A molecular phylogeny of fleas (Insecta: Siphonaptera): origins and host associations

Michael F. Whiting\textsuperscript{a,b,*}, Alison S. Whiting\textsuperscript{a}, Michael W. Hastriter\textsuperscript{b} and Katharina Dittmar\textsuperscript{a,c}

\textsuperscript{a}Department of Biology, Brigham Young University, Provo, UT 84602, USA; \textsuperscript{b}Monte L. Bean Life Science Museum, Brigham Young University, 290 MLBM, PO Box 20200, Provo, UT 84602-0200, USA; \textsuperscript{c}SUNY at Buffalo, Department of Biological Sciences, 109 Cooke Hall, Buffalo, NY 14260, USA

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Abstract

Siphonaptera (fleas) is a highly specialized order of holometabolous insects comprising \(\sim2500\) species placed in 16 families. Despite a long history of extensive work on flea classification and biology, phylogenetic relationships among fleas are virtually unknown. We present the first formal analysis of flea relationships based on a molecular matrix of four loci (18S ribosomal DNA, 28S ribosomal DNA, Cytochrome Oxidase II, and Elongation Factor 1-alpha) for 128 flea taxa from around the world representing 16 families, 25 subfamilies, 26 tribes, and 83 flea genera with eight outgroups. Trees were reconstructed using direct optimization and maximum likelihood techniques. Our analysis supports Tungidae as the most basal flea lineage, sister group to the remainder of the extant fleas. Pygiopsyllomorpha is monophyletic, as are the constituent families Lycopsyllidae, Pygiopsyllidae, and Stivaliidae, with a sister group relationship between the latter two families. Macropsyllidae is resolved as sister group to Coptopsyllidae with moderate nodal support. Stephanociricidae is monophyletic, as are the two constituent subfamilies Stephanociricinae and Craneopsyllinae. Vermipsyllidae is placed as sister group to \textit{Jordanopsylla}. Rhopalopsyllidae is monophyletic as are the two constituent subfamilies Rhopalopsyllinae and Parapsyllinae. Hystrichopsyllidae is paraphyletic with Hystrichopsyllini placed as sister to some species of Anomiopsyllini and Ctenopariini placed as sister to Carterettini. Ctenopariinae is grossly paraphyletic with the family broken into seven lineages dispersed on the tree. Most notably, Anomiopsyllini is paraphyletic. Pulicidae and Chimaeropsyllidae are both monophyletic and these families are sister groups. Ceratophyllomorpha is monophyletic and includes Ischnopsyllidae, Ceratophyllidae, and Leptopsyllidae. Leptopsyllidae is paraphyletic as are its constituent subfamilies Amphipsyllinae and Leptopsyllinae and the tribes Amphipsyllini and Leptopsyllini. Ischnopsyllidae is monophyletic. Ceratophyllidae is monophyletic, with a monophyletic Dactypsyllinae nested within Ceratophyllinae, rendering the latter group paraphyletic. Mapping of general host associations on our topology reveals an early association with mammals with four independent shifts to birds.

\textsuperscript{*}Corresponding author. Fax +1 801 422 0090. E-mail address: Michael_Whiting@byu.edu

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Introduction

Siphonaptera (fleas) is a highly specialized holometabolous insect order, currently comprising 246 genera and approximately 2575 described taxa (including subspecies—modified from Lewis, 1998). 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 and Mardon, 1991). Fleas are obligate parasites (endo- or ectoparasitic) on birds and mammals. Those occurring on birds are only represented in five flea families: Ceratophyllidae (68 spp./84 subspecies), Leptopsyllidae (8/9), Pulicidae (5/5), Pygiopsyllidae (10/12), and Rhopalopsyllidae (17/22), while the remaining species parasitize mammals. 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 medical and economic importance as vectors of several diseases important to human health including bubonic plague, murine typhus, and tularemia (Dunnet and Mardon, 1991). The recognition that fleas were capable of transmitting plague organisms (*Yersinia pestis*), and later murine typhus (*Rickettsia typhi*) stimulated a frenzy of flea studies in the early 20th century. A history of notable flea workers has been described elsewhere (Hastriter and Whiting, 2003).

**Classification**

Compared with most holometabolous insects, the classification of fleas is relatively advanced, and it is not uncommon to extend the classification of fleas to the subspecific level in many groups. Some authors have argued that Siphonaptera is the most completely studied order of insects (Medvedev, 1994), and although this is perhaps true from an alpha level classification point of view, from a phylogenetic standpoint, they have been sorely neglected as a group. G. H. E. Hopkins and M. Rothschild published a five-volume series on flea systematics based on the extensive Rothschild flea collection (Hopkins and Rothschild, 1953, 1956, 1962, 1966, 1971). The series considers 12 families: Ancestropsyllidae, Coptopsyllidae, Hypsophthalmidae, Hystrichopsyllidae, Ischnopsyllidae, Leptopsyllidae, Macropsyllidae, Pulicidae, Stephanocircidae, Tungidae, Vermipsyllidae, and Xiphiopsyllidae. Currently, Hypsophthalmidae is recognized as Chimaeropsyllidae and many of the taxa included under Hystrichopsyllidae are recognized by most (Lewis, 1998), but not all (Medvedev, 1994, 1998) workers as belonging within the family Ctenophthalmidae. Three additional companion volumes were published for the remaining families: Pygopsyllidae by Mardon (1981), Ceratophyllidae by Traub et al. (1983), and Malacopsyllidae and Rhipalopsyllidae by Smit (1987). 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 and Starcke, 1980; Traub et al., 1983; Dunnet and Mardon, 1991; Lewis, 1993; Medvedev, 1994, 1998). Lewis (1993, 1998) recognized 15 families that he placed within five superfamilies: Ceratophyloidea, Hystrichopsyloidea, Malacopsyloidea, Pulicoida, and Vermipsyloidea. Medvedev (1994, 1998) recognized 18 families in the order, which he placed in four major complexes (1994), that were later (1998) formally described as infraorders: Ceratophylophoromorpha, Hystrichopsyllomorpha, Pulicomorpha, and Pygopsyllomorpha. Figure 1 illustrates species representing the morphological and phylogetic diversity within Siphonaptera. Table 1 compares the higher-level classifications of Lewis and Medvedev, and highlights the differences between their placement of subfamilies and families within the higher taxonomic groups. For the purpose of this paper, we chose to follow Medvedev’s treatment (1994, 1998) by recognizing the families Tungidae, Lycopsyllidae, Sti-validae, and Pygiopsyllidae, as well as the four infraorders listed above. However, we follow Lewis in recognizing Ctenophthalmidae and the associated subfamilies as presented in Table 1.

**Phylogeny**

Although 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, a rigorous exploration of phylogenetic relationships among fleas has been a challenge. From a phylogenetic standpoint, Siphonaptera has remained the most neglected of the holometabolous insect orders. In the past 30 years, there have been over 3000 publications dealing with some aspect of fleas (Lewis and Lewis, 1985), but only a few instances of formal cladistic analyses (Cheetham, 1988; Linardi and Guimaraes, 1993; Lu and Wu, 2003; Blank et al., 2007). Although most flea genera and many flea tribes appear to be natural groupings, there are many cases where the assignment of these taxa to a particular family is dubious, and certain families that have been used as a catchall for a wide range of divergent taxa (e.g. Ctenophthalmidae) are almost certainly paraphyletic assemblages.

Classically, the major obstacle in flea phylogenetics has been their extreme morphological specializations associated with ectoparasitism, and the inability of systematists to homologize characters adequately across flea and outgroup 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 and Starcke, 1980; Dunnet and Mardon, 1991; Hastriter and Whiting, 2003). Although these characters are excellent for species diagnoses, they are mostly autopomorphic at the species level and of limited utility for phylogenetic reconstruction. Fleas appear to have many instances of parallel evolution of morphology (via both structural losses and modifications), probably associated with multiple invasions of similar hosts, which further obscures homology (Holland, 1964).

Another complicating factor has been the inability to identify the sister group to fleas in order to polarize characters. 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
Fig. 1. Plate representing morphological and phylogenetic diversity of fleas. (a) *Tunga penetrans*, male (Tungidae); (b) *Xenopsylla cheopis*, male (Pulicidae); (c) *Rhinolophopsylla ectopa*, male (Ischnopsyllidae); (d) *Nearctopsylla traubi*, male (Ctenophthalmidae); (e) *Barneropsylla excelsa*, male (Stephanocircidae); (f) *Deimellonia granti*, male (Chimaeropsyllidae); (g) *Typhloceras favosus favosus*, male (Hystrichopsyllidae); (h) *Dorcadia ioffi*, replete female (Vermipsyllidae).
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. More recently, a sister group relationship between the mectopteran family Boreidae and fleas was pinpointed via molecular data from four genetic markers (Whiting, 2002), and is bolstered by morphological evidence. Morphological synapomorphies supporting this relationship include a similar process of resilin secretion (Rothschild et al., 1975; Schlein, 1980), presence of unusual proventricular spines (Richards and Richards, 1969), and a complex series of characters associated with the ovarioles (Bilinski et al., 1998). The combination of morphological with molecular data provides compelling evidence for a sister group relationship between Boreidae and Siphonaptera.

In the first volume of the catalogue of the Rothschild Collection of Fleas (Hopkins and Rothschild, 1953, 1956, 1962, 1966, 1971), a phylogeny representing relationships among the families is presented and attributed to Karl Jordan (Fig. 2). No discussions of

### Table 1
Comparison of the higher-level classification systems of Lewis (1993) and Medvedev (1998)

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characters or analyses are presented to support this phylogeny, and this topology appears simply to reflect Jordan’s intuitive view of relationships based on his extensive alpha taxonomic work on the group. Most notably, Jordan recognized a sister group relationship between Tungidae and Pulicidae, and placed this clade as sister group to the remaining fleas. Smit (1987) presented a phylogeny which recognized five major superfamilies within Siphonaptera: Hystrichopsylloidea, Ceratophylloidea, Malacopsylloidea, Vermipsylloidea, and Pulicoidea (Fig. 3). Relationships among and within these groups are entirely unresolved, and this phylogeny was not based on any sort of formal quantitative analysis of flea morphology.

