

# **Phylogenetic analysis of partial sequences of elongation factor 1 alpha identifies major groups of lice (Insecta: Phthiraptera)**

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## ABSTRACT

As a first attempt to use molecular data to resolve the relationships between the four suborders of lice and within the suborder Ischnocera, we sequenced a 347bp fragment of the elongation factor 1 alpha gene of 127 lice (Insecta: Phthiraptera) as well as outgroup taxa from the order Psocoptera. A number of well-supported monophyletic groups were found but the relationships among many of these groups could not be resolved. While it is probable that multiple substitutions at high divergences and ancient radiation over a short period of time have contributed to the problem, we attribute most of this lack of resolution to the high ratio of taxa to characters. Nevertheless, the sequence data unequivocally support a number of important relationships that are at variance with the conclusions of morphological taxonomy. These include the sister group relationship of *Chelopistes* and *Oxylipeurus*, two lice occupying different ecological niches on the same host, which have previously been assigned to different families. These results provide evidence in support of the hypothesis that lice have speciated *in situ* on the host in response to niche specialisation, and that this has given rise to convergent morphologies in the lice of different host groups which share similar ecological niches. We discuss our attempts to overcome the limitations of this large data set, including the use leaf stability analysis, a new method for analysing the stability of taxa in a phylogenetic tree, and examine a number of hypotheses of relationships based on both traditional taxonomy and host associations.

**Keywords:** Elongation factor 1 alpha, leaf stability analysis, molecular systematics, niche specialisation, phylogenetics, lice, Phthiraptera, Ischnocera

## INTRODUCTION

Lice (order Phthiraptera) are obligate ectoparasitic insects of birds and mammals. Unlike fleas, they have no free-living stage and of all the insects, are the most completely committed to parasitism (Askew, 1971). Most families of birds and mammals have their own specific lice. Notable exceptions among the mammals are the monotremes, anteaters, armadillos, bats, cetaceans and sirenians, none of which appear to have lice. The sister group of the lice is thought to lie within the paraphyletic order "Psocoptera" (psocids, booklice, barklice), possibly

within the family Liposcelidae (Lyal, 1985a). Psocoptera are free-living insects that feed upon microflora and organic debris. Some are associated with birds and mammals, dwelling in nests or found amongst their plumage or fur, but none are parasitic (Smithers, 1996). The Phthiraptera and Psocoptera together constitute the superorder Psocodea (Lyal, 1985a).

Lice have traditionally been divided into four suborders; [1] Anoplura (532 species of mammal ‘sucking’ lice), [2] Rhyncophthirina (3 species of mammal lice confined to elephants and pigs), [3] Amblycera (1182 species of bird lice and 162 species of mammal lice) and [4] Ischnocera (2683 species of bird lice and 377 species of mammal lice). The relationships among these suborders are poorly understood. Early attempts to resolve this issue placed the Rhyncophthirina, Amblycera and Ischnocera together in a group called the “Mallophaga” (biting or chewing lice), and some entomologists even placed the Anoplura and “Mallophaga” in separate orders (Richards and Davies, 1978). This division was based largely on the morphology of the mouthparts and mode of feeding. The “Mallophaga” have biting mouthparts and feed mostly by chewing feathers or hair and eating the secretions of sebaceous glands, whereas the Anoplura have piercing mouthparts and feed by drawing up blood. More recently (Lyal, 1985a), the “Mallophaga” has been regarded as a paraphyletic group with the basal split within the Phthiraptera being placed between the Amblycera and the remaining three suborders (Fig. 1). These uncertainties provided the motivation for the first aim of our phylogenetic analysis; an investigation of the relationships between the four suborders of lice.

The relationships within the suborders of lice are also poorly understood. The largest suborder is the Ischnocera in which four families are generally recognised, however, only three of these families appear to be monophyletic. These are the Trichodectidae (all ischnoceran lice of mammals with the probable exception of the lemur louse, *Trichophilopterus*), Gonioididae (lice of galliform and columbiform birds) and Heptapsogasteridae (tinamou lice). The remainder of the Ischnocera are placed in the large family “Phlopteridae” which is likely to be paraphyletic. Relationships within this family remain almost entirely unresolved. It has been suggested that ischnoceran lice are particularly host specific and therefore likely to be a rich source of data for cospeciation studies. The desire to identify appropriate outgroups for such studies provided the motivation for the second aim of our phylogenetic analysis; an investigation of the relationships within the suborder Ischnocera.

## **Our current understanding of louse phylogeny**

Current knowledge of louse relationships is summarised in Fig. 1. Every conceivable arrangement of the four suborders of lice has been proposed at some time. Morphological data supporting the monophyly of “Mallophaga” were proposed by Kim and Ludwig (Kim and Ludwig, 1978b; 1982), although these results were controversial (Haub, 1980). Lyal (1985a) conducted a detailed review of the morphological data supporting the monophyly of the four suborders and their relative relationships. His study confirmed the monophyly of all four

suborders, although ischnoceran monophyly was the least well supported. The subordinal phylogeny established by Lyal (1985a) is concordant with comments in Clay (1970) and Konigsmann (1960) who both considered the Amblycera sister taxon to a monophyletic group comprising the Ischnocera, Rhyncophthirina and Anoplura.

Familial classifications within each of the suborders are less problematic, with the notable exception of the Ischnocera. Anopluran lice have a significant medical and veterinary importance, which in part, explains why they are the best studied suborder of Phthiraptera. Ferris, between 1920 and 1935, provided the foundation for modern taxonomic work on the Anoplura in a series of papers entitled “Contributions toward a monograph of the sucking lice”. When fully republished as a monograph (Ferris, 1951) he recognised six families. In the light of new species descriptions, this was expanded to 15 families by Kim and Ludwig (1978a). More recently a morphological phylogeny has been developed (Kim, 1988). Rhyncophthirina comprise just two species in a single genus. The type species was originally designated a sucking louse, however careful study of the mouthparts suggested this assignment was untenable, and the taxon was awarded subordinal status (Ferris, 1931). Amblyceran classification has been the subject of several detailed studies, most notably by Clay (1970) who has done much to stabilise their familial classification. She also considered possible relationships of genera in the largest Amblyceran family, the Menoponidae (Clay, 1969). Phylogenetic relationships between these families have yet to be studied in detail.

The number of families making up the Ischnocera is a matter of some contention. Eichler (1963) recognised 21 families whilst Hopkins and Clay (1952) accepted just three. This discrepancy can partly be explained by the diversity of form exhibited amongst the genera, as ischnoceran lice vary considerably in terms of their size and general morphology. This diversity makes even generic differences hard to define, and comparative morphological studies within this group are exceedingly difficult. No clear justification of the scheme proposed by Eichler (1963) was ever published, and it has subsequently been rejected by most authorities due the assumption that it was unduly biased towards the host classification. More recent studies on Ischnocera recognise at least three monophyletic groups (Lyal, 1985a; Mey, 1994; Smith, 2000); Trichodectidae, Heptapsogasteridae and Gonioididae. A fourth group (the Philopteridae *sensu* Eichler, 1963) comprise some 70% of ischnoceran species and are present on almost all families of birds. It is generally accepted that this is a miscellaneous collection of genera and is almost certainly para- or polyphyletic. However, the relationships amongst these taxa have never been studied. A monotypic taxon (the Trichophilopteridae) represented by a single species present on Madagascan primates (Lemuridae and Indriidae) may be related to the avian Philopteridae. This species bears a number of significant morphological characters in common with the Philopteridae and the mammalian trichodectids. Consequently, it has been variably placed amongst both these groups and in an independent family within Ischnocera (Emerson and Price, 1985; Ferris, 1933; Stobbe, 1913).

