Mecoptera is paraphyletic: multiple genes and phylogeny of Mecoptera and Siphonaptera

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Phylogenetic relationships among members of the Mecoptera and Siphonaptera were inferred from DNA sequence data. Four loci (18S and 28S ribosomal DNA, cytochrome oxidase II and elongation factor-1α) were sequenced for 69 taxa selected to represent major flea and mecopteran lineages. Phylogenetic analyses of these data support a paraphyletic Mecoptera with two major lineages: Nannochoristidae + (Siphonaptera + Boreidae) and Meropidae + ((Choristidae + Apteropanorpidae) (Panorpidae + (Panorpidae + Bittacidae))). The flea family Ctenophthalmidae is paraphyletic, and the Ceratophylloidea is monophyletic. Morphological evidence is discussed which is congruent with the placement of Siphonaptera as sister group to Boreidae.

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Introduction

Mecoptera is a small holometabolous insect order with approximately 600 extant described species placed in nine families and 32 genera (Penny & Byers 1979; Penny 1997). This group is called scorpionflies because the male ninth abdominal (genital) segment of one family (Panorpidae) is enlarged, bulbous, and curves anterodorsally, resembling the stinger of a scorpion. Two families — Panorpidae and Bittacidae — contain 90% of mecopteran species. Panorpidae (377 spp.) is the most speciose family with three described genera: *Panorpa* (254 spp.) is distributed throughout northern continents and Indonesia, but not in Australia; *Neopanorpa* (110 spp.) is distributed throughout India, southern China, Indochina and southward to Java and Borneo; and *Leptopanorpa* (13 spp.) is restricted entirely to Java (Byers & Thornhill 1983). Bittacidae, sometimes known as hangingflies because species hang from plants by the fore or mid legs, comprises 172 species placed in 16 genera. During courtship, males present females with a nuptial meal, and in some species males mimic females to steal the nuptial meal (Thornhill 1979). Bittacidae is the most diverse neotropical mecopteran group, where the ranges of the small genera, *Anabittacus* (1 sp.), *Issikiella* (5 spp.), *Kalobittacus* (8 spp.), *Nannobittacus* (4 spp.), *Neobittacus* (2 spp.) and *Pazius* (8 spp.), overlap within the ranges of neotropical *Bittacus* (25 spp.). *Orobittacus*, *Apterobittacus* and *Hylobittacus* are monotypic genera restricted to North America, and there are seven additional *Bittacus* species in North America. Ten species of *Harpobittacus* and one each of *Austrobittacus*, *Edriobittacus*, *Symbittacus* and *Tytthobittacus* are endemic to Australia. *Anomalobittacus* (1 sp.) and 48 species of *Bittacus* are restricted to Africa, and comprise the entire mecopteran fauna of Africa (Byers 1991). The remaining *Bittacus* species occur in Europe, Japan, Korea, India, Taiwan, China and Thailand (Penny 1997).

The other mecopteran families, although less speciose, show a spectacular degree of variation in morphology and ecology. Boreidae (snow fleas) is a small group of 26 species placed in three genera that is distributed throughout North America, Europe and Japan. Adults emerge in winter and are associated with bryophytes (Penny 1977; Russell 1982). Wings are reduced to small, oval flaps in females, and thin spiny hooks in males, which function to clasp the female during mating. Boreids are unique among Mecoptera in their ability to jump up to 30 cm when disturbed, which not only facilitates escape from predators, but also allows them to cross light, fluffy snow where it is difficult to walk (Penny 1977). In the case of *Hesperoboreus*, the male jumps directly onto the female prior to copulation (Cooper 1972). Panorpodidae, which morphologically resembles Panorpidae except for a much shorter rostrum, consists of two genera, *Brachypanorpa* in the Pacific north-western USA (3 spp.) and in Appalachia (2 spp.), and *Panorpodes* (4 spp.) occurring in Japan. Choristidae consists of 10 species in three genera restricted entirely to Australia, while Nannochoristidae comprises two genera and seven species found in Australia and South America. Meropeidae, 'earwig flies', consists of

two extant species: *Merope tuber* (eastern North America) and *Austromerope poultoni* (Australia), both of which are cockroach-like in general appearance with extremely large forcep-like appendages on the abdomen. Eomeropidae is also cockroach-like and is a monotypic family with one Chilean species, *Notiothauma reedi*. Apteropanorpidae, another apterous mecopteran family adapted to cold climates, has two species known from Tasmania (Byers & Yeates 1999).

The monophyly of each mecopteran family is well established by morphological characters that have been summarized in other studies (Kaltenbach 1978; Willmann 1987; Byers 1991). From a morphological standpoint, some of the families appear to be living fossils (e.g. Eomeropidae and Meropeidae) and may be the sole remnants of what were once more diverse lineages (Kaltenbach 1978; Willmann 1989). Mecoptera have a very well-documented fossil history and are among the most conspicuous part of the insect fauna of the Lower Permian. There are 348 species of Mecoptera described from the Permian, Mesozoic and Tertiary, representing 87 genera in 34 families (see Willmann 1977, 1981 1983, 1984a,b, 1987). There is no other holometabolous insect order that has such a biased distribution of species within families, where 90% of the species occur in ~20% of the families, or where the diversity of the extinct taxa at the familial and generic level is about three times that of the extant taxa.

Siphonaptera (fleas) is a highly specialized holometabolous insect order with 2380 described species placed in 15 families and 238 genera (Lewis & Lewis 1985). Fleas are laterally compressed, wingless insects that range from 1 to 10 mm in length. The head is usually small and shield- or helmet-shaped, compound eyes are absent, and mouthparts are specialized for piercing and sucking (Dunnet & Mardon 1991). Fleas are entirely ectoparasitic, with ~100 species as parasites of birds and the remaining species as parasites of mammals (Holland 1964). Flea distribution extends to all continents, including Antarctica, and fleas inhabit a range of habitats and hosts from equatorial deserts, through tropical rainforests, to the arctic tundra. Fleas are of tremendous economic importance as vectors of several diseases important to human health, including bubonic plague, murine typhus and tularaemia (Dunnet & Mardon 1991).

From a phylogenetic standpoint, Siphonaptera is the most neglected of the holometabolous insect orders. While we have a reasonable knowledge of flea taxonomy at the species and subspecific level, and a relatively good record of their biology and role in disease transmission, phylogenetic relationships among fleas at any level have remained virtually unexplored. Classically, the major obstacle in flea phylogenetics has been their extreme morphological specializations associated with ectoparasitism, and the inability of systematists to adequately homologize characters across taxa. The majority of characters used for species diagnoses are based on the shape and structure of their extraordinarily complex genitalia, or the presence and distribution of setae and spines (Traub & Starcke 1980; Dunnet & Mardon 1991). While these characters are adequate for species diagnoses, they are mostly autapomorphic at the species level and of limited utility for phylogenetic reconstruction. Siphonaptera appears to have many instances of parallel reductions and modifications, probably associated with multiple invasions of similar hosts, which may obscure homology (Holland 1964).

Ordinal phylogeny

While it is clear that Mecoptera and Siphonaptera are holometabolous insect orders, their position relative to the other Holometabola is somewhat controversial. Hennig (1969) placed Mecoptera as sister group to Diptera in Antliophora, but was uncertain as to whether Siphonaptera should be included within Antliophora, or even affiliated with the other mecopteroid orders. Based on similarities of the proventriculus, Ross (1965) argued for a sister group relationship between Mecoptera and Siphonaptera. Alternatively, Boudreaux (1979) placed Mecoptera as sister group to Diptera + Siphonaptera. Kristensen (1981, 1991) favoured a sister group relationship between Mecoptera and Siphonaptera. The sister group to Antliophora is probably Amphiesmenoptera (Lepidoptera + Trichoptera) (Whiting *et al.* 1997; Kristensen 1999). The close association between Mecoptera and Siphonaptera has been borne out in recent molecular studies (Chalwatzis *et al.* 1996; Whiting *et al.* 1997; Whiting 2001, 2002), although the monophyly of Antliophora + Amphiesmenoptera is not well supported by DNA sequence data (see Whiting 2002).

