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Fleas are parasitic scorpionflies

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Abstract

Fleas (Siphonaptera) are medically important blood-feeding insects responsible for spreading pathogens such as plague, murine typhus, and myxomatosis. The peculiar morphology of fleas resulting from their specialised ectoparasitic lifestyle has meant that the phylogenetic position of this diverse and medically important group has remained one of the most persistent problems in insect evolution. Here we test competing hypotheses on the contentious evolutionary relationships of fleas and antliophoran insects using the largest molecular dataset available to date consisting of over 1,400 protein-coding genes, and a smaller mitogenome and Sanger sequence alignment of 16 genes. By removing ambiguously aligned sequence regions and using site-heterogeneous models, we consistently recover fleas nested within scorpionflies (Mecoptera) as sister to the relictual southern hemisphere family Nannochoristidae. Topology tests accounting for compositional heterogeneity strongly favour the proposed topology over previous hypotheses of antliophoran relationships. This clade is diagnosed by shared morphological characters of the head and sperm pump. Fleas may no longer be regarded as a separate insect order and we propose that Siphonaptera should be treated as an infraorder within Mecoptera, reducing the number of extant holometabolan insect orders to ten.

Keywords: Antliophora, Siphonaptera, Mecoptera, phylogenomics, evolution, phylogenetic position

Introduction

Fleas are obligate parasites, feeding on the blood of a variety of birds and mammals, and exhibit one of the most bizarre bodyplans and modes of life among insects (Lewis, 1998). Flea monophyly is strongly supported by siphonate mouthparts formed from the laciniae and labrum, strongly reduced eyes, laterally compressed wingless body, and hind legs adapted for jumping (Beutel *et al*., 2013; Medvedev, 2017). However, the phylogenetic position of fleas among insects has proven to be one of the most persistent problems in insect evolution and systematics (Beutel *et al*., 2017; Hennig, 1969; Kjer *et al*., 2016). The 19th century idea that fleas might be close relatives of beetles (Börner, 1904) based on superficial similarities (Hennig, 1969) was gradually replaced by a mid-20th century consensus that fleas share features in common with scorpionflies (Mecoptera) and true flies (Diptera), together constituting the group Antliophora (Kristensen, 1975; Wille, 1960). Affinity with scorpionflies was first proposed in the 1930s (Tillyard, 1935), supported by larval mouthpart characters and the structure of the proventriculus (Hinton, 1958; Richards, 1965; Ross, 1965), while a closer relationship with flies (Boudreaux, 1979) was argued based on the shared absence of thoracic legs in larvae and one-segmented labial palpi (Byers, 1996). The advent of molecular phylogenetics and phylogenomics has not resolved the systematic position of fleas either (Misof *et al*., 2014), with studies consistently recovering fleas either as a sister group to monophyletic scorpionflies (McKenna & Farrell, 2010; Misof *et al*., 2014; Peters *et al*., 2014; Wiegmann *et al*., 2009) or nested within scorpionflies either as sister to Boreidae (snow scorpionflies) or Nannochoristidae (Chalwatzis *et al*., 1996; Whiting, 2002a; Whiting *et al*., 1997, 2003). These competing hypotheses imply dramatically different

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scenarios for the evolution of parasitism and hematophagy in fleas, as well as for the classification of insects.

Resolution of the 'Siphonaptera problem' requires a quantitative comparison of competing hypotheses of antliophoran relationships and an understanding of why past analyses have arrived at divergent results. Here we use the largest currently available molecular dataset for Antliophora consisting of over 1,400 protein-coding genes (Misof *et al*., 2014) and methods to overcome common sources of phylogenomic error to elucidate the systematic position of fleas.

Material and methods

Transcriptome dataset

We used the comprehensive insect transcriptome dataset of Misof *et al*. (2014), pruned to include only Antliophora, with Lepidoptera and Trichoptera as outgroups. With 1,478 protein-coding genes (PCGs) this represents the most extensive dataset for Antliophora available to date. Sampling among scorpionflies includes representatives of all three major groups recognised based on morphology: Neomecoptera (Boreidae), Nannomecoptera (Nannochoristidae), and Pistillifera (Bittacidae and Panorpidae). We focused on analysis of amino acids (AA) which are more suitable for reconstructing ancient relationships (Inagaki & Roger, 2006; Rota-Stabelli *et al*., 2013; Schwentner *et al*., 2017).

Since phylogenetic noise resulting from errors including poor alignment and incorrect identification of orthologs is a major confounding factor in phylogenomics (Philippe *et al*., 2011), we removed ambiguously aligned sequence regions using BMGE with BLOSUM95 and three different -h settings to produce datasets with varying degrees of noise in descending order: -h 0.5, -h 0.4, and -h 0.3 (Criscuolo & Gribaldo, 2010). Trimming resulted in a reduction of the original dataset by 72.6% (-h 0.5, 163,035 AA sites), 83.1% (-h 0.4, 100,430 AA sites), and 92.9% (-h 0.3, 42,005 AA sites). Data occupancy increased from 59.8% to 83.0% (-h 0.5), 82.9% (-h 0.4), and 90.8% (-h 0.3).