The work of Medvedev (1994, 1998, 2001) stands as currently the most comprehensive attempt to reconstruct phylogeny based upon morphology. Medvedev’s work is important on two fronts: he defined a number of “morphogenetic compartments” which represent the major morphological features of fleas, and attempted to survey these features across the diversity of flea groups, in the hope of providing additional characters to support classification and to establish “evolutionary trends” of the structures. Secondly, he attempts to analyse these data in a systematic fashion and describes a novel methodology for deriving the classification from this character information. This method searches for “correlations between the characters of the body segments—the head, thorax, and abdomen” by analysing characters from “the point of view of their functional interconnection and their possible adaptive importance” (Medvedev, 1994: p. 32). However, this method does not specifically search for synapomorphic evidence for monophyly, nor is there any formal algorithmic analysis of character information as is standard in modern cladistic practice. For example, in his 1994 work, Medvedev provides a list of 50 multistate characters which he codes across the families, but many of these character states are overlapping, continuous features that are difficult to code as discrete characters. He presents a matrix coded at the family level, but it is unclear how that information was transformed into his figure of the “Cladistic relationships of Siphonaptera”. We have analysed this matrix via standard parsimony methods, and obtained a topology that is almost entirely unresolved, in large part because a large percentage of the cells in his matrix are polymorphic, and many of the characters are not phylogenetically informative (topology not shown). Consequently, Medvedev’s hypothesis still lacks a clear connection between character information and phylogeny, and it is unclear how well the data actually support relationships across the topology. Despite these limitations, the Medvedev topology provides a useful comparison for our molecular results. The four infraorders that Medvedev (1998) recognizes are shown on his 1994 topology (Fig. 4). Medvedev agrees with the Jordan topology and Smit topology in placing Tungidae as sister group to Pulicidae, although he nests this clade higher in the tree, rather than at the base as did Jordan.

**Fossil records and flea evolution**

Given the parasitic lifestyle of fleas, it is not surprising that the flea fossil record is sparse. Three extinct species have been reported from Baltic amber (Eocene/Oligocene; c. 40–35 Ma): *Palaeopsylla klebsiana* (Dampf, 1910), *Palaeopsylla dissimilis* (Peus, 1968), and *Palaeopsylla balatica* (Beaucournu and Wunderlich, 2001). One species has been reported from Dominican amber...
(30–35 Ma): *Pulex larimerius* (Lewis and Grimaldi, 1997). The *Palaeopsylla* species belong to an extant Palearctic genus (family: Ctenophthalmidae). *P. larimerius* belongs to the extant genus *Pulex*, which includes five species confined to the Nearctic and Neotropical regions and a sixth that is cosmopolitan (*Pulex irritans*).

Poinar (1995) reported a *Rhopalopsyllus* sp. (Rhopalopsyllidae) from Dominican amber, and seven additional specimens from Dominican amber, although unpublished, are thought to represent the Neotropical family Rhopalopsyllidae (Lewis and Grimaldi, 1997). The amber fossils are so similar to extant taxa that they provide little insight into general trends in flea evolution. Riek (1970) reported two fleas from the lower Cretaceous in Australia (100–125 Ma), but the specimens are lost, and these records cannot be confirmed.

The assignment of certain Mesozoic compression fossils to Siphonaptera, or close relatives of Siphonaptera, provides some intriguing (if not entertaining) scenarios of flea evolution. Rasnitsyn (1992) described *Strashila incredibilis* from the Upper Jurassic of Siberia, which he characterizes as “in general a nasty looking creature” (p. 324). This fossil along with two other fossils (*Saurophthirus longipes* and *Tawinia australis*) are considered as “pre-fleas” by Rasnitsyn (2002) based on the presence of a hypognathous head with a beak, and relatively short moniliform antennae. These fossils also possess widely separated coxae, very long legs, and strong claws, features which Rasnitsyn (2002) suggests are similar to the general appearance of insects that are parasitic on bat wing membranes, notably flies (Nycteribiidae), bugs (Polycenidae), and mites (Sphinturnicidae). Rasnitsyn thus postulates that these insects were parasites of the pterosaur body and wing membrane, and that there was a shift from pterosaur to mammalian fur. Rasnitsyn does not discuss, however, that there is one entire family (Ischnopsyllidae) and one species of *Hectopsylla* that are strictly bat ectoparasites, and these fleas do not exhibit the morphological features of arthropod bat parasites as listed above.

The placement of Boreidae as the extant sister taxon to fleas suggests a different evolutionary scenario (Whiting, 2002). In Boreidae, females are wingless and males have the forewing reduced and modified into hooks, used for grasping the female during copulation. In addition, boreids have the ability to jump in a manner that appears similar to that of fleas, although detailed morphological and functional comparisons have yet to be performed. Boreids emerge as adults only during winter months, are closely associated with mosses, and like many other winter insects, the reduction and loss of wings reduces the body surface area, and may be an adaptation to the cold. The ability of boreids to jump facilitates movement on soft, fluffy snow, and is also probably an adaptation to this extreme environment. When the boreid–flea ancestor shifted from a snowy, mossy habitat to the nest of a mammal host, it had already undergone the loss of wings and acquired the ability to jump. Subsequent modifications to the primitive flea include lateral flattening, the development of sucking mouthparts, and the development of elaborate combs and setae as further adaptations for a parasitic life. If the pterosaur scenario is correct, then one must postulate the less-likely shift from a boreid-like ancestor to the pterosaur, and from the pterosaur to mammal hosts. That would have been a remarkable jump indeed.

**Host associations, ancestral fleas, and host specificity**

The majority of flea species are associated with mammal hosts, with about 74% of described species recorded from rodents. Only 8% of fleas are known from Soricomorpha, 5% each from Metatheria and
Chiroptera, followed by a mere 3% from Carnivora and Lagomorpha. The mammalian orders Monotremata, Cingulata, Pilosa, Pholidota, Hyracoidea and Artiodactyla harbour together only 1% of the flea fauna, whereas 6% of the total diversity is ornithophilic (Marshall, 1981).

Bird fleas of two families (Ceratophyllidae and Leptopsyllidae) are distributed almost exclusively in the Holarctic region, while all representatives in Pygopsyllidae and Rhopalopsyllidae occur in the Australian and Neotropical regions, respectively. Excluding the cosmopolitan species (*Echidnophaga gallinacea* and *Hectopsylla psittaci*), avian pulicids are limited to the Palearctic (*Ornithopsylla laetitae*) and Neotropical regions (*Actenopsylla suavis* and *Hectopsylla narium*).

Although Siphonaptera are rarely monoxenous at the host species level, there appear to be clades of fleas that associate with a particular host group at higher ordinal levels. For instance, Parapsyllini are exclusively associated with birds and Rhadinopsyllini are only recorded from mammals. Generally, mammals that have vast home ranges and do not inhabit dens for rearing their young almost always lack fleas of their own, whereas hosts (mammals or birds) with dens or nests reused seasonally exhibit a more specific flea fauna.

The species richness of fleas on Rodentia has led some researchers to speculate that fleas might have initially coevolved with this species-rich group of mammals, and that shifts to other mammals and birds occurred secondarily (Marshall, 1981). Other researchers have pointed out that primitive siphonapterans are almost always on primitive hosts, such as shrews, moles, and metatherians (Traub and Starcke, 1980). In the absence of a stringent phylogenetic hypothesis, the term “primitive” in this context alludes to the morphologically simplified appearance of fleas (i.e. devoid of combs, spines, or spinelets), and the relatively basal position of their mammalian hosts. The difficulty with this hypothesis is that it is still too broad: two flea families (e.g. Pulicidae and Ancistropsyllidae) are morphologically simplified and thus potential candidates for the basal flea lineage. Although hosts and parasite associations for fleas have been investigated using current flea classification as a basis of comparison (Medvedev, 2000a, b), it has not been investigated from a flea phylogeny point of view. Thus, the degree to which there has been host/parasite coevolution within fleas and their hosts remains unclear.

### Research goals

The main goals of this study were to: (i) use molecular data from multiple loci and a broad sampling of taxa to provide the first formal estimate of flea phylogeny, (ii) decipher which extant taxa or taxon is sister group to the remaining fleas (i.e. is the most basal extant flea lineage), and (iii) use the phylogenetic reconstruction as a framework to understand the evolution of general host associations for Siphonaptera. This work will focus on molecular data as the underlying source of information on flea phylogeny. The formal description and analysis of a comprehensive morphological matrix and revision of classification is ongoing and will be the focus of a future publication.

### Materials and methods

#### Taxon sampling

Eight taxa representing two genera and seven species of Boreidae were used as the outgroup taxa, and the tree was rooted to *Caurinus dectes*. Ingroups included 128 flea taxa comprising 125 species representing a wide diversity of flea groups. Taxonomically, these exemplars represent 16 of 19 families, 25 of 29 described subfamilies, 26 of 43 described tribes, and 83 of 246 described genera (Table 2). Species were obtained from throughout the world through our own collecting efforts and through the assistance of numerous colleagues (see Acknowledgements). Biogeographically, the sparsest taxonomic sampling was from Asia, and the richest was from the Nearctic. Taxa absent in this analysis include exemplars of the families Ancistropsyllidae, Malacopsyllidae, and Xiphiopsyllidae. Appendix I provides a complete list of taxa, host associations, and GenBank accession numbers.

All specimens were preserved in 95–100% ethanol and stored at −80 °C. Specimens were cut at the anterior dorsal part of the abdomen and were incubated in a tissue buffer with gentle rocking at 55 °C for 12 h. Tissue was extracted using the Qiagen DNeasy® Tissue Kit (Qiagen Inc., Valencia, CA, USA). Complete flea exoskeletons from each DNA extraction were retained and mounted on slides as primary voucher specimens. These were prepared and mounted on glass slides using conventional procedures as outlined elsewhere (Hastreiter and Whiting, 2003). Because of the dissolution of soft tissues after extraction, the time required to clear in potassium hydroxide is generally not required or is reduced to only a few hours. Voucher specimens are deposited in the Insect Genomics Collection, Brigham Young University.

#### Gene sampling

Four genes were targeted for amplification and sequencing: small subunit nuclear ribosomal RNA (18S, ~2000 bp), large subunit nuclear ribosomal RNA (28S, ~2400 bp), Elongation Factor-1α (EF-1α, 1065 bp), and mitochondrial Cytochrome Oxidase II gene.
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<th>No. of species</th>
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<td>16 of 19 families</td>
<td>25 of 30 subfamilies</td>
<td>26 of 43 tribes</td>
<td>83 of 246 genera</td>
<td>125 of 2118 spp.</td>
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The number of genera and approximate number of species (including only nominate species and not subspecies) included in this molecular analysis is provided, with the specific species and hosts listed in Appendix 1.

n/a, not applicable.