## **A molecular phylogeny for lice**

As a first attempt to use molecular data to resolve higher order relationships within the lice, we sequenced 347bp of the elongation factor 1 alpha gene (EF1 $\alpha$ ) of 127 individual lice (representing 105 species in 70 genera) as well as outgroup taxa from the order Psocoptera. This nuclear gene was chosen for an initial survey of the phylogeny of lice for a number of reasons including its low copy number, ease of alignment (due to conservation of amino acid sequence and lack of insertions/deletions) and proven phylogenetic utility in other insect groups (Friedlander *et al.*, 1998), as well as the important technical reason that universal primers already available gave reliable PCR amplification in lice.

## **METHODS**

### **Sequence determination**

Total genomic DNA was extracted from single lice using the DNAeasy Tissue Kit (Qiagen). The heads of the lice were removed prior to DNA extraction and both the head and body were incubated in lysis buffer over two nights, after which the exoskeletons of the head and body were removed for slide mounting as vouchers. 347bp of the EF1 $\alpha$  gene were amplified and sequenced using the primers EF1-For3 and Cho10 (Danforth and Ji, 1998). After removal of introns, redundant taxa and two base pairs at the 5' end of the sequence (see below), the data set consists of 345 characters for 111 taxa, of which 158 (46%) are variable and 143 (41%) are parsimony informative. Most of the variation (73% of variable and 80% of informative sites) was at the third codon position. Amplification products were gel purified using the QIAquick Gel Extraction Kit (Qiagen) and sequenced using the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA polymerase, FS (Perkin Elmer). Sequencing products were ethanol precipitated and run on an ABI 373 Stretch automated sequencing machine.

### **Taxon sampling**

We sequenced one individual for 91 species, two individuals for 16 species, and six individuals for a single species. The data set therefore consists of 129 sequences representing 108 species (table 1). Multiple representatives of 17 taxa (16% of the total) were sequenced in order to [1] assess the levels of sequence polymorphism within species (although this is likely to be low anyway, since no heterozygotes were detected) and [2] to check for consistency between the two laboratories in which sequencing was performed.

Since we were interested in examining both the relationships between the four suborders of lice and those within the suborder Ischnocera, all four suborders were sampled with the densest sampling within the Ischnocera (80 taxa). All major groups of Ischnocera were sampled. Although only 4 anopluran taxa were included, these span the root of the tree

published by Kim (1988). Unfortunately the 22 amblyceran taxa sampled come from just two families (Menoponidae and Ricinidae) and therefore a substantial portion of amblyceran diversity remains unsampled. This includes all of the families of amblyceran mammal lice (Gyropidae, Trimenoponidae and Boopidae) as well as one family of amblyceran bird lice (Laemobothriidae).

Outgroups consist of two booklice from the genus *Liposcelis* (Psocoptera: Liposcelidae) which we sequenced ourselves (table 1) and three representatives of the paraneopteran order Hemiptera obtained from GenBank; a leafhopper, *Chiasmus* sp. (Euhemiptera: Cicadellidae) (AF182636), a treehopper, *Glossonotus acuminatus* (Euhemiptera: Membracidae) (AF182617) and an aphid, *Stomaphis fagi* (Sternorrhyncha: Aphidoidea) (AF163880).

## Phylogenetic analysis

An initial unweighted maximum parsimony search found 704 equally most parsimonious trees. The strict consensus of these trees (Fig. 2) represents a conservative estimate of phylogeny, however, the data may contain more resolution than this consensus reveals. For this reason we turned to other methods of analysis in an attempt to improve then resolution of the tree. A test of stationarity of base composition (Rzhetsky and Nei, 1995) was performed on a subset of the data chosen to include representatives of all major clades found in the initial MP trees. Stationarity was rejected ( $p < 0.005$ ) i.e. the base composition differs significantly in different parts of the tree. This can be a problem for some methods of phylogenetic analyses that may group taxa together due to similarity of base composition rather than genuine shared ancestry (Galtier and Gouy, 1995). This suggests that only methods which can correct for non-stationarity of base composition (e.g. LogDet distance based methods) should be used to analyse this data set. It is possible however, that the loss of information due to the use of distances rather than discrete characters could adversely affect the performance of the phylogenetic analyses. This loss of information may have a greater detrimental effect than failure to correct for non-stationarity of base composition. For this reason we used a combined distance and discrete character based approach. An initial neighbour joining (NJ) tree was constructed using LogDet distances, which correct for non-stationarity of base composition (Lockhart *et al.*, 1994). Rate heterogeneity was assumed, with two rate classes i.e. constant sites vs. varying sites. The proportion of invariant sites (0.542) was estimated using maximum likelihood. Inclusion of a relatively large number of taxa for a study of this sort means that rate heterogeneity parameters can be estimated very reliably (i.e., they will have very small confidence intervals) (Sullivan *et al.*, 1999). Constant sites were removed in proportion to base frequencies estimated from constant sites only, according to the suggestion of Swofford *et al.* (1996). This tree was then used as the starting tree for two different kinds of branch swapping; TBR branch swapping under the criterion of minimum evolution and NNI branch swapping under the criterion of maximum likelihood (ML). Maximum likelihood (ML) was chosen as the

discrete method since we considered it least likely to be adversely affected by non-stationarity of base composition and because it is more data inclusive than parsimony which does not consider parsimony uninformative sites. Data inclusiveness may be particularly important in the analysis of this data set, which contains relatively few characters for the number of taxa involved. Parameters for ML branch swapping were estimated using the program MODELTEST (Posada, 1998) (but setting base frequencies to empirical values rather than estimating them due to a bug in PAUP\* version 4.0b2a (PPC)). The model with the minimum information theoretical content is K81uf+I+ which has three substitution rates ( $A \rightarrow C = G \rightarrow T$ ,  $A \rightarrow G = C \rightarrow T$  and  $A \rightarrow T = C \rightarrow G$ ), unequal base frequencies, invariable sites and gamma distributed rates at variable sites. Fig. 3 shows an Adams consensus of the two branch swapping methods with nodes not found in either of the two fundamental trees removed. All phylogenetic analyses were conducted using PAUP\* version 4.0b2a (PPC) (Swofford, 1999). The trees will be deposited in TreeBase <<http://herbaria.harvard.edu/treebase/>>.

## RESULTS

### Introns

Both the fruit fly and the honeybee are known to have two copies of EF1 $\beta$  (F1 and F2) (Danforth and Ji, 1998; Hovemann *et al.*, 1988). In both cases the F2 copies of the gene contain an intron (intron 5) which is not present in the F1 copy. Although we have found only a single copy of EF1 $\beta$  in both lice and booklice, the booklouse (*Liposcelis*) sequences contain an intron not present in any of the louse sequences. This was removed from the data set prior to phylogenetic analysis. The position of this intron is identical to that of intron 5 in the F2 copies of EF1 $\beta$  in the fruit fly and the honeybee. This could mean that the EF1 $\beta$  sequences from *Liposcelis* are paralogous with those from lice. These sequences would still represent the closest available outgroup unless the gene duplication event which gave rise to them predates the last common ancestor of the Psocodea and any alternative outgroup taxon for which a truly homologous sequence is available.

In common with our sequences from lice, the F1 copies of EF1 $\beta$  in the fruit fly and the honeybee (GenBank accession numbers X06869 and X52884 respectively) lack intron 5. If these sequences are genuine homologs of the louse sequences, while the booklouse sequences are paralogs, then the honeybee and fruit fly sequences may represent closer outgroups. However, this seems unlikely since the genetic distances between the lice and the honeybee and fruit fly (24.8% and 22.5% respectively) are both greater than the distance between the lice and the booklice (19.4%). If the *Liposcelis* sequences are indeed paralogous to the sequences from the lice, it seems likely that the gene duplication event which gave rise to them is independent to that/those which gave rise to the duplicate copies in the honeybee and fruit fly, and occurred

closer to the root of the Psocodea. For this reasons we consider the *Liposcelis* sequences to be the closest available outgroup at this time.