Familial phylogeny

The phylogeny of Mecoptera has centred around two problematic families: Nannochoristidae and Boreidae. The Nannochoristidae have unusual, aquatic larvae (Pilgrim 1972), a pigmented larval 'eye spot' (Melzer *et al.* 1994), unique venational characteristics (Kristensen 1989) and a suite of characters that are presumably primitive for Mecoptera (Willmann 1987). Phylogenetically, Nannochoristidae was placed as the most basal mecopteran family (Willmann 1987), sister group to Diptera + Siphonaptera (Wood & Borkent 1989) and even elevated to ordinal status, 'Nannomecoptera' (Hinton 1981). The Boreidae also have unusual morphological features (Penny 1977) and were placed as a highly derived mecopteran sister group to Panorpodidae (Penny 1975), as a relatively basal group placed in a trichotomy with Meropeidae and Panorpomorpha (Willmann 1987: Fig. 1) or elevated to their own order, 'Neomecoptera' (Hinton 1958). Hinton's suggestion that Nannochoristidae and Boreidae should be given their own ordinal status was based exclusively on a phenetic

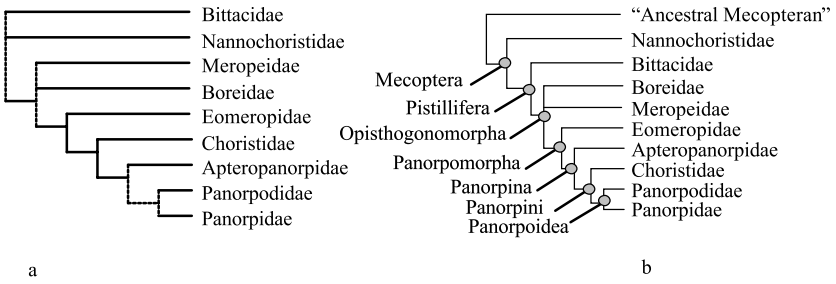

Fig. 1 Phylogeny of Mecoptera based on morphology after Mickoleit (1978) (a) and Willmann (1989) (b).

argument, essentially that these taxa appear so different from other Mecoptera that they deserve ordinal status.

Penny (1975) presented an 'intuitive' phylogeny in which Meropeidae is the basal-most taxon with Boreidae placed as sister group to Panorpodidae. Mickoleit (1978) inferred familial relationships based on characters of genitalia, and proposed a phylogeny in which the Nannochoristidae and Bittacidae are the basal-most taxa (Fig. 1a). Kaltenbach (1978) presented Mecoptera subdivided into three suborders, Protomecoptera (Meropeidae + Eomeropidae), Neomecoptera (Boreidae) and Eumecoptera (remaining families), but did not present a specific phylogeny for these taxa. In a comprehensive analysis of mecopteran morphology from extinct and extant taxa, Willmann (1987, 1989) presented a phylogeny in which Nannochoristidae is the basal-most taxon, with Panorpidae + Panorpodidae forming the most apical clade (Fig. 1b). This phylogeny was not the result of a formal quantitative analysis of a coded character matrix, but Willmann did provide an explicit explanation of the characters supporting each node of the phylogeny. In all cases, these authors are uncertain as to the placement of Meropeidae, and it is possible that its close association with Eomeropidae (i.e. Protomecoptera *sensu* Kaltenbach) is due to symplesiomorphy.

Familial relationships among fleas are much less well resolved and have been less studied than mecopteran families. There is no generally accepted higher classification for Siphonaptera, and several classifications published in recent years have significantly conflicting treatments of superfamilial relationships (Mardon 1978; Smit 1979, 1983, 1987; Traub & Starcke 1980; Traub *et al.* 1983; Lewis & Lewis 1985; Dunnet & Mardon 1991). The monophyly of many flea families is questionable, and certain families that have been used as a catch-all for a wide range of divergent taxa (e.g. Ctenophthalmidae) are almost certainly paraphyletic assemblages. The phylogeny presented by Smit (1979: Fig. 2) is not based on a formal quantitative analysis of flea morphology, and the monophyly of each of these groups is questionable.

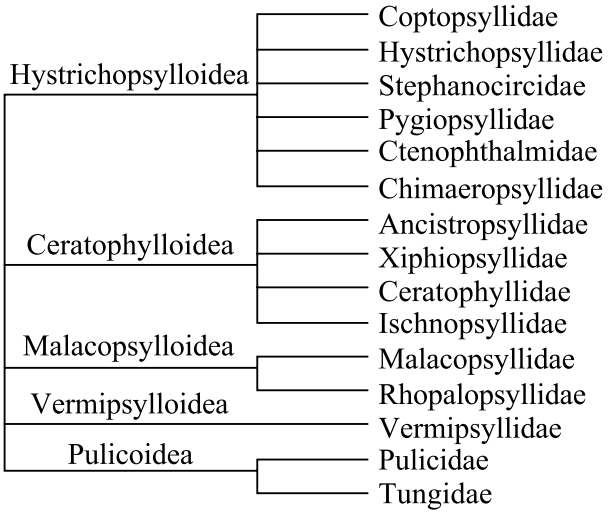

Fig. 2 Phylogeny of Siphonaptera after Smit (1979).

Materials and methods

Sequence data were generated for a total of 69 taxa, representing Amphiesmenoptera (six taxa), Diptera (three taxa), Mecoptera (41 taxa) and Siphonaptera (19 taxa). Although there is morphological and molecular evidence to support the placement of Strepsiptera within Antliophora (Whiting 1998), Strepsiptera was excluded as an outgroup in this analysis because of the difficulty of accurately sequencing the protein-coding genes for strepsipteran exemplars. All mecopteran families, with the exception of Eomeropidae, and the majority of flea families (nine of 15) are included in this analysis (Appendix 1). Thoracic muscle tissue was dissected and incubated in a standard buffer (100 mM ethylenediaminetetraacetic acid (EDTA), 10 mM Tris, 1% sodium dodecylsulphate (SDS), 20 μg proteinase K, pH 7.5) overnight at 55 °C. After buffer incubation, DNA was extracted using standard phenol/chloroform extraction protocols and concentrated by column purification (Centricon-30, Ambion). Four genes were targeted for amplification and sequencing: 18S ribosomal DNA (18S rDNA), 28S ribosomal DNA (28S rDNA), elongation factor-1α (EF-1α) and cytochrome oxidase II (COII). Primer sequences are given in Table 1; relative primer positions and cycling conditions are given in Fig. 3. Genomic DNA templates and controls were

Table 1 Primer sequences. Positions of primers are indicated in Fig. 3.

Primer	Sequence (5′ → 3′)
18S 1.2F	TGCTTGTCTCAAAGATTAAGC
18S ai	CCTGAGAAACGGCTACCACATC
18S a0.7	ATTAAAGTTGTTGCGGTT
18S a0.79	TTAGAGTGCTYAAAGC
18S a1.0	GGTGAAATTCTTGGAYCGTC
18S a2.0	ATGGTTGCAAAGCTGAAAC
18S a3.5	TGGTGCATGGCCGYTCTTAGT
18S 7F	GCAATAACAGGTCTGTGATGCCC
18S 9R	GATCCTTCCGCAGGTTCACCTAC
18S 7R	GCATCACAGACCTGTTATTGC
18S bi	GAGTCTCGTTCGTTATCGGA
18S b0.5	GTTTCAGCTTTGCAACCAT
18S b2.5	TCTTTGGCAAATGCTTTCGC
18S b3.0	GACGGTCCAACAATTTCACC
18S b3.9	TGCTTTRAGCACTCTAA
18S b5.0	TAACCGCAACAACTTTAAT
18S b7.0	ATTTRCGYGCCTGCTGCCTTCCT
28S rD1.2a	CCCSSGTAATTTAAGCATATTA
28S rD3.2a	AGTACGTGAAACCGTTCASGGGT
28S A	GACCCGTCTTGAAGCACG
28S Rd4.2a	CTAGCATGTGYGCRAGTCATTGG
28S Rd4.5a	AAGTTTCCCTCAGGATAGCTG
28S Rd4.8a	ACCTATTCTCAAACTTTAAATGG
28S rD5a	GGYGTTGGTTGCTTAAGACAG
28S Rd6.2a	GAAAGGGAATCYGGTTMMTATTCC
28S rD7b1	GACTTCCCTTACCTACAT
28S Rd6.2b	AATAKKAACCRGATTCCCTTTCGC
28S rD5b	CCACAGCGCCAGTTCTGCTTAC
28S B	TCGGAAGGAACCAGCTAC
28S Rd4.2b	CCTTGGTCCGTGTTTCAAGACGG
28S Rd3.2b	TGAACGGTTTCACGTACTMTTGA
COII-2a	ATAGAKCWTCYCCHTTAATAGAACA
COII-9b	GTACTTGCTTTCAGTCATCTWATG
COII-F-leu	TCTAATATGGCAGATTAGTGC
COII-R-lys	GAGACCAGTACTTGCTTTCAGTCATC
EF-1α M 44–1	GCTGAGCGYGARCGTGGTATCAC
EF-1α M 46–1	GAGGAAATYAARAAGGAAG
EF-1α M 52.7	GTCAAGGARYTGCGTCGTGG
EF-1α rcM 4.0	ACAGVCACKGTYTGYCTCATRTC
EF-1α rcM 53.2	GCAATGTGRGCIGTGTGGCA
EF-1α rcM 53.0	ATRTGRGCNGTGTGGCAATC
EF-1α rcM 52.6	GCYTCGTGGTGCATYTCSAC
EF-1α rcM 51–1	CATRTTGTCKCCGTGCCAKCC
EF-1α rcM 44.9	CTTGATGAAATCYCTGTGTCC