The application of poorly fitting models to analyse the data can yield strongly supported but erroneous relationships (Feuda *et al*., 2017; Pisani *et al*., 2015) resulting from various types of phylogenomic error (*e.g*., long-branch attraction) rather than phylogenetic signal (Feuda *et al*., 2017; Lartillot *et al*., 2007; Simion *et al*., 2017). We therefore compared the fit of compositionally site-heterogeneous (C10, C20) and site-homogeneous (WAG, LG) models to our antliophoran transcriptome dataset using ModelFinder (Kalyaanamoorthy *et al*., 2017) implemented in IQ-Tree 1.6.3 (Nguyen *et al*., 2015).

Mitogenome and Sanger sequence dataset

To test the effect of gene sampling, we mined GenBank for publicly available sequences of mitochondrial PCGs as well as the nuclear genes *18S*, *28S*, and *EF-1* (Table A1). PCGs were aligned by codon in MEGA X 10.0.5 with the 'MUSCLE' tool (Kumar *et al*., 2018) and ribosomal genes were aligned using the Q-INS-i algorithm in MAFFT on XSEDE 7.402 (Katoh & Standley, 2013) via the CIPRES Science Gateway (Miller *et al*., 2010). Three versions of the mitogenome and Sanger-sequence dataset were made: amino acids only (AA, *i.e*., excluding rDNA sequences), nucleotides (PCG123 + rDNA), and nucleotides with the third codon position excluded to reduce data heterogeneity (PCG12) (Rota-Stabelli *et al*., 2013). The sequences were concatenated in SequenceMatrix 1.8 (Vaidya *et al*., 2011). In total, 25 flea and scorpionfly species were sampled belonging to four and nine families, respectively, with two dipterans as outgroups.

Phylogenomic reconstruction

Phylogenomic analyses of the transcriptome dataset were conducted using the compositionally site-heterogeneous empirical mixture model C20+R4 implemented in IQ-Tree, which fitted the transcriptome dataset significantly better than the remaining tested models in ModelFinder (Table A2). We also tested the effect of using the LG4X+R model implemented in IQ-Tree, and the compositionally site-heterogeneous infinite mixture model CAT-GTR+G4 in PhyloBayes MPI 1.7 (Lartillot *et al*., 2009). For the latter, two independent Markov chain Monte Carlo (MCMC) chains were run until convergence (maxdiff < 0.3) and the bpcomp program was used to generate output of the largest (maxdiff) and mean (meandiff) discrepancies observed across all bipartitions. Support values in IQ-Tree were generated using 1,000 ultra-fast bootstraps, while support values in PhyloBayes runs are shown as Bayesian posterior probabilities (BPP). The mitogenome and Sanger-sequence datasets were analysed in PhyloBayes only (Marlétaz *et al*., 2019).

Evaluating alternative hypotheses of Siphonaptera placement

To evaluate support for the different hypotheses concerning the systematic position of fleas within Antliophora proposed by previous morphological and molecular analyses, we ran topology tests in IQ-Tree using the C20+R4 model (Schrempf *et al*., 2020). Statistical analyses included the approximately unbiased (AU), weighted Kishino-Hasegawa (KH), and weighted Shimodaira-Hasegawa (SH) tests. We did not test early hypotheses that considered the twisted wing insects (Strepsiptera) as members of Antliophora (Whiting *et al*., 1997) since the position of this enigmatic order is now well supported as sister to beetles (Misof *et al*., 2014;

