Congruence and Cospeciation:
Morphological and Molecular Phylogenetics of the Amblycera (Phthiraptera)

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Louse Princess

Declaration

I declare that the contents of this thesis are my own, unless otherwise stated, and that it is my own composition. No part of this study has been submitted for any other degree to any other institution.

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September, 2001.

Abstract

Lice (Phthiraptera) are highly host-specific, permanent ectoparasites of birds and mammals. Their long association and close ecological relationship with their hosts is considered to facilitate the cospeciation (or parallel cladogenesis) of louse and host taxa. The high degree of topological congruence that has been found between the phylogenies of some lice (Ischnocera: Trichodectidae) and their hosts, has led to their recognition as the definitive example of cospeciation. However, further empirical studies of this phenomenon in other groups of lice are hampered by a lack of parasite phylogenies. Here, the phylogeny and cospeciation of a suborder of chewing lice (Phthiraptera: Amblycera) with their hosts is investigated.

The first phylogeny reconstructed solely for amblyceran genera is presented. This study, based on an extensive comparison of adult morphology and a rigorous cladistic analysis, considers generic exemplars from 4 families of amblyceran lice (Menoponidae, Boopiidae, Laemobothriidae and Ricinidae). The monophyly and evolutionary relationships of these families are strongly supported and there is good support for the Menoponidae and Boopiidae as sister taxa. The relationships of the families are not concordant with the traditional hypothesis of a basal Menoponidae. The study identifies 4 supra-generic groups within the Menoponidae, which are discussed with reference to previous classifications and studies which have included amblyceran taxa. A preliminary assessment of host-parasite cospeciation is also provided.

Whether a similar phylogeny would be produced from molecular data is investigated. The relationships of genera based on morphology are compared with

phylogenies generated from the nuclear gene elongation factor 1α and the mitochondrial gene cytochrome oxidase I. Different methods of reconstruction used to assess their phylogeny and raw signal find that the data are largely incongruent, although there is little support for the topologies generated from the sequence data. The monophyly and relationships of families are compared between the datasets and differences in rate heterogeneity between the data are also discussed.

A first phylogeny for the genus *Austromenopon* (Amblycera: Menoponidae) and their close allies (based on the results of the morphological analysis) is reconstructed from molecular data using the mitochondrial genes COI and 12S rRNA. The molecular phylogenies obtained are generally incongruent, with most branch support located nearer the tips of the tree. No analysis recovered a monophyletic *Austromenopon*, although there is good support for a subset of the *Austromenopon* taxa, which repeatedly group together. The combined molecular phylogeny for the lice is subsequently compared with a phylogeny constructed for their seabird hosts (Aves: Charadriidae, Laridae, Phaethontidae, Phalacrocoracidae, Procellariidae, Scolopacidae and Sulidae), to evaluate the relative contributions of cospeciation and other processes in the host-parasite association (i.e. duplications, sorting events and host-switching). A significant level of cospeciation is found. A quantitative comparison with results found for another suborder of chewing lice (Phthiraptera: Ischnocera) on similar birds, concludes that both amblyceran and ischnoceran lice have similarly cospeciated with their hosts. However, the amblyceran lineage has undergone more host-switching and less duplication and sorting events than ischnoceran lice. The ecological reasons for these different patters of host association are discussed.

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There are many people who deserve formal recognition for the help and support I have received throughout my studentship at Glasgow.

Firstly, I extend a special thank you to my supervisor in this project, Rod Page, who initially offered me the chance to become one of the few people who have studied amblyceran lice. During my studies, the door to Rod's office was always open, and he readily stopped what he was working on to answer my sometimes inane questions, and gave me invaluable help and direction whenever I felt I needed it. For this I am very grateful. A great deal of thanks must also go to Vince Smith, Rob Cruickshank (Lincoln University, Canterbury, NZ) and Martyn Kennedy. Vince was especially helpful in the earlier stages of my studies, when I was trying to come to terms with louse morphology. Rob was responsible for introducing me to molecular techniques, and he and Kevin Johnson (Illinois Natural History Survey, USA) allowed me access to additional sequence data. Martyn's extensive experience in obtaining sequence data for lice aided me greatly in the generation of molecular phylogenies for my cospeciation analysis, and he helped to improve my writing skills by covering everything that I wrote with red pen. Rod, Martyn, Vince and James Cotton all provided help with computing software and read various chapters of this thesis.

I should also additionally thank my friends and colleagues in the department (past and present), some of whom I have known now for 8 years.

Previous tutors were interested in my progress throughout my PhD, and some of the staff and fellow students made sure they took care of my social development by 'luring' me to the pub of an evening. There are far too many people to mention here individually, but a special note of appreciation must go to Helen Whitaker for listening to my worries during hundreds of lunches, and her caricature of myself as "Louse Princess".

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Finally, I extend many thanks towards Chris Lyal and Jane Beard of The Natural History Museum, London, for the loan of and assistance with the many slide-mounted specimens required for the morphological part of this study, and the numerous different collectors in the field who have provided specimens for the molecular analyses. Without access to these resources, this study would not have been possible.

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Table of Contents

Tables & Figures

CHAPTER 1

Introduction

1.1: Cospeciation

As Page (2002) writes in a book devoted to the study of cophylogeny – Tangled Trees: Phylogeny, Cospeciation, and Coevolution, "these are exciting times in the study of cospeciation". This is indeed the case since the recent advances in molecular techniques have greatly facilitated the collection of genetic data for cospeciation studies, and further advances in tree building software (e.g., Swofford, 2002) have provided faster and more explicit methods for reconstructing phylogenies. Additionally, the development of new comparative tools (e.g., Charleston & Page, 2002; Huelsenbeck & Ronquist, 2001) has allowed the more rigorous evaluation of historical associations between coevolving phylogenies to be carried out.

