site remained as a fixed synapomorphy. In contrast, no synapomorphic sites support either Auchenorrhyncha or a Clypeorrhyncha + Prosorrhyncha clade.

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ings. Composite analysis using datasets of several genes improves phylogenetic inference if sequence datasets are the sole bases of inferring phylogenetic affiliations (Degnan, 1993). One synapomorphic site might be construed as a tenuous inference of a neohemipteran clade that dethrones Auchenorrhyncha. However, parsimony analysis also recognized as a second but equitable possibility a monophyletic relationship between Clypeorrhyncha and Prossorhyncha, which complies with a fossil-based phylogeny (Shcherbakov, 1988). None of the parsimony analyses nor synapomorphies in our molecular studies support a clade recognizing Auchenorrhyncha. Hence, Auchenorrhyncha may be a taxonomically convenient but phylogenetically incoherent assemblage of insects. If this is truly the case, then morphological characters used to validate Auchenorrhyncha are *de facto* homoplasies or symplesiomorphies. As argued in Sorensen *et al.* (1995), auchenorrhynchan synapomorphies of aristate antennae and tymbal (see Carver *et al.*, 1991) are convergent characters. The presence of filiform antennae on a Cretaceous archaeorrhynchan fossil, *Megaleurodes megocellata* Hamilton (a characteristic of sternorrhynchan antennae) (Hamilton, 1990), the presence of a tymbal on a pentatomorph (Chapman, 1971) [a highly derived clade of Heteroptera (Wheeler *et al.*, 1993)] and the absence of a consensus on the homology of archaeorrhynchan and clypeorrhynchan tymbals (Ossiannilsson, 1949) undermine the weight of these characters as auchenorrhynchan synapomorphies. Interpreted fusion of veins ScP + R in forewings of auchenorrhynchans (Kukalova-Peck, 1983; Dworakowska, 1988) may constitute a synapomorphy that outweighs the molecularly based argument presented here. However, this assessment of auchenorrhynchan venation must be thoroughly re-examined in the light of a similar fusion which occurs in the coleopteran hindwing. Also, sclerite-origins of some of these veins are not conspicuous among the auchenorrhynchans studied. Moreover, such fusions may be under selective pressure to strengthen the flying wing (Sorensen *et al.*, 1995).

Considering the homoplasy inherent to hemipterans, including those preserved as fossils, convincing morphological synapomorphies that support Neohemiptera may be difficult to establish (as is the case for Auchenorrhyncha). Sorense *et al.* (1995) offered the following neohemipteran synapomorphies: (1) reduction of the lorum (Hamilton, 1981), (2) wing venation – fusion of forewing veins 1A + 2A and an elongated, longitudinally directed forewing vein CuA (Wootton & Betts, 1986), (3) microspines opposite fold of wing-coupling apparatus (D'Urso & Ippolito, 1994), and (4) of uncertain polarity, features of the alimentary canal associated with absence of a filter chamber (Goodchild, 1966). Bourgoin, independently of our study, speculated that Archaeorrhyncha and Heteroptera have a monophyltic relationship (peloridiids were not studied) and offered a number of anatomically based synapomorphies including: (1) internal pretentorinae: anterior arms of the tentorium join the mandibular levers (Bourgoin, 1986), (2) modifications to the aedeagus (Bourgoin, 1988; Bourgoin & Huang, 1990), (3) absence of accessory glands in female genitalia (Bourgoin, 1993), and (4) spermatheca divided into four chambers with an apical gland (Bourgoin, 1993). Further examinations, including that of the fossil record, may determine that these and other synapomorphies define Neohemiptera as monophyletic.

Although we were unable to obtain an exemplary coleorhynchan to sequence, the partial sequence from fragments of 18S rDNA of a peloridiid (Wheeler *et al.*, 1993) was sufficient to infer its affiliation among hemipteran lineages. Extant coleorhynchans are represented by members of a singular relictual family (Peloridiidae) of small, flattened, moss-feeding insects having a Neotropical-Australasian distribution (Burckhardt & Agosti, 1991). Interpretation of fossils suggests that a coleorhynchan lineage first appeared in the Upper Permian (Popov & Shcherbakov, 1991). The Coleorhyncha has been placed as the sister group of Heteroptera on morphological grounds (Schlee, 1969; Hamilton, 1981). Arguments placing Coleorhyncha in Homoptera include those based on interpretations of fossil history (Popov & Shcherbakov, 1991) and against morphological interpretations (Cobben, 1978). A recent interpretation of parsimony analysis of combined molecular and morphological datasets suggested a Coleorhyncha + Heteroptera (Heteropterodea) clade (Wheeler *et al.*, 1993). However, fulgoroid taxa were not analysed in that study. Our parsimony analysis here strongly supports a monophyletic relationship between peloridiids + heteropterans (viz Prosorrhyncha *sensu* Sorensen *et al.*, the new suborder name to replace Heteropterodea). This support is bolstered by the presence of at least two synapomorphic sites identifying Prosorrhyncha (Table 1). Additional morphological synapomorphies (see Sorensen *et al.*, 1995) supporting a monophyletic relationship between Coleorhyncha and Heteroptera include: (1) a gula or its rudiments (Hamilton, 1981), (2) a distinctive triangular mandibular lever (Hamilton, 1981), (3) non-aristoid reduction of the antennae (Schlee, 1969; Wheeler *et al.*, 1993), (4) certain wing characteristics (Wootton & Betts, 1986; Wheeler *et al.*, 1993), and (5) a number of abdominal characters (Schlee, 1969).