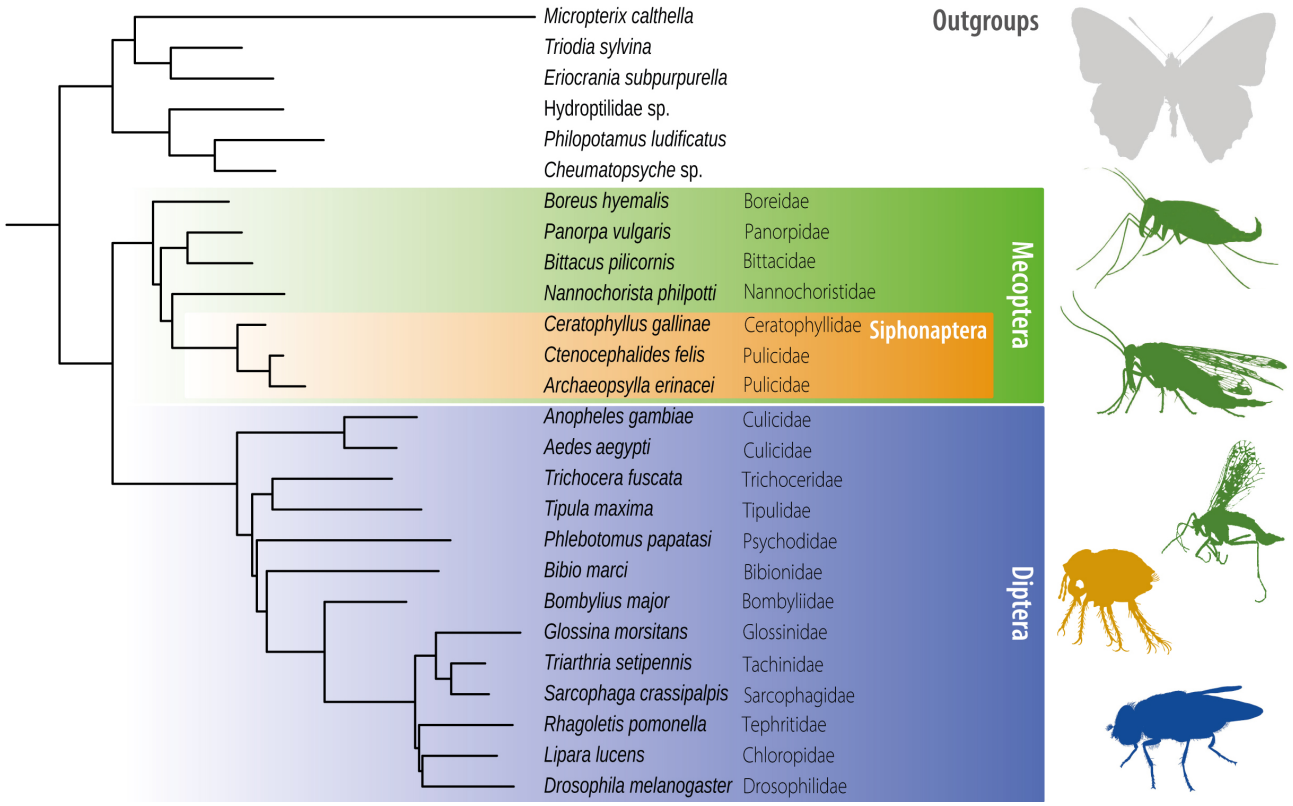

FIGURE 1. Phylogeny of Antliophora based on the conserved (-h 0.4) transcriptome dataset analysed with the CAT-GTR+G4 model. Mecoptera is paraphyletic with respect to Siphonaptera. All nodes are fully supported (BPP = 1). Coloured silhouettes displayed from top down are representatives of major antliophoran clades: *Boreus* (Neomecoptera), *Panorpa* (Pistillifera), *Nannochorista* (Nannomecoptera), *Tunga* (Siphonaptera), and *Chrysomya* (Diptera).

Niehuis *et al*., 2012). We also disregarded historical hypotheses of flea placement within Diptera (Byers, 1996; Rohdendorf, 1957), since these were formulated in a non-cladistic framework and have not been supported in formal phylogenetic analyses.

Result

Transcriptome analyses using the LG4X+R model as well as the site-heterogeneous models C20+R4 and CAT-GTR+G4 yielded well-resolved topologies with an identical position of Siphonaptera (Figs 1, 2). The selection of data filtering methods had no impact on the position of fleas. In line with previous phylogenomic analyses, Diptera is recovered as monophyletic (Misof *et al*., 2014; Wiegmann *et al*., 2011). However, scorpionflies are recovered as paraphyletic. The snow scorpionfly *Boreus hyemalis* is supported as sister to the remaining scorpionflies, while members of families Bittacidae and Panorpidae form a clade. The enigmatic southern hemisphere scorpionfly family Nannochoristidae is supported as sister to fleas.

The position and sister group of fleas was identical in all transcriptome analyses. Within the Mecoptera + Siphonaptera clade, the transcriptome analyses differed on a single node only. The C20+R4 analysis of the -h 0.3 dataset recovered *Boreus* as sister to *Panorpa* + *Bittacus* instead of being sister to the remaining mecopterans and fleas as in the other analyses. This node is however poorly supported (bootstrap value = 63), and so we regard *Boreus* as the earliest diverging mecopterans.

In contrast to the transcriptome runs, the mitogenome and Sanger sequence analyses recovered Boreidae or Eomeropidae as sister to fleas with varying support in different datasets (Fig. A1). Our results thus highlight the importance of extensive gene sampling for recovering the position of fleas.

Topology tests accounting for compositional heterogeneity consistently supported the transcriptome tree with fleas nested within scorpionflies as sister to Nannochoristidae (P_{AU}, P_{KH}, P_{SH} > 0.99) over the remaining hypotheses (Table 1), rejecting all remaining hypotheses with high significance. Only Diptera + Siphonaptera nested within a paraphyletic Mecoptera was supported by the results of the AU test (P_{AU} = 0.2105), but significantly rejected in the KH and SH tests (P_{KH}, P_{SH} < 0.0000).