(COII, ~612 bp). Primer sequences and amplification protocols are given in Whiting (2002). These markers were amplified using AmpliTaq Gold® DNA polymerase (Applied Biosystems, Foster City, CA, USA). Product yield, specificity, and potential contamination were monitored by agarose gel electrophoresis and purified using Gene Clean® III (Qiagen Inc., Carlsbad, CA, USA). Sequencing of purified PCR products was performed using BigDye™ v3.0 (Applied Biosystems). Sequencing reactions were purified using Sephadex™ G-50 gel filtration medium (Amersham Biosciences Corp., Piscataway, NJ, USA) and fractionated on an ABI PRISM® 377 or ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems). Primary sequence data were assembled using Sequencher™ 4.2 (Gene Codes Corp., Ann Arbor, MI, USA), and complementary strands were assembled to verify the identity of the sequences. All genes were screened for potential host contamination by using the Blastx algorithm on GenBank.

Phylogenetic analyses

Analyses—POY

To analyse this dataset we applied two approaches, maximum parsimony as implemented in POY (POY-MP) and maximum likelihood (ML) based on a Muscle alignment. For the POY analysis, ribosomal genes were initially manually aligned in Sequencher® 4.2 and partitioned into regions at the conserved domains. This allows for more efficient analysis in POY (Giribet, 2001) and reduces ambiguity in reconstructing the implied alignment (Wheeler, 2003). This resulted in three regions in 18S and 13 regions in 28S. The genes COII and EF-1α were aligned based on conservation of codon reading frame. Partitioned gene regions were analysed simultaneously via Direct Optimization (DO) in POY 3.0.11 (Wheeler et al., 2003) as implemented in parallel on an IBM SP-2 supercomputer (http://mary lou.byu.edu/resources.htm) containing 316 power3 processors (375 MHz). POY search parameters are as follows for equivalent cost ratios: “-fitchtrees -parallel -noleading -norandomizeoutgroup - impliedalignment -sprmaxtrees 1 -tbrmaxtrees 1 -maxtrees 5 -holdmaxtrees 50 -slop 5 -checkslp 10 -buildspr -buildmaxtrees 2 -random 100 -stopat 25 -multirandom -treefuse -fuselimit 10 -fuseingroup 5 -fusematrix 100 -numdriftchanges 30 -driftspr -numdriftspr 10 -drifttbr -numdrifttbr 10 -slop 10 -checkslop 10 -molecular-matrix 111.txt -seed -1”. Nodal support was calculated for the combined dataset. Partitioned Bremer support values (Baker and DeSalle, 1997) were calculated from POY’s implied alignment using TreeRot.v2b (Sorenson, 1999) and Paup 4.0b10 (Swofford, 2002). Although the implied alignment of POY is not intended to be the same as a standard multiple alignment, it is nonetheless the best estimate of a minimal cost multiple alignment (Ogden and Whiting, 2003; Wheeler, 2003) and is useful for further phylogenetic investigation. Non-parametric bootstrap values were calculated using the implied alignment from POY (1000 replicates, 50 random additions per replicate, gaps treated as 5th state) in PAUP* 4.0b10.

Analysis—maximum likelihood

Alignment was conducted for each gene separately in Muscle 3.6 (Edgar, 2004). Muscle uses a progressive alignment with horizontal refinement. Pairs of profiles are extracted from the progressive alignment and re-aligned, keeping the results only when an objective score is improved. The alignment was calculated using the -maxtiters 16- option, allowing for faster alignment speed. Aligned sequences were then concatenated into one multiple alignment. The alignment was analysed in Modeltest 3.06 PPC (Posada and Crandall, 1999) implementing the Akaike information criterion (AIC) to find the most justified likelihood model for the analysis. AIC reduces the number of unnecessary parameters that contribute little to describing the data by penalizing more complex models (Posada and Buckley, 2004). ML analysis was performed in PAUP* v4.0b10 (Swofford, 2002) with a heuristic search of ten random sequence additions and tree bisection–reconnection (TBR) branch swapping. Nodal support was assessed for the combined dataset with 100 bootstrap replicates, TBR branch swapping, and ten random addition replicates. The analysis was run on a 64-node Debian Linux cluster, with each node powered by two 1.6-GHz 64-bit AMD Opteron processors with 512 MB of RAM (http://babeast.byu.edu).
Sensitivity analysis

Multiple cost parameters for optimization alignment were investigated to test the sensitivity of the phylogenetic conclusions to perturbations in parameter values. The goal of sensitivity analysis is to measure the sensitivity of phylogenetic conclusions against a wide range of biologically meaningful analytical parameters (Wheeler, 1995). Those relationships which are supported under a wide range of parameter values are more robust to perturbations in parameter values than those which are not, and thus provide an additional measure of nodal support (Wheeler et al., 2001; Ogden and Whiting, 2003). We varied the cost ratio of transversion to transition and the cost ratio of gap to transversion in five increments each (0.5, 1, 2, 3, 4). This resulted in 25 combinations of parameter values evaluated across the parameter landscape. Analyses were run under the same POY parameters as given above, but with only 50 replicates per run. The incongruence length difference metric (ILD; Mickevich and Farris, 1981) was used to measure congruence among data partitions across the range of cost parameters. The combination of cost parameter values that maximized dataset congruence by minimizing the ILD value was retained as the best justified parameter values for phylogenetic estimation (Wheeler et al., 2001), and thus underwent a more exhaustive search (200 random additions, with the same POY parameters as above).

Mapping of host associations

The host association information for each flea species used in the molecular analysis was extracted from roughly 15 000 entries in the literature (Hopkins and Rothschild, 1953, 1956, 1962, 1966, 1971; Medvedev, 1997a, b, 2000a, b) and the Australian Biological Resource Study (http://www.deh.gov.au/). We coded host association data for each flea species included in this analysis, and did not try to extend the range of host association by including the host data from congeneric taxa. For mammal hosts, we scored at the level of order, with the exception of rodents (the most common host of fleas) that were scored at the familial level. Bird hosts were scored simply as Aves. Some flea species are widespread on multiple host species, and in these cases the hosts were coded as polymorphic for the states as listed above. Other fleas inhabit an extraordinary range and diversity of hosts (e.g. *Echidnophaga gallinacea* is known from chickens, hedgehogs, mongoose, *Mus, Felis*, and *Rattus*). These fleas were given the character state “promiscuous” to reflect the fact that they are extremely polyxenous. Character states were treated as unordered and mapped in MacClade 4.07 (Maddison and Maddison, 2000) with ACCTRAN optimization on the POY-MP consensus tree. The mammal classification scheme used in this study follows that of Wilson and Reeder (2005).

Results

Sequences and alignment

For 18S, sequences ranged from 1861 to 1869 bp, with an average length of 1868 bp. For 28S, the sequence range was larger (2113–2169 bp) with an average length of 2149 bp. For COII and EF1-alpha, there were no inserts, with respective lengths of 612 bp and 1065 bp. Summary statistics for molecular data based on the implied alignment are given in Table 3. The sum of partitioned Bremer support values indicate that 18S provides 16% of the signal, 28S provides 52% of the signal, COII provides 6% of the signal, and EF1-alpha provides 26% of the signal across the total topology. Across the topology, only 20% of the Bremer support is distributed at the family level or higher, while 80% of the support is for nodes that are below the family level, indicating that interfamilial relationships are not as well supported as relationships within each family. The sum of partitioned Bremer support values divided by the number of parsimony-informative

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<td>37.7</td>
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*Sum of partitioned Bremer values.
†Total Bremer support divided by the number of parsimony-informative characters.
characters for each partition provides a more normalized comparison of the relative signal within each partition. When the values are normalized, 18S provides 24.3% of the signal, 28S provides 37.7% of the signal, COI provides 8.1% of the signal, and EF1-alpha provides 29.9% of the signal.

Phylogenetic analysis

The POY-MP analysis resulted in 96 equally parsimonious trees ($L = 15,512$, $CI = 0.213$, $RI = 0.553$), the strict consensus of which is given in Fig. 5. These 96 trees differ only in the placement of some genera and species within Ceratophyllidae (clade 115, Fig. 5). The concatenated Muscle alignment resulted in 5826 aligned positions, and for the likelihood analysis of this alignment ModelTest selected the TVM + I + Γ model for the combined dataset. The estimated nucleotide frequencies are: $A = 0.2519$, $C = 0.2252$, $G = 0.2741$, and $T = 0.2488$. The substitution model incorporated the following rate matrix: $[A-C] = 1.7855$, $[A-G] = 2.9920$, $[A-T] = 1.6998$, $[C-G] = 0.8179$, $[C-T] = 7.3197$, and $[G-T] = 1.000$. The shape parameter for the discrete gamma distribution was estimated to be 0.4770 with the proportion of invariable sites ratio at 0.5828. ML analysis computed a single best tree with the likelihood of $-\ln L = 98,380.86676975$. This topology differs from the POY-MP analysis in relationships towards the base of the fleas (indicated in red on Fig. 5), but is largely congruent at the family level and lower.

Nodal support measures indicate that this topology is overall well supported towards the middle and tips of the tree, but poorly supported at the base of fleas. A complete list of Bremer and bootstrap values for the POY topology is given in Table 4. Of the 120 nodes on the POY strict consensus topology (Fig. 5), 67 nodes had non-parametric bootstrap values of 90 or greater (indicated by a star) and 58 nodes had Bremer scores of 10 or higher (indicated by a square). For approximately one-third of the nodes, there was no disagreement among any of the partitions (i.e. partition Bremer values were not negative for any partition on that node; indicated by a triangle). On the likelihood topology, roughly one-third of the nodes were supported by bootstrap values of 90 or greater. In both the likelihood and the POY analysis, the majority of the well-supported nodes are at the level of family or below, leaving the backbone of the topology more poorly supported.

The sensitivity landscape for the major flea groups (Fig. 6) likewise indicates that at the level of tribe and subfamily, most groups are robust to perturbations in parameter values. It is only the deeper nodes on the backbone of the ingroup topology where relationships are supported under a more narrow range of parameter values. Of the 25 parameter sets investigated, the set treating transitions, transversions and gaps equally $(1 : 1 : 1)$ yields the most congruent results, with an ILD value of 0.0289.