Cospeciation is defined as the joint speciation of two organisms with a close ecological association. In effect cospeciation is the parallel cladogenesis of two, often very distantly related, lineages. Although cospeciation can occur in other forms of association (e.g., mutualistic or symbiotic relationships), most work on the subject focuses on the cospeciation of host and parasite relationships (Page, 2002). Coevolutionary studies allow a variety of questions to be addressed. We can ask questions such as are the parasites "heirlooms" or

"souvenirs", that is, is the parasite and host coupling an old association, or is the parasite a relatively recent acquisition? We can address issues about the relative importance of different cophylogenetic events in the coevolving system (e.g., cospeciation, host-switching, lineage sorting and duplication). If hosts and parasites show cospeciation, and we have evidence of a molecular clock in both lineages, we may also be able to compare their relative evolutionary rates (given homologous genes) and also test for identical cospeciation times (temporal cospeciation) (see Huelsenbeck, Rannala & Yang, 1997; Page, 1996).

1.2: Lice as a model organism for studies of cospeciation

Lice are an excellent choice of organism for studies of host-parasite cospeciation. As obligate ectoparasites lice spend their entire life cycle on the host, away from which they cannot survive for any great length of time (Clay, 1949). The group is extremely speciose and highly host specific, but comparatively non-pathogenic. These factors, together with the fact that lice have a wide distribution on birds and mammals, have led many authors (e.g., Clay 1949; Hopkins, 1949; Lyal, 1986; Mauersberger & Mey, 1993; Page, Clayton & Paterson, 1996) to the conclusion that lice have had a long affiliation, and have consequently coevolved, with their hosts. Thus, of all organisms that share a close historical association, we might expect to find a high degree of cospeciation between lice and their hosts (Clay 1949; Lyal 1986).

1.3: Classification and origins of lice

Lice (order Phthiraptera) are wingless, dorsoventrally flattened ectoparasites of birds and mammals. The group contains four recognised suborders: Anoplura (sucking lice), Amblycera, Ischnocera and Rhynchophthirina (all forms of chewing lice). The Amblycera and Ischnocera are parasites of birds and mammals, whilst the Anoplura and Rhynchophthirina are exclusive to placental mammals. To date, over 6000 species of lice have been described, nearly 90% of which are contained within the Amblycera and Ischnocera (Price *at al.*, in press).

Rothschild and Clay (1952) proposed that the Phthiraptera were derived from a free-living ancestor that also gave rise to the Psocoptera (psocids, booklice, barklice). Psocopterans are free-living insects that feed on fungi or fragments of animal or vegetable matter. The support for a shared ancestry for these two groups comes from two considerations i) phthirapterans and psocopterans have similar basic morphologies (Snodgrass, 1944), and ii) some members of the group are known to be associated with the nests and feathers of birds and the plumage of mammals (Smithers, 1996; Lyal, 1985a). Rothschild and Clay (1952) held the view that the switch to a parasitic lifestyle (and the consequent reliance on host feathers for food) would not have required the development of any significant modifications in the ancestor of lice.

Recognising their similarity, Königsmann (1960) placed the Phthiraptera and the Psocoptera in the superorder Psocodea. Both he and Clay (1970) shared the view that the Amblycera formed the basal element in the Phthiraptera, standing apart from a clade containing the other three groups (Ischnocera, Rhyncophthirina and Anoplura). To better understand the

evolution of these groups Lyal (1985a) investigated the relationships within the Phthiraptera. He proposed apomorphies for each group and polarised character states with reference to a known outgroup for a monophyletic Psocodea, the Condylognatha (which comprise the Hemiptera and Thysanoptera). Lyal (1985a) resolved the subordinal relationships within the lice, and confirmed that the Amblycera was the basal taxon within the group. Lyal's (1985a) study also found that the sister group to the Phthiraptera was not a monophyletic Psocoptera, but was in fact only a single psocopteran family, the Liposcelididae, thus concluding that the Psocoptera were paraphyletic (Fig. 1).