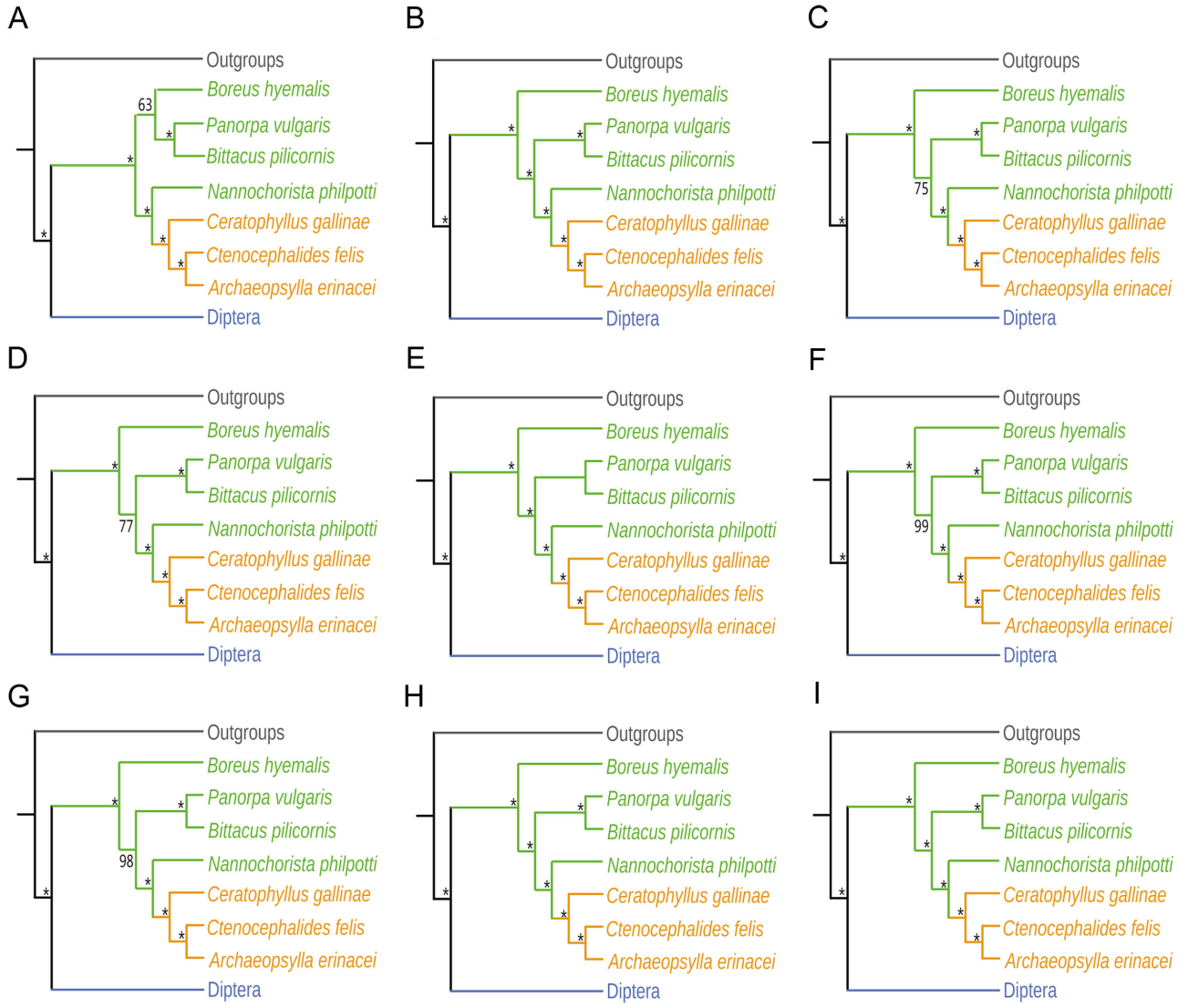

FIGURE 2. Phylogeny of Antliophora based on transcriptome datasets (**A**–**C**) with highly conserved sites (-h 0.3); (**D**–**F**) with conserved sites (-h 0.4); and (**G**–**I**) 'standard noise-free dataset' (-h 0.5). Models include the best fitting compositionally site-heterogeneous C20+R4 implemented in IQ-Tree (**A**, **D**, **I**); compositionally site-heterogeneous CAT-GTR+G4 in PhyloBayes (**B**, **E**, **H**), and LG4X+R implemented in IQ-Tree (**C**, **F**, **I**). Asterisks (*) indicate fully supported nodes (BPP = 1; bootstrap values = 100), numbers indicate nodes with lower than maximal support. Outgroups are in grey, strict sense scorpionflies in green, fleas in orange, and true flies in blue.

Discussion

Which is the sister group to fleas?