All analyses support the monophyly of Siphonaptera (clade 7, Fig. 5) with high support values [MP bootstrap (BS) = 100, Bremer (BR) = 144]. The earliest divergence places the bizarre Tungidae (clade 8, BS = 100, BR = 144) as sister group to the remainder of the fleas, rendering the family Pulicidae *sensu* Lewis (1998) as paraphyletic. A monophyletic flea clade which excludes the Tungidae is moderately supported by bootstrap values (=90) and well supported via Bremer support values (=21). Only two of the superfamilial relationships described by Medvedev (1994, 1998) are supported in these analyses: a monophyletic Ceratophyllomorpha (clade 81, BS = 93, BR = 4) and a monophyletic Pygiopsylloidae (clade 12, BS = 100, BR = 26). Of the 16 families included in this analysis, three have only single exemplars (Macropsyllidae, Coptopsyllidae, and Vermipsyllidae) and so the monophyly of these families could not be addressed. Of the remaining 13 families with multiple exemplars, the MP and ML analyses support the monophyly of ten: Tungidae (BS = 100, BR = 40), Lycopsyllidae (BS = 100, BR = 16), Pygopsyllidae (BS = 85, BR = 9), Stivaliidae (BS = 100, BR = 13), Stephanocircidae (BS = 98, BR = 24), Rhopalopsyllidae (BS = 97, BR = 17), Chimeropsyllidae (BS = 99, BR = 10), Pulicidae (BS = 100, BR = 13), Ischnopsyllidae (BS = 98, BR = 11), and Ceratophyllidae (BS = 70, BR = 4). The families Leptopsyllidae, Hystrichopsyllidae, and Ctenophthalmidae were grossly paraphyletic. At the subfamilial level, our results support the monophyly of Doratopsyllinae (BS = 100, BR = 177), Stephanocercinae (BS = 100, BR = 156), Craneopsyllinae (BS = 96, BR = 16), Rhopalopsyllinae (BS = 100, BR = 47), Parapsyllinae (BS = 100, BR = 11), Steenopiniinae (BS = 100, BR = 75), Rhadinopsyllinae (BS = 98, BR = 21), and Dactypsyllinae (BS = 74, BR = 2). At the level of tribes (with multiple exemplars), our results support the monophyly of Neotyphloceratini (BS = 99, BR = 22), Ctenophthalmini (BS = 100, BR = 57), Doratopsyllini (BS = 100, BR = 177), Hystrichopsyllini (BS = 100, BR = 46), Xenopsyllini (BS = 100, BR = 16), and Spilopsyllini (BS = 87, BR = 9). These results do not support the monophyly of the tribes Anomopsyllini, Phalacropsyllini, Pulicini, Amphipsyllini, or Leptopsyllini. Most notably, in all analyses, Ctenophthalmidae is grossly paraphyletic, with different subclades from this family joined to seven different positions on the topology.

There are some differences in the placement of some of the higher-level clades between the MP and ML analyses. This is not surprising given that the bootstrap and Bremer support values are low on these nodes, and that these are the nodes most sensitive to fluctuations.
Fig. 5. Phylogenetic relationships among fleas based on 28S, 18S, COII, and EF1-α. This is the strict consensus of 96 MP trees found in the POY-MP analysis ($L = 15512$, $CI = 0.213$, $RI = 0.553$). The ML analysis reconstructed a similar topology, except at some deeper branches as indicated in red on the topology. Bootstrap values $\geq 90$ are indicated by stars, Bremer values $\geq 10$ by squares, and congruence among all partitions (i.e. no negative partition Bremer values) is indicated as a triangle on each branch. Nodes are numbered and corresponding support values are given in Table 4. Group names with an asterisk refer to paraphyletic assemblages on this topology.
in the analytical parameters in the POY analysis. For instance, the monophyletic Chimaeropsyllidae + Pulicidae (clade 70) is placed as sister group to Ceratophylloidea (clade 81) in the MP analysis, but as sister to a clade composed of (Doratopsyllini + Macropsyllidae) + Coptopsyllidae in the ML analysis. In the MP topology, Pygiopsylloidea (clade 12) is sister to monophyletic assemblage of Macropsyllidae + Coptopsyllidae (clade 19) plus a clade (no. 20) of ctenophthalmids including Neotyphloceratini,
### Table 4
Nodal support for the phylogeny shown in Fig. 4

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Columns list non-parametric bootstrap values, Bremer support values, and partitioned Bremer support values (the contribution of the specified genes to the total Bremer support at the indicated node) as combined from the combined molecular phylogeny in Fig. 4. Bootstrap support values result from 10 000 bootstrap replicates.
Ctenophthalmini, and Doratopsyllini. In the ML analysis, Pygiopsylloidea is sister group to a monophyletic Stephanocircidae.

Host associations

Results of the mapping of hosts associated with the fleas included in this analysis are presented in Fig. 7. These results suggest that *Tunga*, the sister group to the remainder of the fleas, was a promiscuous lineage that parasitized a wide range of hosts. It appears that host specificity developed only later in flea evolution, and that the primary host was mammalian. Within the first major clade of fleas (node 10), fleas are associated with the mammal hosts Metatheria, Soricomorpha, and Hystricomorpha, but the primary host is Rodentia: Cricetidae, which maps to the base of node 34. In the second major group of fleas (node 69), fleas appear to have diversified largely with Muridae, although there are shifts to cricetids, geomyids, Chiroptera, and birds. Our analysis supports at least four independent shifts from mammal to bird hosts (clades 56, 111, 116, and *Ornithophaga a. anomala*), and one shift to bats with Ischnopsyllidae (node 92).
Fig. 7. Host association data mapped on the flea phylogeny. Hosts were scored at the level of order for the mammals, except for rodents, which were scored at the familial level. Birds were scored at the level of class. Fleas inhabiting multiple hosts were scored as polymorphic. Fleas inhabiting an extraordinary range and diversity of hosts were scored as “promiscuous”. Ambiguous optimizations are indicated on the nodes.
Continued from facing page

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Fig. 7. Continued
Discussion

This is the first formal estimate of broad-scale flea phylogeny based on any character system. Overall, relationships at the family level and below for most groups are generally well supported, with congruence among multiple loci and high nodal support values. The paraphyly of some families appears to be very well supported in this topology. Relationships at the deepest levels of the tree have been more elusive, and these nodes are characterized by lower nodal support and greater sensitivity to parameter perturbation in the sensitivity analysis. Despite these limitations, this study provides important insight into flea classification and evolution, and suggests some general patterns of host association.

Tungidae

The family Tungidae includes a group of fleas that have an unusual morphology, including a characteristic compression of the three thoracic segments, and a unique neosomic lifestyle. Tungidae currently include the genera Tunga (ten spp.) and Hectopsylla (13 spp.); Rhychnopsyllus has been merged recently into Hectopsylla (Hashtirer and Méndez, 2000). Our results consistently placed Tungidae at the base of the phylogeny, as sister to the remaining flea taxa, and this node is well supported (BS = 100, BR = 144). The monophyly of Tunga is also well supported in our analysis (BS = 100, BR = 40). Lewis (1998) considered Tunginae to be a subfamily within Pulicidae, while other authors have treated tungids as a separate family, placing it as sister group to Pulicidae (Hopkins and Rothschild, 1953, 1956, 1962, 1966, 1971; Jordan, 1956, 1962; Smit, 1987; Medvedev, 1994). Our results agree with these latter authors in elevating Tunginae to family status, but disagree with their conclusion that tungids are closely associated with Pulicidae, as no analyses ever place tungids within or as sister to Pulicidae.

The placement of Tunga at the base of flea phylogeny and its association with basal mammal hosts suggests that the origin and diversification of Siphonaptera coincides with basal mammal diversification. The majority of the natural mammalian hosts of the genus Tunga are sloths and armadillos, secondarily evolving on various species of Rodentia. Although humans are the principal host for T. penetrans, from an evolutionary standpoint this is certainly a secondary association. Hectopsylla (which is not included in this analysis) prefers caviomorph rodents, birds, and bats (Hopkins and Rothschild, 1953, 1956, 1962, 1966, 1971; Medvedev, 1997a; Hashtirer and Méndez, 2000). Sloths and armadillos belong to an ancient stock of mammalian orders known as Cingulata and Pilosa (formerly Edentata and later Xenarthra). These orders are closely related to Afrotheria (Afrosoricida, Macroscelidea and Hydracoides; members of these orders are known to harbour numerous flea genera), also at the base of the mammalian tree, which probably has an African origin (Springer et al., 2004). Current hypotheses suggest that these mammalian orders arose at the time of the Gondwanan split (170–100 Ma) and recent molecular studies place the split of these orders from those belonging to the Boreoeutheria at around 100–120 Ma (Murphy et al., 2001; Hasegawa et al., 2003; Springer et al., 2004).

The geographical distribution of its members covers the Neotropic (Tunga and Hectopsylla), the African (Tunga), and the Oriental regions (Tunga). Tunga monositus is the only species occurring in the Nearctic, albeit at the transition zone to the Neotropics. The highest species diversity of Tunginae occurs in the Neotropics (21 of 23 species, two of which are cosmopolitan), mostly in the Brazilian (Tunga) and Chilean subregions (Hectopsylla). The allied taxa for most of the Neotropical flea species occurring outside of South America occur in the Australian and Oriental regions opposed to the Afrotopical region. Tunga is no exception, with two species occurring in the Oriental region (T. caecigena and T. callilda) on murid rodents. Similar to Tunga, the Hectopsylla species apparently crossed over secondarily to Rodentia.

Pygiopsyllomorpha

Previous workers have treated Pygiopsyllidae as either a single family with three subfamilies (Lycopsyllinae, Pygiopsyllinae, and Stivaliinae; Lewis, 1993) or have elevated each subfamily to familial status and placed them in the infraorder Pygiopsyllomorpha (Medvedev, 1998; see Table 1). This group comprises three subfamilies, four tribes, 37 genera, and 167 described species (Table 1). Our analysis supports the monophyly of Pygiopsyllomorpha (BS = 100, BR = 26), and of each family comprising this group; Lycopsyllinae (BS = 100, BR = 16), Pygiopsyllidae (BS = 85, BR = 9), and Stivaliidae (BS = 100, BR = 13), with a sister group relationship between Pygiopsyllidae and Stivaliidae (BS = 90, BR = 16).