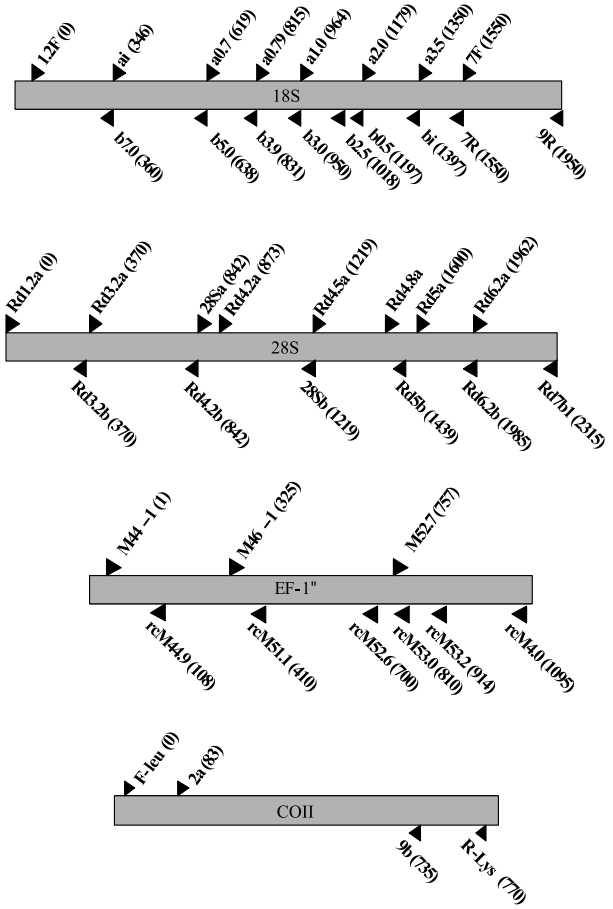

Fig. 3 Map of primer positions for 18S rDNA, 28S rDNA, EF-1α and COII used in this study. Primer sequences are given in Table 1.

amplified using standard polymerase chain reaction (PCR) techniques in a Perkin-Elmer 9600 thermocycler. Product yield, specificity and potential contamination were monitored by agarose gel electrophoresis. The target product was purified and cycle-sequenced using the ABI dRhodamine cycle sequencing kit. The sequencing reactions were column purified and analysed with the ABI 377 automated sequencer. In all cases, DNA was sequenced from complementary strands, with sufficient overlap for the larger genes to ensure the accuracy of all sequence output. Manual correction of chromatography data was facilitated by the program Sequencher™ 3.1.1 (Genecodes 1999), which automatically aligns chromatographs of the sequence output to provide more efficient and accurate sequence correction.

Sequences were assembled in Sequencher™ 3.1.1 (Genecodes 1999). The protein-coding genes (COII and EF-1α) were manually aligned with reference to the amino acid sequences. For the ribosomal genes, a gross alignment was performed by manually aligning the conserved domains across the taxa. Conserved domains, and variable regions between domains, were removed in sections and entered into the computer program POY (Gladstein & Wheeler 1999) to undergo more exhaustive alignment. POY was implemented on a dedicated parallel cluster (64 CPUs, 500 mHz with 1 GB RAM) using gap cost = 2, change cost = 1, with TBR (Tree Bisection and Reconnection), branch swapping on 100 alignments, with the option 'implied alignment' implemented.

While POY is designed to construct a topology while simultaneously performing alignment (Wheeler 1999), the implied alignment option yields a multiple alignment which is more optimal than those typically found by other alignment algorithms, such as MALIGN (Wheeler & Gladstein 1994) or Clustal W (Thompson *et al.* 1994). Variable alignment regions which appeared ambiguously aligned between the ingroup and outgroups, but relatively conserved within each family, were aligned independently within each mecopteran family using POY with the parameters as described above. These variable regions were excluded from the outgroups because resolution among these taxa is not the focus of this study. Each of these regions was considered an alignment block, and the blocks were assembled into a single matrix by scoring the taxa outside the block with missing values, as described elsewhere (see Whiting 2001, 2002). The alignment can be found at http://dnasc.byu.edu/~whitinglab.

Trees were reconstructed under parsimony with gaps treated as missing data using the program NONA (Goloboff 1994) with 50 random addition sequences and TBR branch swapping. Partitioned Bremer support values (Baker & DeSalle 1997) were calculated using the program TreeRot (Sorenson 1999) and PAUP*4.0 (Swofford 2000). The incongruence length difference (ILD) test was performed using the program ARN with 1000 replications, and uninformative characters were removed (Farris *et al.* 1994). Trees were reconstructed with the variable blocked regions included and excluded from the analysis and under a variety of codon weighting schemes (1 : 1 : 0, 1 : 1 : 1, 3 : 5 : 1, and estimated values 5 : 10 : 1 (COII) and 2 : 4 : 1 (EF-1α)) to explore the sensitivity of the phylogenetic results to different weighting parameter values.

Results and discussion

Alignment of the sequence data for 18S resulted in 2137 characters, 522 of which were parsimony informative with one variable blocked region. Hypervariable regions of the alignment (positions 1545–1591 and 1617–1699) were excluded from the analysis. The 28S data consisted of a 6464 base pair (bp) alignment with eight variable blocked regions. The more conserved regions totalled 2114 bp, 739 of which were parsimony informative. The variable blocked regions consisted of 4350 bp, 450 of which were parsimony informative. Hypervariable regions of the alignment (positions 5741–5763, 7053–7084, 7215–7300 and 7527–8222) were excluded from the analysis. The EF-1α data consisted of 1092 bp, 415 of which were parsimony informative, with nucleotide 1 (nt1) = 58 (14%), nt2 = 30 (7%) and nt3 = 327 (78%). The COII data consisted of 599 bp, 326 of which were parsimony informative, with nt1 = 94 (29%), nt2 = 45 (14%) and nt3 = 187 (57%). Results of the ILD test failed to reject the hypothesis of data set incongruence for all combinations except for 18S vs. the protein-coding genes (Table 2). However, as the test was not symmetric (i.e. 18S and 28S were congruent, 28S and the protein-coding genes were congruent, but 18S and the protein-coding genes were incongruent), and because the ILD confounds incongruence due to conflicting signals with incongruence due to homoplasy (Dolphin *et al.* 2000), the molecular data sets were combined in a total evidence analysis.

Table 2 Results from ILD tests among data partitions.

Partition comparison	α value
28S/18S	1.000
EF-1α/28S	1.000
EF-1α/COII	1.000
EF-1α/18S	0.001*
COII/18S	0.001*
COII/28S	0.194
18S/COII + EF-1α	0.001*
18S/COII + EF-1α + 28S	1.000
18S + 28S/COII + EF-1α	0.230

*Values of $\alpha < 0.050$ indicate sufficient evidence to reject the hypothesis of data set congruence.