In order to assess how widespread this intron is within the Psocoptera we sequenced an additional (unidentified) psocopteran that was not included in the phylogeny. This sequence also contained intron 5. Since the genetic distance between *Liposcelis* and the unidentified psocopteran sequences is large (18.6%) compared with the distances within the lice (15.6%) we are likely to have spanned a large part of the diversity within Psocoptera. It therefore seems likely that intron 5 is ubiquitous within the Psocoptera but has been lost in the lice.

## Polymorphism

Of the 31 pairwise comparisons within taxa, 15 are within *Struthiolipeurus struthionis* ex. *Struthio camelus* all six sequences of which are identical. Of the remaining 16 pairs, 11 (69%) are identical. In the remaining five pairs a total of seven pairwise differences were found. The weighted mean sequence divergence within taxa = 0.10%. All differences are due to synonymous substitutions at the third codon position. Two of these (29%) occur at the first codon. While it is possible that this codon represents a mutational hot spot, its position at the very 5' end of the sequence, adjacent to the forward primer, suggests that it may be particularly prone to sequencing errors, for this reason, and also because only the second and third positions of this codon were sequenced, this codon was removed from all subsequent analyses. The weighted mean sequence divergence within taxa after removal of this codon = 0.07%. Since an initial neighbour joining (NJ) tree using uncorrected distances (not shown) put the all of the multiple sequences from each taxon together, only one representative of each was chosen for inclusion in further analyses in order to reduce the time taken for these to complete. In cases where one or more sequences contained missing data, the most complete sequence was used.

## Phylogenetic Relationships

Figs. 2 and 3 show trees derived from a simple unweighted parsimony search and a more sophisticated combined distance and likelihood approach. Neither tree is very well resolved, particularly towards the base, but this is to be expected for a data set with such a high taxon/character ratio. Nevertheless, the two trees do agree on a number of points, which include the following. [1] The anopluran *Echinophthirius horridus* and the ischnoceran *Heptapsogaster temporalis* form a group. We consider this group is likely to be an artifact of character sampling (see below). [2] The Trichodectidae are monophyletic. The relationships within the Trichodectidae are identical in the two trees. [3] *Mulcticola* is consistently found away from the rest of the Quadraceptinae, which are otherwise monophyletic in both trees. The relationships within the Quadraceptinae are also the same in both trees. [4] *Rallicola fuliginosa* is the sister taxon of the Quadraceptinae, but the other two members of the genus *Rallicola* are found elsewhere in the tree. [5] The Degeeriellidae are monophyletic and sister taxon to

*Cuclotogaster*. The relationships within the degeeriellids are the same in both trees. [6] *Oxylipeurus* and *Chelopistes* appear together in a group of cracid lice away from their traditional taxonomic placements. Clay (1976) suggested an association between *Chelopistes* and *Oxylipeurus*. Smith (2000) also cites karyological evidence that *Chelopistes* does not belong in the Gonioididae. This is based on the observation that *Chelopistes* has the typical philopterid chromosome number of 12 (Perrot, 1934), rather than the typical gonioidid chromosome number of 11 (Kettle, 1977) (although since lice have holokinetic chromosomes the karyotype may be particularly plastic in lice). *Chelopistes* is a short fat louse (Kéler, 1939) which appears to be adapted to living amongst the downy feathers of the head and neck where it can avoid preening since the host cannot reach these areas. *Oxylipeurus chiniri*, on the other hand, is a fairly long, thin louse (Carriker, 1944) which may be adapted to living on the wing feathers where it can conceal itself between the feather shafts and avoid being dislodged during preening or flight. This clade represents an interesting case where lice adapted to different microhabitats on the same host, previously thought to be unrelated, in fact form a clade. This is in contrast to the head and wing lice of procellariiform seabirds for example, which are indeed unrelated. (The head lice belong to the Quadraceptinae and the wing lice to the Pseudonirminae.) Another group of birds in which the head and wing lice seem to be related are the ducks. Both head and wing lice of ducks are found in the family Esthiopteridae, head lice in the subfamily Anatoecinae and wing lice in the subfamily Ibioecinae. [7] With the exception of *Chelopistes* the Gonioididae are monophyletic in both trees. [8] The genus *Brueelia* is sister taxon to a monophyletic Paragonicotinae rather than grouping with the rest of the Philopteridae (*sensu* Eichler). This group appears close to Meinertzhageniellidae in both trees. [9] *Pseudolipeurus* consistently appears near the Philopteridae (*sensu* Eichler) rather than the Lipeuridae. [10] The Amblycera are monophyletic and the two trees are compatible with respect to the relationships within the Amblycera although the parsimony tree is more resolved.

## DISCUSSION

### Are the suborders monophyletic?

Of the four suborders of lice, only the Amblycera are unequivocally monophyletic. The Ischnocera appear to be paraphyletic with respect to the Amblycera, although this may be an artifact of character sampling. Since the node that places the Amblycera within the Ischnocera is not supported by bootstrap support above 50% for either maximum parsimony or minimum evolution, no confidence should be placed in this placement. The anopluran seal louse *Echinophthirius* also appears within the Ischnocera, and again this is likely to be an artifact of character sampling. Analysis using split decomposition (see below) indicates that there is conflict in the data resulting in a spurious association of *Echinophthirius* with the heptapsogasterid *Heptapsogaster temporalis*. Moving *H. temporalis* to its traditional place in the

tree, as sister taxon to *H. minuta*, increases the overall tree length from 1750 to 1753, a jump of only three steps.

### **What are the relationships among the suborders?**

Since the position of the Amblycera remains in question, EF1 is unable to resolve this issue unequivocally. However, the gene does appear to favour the old ‘Mallophaga’ scheme that places Amblycera and Ischnocera as sister groups with Anoplura at the base. This scheme has been discredited by more recent taxonomists who have preferred to place Anoplura and Ischnocera together, with Amblycera at the base (Lyal, 1985a).

### **Are the currently recognised families of Ischnocera monophyletic?**

Of the four currently recognised Ischnoceran families, the Trichodectidae and Gonioididae (with the exception of *Chelopistes*) are monophyletic in both trees. Ignoring the spurious position of *Heptapsogaster temporalis*, the Heptapsogasteridae are monophyletic in the distance/likelihood tree and the parsimony tree, although unresolved, is also compatible with this conclusion. The remaining family, Philopteridae *sensu* Hopkins and Clay, appears to constitute a large paraphyletic assemblage.

### **Which philopterid groups could be elevated to familial status?**

The Philopteridae *sensu* Hopkins and Clay represents a large paraphyletic assemblage from which the Gonioididae and Heptapsogasteridae have been raised to the rank of family. While we do not advocate altering the present classification of the lice on the basis of a single gene phylogeny it is interesting to speculate on the extent to which our EF1 phylogeny can be used to identify further groups of philopterids which could be elevated to familial status. Such groups could include the Pseudonirmidae (although the inclusion of *Pectinopygus* in this family remains unresolved), the “cracid lice”, Austrogonioididae, Trichophilopteridae, Docophoroididae, Esthiopteridae (perhaps excluding *Fulicoffula*, *Ardeicola* and *Columbicola*), Colilipeuridae, Degeeriellidae (perhaps including *Cuclotogaster*), Rallicolidae (excluding *Mulcticola*) (although some taxa may have to be removed from the genus *Rallicola*), Meinertzhageniellidae, and Paragonicotidae (perhaps including *Brueelia*). Elevation of these groups to the rank of family would mean that what remains of the Philopteridae (*Penenirmus*, *Philopterus* and *Strigiphilus*) would no longer be paraphyletic. It is unclear from the EF1 data alone where taxa such as *Ardeicola*, *Mulcticola*, *Otidoecus* and *Nyctibicola* belong.