Mecopteran paraphyly with respect to fleas has been recovered in several early molecular studies of insects that used up to eight gene fragments (Kjer, 2004; Kjer *et al*., 2006; Wheeler *et al*., 2001; Whiting, 2002a, b; Whiting *et al*., 2003). Most subsequent studies sampling more genes failed to recover this relationship (Ishiwata *et al*., 2011; McKenna & Farrell, 2010; Peters *et al*., 2014; Wiegmann *et al*., 2009) including the transcriptome analysis of Misof *et al*. (2014) which supported fleas as sister to monophyletic Mecoptera. Misof *et al*. (2014) recovered fleas nested within scorpionflies as sister to Nannochoristidae, but only with low support in some ML analyses; they interpreted this result as a phylogenetic artifact. Nannochoristidae was also recovered as sister to fleas in a parsimony analysis of 18S rDNA (Whiting, 2002b), but rejected for failure to corroborate a contemporaneous study based on four genes (Whiting, 2002a). We show that extensive sampling of genes combined with careful dataset curation resolves fleas as nested within scorpionflies with strong support. Our results are congruent with mitogenome analyses of Song *et al*. (2016) who employed a site-heterogeneous model and found Siphonaptera nested within Mecoptera, albeit with low support for the sister group of fleas.

TABLE 1. Results of approximately unbiased (AU), weighted Kishino-Hasegawa (KH), and weighted Shimodaira-Hasegawa (SH) tests comparing historically proposed hypotheses of flea placement. *P*-value > 0.05: topology not rejected; *P*-value < 0.05: topology rejected significantly (*); *P*-value = 0: topology rejected with high significance.

Topology	Refs.	P_{AU}	P_{KH}	P_{SH}
((Boreidae (Pistillifera (Nannochoristidae, Siphonaptera))) Diptera) *fleas sister to Nannochoristidae, nested within scorpionflies*	Whiting, 2002b	0.9988	0.9988	1.0000
((Pistillifera (Nannochoristidae (Boreidae, Siphonaptera))) Diptera) *fleas sister to Boreidae, next to Nannochoristidae, nested within scorpionflies*	Whiting, 2002a; Whiting *et al.*, 2003	0.0000*	0.0000*	0.0001*
((Nannochoristidae (Pistillifera (Boreidae, Siphonaptera))) Diptera) *fleas sister to Boreidae, nested within scorpionflies, with Nannochoristidae as earliest-diverging mecopteran family*	Whiting, 2003; Whiting *et al.*, 2003	0.0009*	0.0000*	0.0000*
((Mecoptera, Siphonaptera) Diptera) *fleas sister to scorpionflies*	Dallai *et al.*, 2003; Ishiwata *et al.*, 2011; Kristensen, 1975, 1981; McKenna & Farrell, 2010; Misof *et al.*, 2014; Peters *et al.*, 2014; Wiegmann *et al.*, 2009	0.0018*	0.0012*	0.0359*
((Diptera, Siphonaptera) Mecoptera) *fleas sister to true flies*	Beutel *et al.*, 2011; Boudreaux, 1979; Sharov, 1966; Zhao *et al.*, 2020	0.0000*	0.0000*	0.0000*
((Mecoptera, Diptera) Siphonaptera) *fleas sister to scorpionflies and flies*	Kristensen, 1991	0.0021*	0.0000*	0.0000*
(((Diptera, Siphonaptera) Nannochoristidae), remaining Mecoptera) *fleas sister to flies, within a paraphyletic Mecoptera*	Wood & Borkent, 1989	0.2105	0.0000*	0.0000*

Mecoptera are remarkably morphologically heterogeneous and, ever since Hennig (1969), systematists have noted that the monophyly of the order is difficult to support based on morphology alone. In particular, the northern hemisphere Boreidae, and the relictual southern hemisphere family Nannochoristidae have long troubled mecopteran systematists, with some workers even regarding the two as separate orders (Beutel & Friedrich, 2019; Hinton, 1981). The remaining mecopteran families, characterised by a highly specific sperm pump (Willmann, 2003), are strongly supported as a clade referred to as Pistillifera (Beutel & Pohl, 2006). In the 1960s, Ross (1965) noted that fleas and scorpionflies show remarkable similarities in the internal anatomy of the proventriculus. Fleas and scorpionflies also lack outer groups of microtubuli on the sperm flagellum, reduced extrinsic labral muscles, and also lack labial endite lobes (Michelsen, 1997). A close relationship of fleas and scorpionflies is also supported by Mesozoic fossils that combine characters of both groups (Huang *et al*., 2012).