In analyses of full-length (Sorensen *et al.*, 1995) and partial 18S rDNA (Figs 3A, 3B and 4A), internodal distances inferring hemipteran radiation are either short or polytomous. This suggests that divergence of hemipteran lineages at suborder levels (as defined here) occurred near-simultaneously, in an evolutionary time-frame. Based on fossil evidence, this radiation probably occurred some time within or close to the Permian (Wootton, 1981). Whether this radiation is associated with catastrophic events that led to massive Permo-Triassic extinctions (Erwin, 1994) and a concomitant decline in terrestrial plant diversity (Shear, 1991) can only be conjectured. However, a clue to the basis of a punctuated cladogenesis of Hemiptera may be found in various feeding strategies adopted by the disparate hemipteran lineages (Zrzavy, 1990, 1992; Campbell *et al.*, 1994). Stylet-like mouthparts of hemipterans were probably originally adapted for plant feeding (Backus, 1988). This feeding strategy evolved prior to differentiation of plant vasculature in tracheophytes that occurred early in Triassic, followed later by appearance of pollen and seeds in angiosperms of the lower Cretaceous (Crane, 1989; Cronquist, 1988). Nonphloem ingestion may be a plesiomorphic trait in Hemiptera represented in cercopoids and cicadas, which feed on xylem. Psyllids, like other sternorrhynchans, probe plant tissue intercellularly and ingest from phloem but, like cicadas and cercopids, psyllids feed on xylem. Whiteflies, aphids and scales are specialists on phloem sap as are some more recently evolved lineages of archaeorrhynchans and panheteropterans (Backus, 1988; Wilson *et al.*, 1994). Hence, phloem feeding may be derived in hemipterans. Feeding on plant seeds and pollen

(recent phytophagous heteroptera, such as lygus bug) reflects the appearance of flowering plants (angiosperms). Phytophagous heteropteran lineages are the most recent of all hemipteran infraorders (Wheeler *et al.*, 1993). This parallel radiation of hemipteran and tracheophyte lineages probably best explains why few extant hemipterans feed on plants from primitive lignophyte lineages such as gymnosperms or pteridophytes. The few hemipterans that do feed on conifers, etc., are probably derived from angiosperm-feeding ancestors. Alternatively, putative basal lineages of Heteroptera (e.g. enicocephalids; Wheeler *et al.*, 1993) and Archaeorrhyncha (e.g. tettigometrids, cixiids; Wilson *et al.*, 1994) are represented by predatory and root- or fungus-feeding insects, respectively, whereas coleorhynchans are moss feeders. Thus, each major hemipteran lineage manifests an autonomous ancestral feeding strategy that reflects independent adaptation to newly generated niches.

The basal genealogical proximities of major lineages of Hemiptera (*s.l.*) have been an enigma for over a century. Myriad studies that included interpretations of fossils, morphology and anatomy have resulted in numerous incongruent phylogenies (Fig. 1). This quandary may reflect radiation of these lineages within a brief evolutionary time-frame that dispensed few synapomorphies to differentiate basal nodes. Irrefutable resolution of these affiliations, regardless of the technique employed (fossils, morphology, DNA, etc.), may prove to be difficult. The few number of synapomorphic sites which support individual lineages in the basal genealogy of Hemiptera are distributed in a somewhat cluster-like fashion throughout the 18S rRNA gene (Table 1). Consequently, inferring phylogenetic affiliations using short sections (600–800 bp) of the gene may prove to be unreliable for such rapidly radiating and ancient (>200 Mya) lineages, and adding taxa to the dataset will not ameliorate the situation. Our study, based on >1100 homologous nucleotide sites, may offer some resolution and we conclude the following: (1) there are four main monophyletic lineages within Hemiptera (i.e. Sternorrhyncha, Clypeorrhyncha, Archaeorrhyncha and Prosorrhyncha), (2) Homoptera is paraphyletic in that Sternorrhyncha is the sister group of all other Hemiptera (Euhemiptera *sensu* Zrzavy), (3) there is little or no support for a monophyletic Auchenorrhyncha, (4) peloridiids and heteropterans are monophyletic, and (5) genealogical radiation of the four major hemipteran lineages from a theoretical common ancestor was rapid.