Analysis of the 18S rDNA data, with variable blocked regions included, results in a topology where familial relationships are entirely unresolved, except for Panorpidae + Panorpodidae (Fig. 4). These data provide some resolution within the Panorpidae and Ceratophylloidea, but do not provide evidence for the paraphyly of any mecopteran family. Exclusion of the variable blocked regions results in a nearly identical topology. Analysis of the 28S rDNA data results in a topology where Meropeidae is the basal-most clade and Boreidae is sister group to Nannochoristidae + Siphonaptera (Fig. 4). Exclusion of the variable blocked regions results in a less resolved topology, but one which retains the clades (Boreidae (Nannochoristidae + Siphonaptera)) (Panorpidae (Bittacidae + Panorpodidae)), and a basal placement of Meropeidae. Analysis of the COII data for all nucleotide schemes investigated results in topologies which support Boreidae + Siphonaptera as the basal-most clade, with Nannochoristidae in a more derived position (Fig. 4). All COII analyses, rather surprisingly, also support a paraphyletic Panorpidae. Analysis of the EF-1α data with all nucleotide positions weighted equally supports a topology in which fleas, boreids and Meropeidae form a clade, although the first two groups are grossly paraphyletic in respect to each other (Fig. 4). Exclusion of third position nucleotides results in overall less resolution, although relationships among the fleas are fully resolved and more congruent with the other genes.

Phylogenetic analysis of a single gene across the Mecoptera and Siphonaptera appears to be insufficient to resolve the phylogeny of these taxa. 18S results in a poorly resolved topology,

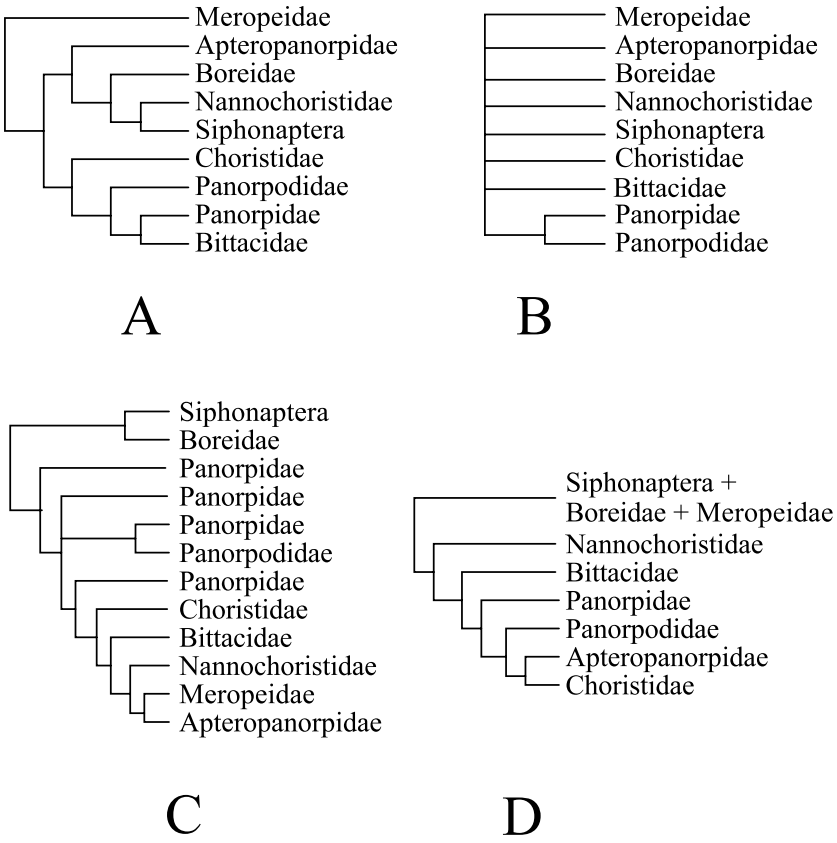

Fig. 4 Summary trees for individual genes used in this analysis based on parsimony analysis: (A) 28S rDNA; (B) 18S rDNA; (C) COII; (D) EF-1α. The 18S tree is based on the entire alignment (conserved and variable regions) and is the strict consensus of 599 trees (L = 1506, CI = 0.58, RI = 0.82). The 28S tree is based on the entire alignment and is the strict consensus of 16 trees (L = 4276, CI = 0.52, RI = 0.81). The COII tree is based on equal weighting of all positions and is the strict consensus of nine trees (L = 2968, CI = 0.30, RI = 0.60). The EF-1α tree is based on equal weighting of all positions, generating only one tree (L = 3436, CI = 0.24, RI = 0.58).

Table 3 Sum of Bremer and partitioned Bremer support values from Table 4 across various nodes on the phylogeny as given in Fig. 5.

		Three partitioned Bremer				Percent partitioned Bremer			
Node partitions	Total Bremer support	18S	28S	EF-1α	COII	18S	28S	EF-1α	COII
Ingroup nodes	1041	119.9	434.7	361.8	124.7	11.5	41.8	34.8	12.0
Interfamilial nodes	319	57.3	138.5	133	–9.8	18.0	43.4	41.7	–3.1
Intrafamilial nodes	722	62.6	296.2	228.8	134.5	8.7	41.0	31.7	18.6
Intrafamilial (flea)	211	52.5	101.5	15.0	42.0	12.1	48.1	7.1	19.9
Intrafamilial (boreids)	103	27.4	37.2	35.6	2.8	26.6	36.1	34.6	2.7
Intrafamilial (bittacids)	65	–31.0	36.5	37.7	21.9	–47.7	56.2	58.0	33.7
Intrafamilial (panorpids)	237	11.2	91.6	88.4	45.8	4.7	38.6	37.3	19.3
All nodes	1608	297.7	785.9	400.5	124	18.5	48.9	24.9	7.7

COII results in a topology where Panorpidae is paraphyletic, EF-1α results in a topology where Boreidae and Siphonaptera are paraphyletic and 28S produces a topology where Meropeidae is the basal-most taxon and Apteropanorpidae is the sister group to fleas + boreids + nannochoristids. Indeed, the topologies from the individual genes are less congruent with phylogeny based on morphology than is the total evidence topology. Summing the Bremer and partitioned Bremer support values for various nodes on the topology reveals at what level the different genes provide a signal and at what level they produce noise across the entire topology (Table 3). Across all the ingroup nodes, about 77% of the signal is derived from 28S and EF-1α, with 23% provided by the other genes. At the interfamilial level, COII provides no signal, whereas EF-1α and 28S provide about 85% of the signal. At the level of intrafamilial relationships, different genes provide different signal strengths in different groups. For instance, EF-1α provides a very limited signal for relationships among fleas (7.1%), although it provides more than half of the signal for relationships among the bittacids (56.5%). COII provides