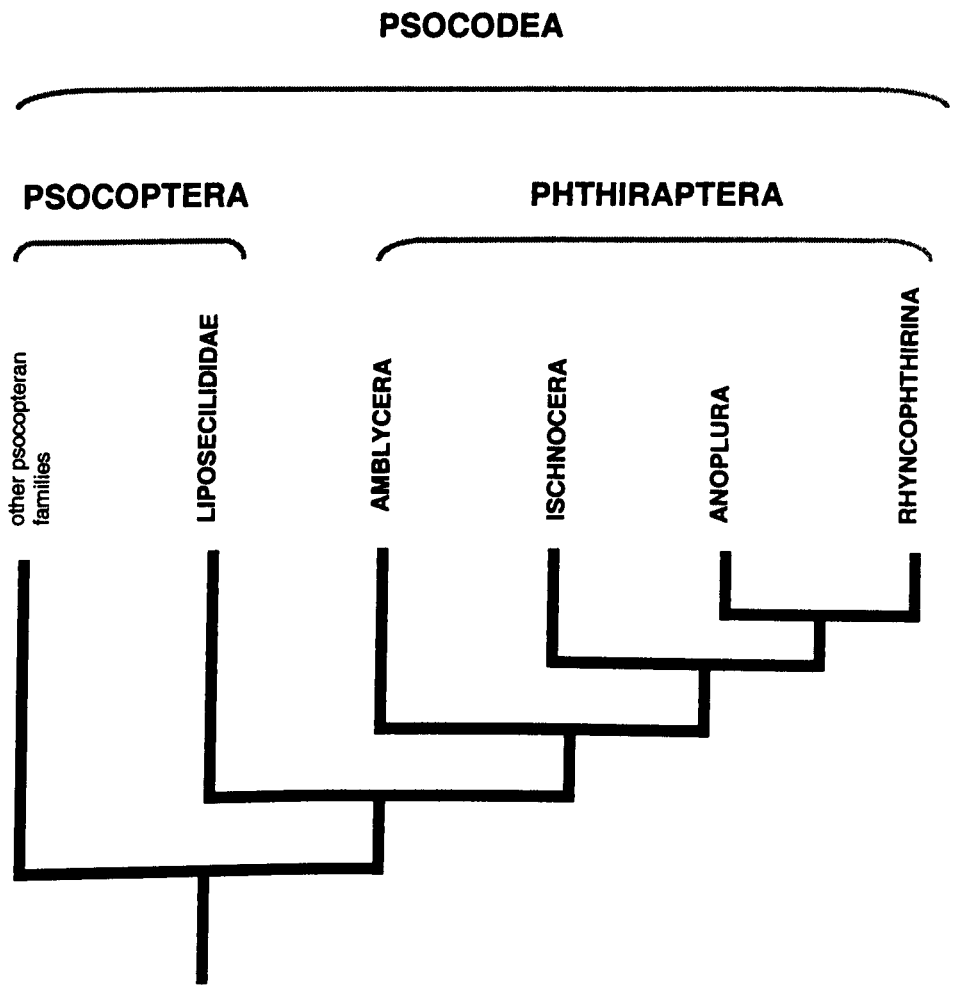

Fig. 1: Phylogenetic relationships within the superorder Psocodea (Psocoptera & Phthiraptera). Figure modified from Lyal (1985a).

1.4: Lack of parasite phylogenies

The main obstacle to using lice for studies of cospeciation is our patchy understanding of their phylogeny (Cruickshank *et al.*, 2001) (Fig. 2). A large amount of alpha-taxonomic work has been conducted on the group, but studies on evolutionary relationships have mainly focused on selected families (e.g., Lyal, 1985b; Smith, 2000) and genera (e.g., Barker, Briscoe and Close, 1992; Clayton, Price & Page, 1996). The exception to this is Kim's (1988) study of the Anoplura.

Much of our knowledge of louse phylogeny has been acquired in a piecemeal fashion from coevolutionary studies. These studies often first require the building of phylogenies for lice so that they can be compared phylogenies for their hosts (which are generally more readily available).