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endix

ignment of 18S rDNA nucleotide sites that served as the full dataset. Top sequence of each block of 100 aligned sites in the consensus ons). Taxa are abbreviated as first letter of genus and first three letters of species names (e.g. *Siphanta acuta* = Sacu, etc.) and are ed as follows: ARCHAEORRHYNCHA: Sacu = flatid; Hsev = issid; Sfum = dictyopharid; Ohes = cixiid; Pmar = delphacid. OSORRHYNCHA: Hlea = peloridiid†, Heteroptera: Arem = gerrid; Ofas = lygaeid; Lhes = mirid. CLYPEORRHYNCHA: Outa = ada; Ppla = cercopid; Pspu = aphrophorid; Sfes = membracid; Evar = cicadellid (deltocephaline); Gatr = cicadellid (cicadelline). TERNORRHYNCHA: Teug = psyllid; Pkel = aleyrodid; Aaur = diaspidid; Apis = aphidid. Coleoptera: Tmol = beetle/outgroup. nambiguously alignable sites (see text) are underlined in consensus sequence and served as attenuated dataset. Nonhomoplasious formative sites (see text) that served as the polarized dataset are designated by an asterisk (*) or as letters to denote synapomorphies r euhemipteran lineages (C = Clypeorrhyncha, N = Neohemiptera, A = Archaeorrhyncha, P = Prosorrhyncha, and H = Heteroptera) bove the consensus sequence. Synapomorphic sites for Sternorrhyncha are designated by S. Insertions or deletions (indels) in alignent are denoted by a hyphen (-). Unavailable character states of Hlea sequence are blank. Nucleotide ambiguity codes: R = A or G; = C or T; M = C or A; K = T or G; W = T or A; S = C or G; B = not A; D = not C; H = not G; V = not T; N = either A, C, G or T. † Partial S rDNA sequence of peloridiid, *Hemiodoecus leai* China, from Wheeler *et al.* (1993).

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                                                                            H     *                  **
Cons  CGCGGYAATT CCAGCTYCAA YMGCGTATAY TAAWGYTRTT GYGGTTAAAA AGCTCGTAGT YRRWWBTGYG TBYKNRYDVY GHMKGWYCRY CMTGTGCKGY
Sacu  .....T.... ......C... TA.......T ...A.T.G.. .C........ .......... TGGATC..T. .CC-CGCACC .TCT.TT.AC .-------.C
Hsev  .....T.... ......C... TA.......T ...A.T.G.. .C........ .......... TGGATC..T. .CC-CGCGCC .TCG.TT.AC .-------.C
Sfum  .....T.... ......C... TA.......T ...A.T.G.. .C........ .......... TGGATC..T. .CC-CGCGCC .TCG.TT.AC .-------.C
Ohes  .....T.... ......C... TA.......T ...A.T.G.. .C........ .......... TGGATC..T. .CC-CGCGCC .TCG.TT.AC .-------.C
Pmar  .....T.... ......C... TA.......T ...A.T.G.. .C........ .......... TGGATC..T. .CC---TTCC .CAG.TT.AC .-------.C
Hlea  .....T.... ......C... TA.......T ...A.T.G.. .C.....  [3'-end of "A" fragment (Wheeler et al., 1993)]
Arem  .....T.... ......C... TA.......T ...A.C.G.. .C........ .......... TGGTTC..T. .GC-CACGCT .TCG.TT.AC .-------.C
Ofas  .....T.... ......C... TA.......T ...A.T.G.. .C........ .......... TGGTTC..C. .GC-CACGCT .TCG.TT.AC .-------.C
Lhes  .....T.... ......T... TA.......T ...A.T.G.. .C........ .......... TGGTTC..C. .GC-CACGCT .TCG.TT.GC .-------.C
Outa  .....T.... ......C... TA.......T ...A.T.G.. .C........ .......... TGGATC..T. .GC-CACGCT .TCG.TT.AC .-------.C
Ppla  .....T.... ......C... TA.......T ...A.T.G.. .C........ .......... TGGACC..T. .TC-CACGCT .TCG.TT.GC .-------.C
Pspu  .....T.... ......C... TA.......T ...A.T.G.. .C........ .......... TGGATC..T. .TC-CACGCT .TCG.TT.GC .-------.C
Sfes  .....C.... ......C... TA.......T ...A.T.G.. .C........ .......... TGGATC..T. .CT-AACGGT .TCG.TT.AC .-------.C
Evar  .....T.... ......C... TA.......T ...A.T.G.. .C........ .......... TGGATC..T. .CT-TACGAT .TCG.TT.AC .-------.C
Gatr  .....T.... ......C... TA.......T ...A.T.G.. .C........ .......... TGGATC..T. .CT-TACGAT .TCG.TT.AC .-------.C
Teug  .....T.... ......C... TA.......T ...A.T.G.. .T........ .......... TGGATG..T. .CT-CGCGGT .TCG.TT.AC .-------.C
Pkel  .....T.... ......C... CA.......C ...T.T.G.. .C........ .......... CAGAAT..T. .TC-CGCGCC .ACG.TT.G- ----------
Aaur  .....T.... ......C... TA.......T ...A.T.G.. .C........ .......... CGGATC..T. .CCTCGCGAC .TCG.AC.AC .A.....T.C
Apis  .....T.... ......C... TC.......T ...A.T.A.. .C........ .......... CGGATC..T. .CTGGACGGT .CCG.AC.AC .C..-..G.T
Tmol  .....T.... ......C... TA.......T ...A.T.G.. .C........ .......... CGAATC..T. .CC-CGCGCC .CCG.TT.AT .-------.T