Both Boreidae and Nannochoristidae have been suggested as potential sister groups to Siphonaptera (Whiting, 2002b, 2003; Whiting *et al*., 2003). Snow scorpionflies are unusual among insects in that their adults with reduced wings emerge during the winter months and are active on snow (hence they are sometimes misleadingly referred to as 'snow fleas'). They share with true fleas their ovariole structure and mouthparts, proventricular spines, and a similar process of resilin secretion (Biliński & Büning, 1998; Kristensen, 1999; Richards & Richards, 1969; Rothschild *et al*., 1975). Although in molecular studies the support for the snow scorpionfly-flea hypothesis has been mixed, Kristensen (1999) pointed out that a transition from wingless moss-feeding snow scorpionflies to fleas that occupy vertebrate nests represents a convincing evolutionary scenario. Members of both groups are able to jump and their overall body plan is superficially similar (Whiting *et al*., 2008). Nonetheless, our analyses indicate that a Boreidae + Siphonaptera clade is only recovered when gene

sampling is limited, and the hypothesis was significantly rejected with the transcriptome dataset analysed in a site-heterogeneous framework (Table 1). This indicates that the apterous body plans of snow scorpionflies and fleas were acquired independently; in the case of boreids, the absence of wings and ability to jump likely represent adaptations for locomotion in snow, while in fleas these adaptations result from their ectoparasitic lifestyle.

Nannochoristids have, unusually for mecopterans, extremely slender predaceous aquatic larvae that feed on immature midges (Byers, 2009). The diet of the adults remains unknown, but probably consists of nectar or fruit juices (Palmer, 2010). Our transcriptome analyses strongly supported a Nannochoristidae + Siphonaptera clade, in contrast to alternative hypotheses. Transcriptomic data are not currently available for all mecopteran families, nevertheless, nannochoristids share with fleas the morphology of their panoistic ovarioles (Simiczyjew, 2002) and a set of characters associated with liquid feeding including: presence of a labral food channel; strongly developed postcerebral pumping apparatus; dorsally concave prelabium forming a trough for the paired mouthparts; exaggerated prementopalpal muscle; and stylet-like mandibles (Beutel *et al*., 2011). The shared presence of salivary channels on the laciniae and a sperm pump with a pistil chamber moved against a fixed pistil, are two possible synapomorphies of the nannochoristid-siphonapteran clade (Beutel & Baum, 2008; Beutel & Friedrich, 2019). The two taxa also share characters of the endophallus and larval terminal appendages, but their homology is difficult to ascertain (Fraulob *et al*., 2012; Mickoleit, 2008).

Fossil evidence and early evolution of fleas

While the fossil record of stem-fleas goes back to the Middle Jurassic (Huang *et al*., 2012, 2013), the earliest nannochoristid is known from the Permian (~272.3 Ma) (Pinto & de Ornellas, 1978). Although Permian fossil fur preserved inside coprolites (Bajdek *et al*., 2016) suggests that the ecological niche inhabited by modern fleas existed by the late Palaeozoic, this does not imply a Permian origin of fleas. The extinct Jurassic–Cretaceous Mesozoic scorpionfly family Aneuretopsychidae has been proposed as a sister-group to fleas based on their similar mouthparts forming an annulated siphonate organ and reduced labial palps (Huang *et al*., 2013; but see Zhao *et al*., 2020). Thus, attempts to constrain the origin of fleas depend on discovering further Mesozoic scorpionflies and unravelling their relationships to fleas.

A sister relationship between Nannochoristidae and Siphonaptera, as well as the reconstructed palaeobiology of Mesozoic siphonate mecopterans (Palmer, 2010; Zhao *et al*., 2020) implies that fleas originated from a liquid-feeding ancestor that may have fed on plant secretions such as pollination drops from gymnosperms. The transition from feeding on plant secretions to blood-feeding is an evolutionary trajectory that occurred independently in at least one other hematophagous insect clade, the dipteran infraorder Culicomorpha. This would suggest that the co-option of mouthparts adapted for feeding on plant secretions to puncturing the cuticle of vertebrate hosts may represent an important step in the evolution of hematophagy and parasitism.

Taxonomic implications

Nested within Mecoptera, fleas no longer warrant the status of a distinct insect order. This situation is not without precedence in entomology; over the past two decades molecular analyses were able to verify earlier morphology-based hypotheses that the highly specialised termites and parasitic lice, both traditionally considered as separate orders, belong among cockroaches, and bark and book lice, respectively (Inward *et al*., 2007; Johnson *et al*., 2018; Yoshizawa & Johnson, 2010). Based on combined phylogenomic and morphological evidence, we propose demoting Siphonaptera to infraordinal level. Taxonomic stability is crucial for medically important groups such as fleas and this treatment retains the widely-recognised and well-defined flea families at their current taxonomic rank. Our newly defined Mecoptera, including Siphonaptera, is equivalent to the Mecoptera *s.l.* of Kristensen (1999) and reduces the total number of extant holometabolan insect orders to ten.

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Data accessibility

Data are available from the Appendix Information and MendeleyData: http://dx.doi.org/10.17632/586yjn978x.