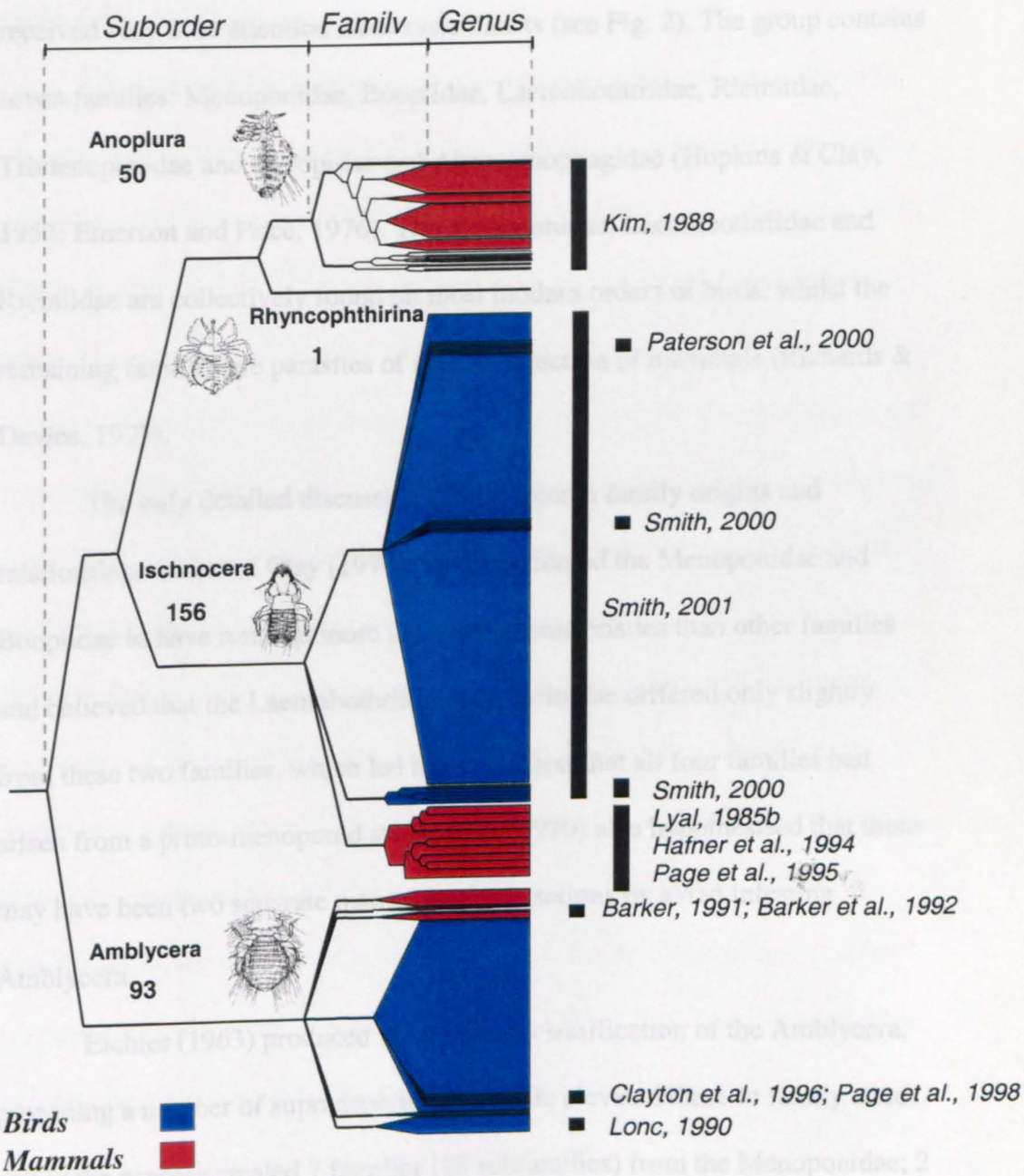

Fig. 2: Our current understanding of louse phylogeny. The subordinal phylogeny is based on Lyal (1985a), amblyceran families as in Clay (1970), ischnoceran families as in Hopkins and Clay (1952), and anopluran families based on Kim (1988). Scale corresponds to most recent estimate of numbers of genera per family, pending the publication of the forthcoming checklist by Price *et al.* (in press). Figure modified from Cruickshank *et al.* (2001).

1.5: Previous studies of the Amblycera

Compared with the Anoplura and Ischnocera, the Amblycera have received very little attention from systematists (see Fig. 2). The group contains seven families: Menoponidae, Boopiidae, Laemobothriidae, Riciniidae, Trimenoponidae and Gyropidae and Abrocomophagidae (Hopkins & Clay, 1952; Emerson and Price, 1976). The Menoponidae, Laemobothriidae and Riciniidae are collectively found on most modern orders of birds, whilst the remaining families are parasites of a small selection of mammals (Richards & Davies, 1977).

The only detailed discussion of amblyceran family origins and relationships is that of Clay (1970). She considered the Menoponidae and Boopiidae to have retained more ancestral characteristics than other families and believed that the Laemobothriidae and Ricinidae differed only slightly from these two families, which led her to suggest that all four families had arisen from a proto-menoponid stock. Clay (1970) also hypothesised that there may have been two separate mammalian infestations by avian infesting Amblycera.

Eichler (1963) produced an extensive classification of the Amblycera, proposing a number of suprageneric groups. He elevated these to family status and in the process created 7 families (18 subfamilies) from the Menoponidae; 2 families from the Ricinidae; 3 families (5 subfamilies) of Gyropidae; 2 families (3 subfamilies) of Boopiidae and 3 subfamilies of the Trimenoponidae. Many of these groups contain, what appears to be, a rather diverse collection of genera, but Eichler's work is the only one to suggest suprageneric classification of the entire Amblycera. However, in the light of our current lack

of knowledge on the phylogeny of these lice, the accepted view is that the Amblycera are divided into 7 distinct families, but without a consensus of opinion on how these groups may be related.

There have been no studies on the relationships between amblyceran genera. A small number of authors have, however, investigated the phylogeny within genera (Barker *et al.* 1992; Clayton *et al.*, 1996; Lonc, 1990; Page *et al.*, 1998). Barker *et al.* (1992) proposed two distinct species groups within the genus *Heterodoxus* (Boopiidae) from a cladistic analysis of 21 allozyme loci. Lonc (1990) completed a phenetic study of morphometric variation between 56 species of *Ricinus* (Ricinidae), in which a consensus dendrogram revealed two major groups. Morphometric data has also been employed to investigate the relationships between 23 representatives of *Dennyus* (*Collodennyus*) (Menoponidae), leading to descriptions of 13 new species and 3 subspecies in the process (Clayton *et al.*, 1996). In a later study, Page *et al.* (1998) constructed a molecular phylogeny based on cytochrome *b* for *Dennyus* lice. They then compared the louse phylogeny with a phylogeny (also from cytochrome *b*) for their swiftlet hosts, finding some evidence of cospeciation (Page *et al.* 1998). Thus, although these studies show that there has been some progress into resolving the relationships within a few amblyceran genera, there remains a definite need for a higher level phylogeny.

1.6: Anatomy and homology

The initial step towards understanding the higher level phylogeny of the Amblycera may be to investigate the group from a morphological perspective. The Amblycera have a different biology to the other three suborders of lice.

They are largely unspecialised parasites and are generally not adapted to host microhabitats. This is in contrast with the other large group of chewing lice, the Ischnocera (see Figs. 3 & 4), who have two morphological forms: a long thin type found on the wings and back of the host (wing lice) and a short rounded type mainly found on the head and neck (body lice) (Smith, 2001).