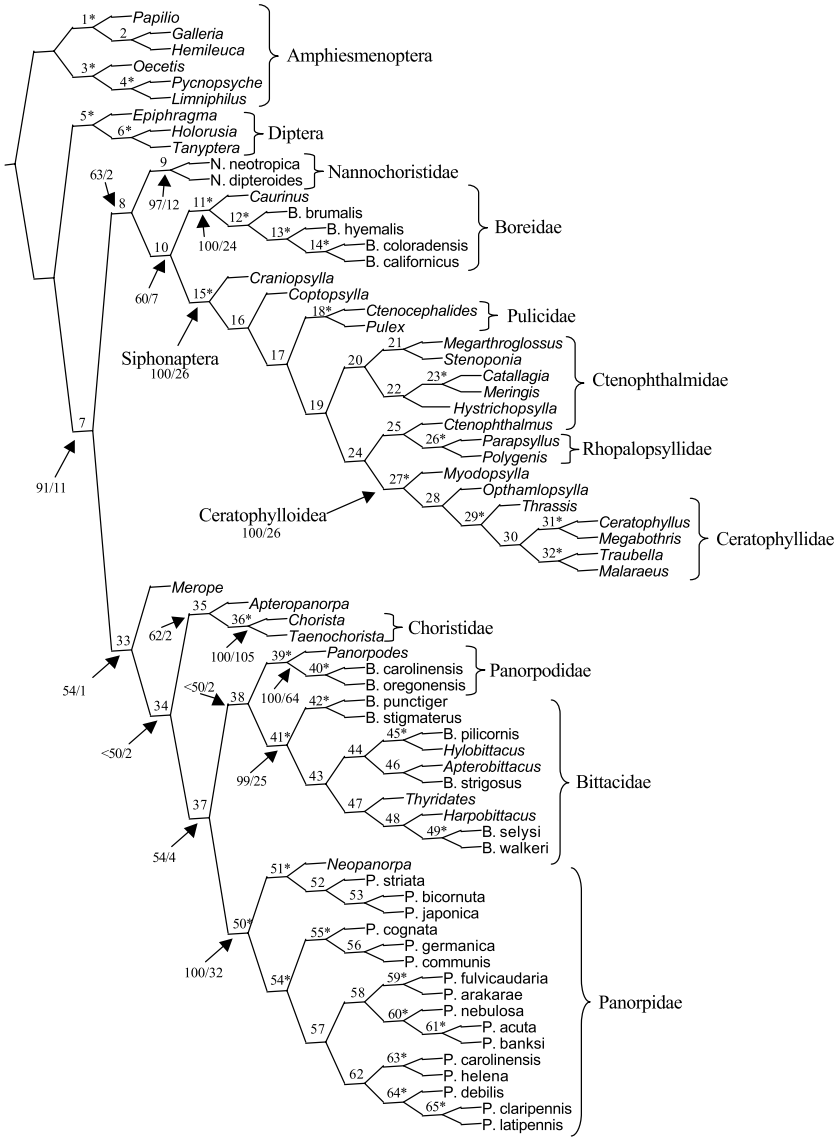

Fig. 5 Total evidence molecular tree based on 18S + 28S + EF-1α + COII with all characters weighted equally. This analysis produces a single most parsimonious tree (L = 12 376; CI = 0.40, RI = 0.66). Nodes are numbered and Bremer and bootstrap values are given in Table 4. Nodes where bootstrap > 98 and Bremer > 10 are indicated with an asterisk. Bootstrap and Bremer values are listed for all interfamilial relationships.

almost no signal for boreid relationships, but it accounts for about 20% of the signal in fleas and panorpids and 33% of the signal in bittacids. 18S provides negative support among the bittacids, but good support within the boreids. 28S appears to be the most useful individual marker as it provides roughly 40% of the signal across all ingroup nodes.

The combination of all these data together in a single analysis with all characters weighted equally produces a single, fully resolved topology (Fig. 5; support values in Table 4). This analysis supports a major division of Mecoptera into two clades: (Nannochoristidae (Boreidae + Siphonaptera)) and the remaining Mecoptera. The clade Siphonaptera + Boreidae is the best supported higher level relationship on the topology (Bremer support = 10; bootstrap = 60). This is congruent with earlier molecular studies which included a much smaller sample of mecopteran and flea taxa and fewer genetic markers (Whiting *et al.* 1997; Whiting 2001, 2002). The position of Nannochoristidae at the base of this clade is supported with less Bremer support (= 2), but a slightly higher bootstrap value (= 63). The basal placement of this family relative to other mecopteran groups accords with morphological evidence (Kristensen 1989; Willmann 1989).

A sister group relationship between Boreidae and Siphonaptera is also supported by morphological evidence. The process of resilin secretion in the flea (pleural arch) and *Boreus* (wing base) is similar, and different from that of the locust and dragonfly (Rothschild 1975; Schlein 1980). The unusual proventricular spines in fleas and boreids are

Table 4 Nodal support for topology in Fig. 5. Columns list non-parametric bootstrap values, Bremer support values and partitioned Bremer support values (the contribution of the specified gene to the total Bremer support at the indicated node) as calculated for the combined molecular data phylogeny in Fig. 4. Bootstrap support values result from 1000 bootstrap replicates.

			Partitioned Bremer							Partitioned Bremer			
Node	Bootstrap support	Bremmer support	18S	28S	EF-1α	COII	Node	Bootstrap support	Bremer support	18S	28S	EF-1α	COII
1	100	83	33.7	49.6	−2.6	2.3	34	< 50	2	0.7	−0.4	−1.6	3.3
2	64	1	0.9	−0.4	3.9	−3.4	35	62	2	−7.1	−3.4	18.9	−6.4
3	100	42	14.0	28.0	0	0	36	100	105	24.4	30.1	57.4	−6.9
4	100	11	1.0	10.0	0	0	37	54	4	−13.6	20.6	7.4	−10.4
5	100	324	118.5	209.4	1.0	−4.9	38	< 50	2	−6.6	7.6	8.4	−7.4
6	100	106	9.7	54.6	36.4	5.3	39	100	64	16.7	18.6	16.4	12.3
7	91	11	10.2	5.6	1.9	−6.7	40	100	60	7.4	19.9	29.4	3.3
8	63	2	−1.1	3.6	−1.1	0.6	41	99	25	−7.3	−2.4	19.4	15.3
9	97	12	8.2	8.6	1.9	−6.7	42	100	44	3.4	10.9	16.6	13.1
10	60	7	0.4	9.6	−6.6	3.6	43	< 50	2	−8.3	−1.4	6.1	5.6
11	100	24	7.9	23.1	−5.1	−1.9	44	57	2	−8.3	−1.4	6.1	5.6
12	100	29	5.9	8.6	17.9	−3.4	45	99	25	−8.6	0.6	16.4	16.6
13	100	32	9.7	12.3	7.4	2.6	46	53	2	0.4	−0.1	−0.4	2.1
14	100	42	11.8	16.3	10.3	3.6	47	63	4	−13.6	20.6	7.4	−10.4
15	100	26	11.9	12.6	3.9	−2.4	48	< 50	2	0.4	−0.1	−0.4	2.1
16	67	10	−2.1	7.6	2.9	1.6	49	100	30	−1.3	16.9	8.6	5.9
17	< 50	3	−0.6	9.6	−11.6	5.6	50	100	32	11.9	5.1	13.4	1.6
18	100	34	4.5	19.7	7.7	2.2	51	98	16	3.1	7.6	9.7	−4.4
19	< 50	3	−0.6	8.1	−9.1	4.6	52	88	9	−4.0	5.0	11.0	−3.0
20	< 50	5	1.2	5.6	4.9	−6.7	53	87	6	4.9	0.6	10.9	−10.4
21	< 50	3	5.4	1.6	−1.6	−2.4	54	100	26	2.2	13.6	15.1	−4.8
22	< 50	1	5.4	0.6	−1.6	−3.4	55	100	21	−1.6	23.6	0.4	−1.4
23	100	27	9.4	3.0	14.8	−0.2	56	< 50	4	−0.3	−2.6	1.1	5.8
24	< 50	3	2.9	2.6	−2.6	0.1	57	65	6	1.7	2.9	−2.9	4.3
25	< 50	2	−1.6	−2.4	0.4	5.6	58	< 50	3	−4.1	3.3	3.4	0.4
26	100	32	6.4	15.6	5.4	4.6	59	100	35	4.9	13.5	9.1	7.4
27	100	26	5.4	13.1	0.4	7.1	60	100	50	3.7	8.2	12.5	25.6
28	< 50	3	2.9	2.6	−2.6	0.1	61	100	11	1.0	6.0	1.0	3.0
29	99	17	4.4	2.1	3.9	6.6	62	84	5	0.0	4.0	0.0	1.0
30	56	3	2.9	2.6	−2.6	0.1	63	100	18	−0.3	2.9	6.1	9.3
31	100	17	4.9	5.6	−1.1	7.6	64	99	9	0.0	0.0	5.0	4.0
32	100	22	1.7	3.9	7.4	8.9	65	100	18	0.0	3.0	6.0	9.0
33	54	1	0.7	−0.4	−1.6	2.3							

morphologically similar (Richards & Richards 1969). Both groups have multiple sex chromosomes (Bayreuther & Brauning 1971) and also have eyes in a 'skeletal socket' (Schlein 1980). Boudreaux (1979) considered the above characters as probable convergences, and favoured a placement of Siphonaptera as sister group to Diptera, and Byers (1996) presented arguments for a close association of fleas with flies. Nonetheless, the most convincing morphological evidence comes from recent research on ovarioles, which demonstrates that boreid ovarioles are fundamentally different from those in other Mecoptera, but similar to those found in fleas. Mecoptera possess polytrophic–meroistic ovarioles, whereas the ovarioles in *Boreus* are devoid of nurse cells and therefore panoistic (Bilinski *et al.* 1998). Fleas and boreids share the following ovariole characteristics: (i) secondary loss of nurse cells; (ii) completion of initial stages of oogenesis during postembryonic development; (iii) occurrence of rDNA amplification and resulting appearance of multiple nucleoli; (iv) differentiation of the late previtellogenic ooplasm into two clearly recognizable regions; and (v) presence of accumulations of membrane-free, clathrin-like cages (Bilinski *et al.* 1998). The combination of morphological with molecular data provides a compelling argument for a sister group relationship between Boreidae and Siphonaptera.