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Appendix: Additional material for molecular phylogenetic analyses of Antliophora.

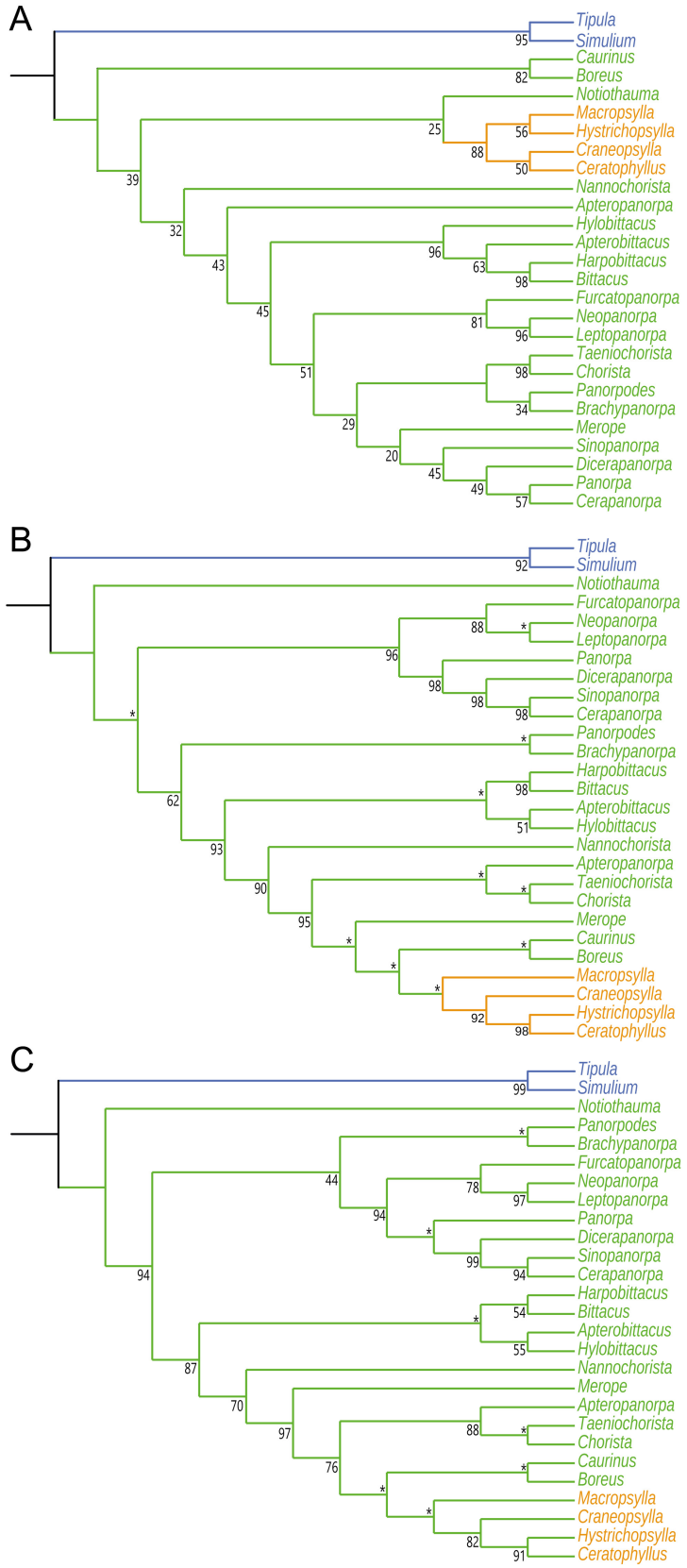

FIGURE A1. Phylogeny of Antliophora based on the mitogenome and Sanger sequence dataset (**A**) amino acids only (AA); (**B**) nucleotides (PCG123 + rDNA); (**C**) nucleotides with the third codon position excluded (PCG12). The compositionally site-heterogeneous CAT-GTR+G4 implemented in PhyloBayes was used to analyse all datasets. Asterisks (*) indicate fully supported nodes (BPP = 1; bootstrap values = 100), numbers indicate nodes with lower than maximal support. Strict sense scorpionflies are in green, fleas in orange, and true flies in blue.

TABLE A1. GenBank accessions for all taxa sampled in the mitogenome and Sanger sequence dataset.