Pygiopsyllomorpha probably originated in the Australian region based on their association with Australian metatherians, and appear to have undergone a radiation with their ancestral metatherian hosts (Traub, 1972). Of all known genera of pygiopsyllomorphs, 63% are distributed in Australia and/or New Guinea. The first author (M.W.H.) has 47 as yet undescribed pygiopsyllomorph taxa and a new genus from Papua New Guinea, Solomon Islands, and the Bismarck Archipelago (material from the late Robert Traub collection housed in the Carnegie Museum of Natural History). Seven genera occur in Indonesia and Borneo, and five in the Philippines. Only one genus (Lentistivalius) has
species which extend into the Palearctic region (Japan) (Traub, 1972).

Exemplars from the families Lycopsyllidae (Lycopsylla and Uropsylla) and Pygiopsyllidae (Bibikovana and Pygiopsylla) included in our analysis are associated with metatherians in Australia proper. Exemplars from Stivaliidae (Parastivalius, Metaaustivallius and Papuapsylla) have their main distribution in New Guinea, where they occur on metatherian hosts and murids. Additional pygiopsyllid hosts in the Indo-Malayan Subregion are callosciurine squirrels and Tupaiidae (tree squirrels, insectivores).

The more apical placement of Stivaliidae within Pygiopsyllomorpha supports an earlier hypothesis by Traub (1972) who believed that pygiopsyllids descended from fleas originating in the Australian Subregion, before they moved into the Indo-Malayan Subregion (Oriental Region) and subsequently switched from metatherians to other hosts.

Based on the numerical majority of metatherian taxa (both familial and generic) in the Australian region, and a corresponding diversity of flea taxa associated with them, a centre of origin for the metatherians may have occurred on the Australian landmass prior to the Gondwanaland split. This is supported by the dearth of pygiopsyllid flea taxa and metatherian hosts in South America. Only a single pygiopsyllid genus (Ctenidiosomus, four spp.) is represented in South America (one of four occurring on metatherians), while there are 35 flea genera from the Australian and Oriental regions. Of the 35 genera, 15 are found exclusively on Metatheria, eight on Rodentia, five a mixture of both, four only from birds, and two predominantly from Scandentia. An additional genus is found only in Africa on Rodentia. As pygiopsyllids radiated from Antarctica across Australia, they evidently evolved on Metatheria and radiated to Rodentia. As they spread north and east through Wallacea, Philippines, Malaysian Archipelago, and into the Indian Subregion of the Oriental region, metatherians were replaced by Sciuroida (Rodentia) and Tupaiidae (Scandentia). The genera Lentistivalius and Medwayella parasitize the latter groups of mammals and to a lesser extent Rodentia.

Macropsyllidae and Coptopsyllidae

Macropsyllidae was treated by Lewis (1998) as a subfamily within Hystrichopsyllidae, although other workers (Hopkins and Rothschild, 1953, 1956, 1962, 1966, 1971; Medvedev, 1988, 1994) have treated it as its own family (Table 1). Jordan (Hopkins and Rothschild, 1953, 1956, 1962, 1966, 1971) considered this family as sister group to Stephanocircidae (Fig. 2). Macropsyllidae comprises two genera: Macropsylla (two spp.) and the monotypic genus Stephanopsylla, all of which occur in the Australian region.

Morphologically, Macropsyllidae appears most similar to Stephanocircidae, the only marked differences being the single, continuous comb on the head of macropsyllids compared with two separate cones in Stephanocircidae. Additionally, female macropsyllids have two spermathecae as compared with the single spermatheca in stephanocircids, and the four abdominal combs in macropsyllids are absent in stephanocircids (except in Stephanocircus jarvisi).

Coptopsyllidae, which is entirely Palearctic, is also a small group (one genus, 19 spp.) and is morphologically distinct from Stephanocircidae and Macropsyllidae. Coptopsyllidae has been recognized by previous workers as its own family, although there has been no clear consensus as to its sister group. Coptopsyllids are completely combless and tergal spinelets are absent. However, just like macropsyllids, the female has two spermathecae. The only other taxa in which a duel spermatheca is found are in Hystrichopsyllinae (Hystrichopsylla, Attypholoceras, Tymphloceras, and Ctenoparia) and Macropsyllidae (Macropsylla and Stephanopsylla). It should be noted that the spermathecae of the Macropsyllidae are of unequal size, a feature Jordan (Hopkins and Rothschild, 1953, 1956, 1962, 1966, 1971) thought might represent a link between those bearing two spermathecae and those bearing only one. All fleas have a “remnant” of the duct to the second (lost spermatheca), and these ducts are of variable stages of development.

The MP analysis supports a sister group relationship between Macropsyllidae and Coptopsyllidae (BS = 88, BR = 21), although it is not particularly robust as gauged by the sensitivity analysis (Fig. 6). The ML analysis places Macropsyllidae as sister to Corrodopsylla (Ctenophthalmidae), and then places this clade as sister to Coptopsyllidae, although this relationship lacks bootstrap support (BS < 70). None of the analyses ever placed Macropsyllidae with Stephanocircidae, although the latter family is well supported in our analyses (see below). Due to our limited taxon sampling in the current analysis, it is not possible to resolve the phylogenetic placement of either family robustly, although both appear to be distinct and do not nest within any of the other monophyletic families.

Stephanocircidae

Stephanocircidae (also called “helmet fleas”) comprises two subfamilies with nine genera and 51 spp. (Table 2). Members of Stephanocircidae are separated into two main geographical regions: the Australian Region (Stephanocircinae) and the Nearctic Region (Craneopsyllinae). Craneopsyllinae is more speciose, with a host range of metatherian and rodent hosts, whereas Stephanocircinae are mainly restricted to metatherians.
Stephanocircids are distinguished from all other fleas by the division of the forward portion of the head which forms a “helmet”. The helmet, or frons, is adorned with more or less vertical combs along the posterior margin. A second vertical comb is present along the genal margin. Smitella thambetosa (a New Guinea pygopsyllid) appears to have a helmet with a well-developed vertical comb, but the articulation with the head differs and is an unrelated convergent feature. Our results support the monophyly of Stephanocircidae (BS = 98, BR = 24) and the subfamilies Stephanocircinae (BS = 100, BR = 156) and Crancopsyllinae (BS = 96, BR = 16); these groups appear robust in the sensitivity analysis (Fig. 6). Our result agrees with the subfamily classification proposed by Hopkins and Rothschild (Hopkins and Rothschild, 1956) and with the biogeographical distribution of the two subfamilies. These data validate the zoogeographical premises put forth by Traub and Starcke (1980) regarding austral connection of South America and Australia prior to the Cretaceous period and that the development of the unique head capsules adorned with the “crown of thorns” occurred very early, prior to separation of the continents.

Vermipsyllidae + Jordanopsylla

Vermipsyllidae is a small family comprising three genera and 42 spp. Vermipsyllids are found on carnivores, mustelids (Chaeotopsylla), and ungulates (Vermipsylla and Dorcadia). The single exemplar Chaeotopsylla (Vermipsyllidae) is placed as sister to the unusual flea Jordanopsylla becki (BS = 84, BR = 10). This relationship is robust in the sensitivity analysis (Fig. 6).

Medvedev (1994) placed Vermipsyllidae as sister group to Rhopalopsyllidae + Malacopsyllidae (Fig. 4). In our topology, the vermipsyllid Chaeotopsylla lotoris is associated with Rhopalopsyllidae, in partial agreement with Medvedev’s topology. However, we have found no morphological characters to support the placement of Jordanopsylla to either a vermipsyllid or a rhopalopsyllid. Traub and Tipton (1951) and Hastriter et al. (1998) provided a detailed analysis of the morphological relationships of Jordanopsylla with other flea taxa, and concluded that this unique genus represented a distinct subfamily of Ctenophthalmidae. However, given that Ctenophthalmidae is a grossly paraphyletic group (as discussed below), there is no expectation as to where this taxon should be placed in flea phylogeny. Obvious and gross morphological features that are present in vermipsyllids and absent in the ctenophthalmids include the absence of an anal stylet in females, presence of a frontal tubercle, antenypigidal bristles lacking, spiracles very large, and tergites and sternites reduced, especially in females.

Rhopalopsyllidae

Rhopalopsyllidae currently comprises two subfamilies, 14 genera, and 126 spp. Our analysis supports the monophyly of this family (BS = 95, BR = 17) and of both subfamilies Rhopalopsyllinae (BS = 100, BR = 47) and Parapsyllinae (BS = 100, BR = 11). Species within the subfamily Rhopalopsyllinae mainly infest small cricetid and octodontid rodents in the Neotropical region. Most genera of Parapsyllinae are likewise associated with cricetid and octodontid rodents on continental South America with one notable exception: the genus Parapsyllus has adapted to penguins and sea birds (albatrosses, fulmars, petrels, shags, prions, and shearwaters) and has a panantarctic distribution primarily on Antarctica, southern hemisphere islands, and the southern coastal areas of the southern continents.

Hystrichopsyllidae

The family Hystrichopsyllidae (Lewis, 1993; Table 1) comprises two subfamilies: Hystrichopsyllinae and Macropsyllinae. Hystrichopsyllinae is composed of the tribes Ctenopariini with one neotropical genus, and Hystrichopsyllini with two nearctic genera and one palaeartic genus. Macropsyllinae comprises two Australian genera, Macropsylla and Stephanopsylla. The head combs of Macropsyllinae (especially Macropsylla) are also remarkably like those of all species of Hystrichopsylla, although our phylogenetic analysis demonstrates that this is a convergent feature.

Our results support the monophyly of Hystrichopsyllini by grouping all three Hystrichopsylla exemplars together in a clade (BS = 100, BR = 46). It does not support the monophyly of Hystriopsyllinae because Ctenopariini is placed with Carterettini (BS < 50, BR = 5). The Hystrichopsyllini is placed as sister group to two ctenophthalmid species from the tribe Anomopsyllini, although this relationship is poorly supported (BS < 50, BR = 2). Ctenoparini + Carterettini is placed as sister to a clade of ctenophthalmids (node 37), although this is also poorly supported (BS < 50, BR = 7). Note that Medvedev (1998) treats Hystrichopsyllidae as a large family that includes Hystriopsyllinae and subfamilies that are traditionally placed within Ctenophthalmidae (Table 1). Our results agree with Medvedev in so far that they suggest that Hystriopsyllinae is a lineage comparable with some subfamilies traditionally placed within Ctenophthalmidae, although ctenophthalmids are grossly paraphyletic.