## **What is the relationship of the Trichodectidae to the avian Ischnocera?**

Many taxonomists place the Trichodectidae (ischnoceran lice of mammals) at the base of the Ischnocera. This issue remains unresolved, however, the divergences within the Trichodectidae are much smaller than those within the avian Ischnocera. This suggests that either [1] the avian Ischnocera is a great deal older than the Trichodectidae, [2] the avian Ischnocera is evolving more quickly than the Trichodectidae or [3] the modern radiation of the Trichodectidae considerably post-dates its origin. The fact that the Trichodectidae is at the end of one of the longest internal branches in the tree favours option 3.

## **Do the lemur lice, *Trichophlopterus*, belong in the Trichodectidae?**

It has been suggested that the lemur lice, *Trichophlopterus*, do not belong in the Trichodectidae with the other ischnoceran lice of mammals, but have more affinities with the avian Ischnocera. To the extent that EF1 says anything at all about this issue, it suggests that *Trichophlopterus* belongs in a family of its own, but this may be an artifact of the poor resolution at the base of the tree. Although the exact placement of this genus remains in question we can be confident that it does not belong in the Trichodectidae.

## **Do the penguin lice, *Austrogoniodes*, belong in the Gonioididae?**

The penguin lice, *Austrogoniodes*, have often been considered to have affinities with the Gonioididae, however, Mey (1994) and Smith (2000) consider that the morphological similarities on which this assumption has been based are due to convergence. EF1 does indeed place *Austrogoniodes* well outside the Gonioididae, however, since the base of the tree is completely unresolved the true position of this genus remains in question. Although we cannot place *Austrogoniodes* precisely in the tree, we can be confident that it does not belong in the Gonioididae.

## **How can we account for the spurious positions of *Echinophthirius* and *Heptapsogaster temporalis*?**

Data from the mitochondrial gene, COI (Johnson, unpublished), place *Echinophthirius* near other anoplurans, suggesting that the relationship will break down once more data are added. Smith (2000) is in no doubt as to the monophyly of the Heptapsogasteridae, let alone the monophyly of the genus *Heptapsogaster* and Lyal (1985a) seems almost as confident about the monophyly of the Anoplura, so why do these two taxa consistently group together? Worse still, if such a spurious grouping can occur between two taxa we are almost certain belong in

other parts of the tree, how can we be sure about the relationships of the taxa for which we have no prior hypothesis? Is there an independent method which would identify these two taxa as particularly prone to appearing in the wrong part of the tree, or must we call the entire phylogeny into question?

Using SplitsTree (Huson, 1998) we can visualise the conflicting signals in the data. Fig. 4 shows a splits graph diagram for a subset of louse sequences. A large parallelogram connects the sequence for the tinamou louse *Heptapsogaster temporalis* with the seal louse *Echinophthirius horridus* on one hand, and the other tinamou louse sequences (including the congeneric *H. minuta*) on the other. The signal grouping all the tinamou lice together is supported by morphological data (Smith, 2000), hence we regard the signal grouping *H. temporalis* with *E. horridus* as spurious. Inspecting the data reveals that the latter two taxa share a number of substitutions at the third codon position at sites where the amino acid is highly conserved. These are probably convergent changes that have occurred sufficiently often to mislead our tree construction methods into grouping these two taxa together.

In the case of *H. temporalis*, we had *a priori* expectations of its correct relationships (Smith, 2000) and hence when confronted with its strange placement in our EF1 trees we were able to investigate this further, uncovering the conflicting signals in our data. However, for many lice we have no previous hypotheses of relationship, which raises the question of whether other taxa might be as misplaced as *H. temporalis*. To investigate this we used the program RadCon (Thorley and Page, 2000) to compute leaf stability measures for all the lice taxa. When presented with a number of different trees for the same taxa, RadCon computes three different measures of the degree to which taxa move around in the tree (Thorley and Wilkinson, 1999). Using 100 LogDet NJ bootstrap replicates, the least stable taxa (those within the least stable 10% of taxa for at least one of the three methods) are (in increasing order of stability according to the entropy criterion); *Heptapsogaster temporalis*, *Ardeicola* sp., *Colilipeurus colius*, *Strigiphilus rostratus*, *Haematomyzus elephantis*, *Echinophthirius horridus*, *Pseudolipeurus similis*, *Trichophlopterus babakotophilus*, *Liposcelis* sp., *Fulicoffula heliornis*, *Penenirmus zumpti* and *Neohaematopinus sciuri*. There is considerable agreement between the three methods for estimating leaf stability. *Echinophthirius horridus* and *Heptapsogaster temporalis* are indeed amongst the least stable taxa, however, the leaf stability analysis also calls into question the status of a number of other groups for which we had no prior hypothesis of relationships. The position of the root, which has already been called into question on other grounds (see above), is also in doubt.

### **Are host assemblages monophyletic?**

The ischnoceran lice from some host groups appear to form monophyletic groups. In addition to these ischnoceran lice, many host groups also have one or more clades of amblyceran lice. However, due to the paucity of amblyceran taxa sampled, this discussion will

consider only ischnoceran lice. The classic examples of a monophyletic host assemblage are the tinamou lice of the family Heptapsogasteridae. Other host groups which appear to have monophyletic assemblages of Ischnocera according to the EF1 tree include the Cracidae (*Oxylipeurus* and *Chelopistes*), ratites (Meinertzhageniellidae), and pelecaniform seabirds (Pectinopyginae). There is also some evidence for a clade of duck lice.

Other host groups appear to have lice that are restricted to a few separate clades. For example, lice of charadriiform seabirds occur in two clades; the Pseudonirminae and the Quadraceptinae. In this case it seems that the Quadraceptinae is a genuine charadriiform clade, whereas the charadriiform lice of the Pseudonirminae represent a host switch in an otherwise procellariiform clade. Similarly, lice of procellariiform seabirds are found in these same two clades, however, whilst the Pseudonirminae appears to represent a genuine procellariiform clade, the procellariiform lice of the Quadraceptinae represent a host switch in an otherwise charadriiform clade. These reciprocal host switches were probably facilitated by the fact that these groups of lice are adapted to a different part of the host anatomy. The Pseudonirminae tend to be found on the wings where their elongated shape allows them to fit between the feather shafts (Edwards, 1951). This may give them protection against preening (Clayton, 1991) or from air currents created during flight (Sternram, 1956). The Quadraceptinae appear to specialise in living in the soft downy feathers of the head and neck (*Saemundssonina*) and body (*Quadriceps*) (Choe and Kim, 1988). These adaptations to different microhabitats appear to have allowed these two groups of birds to coexist on the same hosts without competing for resources. Procellariiform lice also belong in a third clade, the Docophoroididae, which is restricted to albatrosses. The lice of columbiform birds (pigeons and doves) also occur in two distinct clades; Columbicolinae and Physconelloidinae. Piciform lice appear to form a clade within the Degeeriellidae, however, another piciform louse, *Penenirmus zumpti*, appears elsewhere in the tree. Other host groups, such as passerines, are more widely represented in the tree.