Taxon	GenBank accessions mitochondrial			nuclear		
	mtDNA	*COI*	*COII*	*18S*	*28S*	*EF-1*
MECOPTERA						
Apteropanorpidae						
Apteropanorpa evansi		AF423987		AF286284	AF423925	AF423816
Bittacidae						
Apterobittacus apterus			AF423988	AF423875	AF423926	AF423817
Bittacus sp.	NC_044741			L10184	U65204	AF423818
Harpobittacus australis			AF423990	AF423877	AF423928	AF423819
Hylobittacus apicalis		MN344499	AF423994	AF423880	AF423931	AF423823
Boreidae						
Boreus sp.	HQ696579			X89487	EU426882	AF423827
Caurinus dectes			KF282717	AF286288	AF423937	AF423830
Choristidae						
Chorista australis			AF424007	AF286289	AF423943	AF423836
Taeniochorista pallida			AF424008	AF423889	AF423944	AF423837
Eomeropidae						
Notiothauma reedi		MN345521				
Meropeidae						
Merope tuber		AF436484	AF424017	AF286287	DQ202351	AF423847
Nannochoristidae						
Nannochorista sp.	HQ696580			KC177275	KC177635	AF423849
Panorpidae						
Cerapanorpa sp.	KX091860				KY582989	KT943360
Dicerapanorpa sp.		MK155218	MK175492		MK153733	
Furcatopanorpa longihypovalva		JN223455	GU722407		MG775026	
Leptopanorpa cingulata		MH592629	MH592605		MH592553	
Neopanorpa sp.	KX091857			AF423903	AF423961	AF423856
Panorpa sp.	NC_044742			X89493	AF423954	AF423851
Sinopanorpa tincta		MK155248	MK175491		HM061595	
Panorpodidae						
Brachypanorpa sp.		MN344636	EF050569	AF423912	AF423972	AF423867
Panorpodes sp.		JN223473	EF050568	AF423913	AF423973	AF423869
"SIPHONAPTERA"						
Ceratophyllidae						
Ceratophyllus sp.	MG886872			EU336130	KM891088	EU336258
Hystrichopsyllidae						
Hystrichopsylla sp.	MH259703			KM891245	KM891029	EU336297
Macropsyllidae						
Macropsylla novaehollandiae			EU336001	EU336087	EU336195	EU336300
Stephanocircidae						
Craneopsylla sp.		KM891000	AF424044	EU336118	KM891122	KM890565
DIPTERA						
Simuliidae						
Simulium sp.	KP793690			JQ793854	KP661560	AF003580
Tipulidae						
Tipula sp.	NC_030520			U65156	AY456152	

TABLE A2. Fit of maximum likelihood (ML) models to the antliophoran transcriptome dataset analysed in ModelFinder. Plus signs denote the 95% confidence sets, while minus signs denote significant exclusion. Abbreviations: AIC, Akaike information criterion scores.

Model	AIC
C20+R4	1881039.9786 +
C20+R5	1881041.5004 +
C20+R3	1881341.5460 –
C20+I+G	1883302.4680 –
C20+I	1883302.6316 –
C20+G	1885395.1112 –
C20	1885395.1136 –
C20+R2	1886603.2846 –
C20+F+R4	1925365.7360 –
C20+F+R5	1925367.0068 –
C20+F+R3	1925654.5828 –
C20+F+G	1929531.5998 –
C20+F	1929562.6818 –
C20+F+R2	1930796.4298 –
LG+F+R4	1931726.8104 –
LG+F+R5	1931729.0944 –
LG+F+R3	1932387.6026 –
LG+R4	1933688.1048 –
LG+R5	1933689.7822 –
LG+R3	1934330.5638 –
LG+F+I+G4	1934537.6278 –
LG+F+G4	1936271.7918 –
LG+I+G4	1936490.8632 –
LG+F+R2	1937959.1518 –
LG+G4	1938246.8296 –
LG+R2	1939858.3062 –
WAG+F+R4	1948032.1170 –
WAG+F+R5	1948033.5574 –
WAG+F+R3	1948503.1394 –
WAG+F+I+G4	1950257.9792 –
WAG+F+G4	1951847.5078 –
WAG+R5	1953041.5218 –
WAG+R4	1953077.7234 –
WAG+R6	1953044.8092 –
WAG+R3	1953486.4096 –
WAG+F+R2	1953346.7444 –
C10+F+R4	1953243.2674 –
C10+F+R5	1953244.5158 –
C10+F+R3	1953532.1786 –
WAG+I+G4	1955224.5322 –
C10+R4	1956316.4356 –
C10+R5	1956317.8178 –
C10+R3	1956599.4424 –
WAG+G4	1956840.4352 –
C10+F+G	1957502.3048 –
C10+F	1957518.9550 –
LG+F+I	1957990.6730 –
WAG+R2	1958371.2730 –
C10+I+G	1958434.7098 –
C10+I	1958435.4318 –
C10+F+R2	1958823.8172 –
LG+I	1959769.9022 –
C10+G	1960575.1578 –
C10	1960575.1602 –
C10+R2	1961876.6264 –
WAG+F+I	1972363.8770 –
WAG+I	1977462.8388 –
LG+F	2031678.4620 –
LG	2034044.8160 –
C20+F+I	2037681.7800 –
WAG+F	2046704.0900 –
C20+F+I+G	2048537.7400 –
WAG	2052574.4480 –
C10+F+I	2062396.8000 –
C10+F+I+G	2074148.8800 –