The second major clade supported by the combined data includes the remainder of Mecoptera, with Meropeidae as the basal-most member of this clade. There were no sequences included from Eomeropidae, and so it is not clear whether 'Protomecoptera' *sensu* Kaltenbach (1978) is supported. These data support a sister group relationship between Apteropanorpidae and Choristidae. The combined analysis favours a sister group relationship between Panorpidae and

Bittacidae, and this finding contradicts results from previous morphological analyses which favour Panorpidae + Panorpodidae, although the position of Bittacidae has always been open to question. It is interesting that the Panorpidae + Panorpodidae clade, which is thought to be well supported via morphological data (Willman 1987), was never well supported in any of the gene partitions. Three gene partitions directly contradict Panorpidae + Panorpodidae, and, in the fourth (18S rDNA), the relationship is poorly supported. Likewise, the Bittacidae + Panorpidae relationship in the combined analysis is poorly supported, and Bittacidae are placed with different clades for every gene partition in this analysis. These observations suggest that further data are needed to establish a robust placement for Bittacidae.

In contrast to the marginally supported interfamilial relationships, the monophyly of every mecopteran family is very well supported (minimum bootstrap = 97; minimum Bremer = 12), as are many of the generic and species group relationships within Mecoptera and Siphonaptera. Within Siphonaptera, the families Ceratophyllidae, Rhopalopsyllidae and Pulicidae, and the superfamilial group Ceratophylloidea, are well supported, but the data suggest that Ctenophthalmidae is paraphyletic. This analysis supports *Craniopsylla* as the most basal flea taxon and *Caurinus* as the most basal boreid. Although there has been no previous formal analysis of phylogenetic relationships within Panorpidae, the species group designations suggested by Carpenter (1931) and Issiki (1935) are supported in this analysis, including the Japonica group (*P. striata*, *bicornuta* and *japonica*), the Communis group (*P. cognata*, *germanica* and *communis*), the Fulvicaudaria group (*P. fulvicaudaria* and *arakarae*), the Nebulosa group (*P. nebulosa*, *acuta* and *banksi*), the Helena group (*P. carolinensis* and *helena*) and the Claripennis group (*P. claripennis* and *latipennis*). The genus *Panorpa* is paraphyletic, as *Neopanorpa* is placed as sister taxon to the Japonica species group. Likewise, within Bittacidae, the genus *Bittacus* is grossly paraphyletic with regard to the other bittacid genera. The fact that these two genera are paraphyletic is not particularly surprising as both are catch-all genera that include a wide range of species from throughout the world. Within Panorpodidae, the two *Brachypanorpa* species are sister taxa as expected from morphology.

These data suggest that Mecoptera, as currently constituted, is a paraphyletic assemblage. While it seems certain that Boreidae and Siphonaptera are sister groups, their placement relative to the other Mecoptera is not as well supported by the data. Likewise, while it seems clear that Nannochoristidae should occupy a basal position, it is not clear whether it is sister group to the flea + boreid clade or sister to the remainder of Mecoptera. Additional data in the form of increased taxon sampling for the molecular data and a coded morphological matrix are needed to provide a more robust estimate of mecopteran and flea relationships.

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References

Baker, R. H. & DeSalle, R. (1997). Multiple sources of character information and the phylogeny of Hawaiian drosophilids. *Systematic Biology*, *46*, 654–673.

Bayreuther, K. & Brauning, S. (1971). Die Cytogenetik der Flohe (Aphaniptera). *Chromosoma*, *33*, 19–29.

Bilinski, S., Bünnig, J. & Simiczyjew, B. (1998). The ovaries of Mecoptera: basic similarities and one exception to the rule. *Folia Histochemica et Cytobiologica*, *36*, 189–195.

Boudreaux, H. B. (1979). *Arthropod Phylogeny with Special Reference to Insects*. New York: John Wiley & Sons.

Byers, G. W. (1991). Mecoptera. In CSIRO (Ed.) *The Insects of Australia: a Textbook for Students and Research Workers*, 2nd edn. (pp. 696–704). Melbourne: CSIRO and Melbourne University Press.

Byers, G. W. (1996). More on the origin of Siphonaptera. *Journal of the Kansas Entomological Society*, *69*, 274–277.

Byers, G. W. & Thornhill, R. (1983). Biology of the Mecoptera. *Annual Review of Entomology*, *28*, 203–228.

Byers, G. W. & Yeates, D. K. (1999). A second species of *Apteropanorpa* Carpenter from Tasmania (Mecoptera: Apteropanorpidae). *Australian Journal of Entomology*, *38*, 60–65.

Carpenter, F. M. (1931). Revision of the Nearctic Mecoptera. *Bulletin of the Museum of Comparative Zoology*, *72*, 205–277.

Chalwatzis, N., Hauf, J., Peer, Y. V. D., Kinzelbach, R. & Zimmerman, F. K. (1996). 18S ribosomal RNA genes of insects: primary structure of the genes and molecular phylogeny of the Holometabola. *Annals of the Entomological Society of America*, *89*, 788–803.

Cooper, K. W. (1972). A southern California *Boreus*, *B. notoperates* n. sp. I. Comparative morphology and systematics (Mecoptera: Boreidae). *Psyche*, *79*, 269–283.

Dolphin, K., Belshaw, R., Orme, C. D. L. & Quicke, D. L. J. (2000). Noise and incongruence: interpreting results of the Incongruence Length Difference Test. *Molecular Phylogenetics and Evolution*, *17*, 401–406.

Dunnet, G. M. & Mardon, D. K. (1991). Siphonaptera. In CSIRO (Ed.) *The Insects of Australia: a Textbook for Students and Research Workers*, 2nd edn (pp. 125–140). Melbourne: CSIRO and Melbourne University Press.

Farris, J. S., Kallersjo, M., Kluge, A. G. & Bult, C. (1994). Testing significance of incongruence. *Cladistics*, *10*, 315–320.

Genecodes (1999). *Sequencher* (Version 3.1.1). Ann Arbor, MI: Genecodes Co.

Gladstein, D. S. & Wheeler, W. C. (1999). *POY: Phylogeny Reconstruction Via Direct Optimization of DNA Data* (Version 2.0). New York: American Museum of Natural History.

Goloboff, P. A. (1994). *NONA: a Tree Searching Program. Program and Documentation*. Tucuman, Argentina: Fundacion e Instituto Miguel Lillo.

Hennig, W. (1969). *Die Stammesgeschichte der Insekten*. Frankfurt am Main: Krammer.

Hinton, H. E. (1958). The phylogeny of the Panorpoid orders. *Annual Review of Entomology*, *3*, 181–206.

Hinton, H. E. (1981). *Biology of Insect Eggs*, 1st edn, Vol. II. New York: Pergamon Press.

Holland, G. P. (1964). Evolution, classification, and host relationships of Siphonaptera. *Annual Review of Entomology*, *9*, 123–146.

Issiki, S. (1935). Morphological studies on the Panorpidae of Japan and adjoining countries and comparison with American and European forms. *Japanese Journal of Zoology*, *Vol. 4*, 315–416.

Kaltenbach, A. (1978). Mecoptera (Schnabelhafte, Schnabelfliegen). In Helmcke, J., Starck, D. & Wermuth, H. (Eds) *Handbuch der Zoologie Eine Naturgeschichte der Stamme des Tierreiches* (pp. 1–111). New York: Walter de Gruyter.