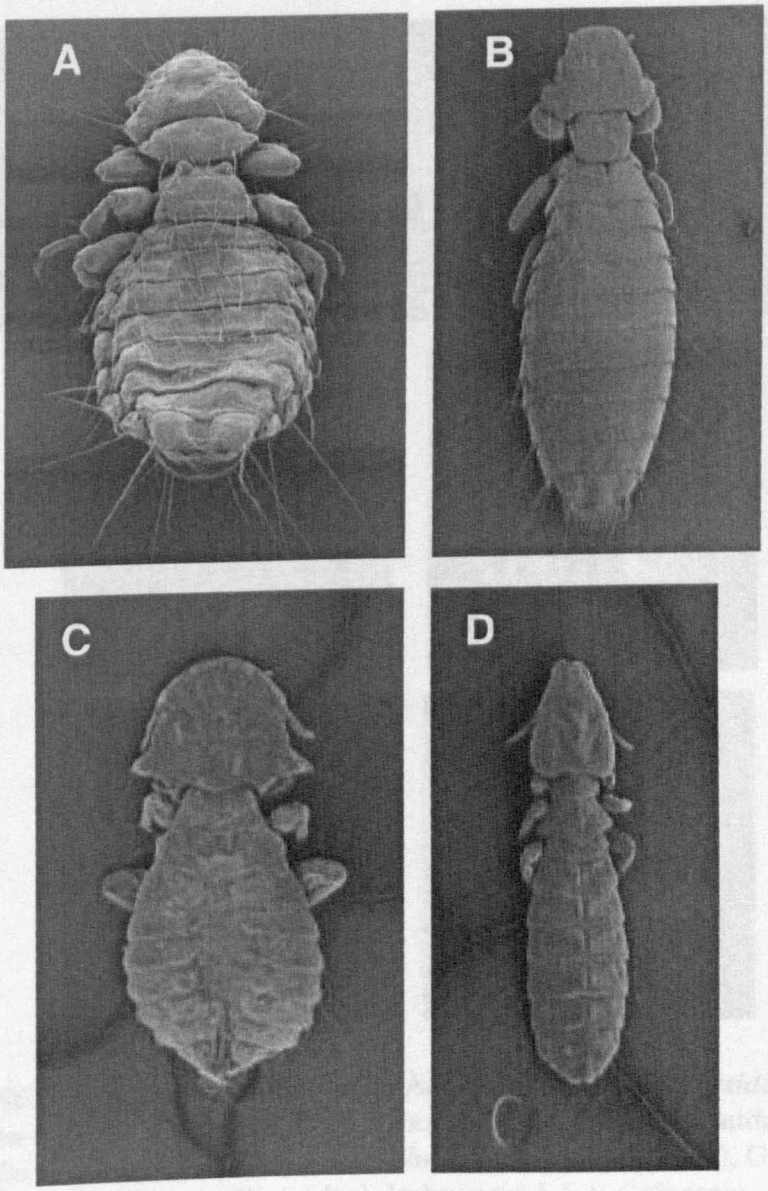

Fig. 3: Differences in the body plan of amblyceran and ischnoceran lice. A-B (Amblycera). A: *Boopia* (Boopiidae), B: *Dennyus* (Menoponidae). C-D (Ischnocera). C: *Goniodes* (Goniodidae), a body louse and D: *Quadraceps* (Philopteridae) a wing louse. SEM images by Isabel Marshall (A) and Vince Smith (B-D).