Ctenophthalmidae

The family Ctenophthalmidae (sensu Lewis, 1993) consists of nine subfamilies and 17 described tribes, with
42 genera and 664 species. Medvedev (1998) joins these subfamilies with Hystrichopsyllinae and places them within Hystrichopsyllidae. For clarity, we will follow Lewis' treatment of these groups. Roughly one-quarter of flea species are placed within this group, and Ctenophthalmidae has been traditionally the “catch-all” family for fleas that have been difficult to assign to other families. Consequently, it is no surprise that this family is grossly paraphyletic in our analyses. Our analyses place ctenophthalmids in seven positions on the topology. Doratopsyllini (BS = 100, BR = 177; node 25) and Ctenophthalmini (BS = 100, BR = 57; node 23) are each monophyletic and sister groups (BS = 90, BR = 21; node 22). This clade is sister group to a monophyletic Neotyphloceratini (BS = 99, BR = 22; node 21), but the support for this relationship is rather poor (BS = 76, BR = 7; node 20). The monophyly of these tribes was supported across all parameters investigated in the sensitivity analysis (Fig. 6), although relationships among them were supported under only five parameter combinations.

The tribe Anomiopsyllini is paraphyletic. The genera Anomiopsyllus and Stenostomera are sister groups (BS = 100, BR = 58; node 38) and this clade is placed as sister to Phalacropsyllini. The other exemplars of Anomiopsyllini in this analysis (Conorhinopsylla and Megarthroglossus) are sister to each other (BS = 99, BR = 23; node 59) and this clade is placed as sister group to Hystrichopsyllini, although this relationship is very poorly supported (BS < 50, BR = 2; node 58). The single exemplar of Neopsyllini (Neopsylla) is nested within Phalacropsyllini (node 43). Carterettini is represented by a single exemplar (Carteretta), which is placed as sister to the hystrichopsyllid Ctenoparia (node 36). Dinopsyllini is represented by the single exemplar Dinopsyllus, which is placed as sister to a clade comprising Rhopalopsyllinae, Vermipsyllidae, and Jordanopsylla with limited support (BS < 50, BR = 7; node 46). Stenoponiinae (BS = 100, BR = 75; node 63) and Rhadinopsyllinae (BS = 98, BR = 21; node 65) are each monophyletic, are robust in the sensitivity analysis, and are placed as sister groups, but with limited support (BS < 50, BR = 5). It is interesting to note that although the various clades of ctenophthalmids are scattered among other flea groups, none is placed within Ceratophyllomorpha (node 69).

The current arrangement of Ctenophthalmidae is clearly in a state of disarray; however, if one assesses the phylogeny on the basis of the subfamily, four natural groupings may be seen: Ctenophthalminae/Doratopsyllinae (node 20), Neopsyllinae (node 39), Stenoponiinae (node 40), and the Rhadinopsyllinae (node 65). Neotyphloceras crassispina chilensis and Chilopsylla a. allophyla occur on sigmodontid rodents and on the metatherian Dromeciops australis in the Chilean Subregion, and are frequently found on the same host animal. Corrodopsylla spp. (C. birulai and C. c. curvata) are found on shrews (Soricidae) and occur in the Palearctic and Nearctic regions, respectively. They each bear a common genal comb of four broad spatulate spines. The Ctenophthalmus spp. in our analysis include exemplars from both the Nearctic and the Oriental regions. The second subfamily, Neopsyllinae, has a characteristic genal comb of two sharp overlapping spines. It is of interest that all but one species of Neopsylla occur in the Oriental Region. The only exemplar of Neopsylla in our analysis is a Chinese species (N. bidentatiformis) and the Nearctic species (Neopsylla inopina, not included) occurs in the western United States along with Epitedia, Catallagia, and Meringis spp. The holarctic subfamily Stenoponiinae are all very large and darkly pigmented fleas with a striking genal comb spanning most of the lateral portion of the head. Exemplars from the Nearctic (Stenoponia americana), Palearctic (Stenoponia trpectina nata medialis), and the Oriental (Stenoponia sidimi) regions are represented and all parasitize murid rodents. Also represented from the Nearctic and Palearctic regions, representatives of the Rhadinopsyllinae are associated with Soricidae (Neactropsylla trauibi and Corypsylla ornata), Muridae (Rhadinosylla masculana, Rhadinopsylla difficilis), and Sciaridae (Rhadinosylla heiseri). Although further detailed taxon sampling will be required to elucidate these relationships, our data clearly demonstrate the gross polyphyly of Ctenophthalmidae.

Chimaeropsyllidae + Pulicidae

Pulicidae consists of four tribes, 21 genera, and 167 spp. As described above, some workers have treated Pulicidae as including Tungidae (Lewis, 1998). In our analysis, Pulicidae is well supported as a monophyletic group (BS = 100, BR = 13; node 72) that is phylogenetically distant from Tungidae. Xenopsyllini is well supported (BS = 100, BR = 16) and placed as sister to the remaining pulicines, with Parapulex nested within Xenopsylla. Spilopsyllini is monophyletic (BS = 87, BR = 9; node 79), but it is nested within Pulicini, rendering the latter tribe paraphyletic. Chimaeropsyllidae comprises three subfamilies, eight genera, and 27 spp. This family is well supported as a monophyletic group (BS = 99, BR = 10; node 71), and the sister group relationship with Pulicidae is also well supported (BS = 100, BR = 19; node 70). Relationships within Chimaeropsyllidae + Pulicidae are very robust in the sensitivity analysis (Fig. 6).

Species of Chimaeropsyllidae are found exclusively in the Ethiopian Region, where they occur on elephant shrews (Macroscelidae) and small rodents. Chimaeropsyllids share characters with the family Pulicidae that may be potential synapomorphies. These include sensillum with 14 pits per side, inner side of hind coxa with
spiniform setae, generally one row of setae per tergite, and setae are fine (exceptional in Parapulex) and rather sparse. Additionally, both families have a propensity for living in xeric environmental conditions.

Pulicidae are mostly of a Palearctic, Nearctic, or cosmopolitan distribution, the last attributable to the secondary dispersal of their hosts (e.g. Rattus norvegicus and Mus musculus). Pulicidae exhibit an interesting diversity of host specificity patterns and ecological habits. The pulicid flea Spilopsyllus cuniculi and Cediopsylla spp. are monoxenous upon the rabbit, presumably because of its complete dependence on the reproductive hormone cycle of its host (Oryctolagus cuniculus) (Rothschild and Ford, 1972, 1973). In contrast, Ctenocephalides felis, occupying a more basal position in our phylogeny, is highly promiscuous, and occurs on a wide variety of Carnivora. The genus Echidnophaga includes stick-tight fleas in which the female exhibits a moderate degree of neosomy. Echidnophaga has 23 species: five Ethiopian, 11 Australian, six Palearctic, and one cosmopolitan.

The monotypic genus Neotunga (not included in our analysis) is restricted to pangolins in East Africa. Smit (1962) stated that Neotunga euloidea was “an echnidnophagoid flea with tungoid habits” and considered the species as an example of tungid evolution. As Tungidae and Pulicidae are so distant phylogenetically, Neotunga euloidea may well exemplify the evolutionary pattern of Tunga but is a representative of the family Pulicidae. This appears to be an interesting case of convergence in neosomy and associated morphological features between these phylogenetically distant groups (discussed below).

The centre of origin for Xenopsyllinae is generally believed to be the Ethiopian Region and secondarily the warmer parts of the Palearctic (Hopkins and Rothschild, 1953, 1956, 1962, 1966, 1971). There are no indigenous Xenopsylla in the Nearctic or Neotropical regions, and only a few species in the Oriental and Australian regions. In the Australian region, species such as X. papuensis in Papua New Guinea and X. vexabilis in Australia are probably the result of Muridae radiating from Asia to Australasian areas. The majority of Xenopsyllinae occur on Murinae and Gerbillinae.

**Leptopsyllidae**

The family Leptopsyllidae currently consists of two subfamilies (Amphipsyllinae and Leptopsyllinae), six tribes, 29 genera, and 260 species. Hopkins and Traub (1955) suggested that too much emphasis had been placed on the presence or absence of genital combs and the fracticpit or integricipit nature of the head, and that clearly both groups are closely related to the Ceratophyllidae, and to each other. Our analyses place leptopsyllids within Ceratophyllomorpha, but they do not support the monophyly of the family Leptopsyllidae, the subfamilies Amphipsyllinae and Leptopsyllinae, nor the tribes Amphipsyllini and Leptopsyllini. Our analyses place leptopsyllids in three positions on the tree. The first group (node 82) comprises five species all placed within Amphipsyllini, and this clade is relatively well supported (BS = 100, BR = 29). The second group (node 87) includes six species currently placed within Leptopsyllini, Amphipsyllini, and Ornithopha- gini, and this clade is also well supported (BS = 97, BR = 15). Both these major groups (nodes 82 and 87) were recovered across all sensitivity analyses (Fig. 6) and are supported by the morphological discussions presented by Traub and Starcke, (1980). The tribe Dolichopsyllini represented by Dolichopsyllus stylosus is placed as sister group to Ceratophyllidae. Although the nodal support for this relationship is somewhat low (BS = 60, BR = 4; node 95), the group is recovered in 20 of the 25 parameter combinations in the sensitivity analysis (Fig. 6).

Traub and Starcke (1980) suggested a Palearctic origin for the group, based on the majority of taxa which are associated with Palearctic hosts. Although leptopsyllids do not occur in the Neotropics, they are abundant in the Nearctic. Dolichopsyllus stylosus is a flea of the ancient rodent Aplodontia rufa. Recent molecular studies have found strong support for a sister-group relationship of Aplodontidae to Sciuridae, lending support to the positioning of the aplodontid flea at the base of the Ceratophyllidae, whose ancestral hosts were Sciuridae (Adkins et al., 2001).