Kristensen, N. P. (1981). Phylogeny of insect orders. *Annual Review of Entomology*, *26*, 135–157.

Kristensen, N. P. (1989). The New Zealand scorpionfly (*Nannochorista philpotti* comb.n.): wing morphology and its phylogenetic significance. *Zeitschrift für die Zoologische Systematik und Evolutionforschung*, *27*, 106–114.

Kristensen, N. P. (1991). Phylogeny of extant hexapods. In CSIRO (Ed.) *The Insects of Australia: a Textbook for Students and Research Workers*, 2nd edn (pp. 125–140). Melbourne: CSIRO and Melbourne University Press.

Kristensen, N. P. (1999). Phylogeny of endopterygote insects, the most successful lineage of living organisms. *European Journal of Entomology*, *96*, 237–253.

Lewis, R. E. & Lewis, J. H. (1985). Notes on the geographical distribution and host preferences in the order Siphonaptera. *Journal of Medical Entomology*, *22*, 134–152.

Mardon, D. K. (1978). On the relationships, classification, aedeagal morphology and zoogeography of the genera of Pygiopsyllidae (Insecta: Siphonaptera). *Australian Journal of Zoology, Supplementary Series*, *64*, 1–69.

Melzer, R. R., Paulus, H. F. & Kristensen, N. P. (1994). The larval eye of nannochoristid scorpionflies (Insecta, Mecoptera). *Acta Zoologica*, *75*, 201–208.

Mickoleit, G. (1978). Die phylogenetischen beziehungen der schnabelfliegen-familien aufgrund morphologischer ausprägungen der weiblichen genital und postgenitalsegmente (Mecoptera). *Entomologica Germanica*, *4*, 258–271.

Penny, N. D. (1975). Evolution of the extant Mecoptera. *Journal of the Kansas Entomological Society*, *48* (*3*), 331–350.

Penny, N. D. (1977). A systematic study of the family Boreidae (Mecoptera). *Kansas University Scientific Bulletin*, *51*, 141–217.

Penny, N. D. (1997). *World Checklist of Extant Mecoptera Species*. California: California Academy of Sciences. Available: http://www.calacademy.org/research/entomology/mecoptera.

Penny, N. D. & Byers, G. W. (1979). A check-list of the Mecoptera of the world. *Acta Amazonica*, *9* (*2*), 365–388.

Pilgrim, R. L. C. (1972). The aquatic larva and the pupa of *Choristella philpotti* Tillyard, 1917 (Mecoptera: Nannochoristidae). *Pacific Insects*, *14*, 151–168.

Richards, P. A. & Richards, A. G. (1969). Acanthae: a new type of cuticular process in the proventriculus of Mecoptera and Siphonaptera. *Zoologische Jahrbücher Anatomie und Ontogenie der Tiere*, *86*, 158–176.

Ross, H. H. (1965). *A Textbook of Entomology*, 3rd edn. New York: John Wiley & Sons.

Rothschild, M. (1975). Recent advances in our knowledge of the order Siphonaptera. *Annual Review of Entomology*, *20*, 241–259.

Russell, L. K. (1982). The life history of *Caurinus dectes* Russell, with a description of the immature stages (Mecoptera: Boreidae). *Entomologica Scandinavica*, *13*, 225–235.

Schlein, Y. (1980). Morphological similarities between the skeletal structures of Siphonaptera and Mecoptera. In: Traub, R. & Starck, H. (Eds) *Proceedings of the International Conference on Fleas* (pp. 359–367). Rotterdam: A. A. Balkema.

Smit, F. G. A. M. (1979). The fleas of New Zealand (Siphonaptera). *Journal of Royal Society of New Zealand*, *9*, 143–232.

Smit, F. G. A. M. (1983). Key to the genera and subgenera of Ceratophyllidae. In R. Traub, M. Rothschild & J. Haddow (Eds) *The Ceratophyllidae, Key to the Genera and Host Relationships*. New York: Academic Press.

Smit, F. G. A. M. (1987). *An Illustrated Catalogue of the Rothschild Collection of Fleas (Siphonaptera) in the British Museum (Natural History), Malacopsylloidea (Malacopsyllidae and Rhopalopsyllidae)*, Vol. VII. Oxford: Oxford University Press.

Sorenson, M. D. (1999). *Treerot* (Version 2). Boston, MA: Boston University.

Swofford, D. L. (2000). *PAUP*: Phylogenetic Analysis Using Parsimony (*and Other Methods)* (Version 4.0b4). Sunderland, MA: Sinauer Associates.

Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994) Clustal W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, positions specific gap penalties and weight matrix choice. *Nucleic Acids Research*, *22*, (4673–4680).

Thornhill, R. (1979). Adaptive female-mimicking behavior in a scorpionfly. *Science*, *205*, 412–414.

Traub, R. M., Rothschild, M. & Haddow, J. (1983). *The Ceratophyllidae, Key to the Genera and Host Relationships*. New York: Academic Press.

Traub, R. & Starcke, H. (1980). Fleas. In Traub, R. & Starck, H. (Eds) *Proceedings of the International Conference on Fleas*. Rotterdam: A. A. Balkema.

Wheeler, W. (1999). Fixed character states and the optimization of molecular sequence data. *Cladistics*, *15*, 379–386.

Wheeler, W. C. & Gladstein, D. L. (1994). *MALIGN* (Version 1.93). New York: American Museum of Natural History.

Whiting, M. F. (1998). Phylogenetic position of the Strepsiptera: review of molecular and morphological evidence. *International Journal of Insect Morphology and Embryology*, *27*, 53–60.

Whiting, M. F. (2001). Phylogeny of the holometabolous insect orders based on 18S ribosomal data: when bad things happen to good data. In: R. DeSalle, G. Giribet & W. C. Wheeler (Eds) *Molecular Systematics and Evolution: Theory and Practice*, in press.

Whiting, M. F. (2002). Phylogeny of the holometabolous insect orders: molecular evidence. *Zoologica Scripta*, *31*, 3–15.

Whiting, M. F., Carpenter, J. C., Wheeler, Q. D. & Wheeler, W. C. (1997). The Strepsiptera problem: phylogeny of the holometabolous insect orders inferred from 18S and 28S ribosomal DNA sequences and morphology. *Systematic Biology*, *46*, 1–68.

Willmann, R. (1977). Zur systematischen Stellung von Austropanorpa (Insecta, Mecoptera) aus dem Alttertiar Australiens. *Paläontologische Zeitschrift*, *51* (*1/2*), 12–18.

Willmann, R. (1981). Phylogenie und Verbreitungsgeschichte der Eomeropidae (Insecta: Mecoptera) Ein Beispiel für die Anwendung der phylogenetischen Systematik in der Palaontologie. *Paläontologische Zeitschrift*, *55* (*1*), 31–49.

Willmann, R. (1983). Die phylogenetischen Beziehungen unter den sudamerikanischen Bittacidae (Insecta: Mecoptera). *Zoolischer Beiträge*, *28* (*12*), 47–65.

Willmann, R. (1984a). Mecopteren aus dem Lias von Niedersachsen (Insecta, Holometabola). *Neues Jahrbuch für Geologie und Paläontologie*, *Monatshefte*, *1984* (7), 437–448.

Willmann, R. (1984b). Zur systematischen Stellung mesozoischer und tertiarer Mecopteren einschliesslich *Eoses triassica* Tindale (angeblich Lepidoptera) (Insecta, Holometabola). *Paläontologische Zeitschrift*, *58* (3/4), 231–246.

Willmann, R. (1987). The phylogenetic system of the Mecoptera. *Systematic Entomology*, *12*, 519–524.

Willmann, R. (1989). Evolution und phylogenetisches system der Mecoptera (Insecta: Holometabola). *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft*, *544*, 1–153.

Wood, D. M. & Borkent, A. (1989). Phylogeny and classification of the Nematocera. In: J. F. McAlpine & D. M. Wood (Eds) *Manual of Nearctic Diptera*, *Vol. 3* (pp. 1333–1370). Ottawa: Agriculture Canada.

Appendix 1 List of taxa used in this analysis with accession numbers.