Cons  YACGGYCTBY GYGHGCSGYC AGGTGYYYGH NHCYGRCRYG BHYNRYKRVA YRTCYBGYCG GWGVCBGKDN NGDCDCGYNN NYNMGYGBGY KYRNNNCNYR
Sacu  C--------- -T.T-.G.T- --..-T---C AA.T.G.GT. TA-TGCGGG. CG..CT.C.. .T.G-C.GTC C.G-----TT CCCC.----- TTGGCA.GTA
Hsev  C--------- -T.T-.G.T- --..-T---C AA.T.G.GT. TA-TGCGGG. CG..CT.C.. .T.G-C.GTC C.G-----TT CCCC.----- GCGGTA.GTA
Sfum  C--------- -T.T-.G.T- --..-T---C AA.T.G.GT. TA-TGCGGG. CG..CT.C.. .T.G-C.GTT C.G-----TC CCGC.----- GTAGCA.GTA
Ohes  C--------- -T.T-.G.T- --..-T---A AA.T.G.GT. TA-TGCGGG. CG..CT.T.. .T.G-C.GAA C.G-----GG CTTC.----- TCAAAC.GTA
Pmar  C--------- -T.T-.G.T- --.--C---C AA.T.G.GT- TT-TGCGGG. CG..CT.T.. .T.G-T.--- T.-------- -TCA.----- --GAAT.TTA
Hlea
Arem  C--------- -T.C-.G.T- --.------C AA.T.A.AT. T--CGTGGG. TG..TT.C.. .A.G--.GGC C.A-----AA CCC------- ----TT.--A
Ofas  C--------- -T.C-.G.T- --.------C AA.T.G.AT. C--CGTGGC. TG..CT.T.. .T.G-C.GAT A.G-----CC TCCC.----- GCGTGC.CTA
Lhes  C--------- -T.C-.G.T- --.------  AA.T.G.AT. C--CGTGGC. TG..CT.T.. .T.G-C.TTC G.G-----AG CCTC.----- TC------TA
Outa  C--------- -C.T-.G.T- --.------C AA.T.G.AT. TC-TGTGGG. CG..CT.C.. .T.G-C.G-- ---------- .CAC.----- G---------
Ppla  C--------- -C.T-.G.T- --.------C AA.T.G.AT. TCCTGTGGA. CG..CT.C.. .T.G-C.GTC C.G-----GC TTTC.----- GCT-TG.GTA
Pspu  C--------- -C.T-.G.T- --.------T AA.T.G.AT. CC-TGTGGA. CG..CT.C.. .T.C-C.GTC C.G-----GC TCTC.----- GTG-AG.GTA
Sfes  T--------- -C.--.G.T- --.------  AA.T.G.GC. CC-GGTTGG. CG..CT.C.. .T.G-G.GTG C.G-----GC TCTC.----- G----T.---
Evar  T--------- -C.--.G.T- ---------C AA.T.G.AT. TCTTGTGGG. CG..CT.T.. .T.G-T.GTC C.T-----GC CCTC.----- G------ATA
Gatr  T--------- -C.--.G.T- ---------T AA.T.G.AT. TCCTGTGGG. CG..CT.C.. .T.G-T.GTC C.G-----GC TCGC.----- GT------TA
Teug  T--------- -C.A-.G.C- -....T---T AA.T.G.GC. TC-AGCGAA. TG..CT.C.. .T.G.C.GTT C.G.T..CTT CTTC.T.C.C GCACGA.GCA
Pkel  -....C..T- -C.T..C.T- ---..C---A CA.C.A.GC. T--TACGA-. CA..CG.C.. .T.G.-.GTA C.G.G.---C GTCC.C.G.C TCG---.CCG
Aaur  C....T..CC .T.C..G.C. .....TTT.T TC.C.G.GT. TC-AGCGGG. CG..CT.C.. .T-A.-.G-- -.A.A..TG- -TCC.T-T.T G----G.G--
Apis  C-.-.T.-GT .T-C..G.C. -..---CC.T GT.--G.G-. GACCACGG-- TG..CC.T.. .--------- ---------- ---------- ----------
Tmol  T--------- -C.--.G.T- --.------T AA.T.G.GT. C--CGCGGG. CG..CT.C.. .T.G------ ---------- ----.----- ------.TTA