Hopkins and Traub (1955) present a detailed discussion justifying the systematic arrangement of Ceratophyllidae, Leptopsyllidae, and subfamilies of the latter. With the exception of Hopkins and Rothschild (1971), taxonomists have not formally addressed the systematic position of Dolichopsyllus. Most accounts only note new records and host affiliations. Although this flea has been historically placed within Leptopsyllidae, it has features of both this family and Ceratophyllidae, further supporting its placement as a link (Fig. 5, node 95) between these two groups. For instance, leptopsyllid features include an arch of the tenorium that is visible in front of the eye, dorsalmost ocular bristle above level of eye, and the Wagner’s gland is absent. Ceratophyllid features include the absence of genital combs (although 3 min and unusual setulae are present on the posterior margin of the antenatal fossa that may represent primordial genital ctenidia), a vestigial eye, absence of a genital comb, sternum VIII is greatly reduced to a fimbriated intersegmental membrane, and the hind capsule is integricipit.

**Ischnopsyllidae**

The family Ischnopsyllidae (bat fleas) comprises two subfamilies, five tribes, 20 genera, and 125 species.
Currently, two subfamilies are described: Thaumapsyllinae, occurring on Megachiroptera, and Ischnopsyllinae, being exclusively associated with Microchiroptera. These fleas are distinct in having the preoral position of the genal comb placed at the extreme anterior end of the ventral margin of the head. This comb is typically composed of two broad, flattened spines, with the exception of Thaumapsylla dina (Thaumapsyllinae) with three spines and Nycteridopsylla quadrispina (Lu and Wu, 2003) (Ischnopsyllinae) with four spines. Our analyses support the monophyly of this family (BS = 98, BR = 11; node 92), and places it as sister group to Dolichopsyllus + Ceratophyllidae (BS = 76, BR = 2; node 91). The monophyly of this family was recovered across all sensitivity analyses and its placement as sister group to Dolichopsyllus + Ceratophyllidae was reconstructed in 14 of 25 parameter combinations (Fig. 6).

Because of the volant nature of their bat hosts, Ischnopsyllinae are found on every continent (except Antarctica) and the distribution of genera follow that of their hosts on which they coevolved. These fleas are highly host-specific (e.g. Thaumapsyllinae occur on Rousettus spp. and Myodopsylla are found almost exclusively on Vespremilionidae), a trait shared by dipteran ectoparasites (Nycterobiidae and Streblidae) and endoparasites (Streblidae: Ascodipteronidae).

**Ceratophyllidae**

The family Ceratophyllidae comprises two subfamilies (Ceratophyllinae and Dactyloropsyllinae), 47 genera, and 414 species. Although the monophyly of this family was recovered with limited support (BS = 70, BR = 4; node 96), it was recovered in 23 of the 25 parameter combinations in the sensitivity analysis (Fig. 6), suggesting it is a robust result. Our analyses support the monophyly of Dactyloropsyllinae (BS = 85, BR = 4; node 101), but place it within a paraphyletic Ceratophyllinae.

Ceratophyllidae are predominantly rodent fleas that mainly associate with sciurids and certain cricetids. Ceratophyllids are never found on the more basal groups of mammals. Their derived position on the topology indicates a more a recent origin, which coincides with an association with more recent hosts (e.g. Sciuridae or Cricetidae). The mapping of the available host records of Ceratophyllidae on the phylogeny strongly indicated that the Sciurids are the ancestral hosts of this clade, supporting earlier hypotheses to that effect (Hopkins and Rothschild, 1956; Traub and Starcke, 1980).

**Neosomy**

Neosomy is radical intrastadial metamorphosis characterized by cuticular growth in unsclerotized parts of the abdomen, without correlated moulting. Neosomy is a fundamental concept of symbiosis with adaptive parallels to parasitism (Audy et al., 1972). The presence of neosomy in the basal Tunga flea lineage is very surprising, challenging our prevailing notion of neosomy being a derived trait. In fleas, only Tunga and the pulicid Neotunga (host: pangolins) exhibit neosomy coupled with burrowing and tachygenesis. Neosomy greatly increases the reproductive potential of females (>1000 egg/female/lifetime), results in the loss of the feeding requirement in larvae (e.g. Tunga monositus), and provides maximum advantage of food resources.

Cingulata and Pilosa, the primitive host orders on which Tunga seemingly evolved, are restricted to South and Central America, while Manis temminckii (Pholidota), the sole host for Neotunga euloidea, is found only in East Africa. As previously discussed, if Neotunga is indeed a pulicid then it is distantly related to Tunga, and the mode of evolving an endoparasitic lifestyle is apparently convergent. These results suggest the interesting possibility that tachygenesis and neosomy are possible precursors to an ectoparasitic mode of existence in insects. Representatives of Ascodipteroninae (Ascodipteron spp. and Maabella stomalata) have also evolved an endoparasitic mode of existence, and are each basal to the ectoparasitic Streblidae (Dittmar et al., 2006).

**Patterns of host association**

The history and dispersal of ectoparasites is intricately tied to their hosts, and thus host association patterns are an important factor in understanding the evolutionary history of Siphonaptera. Flea host association data are typically analysed from the standpoint of host ranges in different zoogeographical regions of the world (Medvedev, 1997a, b, 2000a, b). However, the degree to which flea evolution has been influenced by the evolution of their hosts has never been analysed in light of a flea phylogeny. This is not only because we have lacked an accurate phylogeny for fleas, but even with a robust topology in place, the coding and scoring of host association data is not as straightforward as in other insect groups that parasite vertebrates and are generally more specific in their associations (e.g. lice, some bat flies). Ideally, we would prefer a phylogeny which includes all extant flea species, and for each flea species we would have an extensive knowledge as to its host species range. However, given the limits of our current taxon sampling, our goal is to attempt to detect broad-scale, general patterns in flea–host evolution that will form working hypotheses for subsequent studies. In particular, we are interested in addressing the question of whether flea diversification coincides with the diversi-
fication of basal mammals, or whether fleas diversified after the mammals had been established in higher mammalian groups such as rodents.

Basic assumptions

In applying our molecular phylogeny to decipher ancient patterns of host association, we recognize that there are difficulties in interpreting these patterns from a study primarily designed to recover deep flea phylogeny. So although we have gone to considerable effort to include a wide taxonomic range of fleas to best represent flea phylogenetic diversity, we have not specifically selected flea taxa in order to best maximize host diversity. For example, we have included 37 species of ceratophyllids in the analysis, which taxonomically represent one half of the described ceratophyllid genera. From a host standpoint, this includes taxa that are associated with Sciuridae, Muridae, Geomyidae, Lagomorpha, Cricetidae, and Aves. However, there are other ceratophyllid species (e.g. Paraceras spp. on Carnivora) that are not included in this analysis, and their inclusion may potentially change the host optimization of some of the deeper nodes.

For some common flea species, we have an extensive record of host association data. However, other flea species are less well known, and even though there may only be a single host record known for that species, additional investigation may reveal a broader range of hosts than originally recorded. Additionally, accidental host associations appear to be more common in fleas than other insects that are vertebrate parasites. For instance, it is not uncommon to collect a rabbit flea on a fox that regularly feeds on rabbits; in this case the rabbit is the primary host and the fox is an accidental association. For some flea taxa we made judgment calls as to their primary host(s) based on the literature and our own experience in working with the flea species. We consider these to be our best estimate of the range of host species for that particular flea species and we feel this is a good starting point for deriving hypotheses that can be subject to additional testing with more taxon sampling and host association data.

The beginnings

Our data suggest that flea diversification began when the boreid ancestor shifted from living on moss to living as an ectoparasite on mammals. According to our phylogeny, all inferred associations to the evolutionarily much older birds, which are probably derived from the dinosaur–pterosaur–Archaeopteryx ancestry (Brush, 1996; Gatesy and Dial, 1996; Ackerman, 1998), happened secondarily. Consequently, we consider it unlikely that fleas first diversified with pterosaurs as has been hypothesized by others (Rasnitsyn, 1992). The basal placement of tungids in our phylogeny is interesting on two accounts: it suggests that the primitive flea had a broad range of hosts as do modern tungids and that neosomy is actually a primitive rather than a derived lifestyle within fleas.

Prior to our study, one candidate group for the “ancestral flea” was the Pygiopsyllomorpha. This notion was based on the “primitiveness” of their morphological appearance and their associations to Australian metatherians, which are clearly of ancient origin in respect to mammal diversification (Traub, 1972; Springer et al., 2004). However, because this group is not placed at the base of our tree, our study does not confirm the hypothesis that the earliest fleas occurred on metatherians, although the radiation of pygiopsyllomorphs as a group appears to be closely linked to the radiation of metatherians, as discussed above.

The mammals

Our data support the placement of non-tungid fleas into two major clades. In the first clade (node 10, Fig. 5), fleas are associated with the mammal hosts Metatheria, Soricomorpha, Hystricomorpha, Carnivora, Soricomorpha, Lagomorpha, and a variety of rodent families including Muridae, Cricetidae, Heteromyidae, Sciuridae, and Aplodontidae. There is also one lineage (Parapsyllus, discussed below) that has shifted to birds. The association with metatherians is linked to the radiation of Pygiopsyllomorpha, but Chilopsylla a. allophylla and Stephanocircus dasyuri are also found on metatherians, although given the ambiguity of the optimization of the deeper nodes, the directionality of this shift is unclear.

Among the rodent families, taxa associated with cricetids are the most phylogenetically widespread and one of the deeper nodes (Fig. 5, node 34) is unambiguously optimized as being a cricetid ancestor. Our analysis supports subsequent shifts to hystrichomorphs in Tiarapsylla titschacki, Tiamastus cavirostris and Ectinosurus spp.; carnivores in Chaetopsylla lotorii; murids in Rhadinopsylla difficilis; soricomorphs in Nearctopsylla traubi, Corypsylla ornata and Hystrichopsylla t. talpae; Heteromyidae in Meringis spp.; Muridae in Dinopsyllus ellobius, Stenoponia tripectinata medialis, and Rhadinopsylla heiseri; and Aplodontidae in Hystrichopsylla schefferi. The second major non-tungid flea group (Fig. 5, node 69) is optimized as a murid ancestor with subsequent shifts to lagomorphs, carnivores, sciurids, cricetids, aplodontids, geomyids, and birds in many flea lineages (Fig. 6).