Family	Name	18S	28S	EF-1	COII
Papilionidae	*Papilio troilus* L. 1758	AF286299	AF423920	AF423810	AF423981
Pyralidae	*Galleria melonella* (L. 1758)	AF286298	AF423921	AF423811	AF423982
Saturniidae	*Hemileuca* sp. Walker 1855	AF286273	AF423922	AF423812	AF423983
Leptoceridae	*Oecetis avara* Banks 1895	AF286300	AF423917	AF423815	AF423986
Limniphilidae	*Pycnopsyche lepida* (Hagen 1861)	AF286292	AF423923	AF423813	AF423984
Limniphilidae	*Limnephilus* sp. Leach 1815	AF286291	AF338267	AF423814	AF423985
Tipulidae	*Epiphragma fasciapenne* (Say 1823)	AF286294	AF423919	AF423808	AF423979
Tipulidae	*Holorusia rubiginosa* Loew 1863	AF423778	AF423924	AF423809	AF423980
Tipulidae	*Tanyptera dorsalis* (Walker 1848)	AF286295	AF423918	AF423807	AF423978
Nannochoristidae	*Nannochorista neotropica* Navas 1928	AF334799	AF338261	AF423848	AF424018
Nannochoristidae	*Nannochorista dipteroides* Tillyard 1917	AF334796	AF338262	AF423849	AF424019
Boreidae	*Caurinus dectes* Russell 1979	AF286288	AF423937	AF423830	AF424001
Boreidae	*Boreus brumalis* Fitch 1847	AF423883	AF423936	AF423828	AF423999
Boreidae	*Boreus hyemalis* (L. 1767)	AF423882	AF423935	AF423827	AF423998
Boreidae	*Boreus colouradensis* Byers 1955	AF286285	AF423934	AF423826	AF423997
Boreidae	*Boreus californicus* Packard 1870	AF334795	AF338257	AF423829	AF424000
Meropeidae	*Merope tuber* Newman 1838	AF286287	AF338260	AF423847	AF424017
Apteropanorpidae	*Apteropanorpa evansi* Byers and Yeates 1999	AF286284	AF423925	AF423816	AF423987
Choristidae	*Chorista australis* Klug 1838	AF286289	AF423943	AF423836	AF424007
Choristidae	*Taeniochorista pallida* Esben-Petersen 1914	AF423889	AF423944	AF423837	AF424008
Panorpodidae	*Brachypanorpa carolinensis* Banks 1905	AF286296	AF423971	AF423867	AF424037
Panorpodidae	*Brachypanorpa oregonensis* (McLachlan 1881)	AF423912	AF423972	AF423868	AF424038
Panorpodidae	*Panorpodes pulcher* Issiki 1927	AF423913	AF423973	AF423869	AF424039
Bittacidae	*Apterobittacus apterus* (McLachlan 1871)	AF423875	AF423926	AF423817	AF423988
Bittacidae	*Bittacus pillicornis* Westwood 1846	AF334800	AF338256	AF423822	AF423993
Bittacidae	*Bittacus punctiger* Westwood 1846	AF423876	AF423927	AF423818	AF423989
Bittacidae	*Bittacus selysi* Esben-Petersen 1917	AF423878	AF423929	AF423820	AF423991
Bittacidae	*Bittacus stigmaterus* Say 1823	AF423881	AF423932	AF423824	AF423995
Bittacidae	*Bittacus strigosus* Hagen 1861	AF286290	AF423933	AF423825	AF423996
Bittacidae	*Bittacus walkeri* Esben-Petersen 1915	AF423879	AF423930	AF423821	AF423992
Bittacidae	*Harpobittacus australis rubipes* Riek 1954	AF423877	AF423928	AF423819	AF423990
Bittacidae	*Hylobittacus apicalis* (Hagen 1861)	AF423880	AF423931	AF423823	AF423994
Panorpidae	*Neopanorpa harmandi* (Navas 1908)	AF423903	AF423961	AF423856	AF424027
Panorpidae	*Panorpa acuta* Carpenter 1931	AF423908	AF423967	AF423863	AF424033
Panorpidae	*Panorpa arakavae* Miyake 1913	AF423901	AF423959	AF423854	AF424025
Panorpidae	*Panorpa banksi* Hine 1901	AF423909	AF423968	AF423864	AF424034
Panorpidae	*Panorpa bicornuta* McLachlan 1887	AF423902	AF423960	AF423855	AF424026
Panorpidae	*Panorpa carolinensis* Banks 1905	AF423898	AF423955	AF423852	AF424022
Panorpidae	*Panorpa claripennis* Hine 1901	AF423904	AF423962	AF423858	AF424028
Panorpidae	*Panorpa cognata* Rambur 1842	AF423897	AF423954	AF423851	AF424021
Panorpidae	*Panorpa communis* L. 1758	AF423900	AF423957	AF423857	AF424024
Panorpidae	*Panorpa debilis* Westwood 1846	AF423899	AF423956	AF423853	AF424023
Panorpidae	*Panorpa fluvicaudaria* Miyake 1910	AF423896	AF423953	AF423850	AF424020

Appendix 1 *Continued*

Family	Name	18S	28S	EF-1	COII
Panorpidae	*Panorpa germanica* L. 1758	AF423907	AF423965	AF423862	AF424032
Panorpidae	*Panorpa helena* Byers 1962	AF334798	AF338264	AF423859	AF424029
Panorpidae	*Panorpa japonica* Thunberg 1784	AF423910	AF423969	AF423865	AF424035
Panorpidae	*Panorpa latipennis* Hine 1901	AF423906	AF423964	AF423861	AF424031
Panorpidae	*Panorpa nebulosa* Westwood 1846	AF423905	AF423963	AF423860	AF424030
Panorpidae	*Panorpa striata* Miyake 1908	AF423911	AF423970	AF423866	AF424036
Stephanocircidae	*Craneopsylla minerva wolffheuglia* (Rothschild 1909)	AF286286	AF338266	AF423874	AF424044
Coptopsyllidae	*Coptopsylla africana* Wagner 1932	AF286275	AF423945	AF423838	AF424009
Pulicidae	*Ctenocephalides canis* (Curtis 1826)	AF423914	AF423974	AF423870	AF424040
Pulicidae	*Pulex irritans* L. 1758	AF423915	AF423975	AF423871	AF424041
Ctenophthalmidae	*Megarthroglossus divisus* (Baker 1898)	AF286276	AF338258	AF423839	AF424010
Ctenophthalmidae	*Stenoponia americana* (Baker 1899)	AF423893	AF423949	AF423843	AF424014
Ctenophthalmidae	*Catallagia* sp.	AF423890	AF423946	AF423840	AF424011
Ctenophthalmidae	*Meringis hubbardi* Kohls 1938	AF423891	AF423947	AF423841	AF424012
Hystrichopsyllidae	*Hystrichopsylla talpae talpae* (Curtis 1826)	AF286281	AF423950	AF423844	AF424015
Ctenophthalmidae	*Ctenopthalmus p. pseudagyrtes* Baker 1904	AF423892	AF423948	AF423842	AF424013
Rhopalopsyllidae	*Parapsyllus magellanicus largificus* Smit 1984	AF423916	AF423976	AF423872	AF424042
Rhopalopsyllidae	*Polygenis pradoi* (Wagner 1937)	AF286277	AF423977	AF423873	AF424043
Ischnopsyllidae	*Myodopsylla gentilis* Jordan & Rothschild 1921	AF423894	AF423951	AF423845	
Leptopsyllidae	*Opthalmopsylla volgensis palestinica* Smit 1960	AF423895	AF423952	AF423846	AF424016
Ceratophyllidae	*Thrassis bacchi gladiolus* (Jordan 1925)	AF423886	AF423940	AF423833	AF424004
Ceratophyllidae	*Ceratophyllus petrochelidoni* Wagner 1936	AF423888	AF423942	AF423835	AF424006
Ceratophyllidae	*Megabothris calcarifer* (Wagner 1913)	AF423887	AF423941	AF423834	AF424005
Ceratophyllidae	*Traubella grundmanni* Egoscue 1989	AF423884	AF423938	AF423831	AF424002
Ceratophyllidae	*Malaraeus sinomus* (Jordan 1925)	AF423885	AF423939	AF423832	AF424003