### **Are there distinct clades of ischnoceran wing lice and body lice?**

Many groups of birds harbour two distinct groups of ischnoceran lice; wing lice, which tend to be long and thin, and body lice, which tend to be short and fat. Are the wing lice from different birds all related to each other forming a wing louse clade? Is the same true of their body lice? For some groups of birds this seems to be true, for example, the wing lice of procellariiform and charadriiform seabirds (Pseudonirminae), and the wing lice of columbiform birds (*Columbicola*) are all found in the basal polytomy. There is some evidence, particularly in the parsimony tree, to suggest that the body lice of these groups (Quadraceptinae and Physconelloidinae respectively) are more closely related to each other than they are to the wing lice of their own hosts. However, this relies on nodes that are not supported by bootstrap proportions above 50% and should therefore be treated with caution. In other host groups,

however, there is more solid evidence that body and wing lice form a clade. For example, the ischnoceran wing and body lice of birds in the family Cracidae (*Oxylipeurus* and *Chelopistes* respectively) are closely related, although even in this case the bootstrap support is not high (59% likelihood and 68% for and distance). In this case though, there is other evidence in support of this clade (see above) despite the fact that it disagrees with the traditional taxonomy. Similarly, wing and body lice of ducks (Ibidoecinae and Anatoecinae respectively) appear to be closely related. It is not clear whether these cases represent independent origins of wing lice from an ancestral stock of body lice or vice versa. More data will be required to resolve this issue. These two cases, in which head and body lice from related hosts are themselves related, suggest that transformation between these body forms is relatively easily achieved. This further suggests that overall body shape as well as other morphological features likely to be associated with adaptation to life on different parts of the host anatomy, are likely to be poor phylogenetic characters due to excessive homoplasy.

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**Table 1: Taxa Sampled**

Louse	Host	Number Sequenced	Species Determination	Sequencing Laboratory	GenBank Accession
<i>Actornithophilus ceruleus</i>	<i>Anous tenuirostris</i> (Lesser noddy)	1	RLP	G	AF320349
<i>Actornithophilus piceus</i>	<i>Larus</i> sp. (Gull)*	1	RJA	U	AF320350
<i>Amyrsidea spicula</i>	<i>Ortalis vetula</i> (Plain chachalaca)	2	RDP	G/U	AF320351/2
<i>Anaticola crassicornis</i>	<i>Anas platyrhynchos</i> (Mallard duck)	1	RJA	U	AF320353
<i>Anaticola crassicornis</i>	<i>Somateria mollissima</i> (Eider duck)	1	VSS	G	AF320354
<i>Anatoecus</i> sp.	<i>Anas platyrhynchos</i> (Mallard duck)	1	RJA	U	AF320355
<i>Anatoecus</i> sp.	<i>Somateria mollissima</i> (Eider duck)	1	VSS	G	AF320356
<i>Ancistrona vagelli</i>	<i>Fulmarus glacialis</i> (Northern fulmar)	1	VSS	G	AF320357
<i>Ancistrona vagelli</i>	<i>Puffinus tenuirostris</i> (Short-tailed shearwater)	1	VSS	G	AF320358
<i>Aquanirmus occidentalis</i>	<i>Aechmophorus occidentalis</i> (Western grebe)	1	RJA	U	AF320359
<i>Archolipeurus nandu</i>	<i>Rhea americana</i> (Greater rhea)	1	EM	G	AF320360
<i>Ardeicola ardeae</i>	<i>Ardea cinerea</i> (Grey heron)	1	VSS	G	AF320361
<i>Austrogoniodes watersoni</i>	<i>Eudyptula minor</i> (Little penguin)	1	AMP	G	AF320362
<i>Austromenopon echinatum</i>	<i>Calonectris diomedea</i> (Cory's shearwater)	1	VSS	G	AF320363
<i>Austromenopon merguli</i>	<i>Alle alle</i> (Little auk)	1	VSS	G	AF320364
<i>Austrophilopterus subsimilis</i>	<i>Ramphastos sulfuratus</i> (Keel-billed toucan)	2	RDP	G/U	AF320365/6
<i>Austrophilopterus</i> sp.	<i>Pteroglossus torquatus</i> (Collared aracari)	1	RDP	U	AF320367
<i>Bedfordiella unica</i>	<i>Lugensa brevirostris</i> (Kerguelen petrel)	2	VSS / RLP	G	AF320368/9
<i>Bovicola bovis</i>	<i>Bos taurus</i> (Domestic cattle)	1	VSS	G	AF320370
<i>Brueelia marginella</i>	<i>Momotus momota</i> (Blue-crowned motmot)	1	RDP	G	AF320371
<i>Brueelia</i> sp.	<i>Parus niger</i> (Black tit)	1	RJA	U	AF320372
<i>Brueelia</i> sp.	<i>Ploceus velatus</i> (Southern masked-weaver)	1	RJA	U	AF320373
<i>Brueelia</i> sp.	<i>Pycnonotus nigricans</i> (Black-fronted bulbul)	1	RJA	U	AF320374
<i>Brueelia</i> sp.	<i>Sylvia subcaeruleum</i> (Rufous-vented warbler)	1	RJA	U	AF320375
<i>Brueelia</i> sp.	<i>Trogon massena</i> (Slaty-tailed trogon)	1	RJA	U	AF320376
<i>Campanulotes compar</i>	<i>Columba livia</i> (Feral pigeon)	2	VSS / RJA	G/U	AF320377/8
<i>Chelopistes oculari</i>	<i>Penelope purpurescens</i> (Crested guan)	1	RJA	U	AF320379
<i>Chelopistes texanus</i>	<i>Ortalis vetula</i> (Plain chachalaca)	1	RJA	U	AF320380
<i>Colilipeurus colius</i>	<i>Urocolius indicus</i> (Red-faced mousebird)	1	RJA	U	AF320381
<i>Colimenopon urocolius</i>	<i>Urocolius indicus</i> (Red-faced mousebird)	1	RJA	U	AF320382