Cons  TYCYTCDGAT BRNYNKNRSY DBYGNBYHBS VNGBHSSYYD NNGYYHBNRN HDBNYKBCSN BRYVYHRHYB BDYYRMGGTG CRKWGAYNDN GHNNDNCYYD
Sacu  -------.-- GGGTCGT-CC -CC.--CTCG C----G--CG TC.TCCCCGA AGGCCTT.GC GGTGTA-TCC GGCCGC---- ------CTTT -TCGAT.CCG
Hsev  -------.-- GGGTCGT-CC -TT.-CCTTG C--TTG--CG TC.T-CCCGA AGGCCGC.GC GGTGTT-TCC GGCCGC--.-- ------CTTT -TCGTA.CCG
Sfum  -------.-- GGTTCGG-CC AGC.-GTTCG CC.TTG--CG TT.T-CCTGA AGTCCTT.GC GGTGCA-TCC GGCTGC---- ------CTTT -TCAAT.CCG
Ohes  -------.-- GGGCAGT-CC AGT--CCTC- ----TG--CG AC-TCCCTGA AGGCTGG.GT -ATGCT-TCC GGCCGC---- ------CTTG -TCAAT.CCG
Pmar  -------.-- ---TTG---- --T.-CTATG CT-TTG--CT TA.T----GC AG--C-T.-T G--GTT-TCG GGCCAC---- ------TTTC -ATAAT.CCG
Hlea
Arem  -------.-- CGGTC-T-CC GTT.---TTG -G.CT---CA -G.TCCTCGC A--------- -----A-T-- -GCTGC---- ------C--- ----A-----