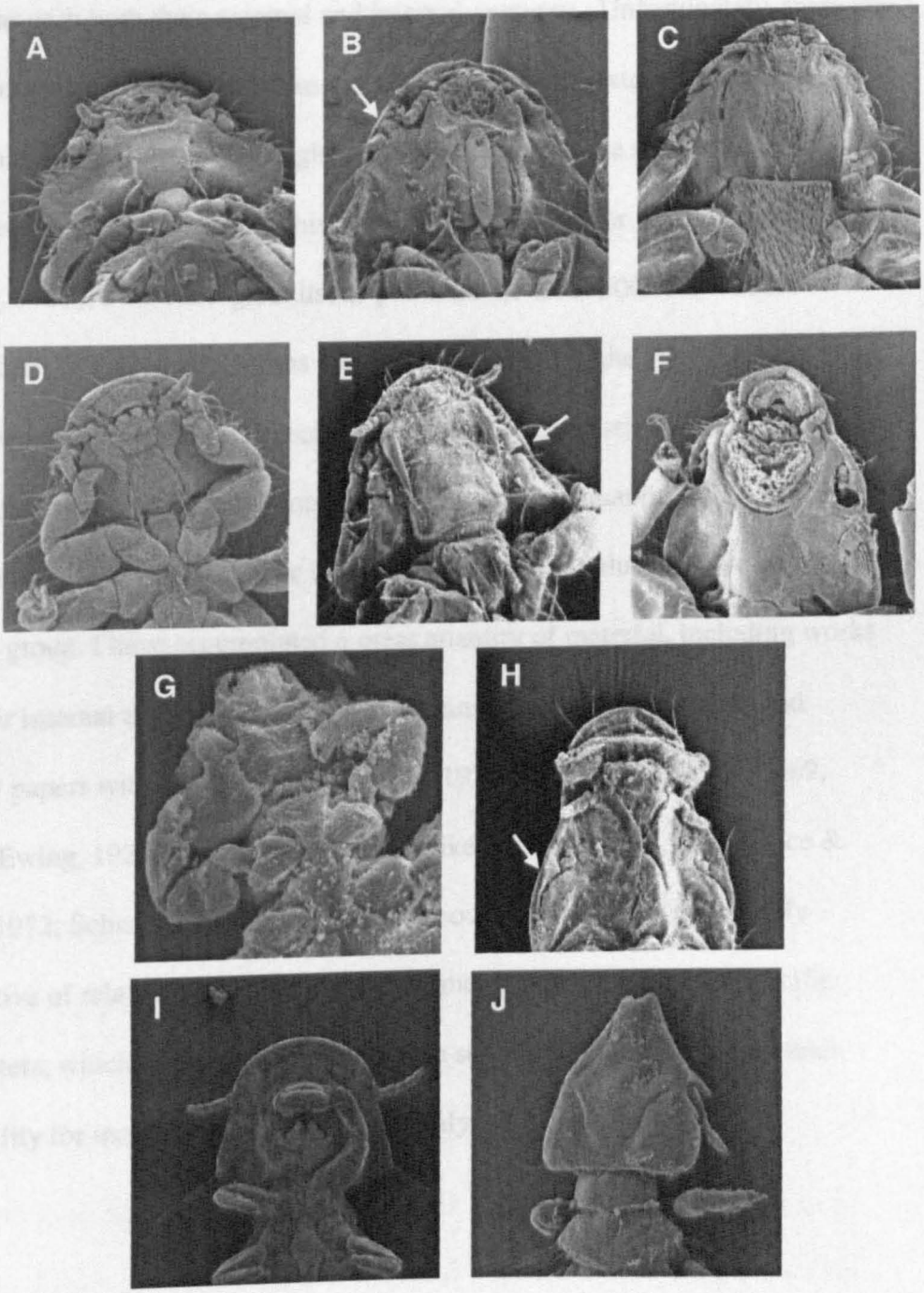

Fig. 4: SEM images of the louse head. Amblycera A-H. *A*: *Myrsidea*, B: *Pseudomenopon*, C: *Trinoton*, D: *Actornithophilus*, all Menoponidae. E: *Heterodoxus* (Boopiidae), F: *Laemobothrion* (Laemobothriidae), G: *Gyropus* (Gyropidae), H: *Ricinus* (Ricinidae). Ischnocera I-J. I: *Coloceras* (Goniodidae), J: *Brueelia* (Philopteridae). The amblyceran antennae are contained within deep fossae on the ventrolateral surface of the head (see arrows), which are thought to protect the antennae whilst running through the feathers/fur of the host. The loss of this characteristic in the Ischnocera may reflect their microhabitat specialisation. SEM images by Isabel Marshall (A-H) and Vince Smith (I-J).

In order to identify homologies within the Amblycera it is necessary to become familiar with both their external and internal anatomy. Unfortunately, there are no works which give a complete outline of the general external anatomy. Such information must therefore be gleaned from the extensive study of a variety of sources. An essential tool for this is the "Bibiographie der Mallophagen" (Kéler, 1960), a chronological list of publications from 500B.C. – 1959. Almost all of these publications have been produced in the last 200 years, with many works (as would be expected) on the lice of domestic animals. This bibliography has aided the identification of a sizeable quantity of literature on amblyceran lice. To investigate the morphology and evolutionary relationships of this group, I have accumulated a great quantity of material, including works on their internal and external anatomy, original generic descriptions, and review papers with keys to families (Clay, 1970), genera (e.g., Clay, 1969, 1970; Ewing, 1924) and species (e.g., Carriker, 1954; Ewing, 1930; Price & Clay, 1972; Scharf & Emerson, 1984). Although keys are not necessarily indicative of relationships, they can sometimes provide a source of specific characters, which can then be scored over a sample of genera to assess their suitability for inclusion in phylogenetic analyses.

1.7: Aims and content of thesis

This thesis has three main aims:

To construct phylogenies for amblyceran lice using morphological and molecular methods at a range of different taxonomic levels.

To compare louse phylogenies with the phylogeny of their hosts and quantitatively assess the degree of cospeciation between parasites and hosts.

To explore whether there are differences in the degree of cospeciation shown by amblyceran and ischnoceran lice.

Content of thesis

The thesis is presented as a series of stand-alone, but interconnected, chapters which have been written for submission to various journals. The *Zoological Journal of the Linnean Society* has accepted chapter two for publication under the sole authorship of I. K. Marshall. Chapter three is to be submitted with the co-authorship of Kevin P. Johnson and Roderic D. M. Page. Chapter four will be co-authored with Martyn Kennedy and Roderic D. M. Page.

As has been discussed above, the phylogeny of the Amblycera is largely unknown. There have been only a small number of studies which have

focused on these lice and the need for a higher-level phylogeny for the group is clearly apparent. This issue is addressed in chapter two, where a phylogeny is presented for 44 genera of amblyceran lice from four families (Menoponidae, Boopiidae, Laemobothriidae and Ricinidae) based on a comprehensive study of their morphology. This chapter represents the first phylogeny generated solely for amblyceran genera, and the morphological characters developed for this study are extensively described and illustrated. The phylogeny presented here is also discussed within the context of previous supra-generic classifications of amblyceran lice.

After generating a morphological phylogeny for four of the families of the Amblycera, the question naturally arises how does it compare with phylogenies based on molecular data? Chapter three is the first study to addresses this issue for amblyceran genera by investigating the level of conflict between molecules and morphology for 22 genera from the Menoponidae, Laemobothriidae and Ricinidae. The molecular phylogenies are reconstructed using the nuclear gene encoding elongation factor 1 alpha (EF1α) and the mitochondrial gene cytochrome oxidase subunit 1 (COI). The molecular phylogenies are then compared with a phylogeny generated from a subset of taxa extracted from the morphological dataset presented in chapter two. Any conflict identified between the competing trees and datasets is discussed and the phylogenetic congruence of the morphological tree with the tree presented in chapter two is assessed. This comparison will provide an additional examination of support for the tree presented in chapter two.