<i>Coloceras</i> sp.	<i>Streptopelia capicola</i> (Ring-necked dove)	1	RJA	U	AF320383
<i>Columbicola baculoides</i>	<i>Zenaida macroura</i> (Mourning dove)	1	RJA	U	AF320384
<i>Columbicola columbae</i>	<i>Columba livia</i> (Feral pigeon)	2	VSS / RJA	G/U	AF320385/6
<i>Cuclotogaster hopkinsi</i>	<i>Scleroptila africanus</i> (Grey-winged francolin)	1	RJA	U	AF320387
<i>Cuculicola atopus</i>	<i>Piaya cayana</i> (Squirrel cuckoo)	1	RDP	G	AF320388
<i>Dennyus cypsiurus</i>	<i>Cypsiurus parvus</i> (African palm-swift)	1	RJA	U	AF320389
<i>Dennyus hirundinis</i>	<i>Apus apus</i> (Common swift)	2	VSS / DHC	G/U	AF320390/1
<i>Discocorpus mexicanus</i>	<i>Crypturellus cinnamomeus</i> (Thicket tinamou)	2	RDP	G/U	AF320392/3
<i>Docophoroides brevis</i>	<i>Diomedea epomophora</i> (Royal albatross)	1	AMP	G	AF320394
<i>Docophoroides harrisoni</i>	<i>Diomedea bulleri</i> (Buller's albatross)	1	AMP	G	AF320395
<i>Echinophthirius horridus</i>	<i>Phoca vitulina</i> (Harbour seal)	2	VSS	G/U	AF320396/7
<i>Felicola subrostratus</i>	<i>Felis catus</i> (Domestic cat)	1	VSS	G	AF320398
<i>Formicariicola analoides</i>	<i>Formicarius moniliger</i> (Mexican ant-thrush)	1	RDP	G	AF320399
<i>Fulicoffula heliornis</i>	<i>Heliornis fulica</i> (Sungrebe)	1	RDP	U	AF3203400
<i>Geomydoecus chapini</i>	<i>Orthogeomys hispidus</i> (Hipsid pocket gopher)	1	MSH	G	AF3203401
<i>Geomydoecus costaricensis</i>	<i>Orthogeomys heterodus</i> (Variable pocket gopher)	1	MSH	G	AF3203402
<i>Goniocotes</i> sp.	<i>Scleroptila africanus</i> (Grey-winged francolin)	1	RJA	U	AF3203403
<i>Goniodes isogenos</i>	<i>Scleroptila africanus</i> (Grey-winged francolin)	1	RJA	U	AF3203404
<i>Haematomyzus elephantis</i>	<i>Elephas maximus</i> (Asian elephant)	1	LAD	U	AF3203405
<i>Haffneria grandis</i>	<i>Catharacta skua</i> (Great skua)	1	VSS	G	AF3203406
<i>Halipeurus bulweriae</i>	<i>Bulweria bulwerii</i> (Bulwer's petrel)	1	VSS	G	AF3203407
<i>Halipeurus pelagicus**</i>	<i>Bulweria bulwerii</i> (Bulwer's petrel)	1	VSS	G	AF3203408
<i>Halipeurus pelagicus</i>	<i>Oceanodroma castro</i> (Madeiran storm-petrel)	1	VSS	G	AF3203409
<i>Harrisoniella densa</i>	<i>Diomedea immutabilis</i> (Laysan albatross)	1	VSS	G	AF3203410
<i>Heptapsogaster minuta</i>	<i>Nothura maculosa</i> (Spotted nothura)	1	VSS	G	AF3203411
<i>Heptapsogaster temporalis</i>	<i>Crypturellus cinnamomeus</i> (Thicket tinamou)	2	VSS	G/U	AF3203412/3
<i>Hohorstiella lata</i>	<i>Columba livia</i> (Feral pigeon)	1	VSS	G	AF3203414
<i>Hohorstiella passerinae</i>	<i>Columbina inca</i> (Inca dove)	1	RJA	U	AF3203415
<i>Hoplopleura sciuricola</i>	<i>Sciurus carolinensis</i> (Grey squirrel)	1	LAD	U	AF3203416
<i>Kurodaia</i> sp.	<i>Otus guatemalae</i> (Middle-American screech owl)	1	RDP	U	AF3203417
<i>Linognathoides marmotae</i>	<i>Marmota flaviventris</i> (Yellow-bellied marmot)	1	VSS	G	AF3203418
<i>Liposcelis</i> sp.	none	2	RLP	G	AF3203419/20
<i>Machaerilaemus</i> sp.	<i>Hirundo abyssinica</i> (Lesser striped-swallow)	1	RJA	U	AF3203421
<i>Megapeostus asymmetricus</i>	<i>Crypturellus cinnamomeus</i> (Thicket tinamou)	2	RDP	G/U	AF3203422/3

<i>Menacanthus eurysternus</i>	<i>Lybius torquatus</i> (Black-collared barbet)	1	RJA	U	AF3203424
<i>Menacanthus</i> sp.	<i>Atilla spadiceus</i> (Bright-rumped attila)	1	RDP	U	AF3203425
<i>Menacanthus</i> sp.	<i>Penelope purpurescens</i> (Crested guan)	1	RDP	U	AF3203426
<i>Multicola</i> sp.	<i>Nyctiphrynus yucatanicus</i> (Yucatan poorwill)	1	RDP	U	AF3203427
<i>Myrsidea eisentrauti</i>	<i>Sporopipes squamifrons</i> (Scaly weaver)	1	RJA	U	AF3203428
<i>Myrsidea ledgeri</i>	<i>Philetarius socius</i> (Social weaver)	1	RJA	U	AF3203429
<i>Myrsidea</i> sp.	<i>Psilorhinus morio</i> (Brown jay)	2	RDP	G/U	AF3203430/1
<i>Naubates harrisoni</i>	<i>Puffinus assimilis</i> (Little shearwater)	1	VSS	G	AF3203432
<i>Neohaematopinus sciuri</i>	<i>Sciurus carolinensis</i> (Grey squirrel)	1	LAD	U	AF3203433
<i>Nyctibicola longirostris</i>	<i>Nyctibius jamaicensis</i> (Northern potoo)	1	RDP	U	AF3203434
<i>Otidocercus houbarae</i>	<i>Chlamydotis undulata</i> (Houbara bustard)	1	VSS	G	AF3203435
<i>Oxylipeurus chiniri</i>	<i>Ortalis vetula</i> (Plain chachalaca)	2	RDP	G/U	AF3203436/7
<i>Paragonicotes microgaster</i>	<i>Amazona albifrons</i> (White-fronted parrot)	1	RDP	U	AF3203438
<i>Paragonicotes</i> sp.	<i>Aratinga astec</i> (Aztec parakeet)	1	RDP	U	AF3203439
<i>Pectenosoma verrucosa</i>	<i>Crypturellus cinnamomeus</i> (Thicket tinamou)	2	VSS	G/U	AF3203440/1
<i>Pectinopygus brevicornis</i>	<i>Phalacrocorax aristotelis</i> (European shag)	1	VSS	G	AF3203442
<i>Pectinopygus bassani</i>	<i>Sula bassana</i> (Northern gannet)	1	VSS	G	AF3203443
<i>Pectinopygus</i> sp.	<i>Sula sula</i> (Red-footed booby)	1	VSS	G	AF3203444
<i>Penenirmus zumpti</i>	<i>Lybius torquatus</i> (Black-collared barbet)	1	RJA	U	AF3203445
<i>Penenirmus</i> sp.	<i>Myrmecocichla formicivora</i> (Southern anteater-chat)	1	RJA	U	AF3203446
<i>Penenirmus</i> sp.	<i>Serinus atrogularis</i> (Black-throated canary)	1	RJA	U	AF3203447
<i>Perineus nigrolimbatus</i>	<i>Fulmarus glacialis</i> (Northern fulmar)	1	VSS	G	AF3203448
<i>Philopterus</i> sp.	<i>Batis pririt</i> (Pirit batis)	1	RJA	U	AF3203449
<i>Philopterus</i> sp.	<i>Habia</i> sp. (Ant-tanager)	1	RDP	G	AF3203450
<i>Philopterus</i> sp.	<i>Momotus momota</i> (Blue-crowned motmot)	1	RDP	U	AF3203451
<i>Physconelloides cubanus</i>	<i>Geotrygon montana</i> (Ruddy quail-dove)	1	RDP	G	AF3203452
<i>Physconelloides eurysema</i>	<i>Columbina passerina</i> (Common ground-dove)	1	RJA	U	AF3203453
<i>Picicola capitatus</i>	<i>Dendropicos fuscescens</i> (Cardinal woodpecker)	1	RJA	U	AF3203454
<i>Pseudolipeurus similis</i>	<i>Crypturellus cinnamomeus</i> (Thicket tinamou)	1	RDP	G	AF3203455
<i>Pseudomenopon carrikeri</i>	<i>Heliornis fulica</i> (Sungrebe)	1	RDP	G	AF3203456
<i>Quadriceps punctatus</i>	<i>Larus cirrocephalus</i> (Grey-headed gull)	1	RJA	U	AF3203457
<i>Quadriceps</i> sp.	<i>Uria aalge</i> (Guillemot)	1	VSS	G	AF3203458
<i>Rallicola columbiana</i>	<i>Dendrocolaptes certhia</i> (Barred woodcreeper)	1	RDP	G	AF3203459
<i>Rallicola fuliginosa</i>	<i>Dendrocincla anabatina</i> (Tawny-winged woodcreeper)	1	RDP	U	AF3203460