Ofas  ------T.-- CGGTATA-CC AGC.TGTAGG CT.CTG--CG CC.-----G- -GGGTGT.CT --TGTA--C- -GCCGA---- ------CT-- --CAAT.CTA
Lhes  ------G.-- TGACGGC-CC -TT.-CCAC- AA.CT---TG GT.TCC--GG AGGCCG--GG GGTATA-CTC GTTCGC---- ------CTAC -TCTAT.CTA
Outa  -------.-- CGCCCGG-CC -GT.CGTAGG --.--G--CA -C.TCCCCGA AGCACGC.GG CGCGTC-CCG GTCCGC---- ------CGAT -CCAAT.CCG
Ppla  ------A.-- GGCCCACGCC --------CG AA.CAG---- --.C-CGAAC AG-------- -----T-ACC GGCCGC---- ------CGGT -CCAAT.CCG
Pspu  -------.-- GGTTC--GCC --------TG AA.CTG---- --.T-CTTGT AG-------- -----T-TCC GGCCGC---- ------CGGT -CATAT.CCG
Sfes  ---------- ---TTGT-CT A--------G --.TT----- -C.---CCGA AGCATGC.GT GA-GT--TCC GGCCGC---- ------CGAT -TCAAT.CCG
Evar  ------T.-- TG-TAG---- ---------G --.TT----- -C.--CCCGA AGCATGC.GT GG-GT--TCC GGCTGC---- ------CGAT -TCAAT.CCG