With the knowledge gained from the morphological and molecular studies in chapters two and three it is possible to identify a sensible amblyceran

group to use as the basis of a cophylogenetic study. By choosing an appropriate group it is also possible to compare the cophylogenetic history of the Amblycera with levels of cospeciation already found between the Ischnocera and their hosts. To achieve this aim, chapter four focuses on the genus *Austromenopon* (Menoponidae). This chapter reconstructs the phylogeny for 11 species of the genus and several of their close relatives using the mitochondrial genes 12S rRNA and COI. The louse tree is compared with a tree for their seabird hosts in a cospeciation analysis and the results are discussed in comparison with a study of cospeciation between ischnoceran lice and similar hosts. This chapter provides the first comparison of levels of cospeciation between different suborders of lice and their hosts.

The conclusions of the thesis are presented in chapter five. This chapter restates the main aims of this thesis, summarises how the work presented here has met those aims, and places these studies in context with previous studies on the Amblycera. Suggestions for future work to build on the results presented here are also discussed.

1.8: Thesis motivation

When I started this project the phylogeny of the Amblycera was largely unknown. Few authors had investigated the phylogenetic relationships between amblyceran taxa and instead there existed a huge number of purely descriptive publications. My initial examination of the morphology of this group suggested that they were remarkably similar to one another but with experience it became apparent that the Amblycera actually contain an enormous amount of variation. Coming to terms with many of the terminologies and identifying the

structures involved in phthirapteran morphology was a challenge that took many months, but it became an enjoyable one. Once the morphology and the relationships within the Amblycera were better known, it would be possible to identify further areas for consideration and study.

Despite the huge amount of variation that exists within the Amblycera (and all lice) and the added potential for them to be used as model organisms in studies of host-parasite cospeciation, it is regrettable that there are not more workers seeking to uncover the phylogeny of these most fascinating of insects.

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CHAPTER 2

A morphological phylogeny for four families of amblyceran lice (Phthiraptera: Amblycera: Menoponidae, Boopiidae, Laemobothriidae, Ricnidae).

2.1: Abstract

The suborder Amblycera (Insecta: Phthiraptera) comprises seven recognised families of lice. Three of these families (the Menoponidae, Laemobothriidae and Ricinidae) are parasitic on a wide range of avian hosts. The four remaining families are restricted to a small section of mammals (the Boopiidae are parasites of Australian and New Guinean marsupials, and the Gyropidae, Trimenoponidae and Abrocomophagidae parasitise South and Central American rodents). This study uses a morphological approach to examine the evolutionary relationships between genera from four amblyceran families: Menoponidae, Boopiidae, Laemobothriidae and Ricinidae. Genera are represented by exemplars and a total of 44 louse taxa and one outgroup taxon were included. A cladistic analysis of 147 unordered characters recovered six equally parsimonious trees. Bootstrap, Jackknife and Bremer support analyses were undertaken to assess the level of support for each resolved node in the strict consensus topology. Strong support was found for deep branch relationships between the families and in some cases for supra-generic groupings within families. The clades present in the strict consensus tree are

discussed with reference to supra-generic and inter-family relationships, character choice, morphological convergence and host distribution. This study is the first phylogeny presented solely for amblyceran genera.

2.2: Introduction

Members of the order Phthiraptera (lice) are wingless insects, parasitic on most orders of birds and mammals. There are four recognised suborders: Amblycera, Ischnocera, Anoplura and Rhyncophthirina, of which the Amblycera are considered the most primitive (Clay, 1970; Königsmann, 1960; Lyal, 1985). The Amblycera contains seven families: the Menoponidae, Laemobothriidae and Ricinidae are distributed across a wide range of avian host orders, whilst the four remaining families are confined to a small selection of mammals. The Boopiidae are found on Australian and New Guinean marsupials with the exception of *Therodoxus oweni* Clay on the cassowary, and *Heterodoxus spiniger* Enderlein which is thought to have secondarily parasitised the domestic dog. The Gyropidae, Trimenoponidae and Abrocomophagidae are parasites of South and Central American rodents, although *Macrogyropus dycotylis* MacAlister (Gyropidae) is also found on peccaries. The size of families varies greatly, with around 70 genera in the Menoponidae compared to just a single genus in the Abrocomophagidae.

Most amblyceran genera were erected sometime between 1800 - 1950. In an age of high production of taxonomic descriptions, the Amblycera suffered the same fate as many other groups during this period: the literature became littered with duplicated descriptions, resulting in many generic and specific synonyms. Hopkins and Clay (1952; 1953; 1955) reviewed this situation, placing many taxa in synonymy, and recognised 69 distinct amblyceran genera in their checklist of Mallophaga. To date, there are over 90 amblyceran genera recognised as valid, containing some 1350 valid species and

subspecies (Price *et al.*, in press). Most work has focused on the production of detailed taxonomic reviews and new species descriptions (e.g., Carriker, 1954; Price & Emerson, 1977), identification keys to a particular genus (Clay, 1962; Price, 1970; Price & Beer, 1965b) or to the Amblycera of a defined geographical area (Ledger, 1980; Uchida, 1926). A small number of workers have published works proposing species groups within genera (e.g., Price, 1970; 1971; Scharf & Price, 1977) and, some have begun addressing phylogeny within individual genera, employing both morphological (e.g. Nelson, 1972) and molecular methods (e.g. Barker, Briscoe & Close, 1992).