<i>Rallicola</i> sp.	<i>Aramides cajanea</i> (Grey-necked wood-rail)	1	RDP	U	AF3203461
<i>Ricinus</i> sp.	<i>Cyanocompsa parellina</i> (Blue bunting)	2	RDP	G/U	AF3203462/3
<i>Saemundssonina lari</i>	<i>Larus argentatus</i> (Herring gull)	1	VSS	G	AF3203464
<i>Saemundssonina peusi</i>	<i>Calonectris diomedea</i> (Cory's Shearwater)	1	VSS	G	AF3203465
<i>Saemundssonina stresemanni</i>	<i>Catharacta skua</i> (Great skua)	1	VSS	G	AF3203466
<i>Strigiphilus crucigerus</i>	<i>Otus guatemalae</i> (Middle-American screech owl)	2	RDP	G/U	AF3203467/8
<i>Strigiphilus rostratus</i>	<i>Tyto alba</i> (Barn owl)	1	VSS	G	AF3203469
<i>Struthiolipeurus struthionis</i>	<i>Struthio camelus</i> (Common Ostrich)	6	EM	G	AF3203470-5
<i>Trichopholopterus babakotophilus</i>	<i>Propithecus verreauxi</i> (Verreaux's sifaka)	1	VSS	G	AF3203476
<i>Trogoninirmus</i> sp.	<i>Trogon melanocephalus</i> (Black-headed trogon)	1	RDP	G	AF3203477

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\**Larus glaucescens* (Glaucous-winged gull) x *Larus occidentalis* (Western gull) hybrid.

\*\* This is a straggler, i.e. *Halipeurus pelagicus* is not normally found on this host.

Species determination; RLP = Ricardo Palma, RJA = Richard Adams, RDP = Roger Price, VSS = Vince Smith, EM = Eberhard Mey, AMP = Adrian Paterson, DHC = Dale Clayton, MSH = Mark Hafner, LAD = Lance Durden.

Sequencing laboratory; G = Glasgow, U = Utah.

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## Figure Legends

### FIG. 1

Our current understanding of louse phylogeny. Cladogram illustrating phthirapteran familial relationships. Subordinal phylogeny based on Lyal (1985a); amblyceran families as diagnosed by Clay (1970) and R. Price (pers. comm.); ischnoceran families modified from Hopkins and Clay (1952) (Trichodectidae [Lyal, 1985b], Heptapsogasteridae [Smith, 2000], Gonioididae [Smith, 2000]); and anopluran families based on Kim (1988). Scale corresponds to the number of species per family (numbers supplied by R. Price pers. comm., see text).

### FIG. 2

Strict consensus of 704 maximum parsimony trees. Numbers above the nodes represent bootstrap proportions (10000 replicates using stepwise addition under the criterion of maximum parsimony with no branch swapping). Internal branches drawn with thin lines occur in the optimal tree but have bootstrap support below 50%. Names of families and subfamilies of Ischnocera are from Eichler (1963). Since *Nyctibicola* was not included in Eichler's phylogeny it does not have a family label. *Archolipeurus* was recently split from *Struthiolipeurus* (Mey, 1998) and is therefore labelled as belonging to the Meinertzhageniellidae, although it did not appear in Eichler's phylogeny.

### FIG. 3

Adams consensus of the minimum evolution and maximum likelihood trees with branches that do not appear in either tree collapsed. Numbers above the nodes represent bootstrap proportions (10000 replicates using neighbour joining with LogDet distances incorporating a correction for rate heterogeneity). Internal branches drawn with thin lines represent nodes found in either the minimum evolution tree or the likelihood tree but not both. Branches drawn with thick lines represent nodes found in both trees i.e. in the strict consensus of the two trees.

### FIG. 4

A splits graph for a subset of lice EF1- sequences. Morphological data (Smith, 2000) suggest *Heptapsogaster temporalis* belongs with the other tinamou lice, rather than the seal louse *Echinophthirius horridus*. Hence the signal linking *H. temporalis* with the seal louse is probably spurious, even though this signal predominates in the phylogenetic analyses that yielded the trees in Figs. 2-3.