Gatr  ------T.-- TG-TAG---- ---------G --.TT----- -C.--CCCGA AGCATTC.GA GG-G---TCT GGCCGC---- ------CGAT -TCAAT.CCG
Teug  .T.T..G... TGCTCGTGCC GCC.AGCTGC GC.TCGGCCG TC.CCCTGGC TTCGCGG.CA GGTGTTGTCC TGTCGC.... .GGA..CGAC .TCGGC.TCG
Pkel  .C.T..T.-- GACTCGGGGT TGC.GTTCCG --.CCCCTCG CC.TCAGTGT CGTTCGC.CA TATCCCATTG CACT-C-... .ATT..TCAA .CGCGG.CTT
Aaur  --.C..-.-- CG-TCGCAC- ---.-----G AA.GCGGCCG CC.TTT---A AA-------- -------TCT G--CGC-... .G------AT .-----.CC-
Apis  ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---CGC...- .G-------- -------CCG
Tmol  ---------- ---------- ---------- --.CT----- -C.T----G- AGG------- ---G-----C GGC--C---- ------CAAC -TCAAT.CCG
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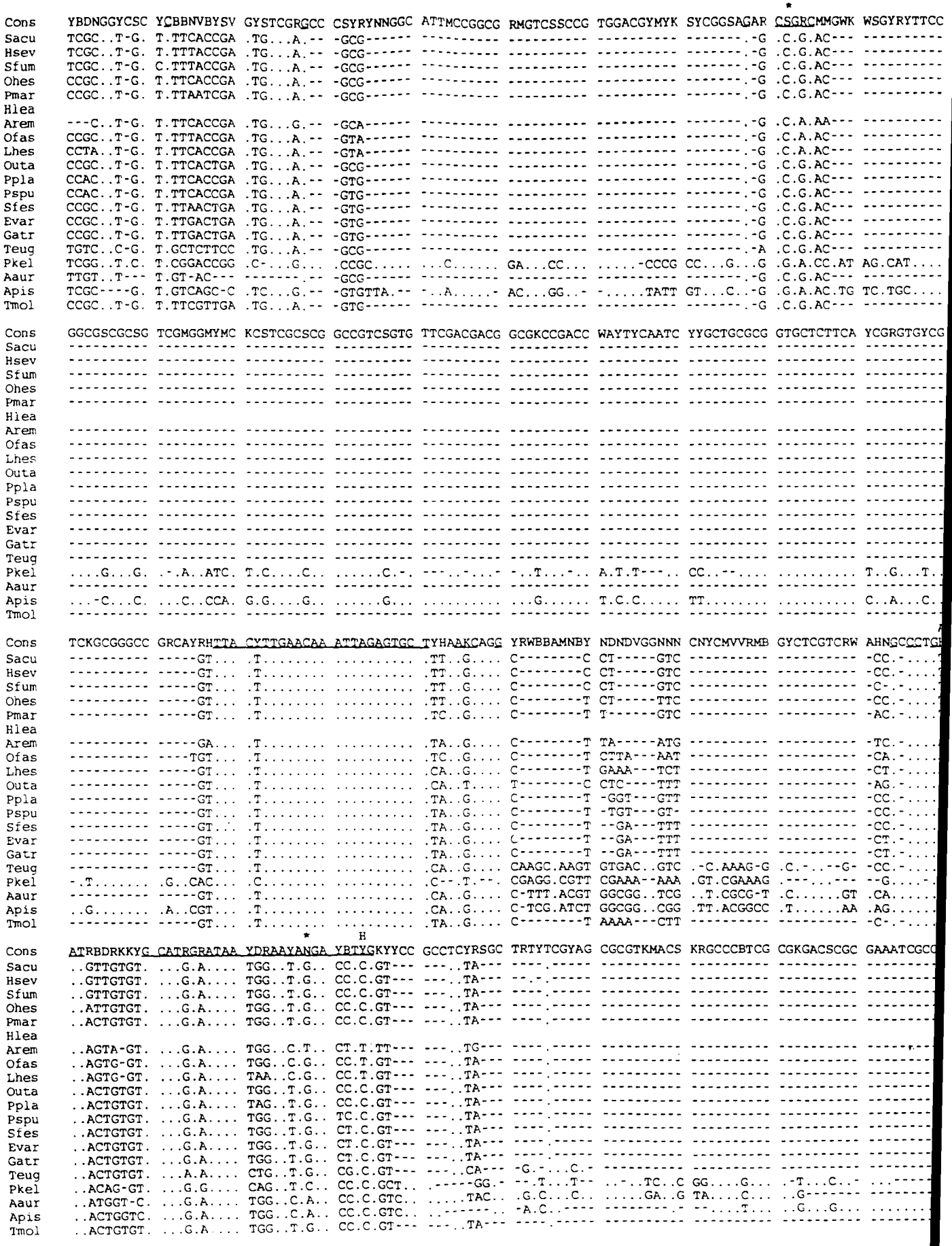
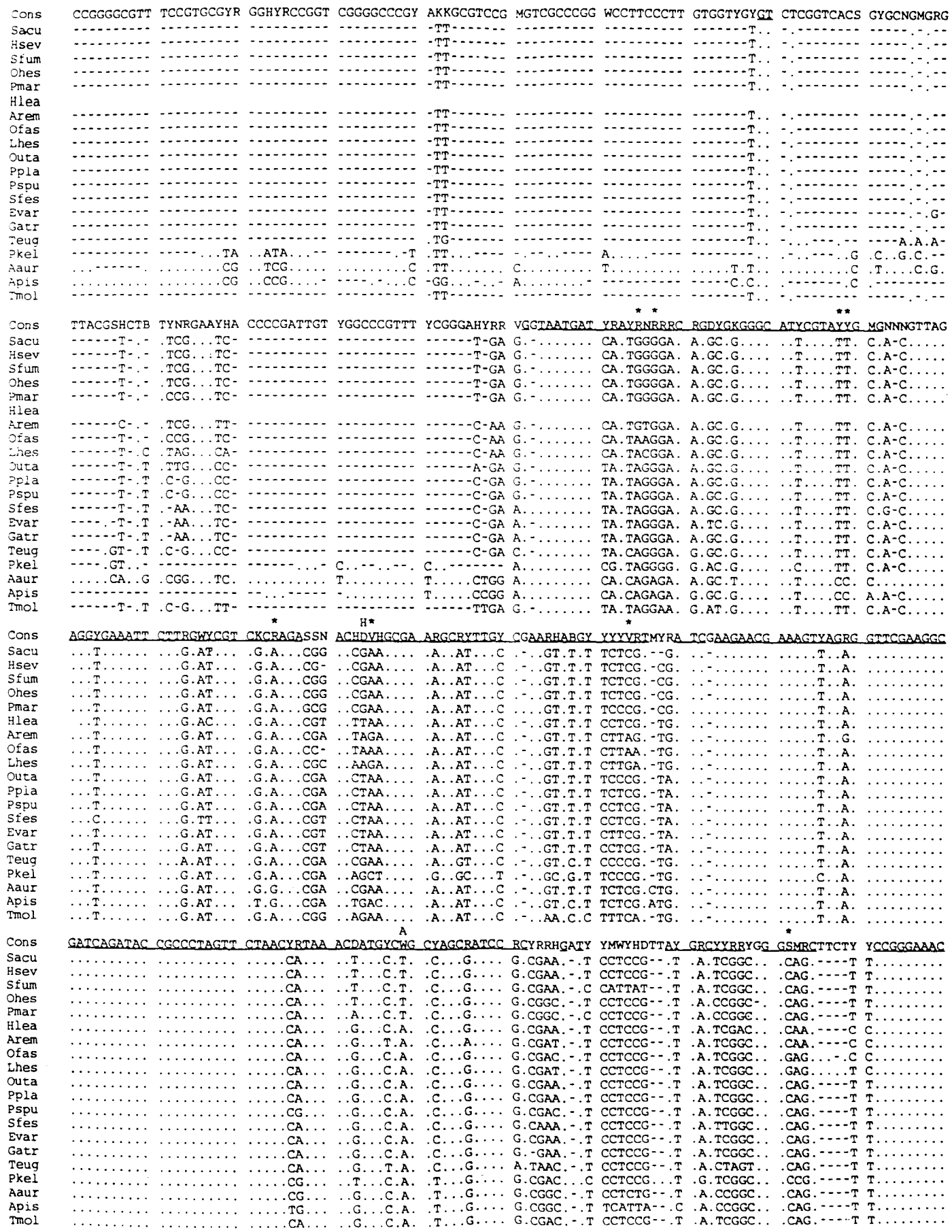
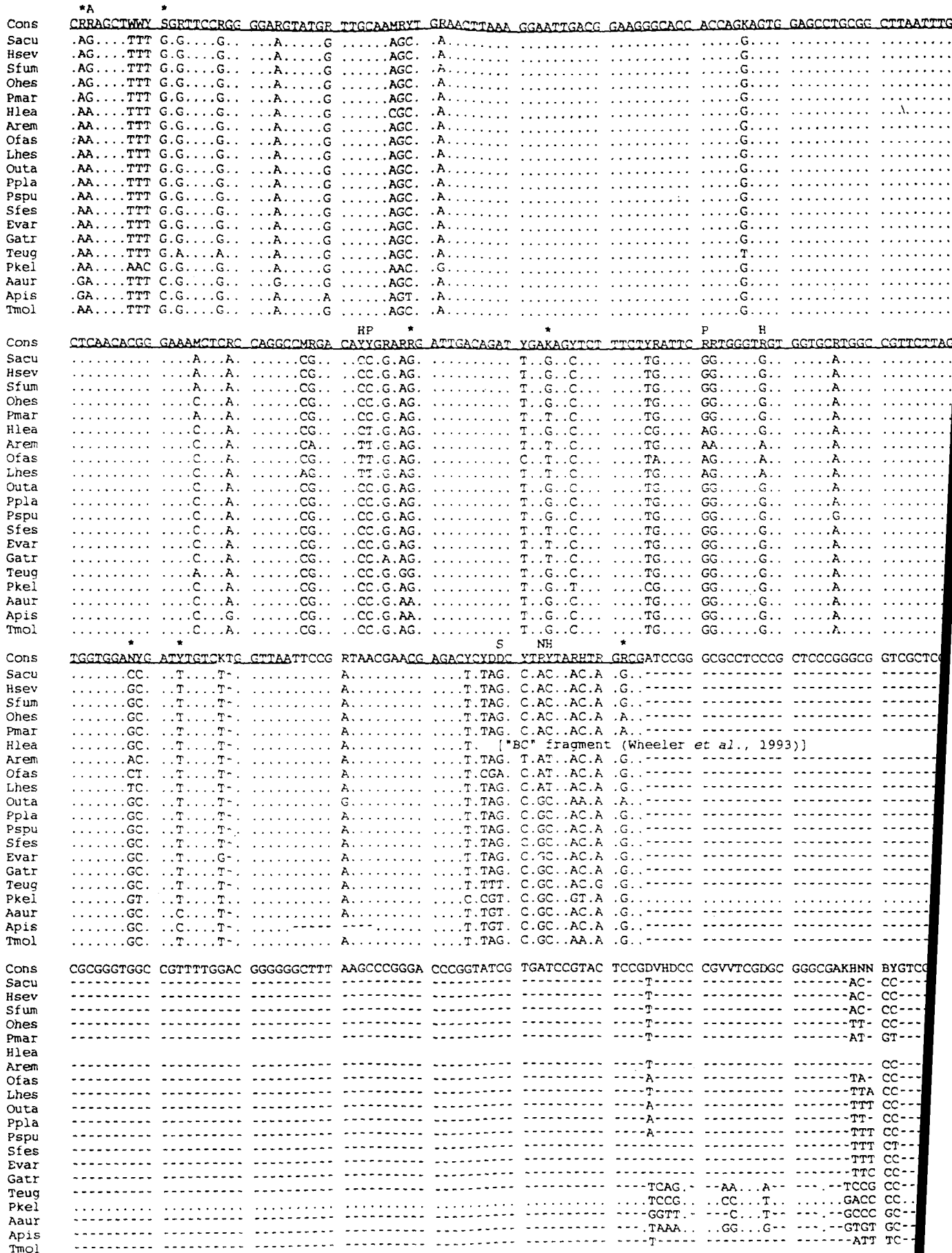
Appendix (*continued*)