Fleas take flight

We detected four independent shifts from a mammal host to a bird host during flea evolution, although this
number is likely to increase with extended taxon sampling. Six per cent of all flea species are parasitic on birds. The majority of them, however, do not live on the host, but rather in the nest of the bird. Thus, it has long been hypothesized that the shift from mammals to birds probably began with burrow-nesting birds sharing the same habitat as the flea-infested burrowing rodents (Marshall, 1981).

This hypothesis is supported by the inferred shift from rodents to birds in the Parapsyllinae clade (node 56, *Parapsyllus longicornis* and *P. magellanicus largificus*). The flea *Ectinorus alejoi* parasitizes the Northern Vischa-cha (*Lagidium peruanum*) and is placed as sister group (node 55) to a clade composed of *Parapsyllus longicornis* and *P. magellanicus largificus*. *Parapsyllus longicornis* is found in the nest of the blue penguin (*Eudyptula minor*) and *P. magellanicus largificus* parasitizes the fulmar prion (*Pachyptila crassirostris*). Both of these *Parapsyllus* species occur in the Australian region, including all major islands surrounding the tip of South America [i.e. Falklands (Islas Malvinas), St. Paul Island, Amsterdam Island]. The shift from mammals to birds probably began with burrow-nesting birds (e.g. penguins) sharing the same habitat as the flea-infested burrowing rodents. *Listronius*, a genus within the Parapsyllinae (not represented in our analysis), parasitizes both small mammals and birds, whereas *Parapsyllus* (which is morphologically extremely similar to *Listronius*) has switched almost entirely to birds.

The other three instances of host shifts to birds are similarly derived from fleas previously associated with rodents. For instance, the leptopsyllid *Ornithophaga a. anomala* (together with all other *Ornithophaga* spp.) occurs on tree-hole-nesting birds, and is placed in a clade (node 82, Fig. 5) that includes highly host-opportunistic fleas (Krasnov et al., 2004) such as *Amphipsylla* spp., *Peromyscopsylla* spp., and *Sigmactenus toxopeusi* that occur on murids and cricetids. In Ceratophyllidae, there are two instances of host shifts to birds: *Dasypyllus gallinulae perpinatus* + *Dasypyllus stefnegeri* are nested in a clade of fleas that were ancestrally associated with cricetids, and *Ceratophyllus petrochelidonii* + *Ceratophyllus gallinacae* are placed together in an unresolved clade that largely includes fleas also associated with cricetids.

Our phylogeny supports a single shift to bats within the monophyletic Ischnopsyllidae. We were only able to include exemplars from the subfamily Ischnopsyllinae, which have a nearly worldwide distribution and are mainly associated with Microchiroptera. The other subfamily Thaumapsyllinae is only known from megachiropteran bats of the Ethiopian and Oriental regions. In addition to ischnopsyllids, there is another genus of fleas known to occur on bats (*Rhynchopsylla*—now *Hectopsylla*) (Hastriter and Méndez, 2000) that were not included in this analysis. Their inclusion will probably lead to a second, independent shift to bats.

Our analysis confirms the notion that there do not appear to be any tight links between flea diversification and host diversification from a strict coevolutionary standpoint. Some authors have suggested that the microhabitat of the vertebrate host and the geographical distribution of the hosts are better predictors of patterns of flea and host associations (Krasnov et al., 1998; Sheeler-Gordon and Owen, 1999; Patterson et al., 2007). The only monophyletic flea groups that are associated with a single host group are pygiopsyllomorphs found on metatherians, the group *Dactyllopsylla + Foxxella* on geomyids, and most notably, Ischnopsyllidae found exclusively on bats. It will be interesting to see whether this lack of correlation is a result of under sampling of flea taxa in the current analysis, or whether our results accurately capture the chaotic nature of host switching during flea evolution.

Conclusions

Our study represents the first comprehensive attempt to reconstruct deep level evolutionary relationships for fleas using a formal analysis of character data from multiple loci. These data and analyses have resulted in a robust phylogenetic hypothesis for fleas, although relationships at the deepest nodes have limited support and will be the subject of additional investigation. From the superfamilial standpoint, our analysis supports the monophyly of Pygiopsyllomorpha and Ceratophyllomorpha, but does not support Pulicomorpha and Hystrichopsyllomorpha. Of the 16 flea families examined in this analysis, ten are clearly monophyletic: Tungidae, Lycopsyllidae, Pygiopsyllidae, Stivaliidae, Stephanocircidae, Rhopalopsyllidae, Chimaeropsyllidae, Pulicidae, Ischnopsyllidae, and Ceratophyllidae. The families Leptopsyllidae, Hystrichopsyllidae, and Ctenophasmatidae were grossly paraphyletic. Three families have only single exemplars (Macropsyllidae, Coptopsyllidae, and Vermipsyllidae) and their monophyly could not be assessed.

Our data and analyses suggest that while the classification of fleas at the level of tribe and genus largely reflects phylogeny, the higher classification of fleas is in need of serious revision to reflect flea evolution better. In particular, the catch-all group Ctenophasmatidae is clearly an unnatural grouping of fleas, and elevating each of its constituent subfamilies to family level would be a closer reflection of phylogeny. Likewise, a re-examination of flea morphology in light of this phylogeny will probably reveal the homoplasic nature of many morphological characters on which the higher classification is based.
We see this study as a first step towards understanding the charismatic fleas, and work is currently underway to generate a more extensive molecular data matrix that will include slower genes to get at the deeper relationships. Concurrent with this effort, we are coding morphology to be used in a formal cladistic analysis, with the ultimate goal of revising flea classification based on a combination of these data.

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We wish to thank Bob Lewis, Sergei Medvedev, and Boris Krasnov who have been supportive of our efforts and discussed many of their views of flea phylogeny with us. We thank Duke Rogers for providing guidance with many aspects of mammal systematics and for providing critical specimens. We express our sincere gratitude to the many scientists who contributed flea specimens from all over the world, without whom this work would not have been possible. Anthony Abley and Rob Gregory, Warranwong Sanctuary, Adelaide, South Australia; Andres Angula, Mauricio Alarcón and Patricia Chandia, Universidad de Concepcion, Casilla, Concepcion, Chile; James Bacon, McCall, Idaho; Rod Baxter, University of Fort Hare, Alice, South Africa; Ellen Bronson, National Zoological Park, Washington, DC; Sarah E. Bush and Dale Clayton, University of Utah, Salt Lake City, Utah; Charles H. Calisher, Colorado State University, Ft. Collins, Colorado; Kevin L. Campbell, University of British Columbia, Vancouver, British Columbia; Robert S. Copeland, International Centre of Insect Physiology and Ecology, Nairobi, Kenya; Jeroen Creuwels, University of Groningen, Groningen, The Netherlands; Justine B. de Cruz, University of Otago, Dunedin, New Zealand; Jan Decher, University of Vermont, Burlington, Vermont; Michael Driessen, Parks and Wildlife Service, Hobart, Tasmania; Jean-Marc Duplantier, Antananarivo, Madagascar; Ralph P. Eckerlin, Northern Virginia Community College, Annandale, Virginia; Robert Elbel, Brad Lengus, and John Vincent, University of Utah, Salt Lake City, Utah; Richard Fagerlund, University of New Mexico, Albuquerque, New Mexico; Karl Frajford, Tromsø Museum, University of Tromsø, Norway; Milton H. Gallardo, Universidad Austral de Chile, Valdivia, Chile; William L. Gannon, University of New Mexico, Albuquerque, New Mexico; Maria S. Gómez, Universidad de Barcelona, Barcelona, Spain; Ricardo Guerrero, University of Caracas, Caracas, Venezuela; Francisco González-Cózatl and Rachel Mercado-Veléz, Universidad Autonoma del Estado de Morelos, Mexico; Werner Haberl, Vienna, Austria; Menna Jones and B. Lazenby, University of Tasmania, Tasmania, Australia; Shin-ichiro Kawada, National Science Museum, Tokyo, Japan; Boris Krasnov, Ben-Gurion University of the Negev, Mizpe Ramon, Israel; James Kucera, Murray, Utah; Juha Laakkonen, University of Helsinki, Finland; Robert E. Lewis, Professor Emeritus, Iowa State University, Ames, Iowa; Li Chao, Institute for Endemic Disease Prevention and Control, Xining, China; Pedro M. Linardi, Universidade Federal de Minas Gerais, Horizonte/Minas Gerais, Brazil; Javier Lucentes, Universidad de Zaragoza, Spain; Lu Liang and Guo Tianyu, Institute of Microbiology and Epidemiology, Beijing, China; Anthony Maddock, KwaZulu-Natal Nature Conservation Service, South Africa; Tom Manning, Leslie Carraway, and Sam Gilman, Oregon State University, Corvalis, Oregon; Leonardo Mendoza-Urrie, Instituto Nacional de Salud, Lima, Peru; Doug McCauley, Stanford University, Stanford, California; Michael Patrick and Michael Gannon, The Pennsylvania State University, Altoona, Pennsylvania; Mary Peacock, University of Nevada, Reno, Nevada; Stephen Petersen, University of Alberta, Edmonton, Alberta, Canada; R.L.C Pilgrim and Andrea Booth, University of Canterbury, Christchurch, New Zealand; Megan Porter, University of Maryland Baltimore County, Baltimore, Maryland; Hugo A. Ruiz-Piña, University of Merida, Merida, Yucatán, Mexico; Richard G. Robbins, Walter Reed Army Medical Center, Washington, D.C.; Duke S. Rogers, Shellye Godell, Taylor Maxwell, James Robertson, Gavin Svenson, and Craig White, Brigham Young University, Provo, Utah; Richard D. Sage and Annaliese K. Beery, University of California, Berkeley, California; Jarrold J. Scharninghausen, College Station, Texas; Shi Gao, Chifeng Center for Disease Control and Prevention, Chifeng, China; Vince Smith, University of Glasgow, Glasgow, Scotland; Mikal Stanko, Institute of Zoology and Ecosozology of the Slovak Academy of Sciences, Košice, The Slovak Republic; Paul Stapp, University of California, Davis, California; and Andrei Tchabovsky, Svertsov Institute of Ecology and Evolution, Moscow, Russia. We thank the National Research Institute of Papua New Guinea for providing field permits for the PNG work. This work was supported by NSF Career Grant # DEB 9983195 with multiple NSF REU supplements to M.F.W.

References


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## Appendix 1

List of taxa used in the analysis, including GenBank accession numbers and host information.

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