**FIG. 1**

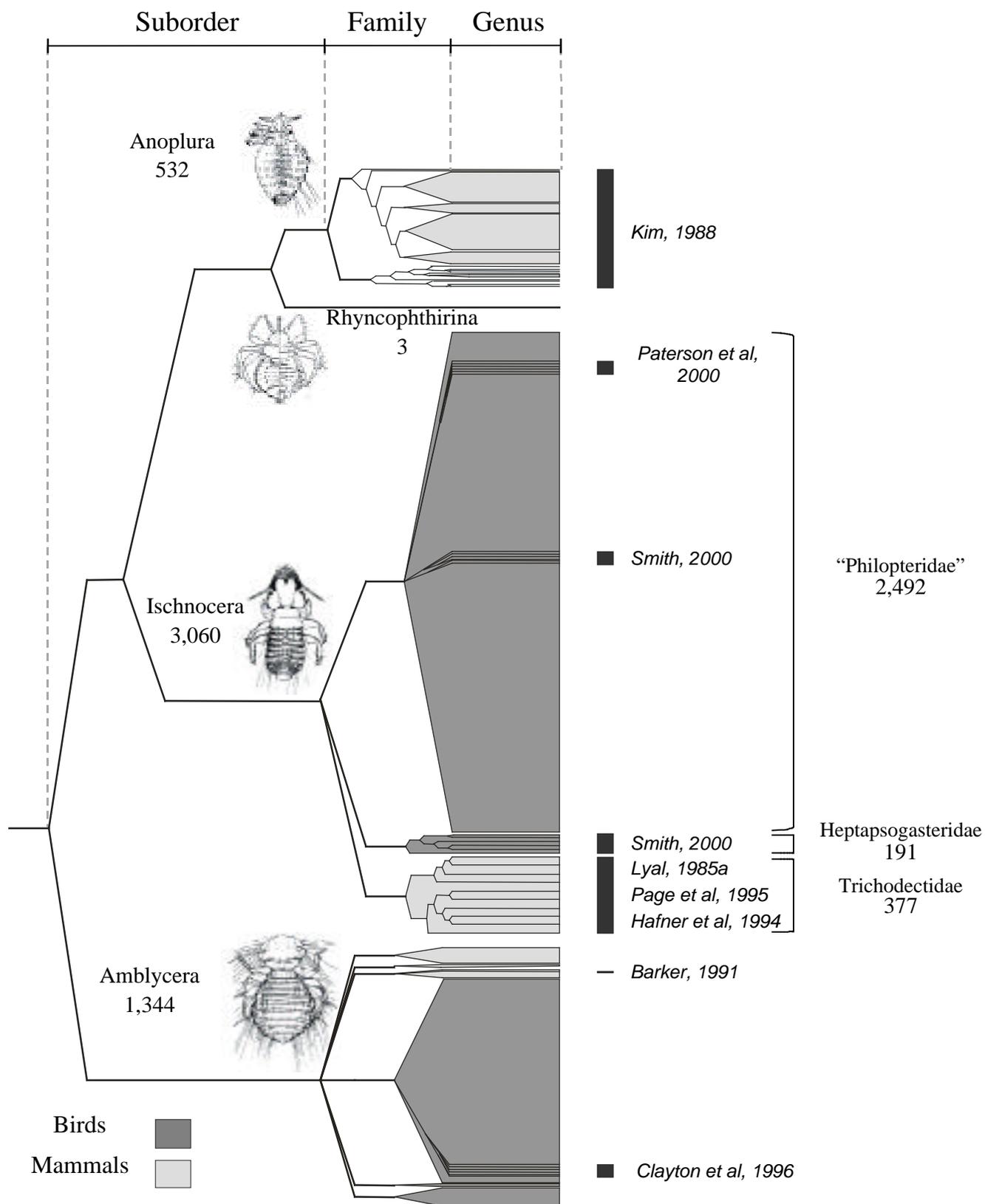


FIG. 2

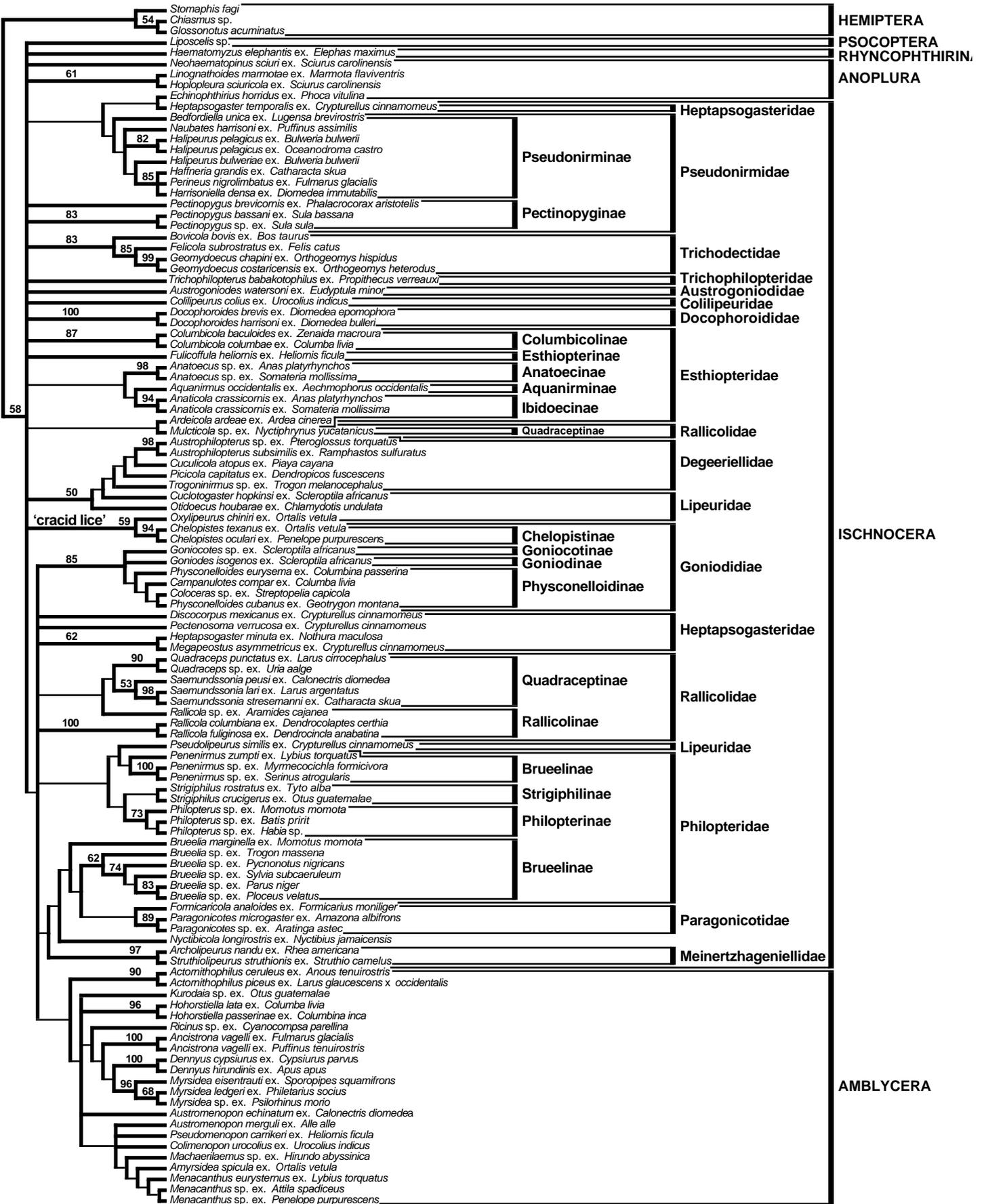


FIG. 3

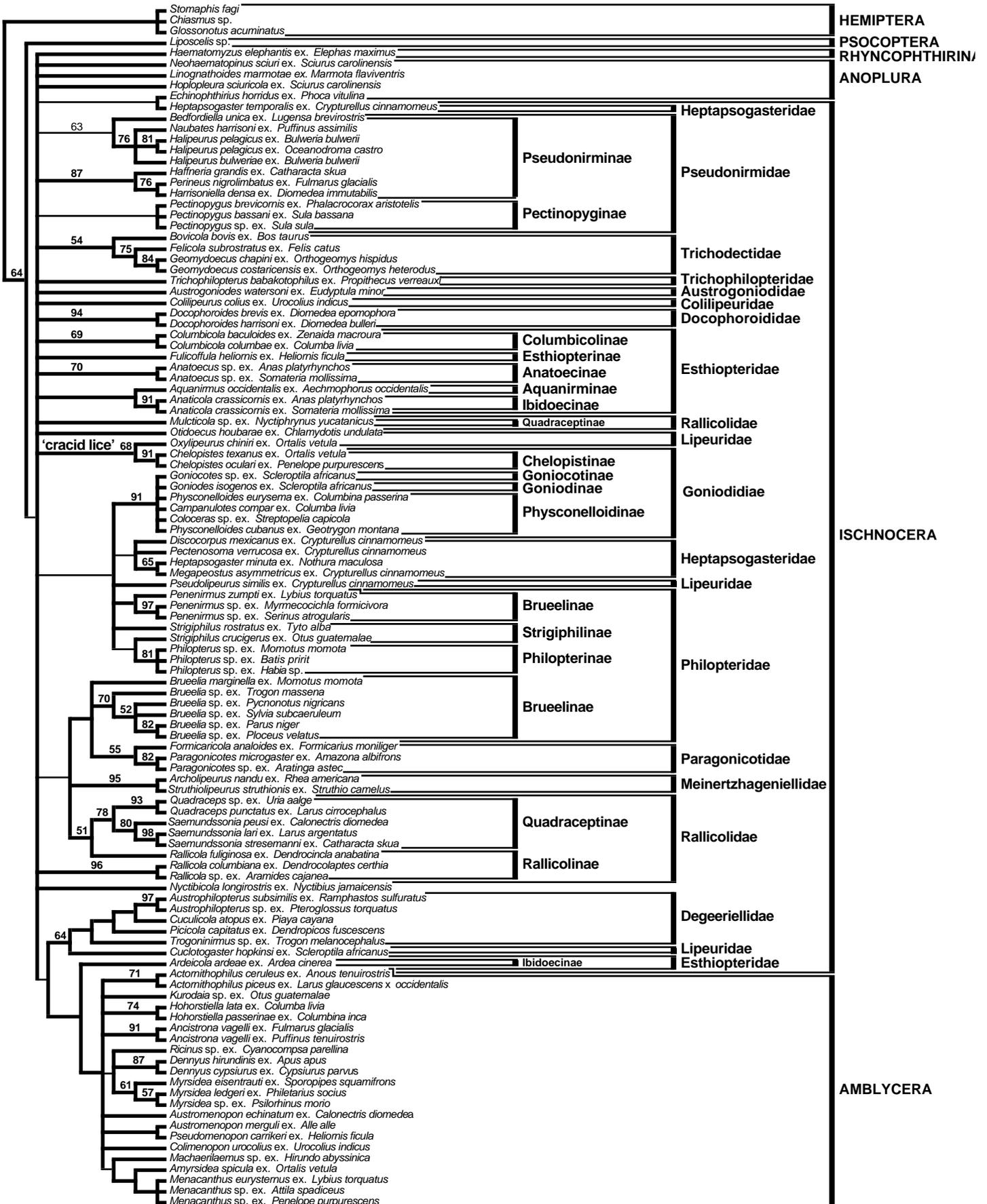


FIG. 4