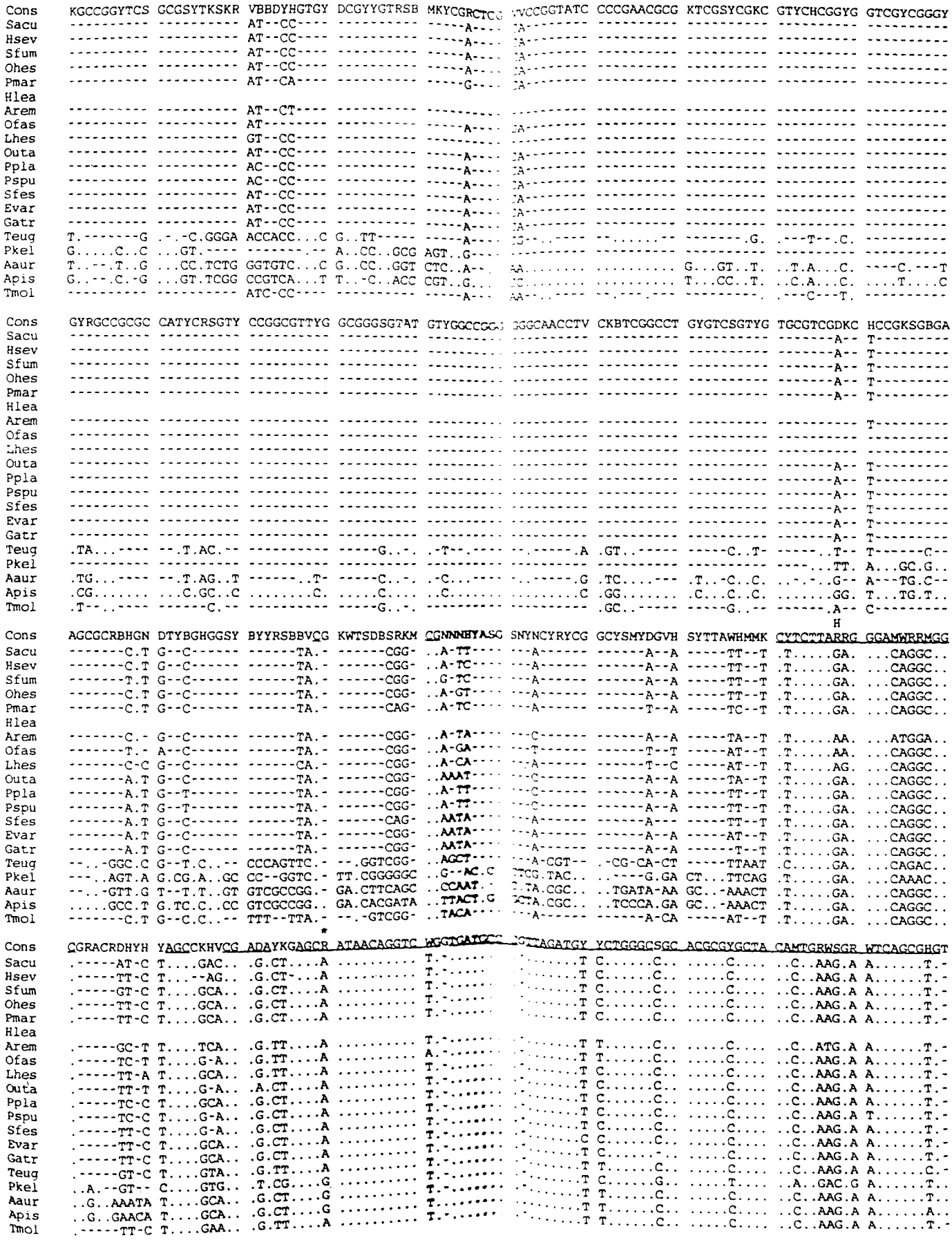
Appendix (*continued*)



Appendix (*continued*)



Appendix (*continued*)



Appendix (*continued*)