Very few authors have considered the broader relationships between amblyceran lice. In an attempt to address this question, Clay (1970) tabulated the distribution of 19 morphological characters across the suborder. There was no explicit phylogenetic analysis in this paper but Clay presented a detailed discussion on what she considered to be the evolutionary relationships of the six amblyceran families (the monogeneric Abrocomophagidae was as yet undescribed). She suggested that the establishment of parasitism by an avian louse on the marsupials gave rise to the Boopiidae and that the mammalian Amblycera were the therefore the result of two major host colonisations. In Clay's (1970) study, the Gyropidae were represented as three independent subfamilies (Gyropinae, Protogyropinae and Grilicolinae) as she had considered that the Gyropidae may not be a monophyletic group. Figure 1 shows the results of a preliminary cladistic analysis of Clay's (1970) data matrix of 19 characters, with the addition of the outgroup taxon to be used in this present study, the psocopteran (or free-living booklouse) *Liposcelis bostrychophilus* Badonnel. The tree presented (see Fig. 1) displays strong

bootstrap support for only two general clades of lice ("A" and "B"). Clade "A" contains the avian-infesting families Menoponidae, Laemobothriidae, Ricinidae and also the mainly marsupial-infesting Boopiidae. Clade "B" contains the rodent-infesting genera Trimenoponidae and the gyropid subfamilies (Protogyropinae, Gyropinae and Grilicolinae). Clay's (1970) proposal for two independent colonisations of mammals by amblyceran lice is not supported by the analysis of her data (Fig. 1) and in fact given the low resolution of the tree presented, a more parsimonious interpretation of evolutionary events could be explained as the single colonisation of birds from mammals. Clay (1970) also proposed that the Menoponidae and Boopiidae were sister taxa, which the cladistic analysis of her data (see Fig. 1) does not resolve.

There have been few studies, which have examined the supra-generic relationships within amblyceran families. Symmons (1952), in an investigation primarily aimed at establishing some main types within the Amblycera, compared the tentorium (an endoskeleton of the head) across fourteen louse genera, with the condition found in the Psocoptera (or free-living booklice). She described four distinct forms of amblyceran tentorium, differing mainly in their degree of reduction and schematisation. Symmons (1952) then placed the genera, on the basis of their tentorial type, into four groups: the Laemobothriidae, Gyropidae and Trimenoponidae constituted a single group; the Boopiidae were the second group and two supra-generic groups of Menoponidae were presented as groups three and four. In a much more in-depth work and entirely on the morphology of the Menoponidae, Clay (1969) indicated which characters she considered to define genera and supra-generic

groups and discussed the stability of the character states. Clay (1969) also suggested that there were two distinct groups of menoponid genera (which contradicted those identified by Symmons, 1952) - the "*Colpocephalum* complex" and the "*Menacanthus* complex" (see Table 1). These two complexes both possess a large number of distinct, exclusive, characteristics but the groups contain only six and five genera respectively, a very small proportion of the Menoponidae. Clay (1969) gave no indication towards any ideas she may have had regarding phylogeny within these "complexes" or of how they might be related to other menoponid taxa. Eichler (1963) took a much less conservative approach to this problem. He produced a very detailed classification of amblyceran genera, where previously recognised families were elevated to interfamily status and created a series of nested sets of taxa down to subfamily level (see Table 2). However, Eichler gave little justification for these hierarchical subdivisions and so most louse taxonomists still follow the more conservative classifications of Hopkins and Clay (1952) and Clay (1970), recognising family groups but with no consensus of opinion on the evolutionary relationships of taxa below this rank.

The cladistic analysis of Clay's (1970) data on the morphology of the Amblycera segregates the avian and marsupial lice from the rodent lice (Fig. 1). The Amblycera are a large group (with over 90 genera) and as a consequence only genera from the families in clade "A" (see Fig. 1) were included in this analysis.

This study set out to: construct a morphologically based phylogeny for genera selected from four amblyceran families (Menoponidae, Boopiidae,

Laemobothriidae and Ricinidae) using the exemplar approach, evaluate the monophyly and stability of families, evaluate the hypothesis that the Boopiidae and Menoponidae are sister taxa, and discuss any support for the alternative supra-generic groups proposed by Clay (1969) and Eichler (1963).

2.3: Materials and methods

Four hundred and twenty nine specimens representing 44 genera (in 4 families) of the suborder Amblycera were obtained for study from the slide-mounted Phthiraptera collection at The Natural History Museum (NHM), London. Since the four families in question comprise a large number of genera, specimens were chosen from a subset, which reflected both the Eichler (1963) and Clay (1969) classifications. This approach to taxa selection also offered an opportunity for comparing the different classifications of these two authors. Due to the large number of species in some genera (e.g. *Colpocephalum* Nitzsch contains in excess of 70 species) exemplars were selected using the type species for the genus, where possible. To assess a type species as a suitable typical representative, original taxonomic descriptions and generic review papers were employed and specimens of the type species were compared to other species within the genus before the final selection. In the few genera where types were rare or absent from the collection, the species morphologically most similar to the type species were included, either to increase the sample size or in some cases, as a substitute for the type species. Adult male and female specimens were favoured over juveniles, as some features present in adults have been shown to appear at different instar

developmental stages in the Ischnocera (Clay, 1951). For one genus (*Neomenopon* Bedford) only 3rd instar juveniles were available and additional information on the morphology of the adult was obtained from the literature. A final limiting factor in selection was specimen condition and only the clearest and best-mounted material was included. A list of study specimens is provided in Appendix 1.

2.3.1: Outgroup

Outgroup selection was influenced by two major factors. Firstly, the Amblycera are remarkably character rich in contrast to the other three recognised suborders of the Phthiraptera. There are numerous structures and characteristics largely absent in the more specialised Ischnocera, Anoplura and Rhyncophthirina, making strong character homologies with other suborders difficult to determine. The second major factor concerns the findings of Lyal (1985), who in a morphological analysis of the Psocodea compared the phthirapteran groups to the Psocoptera (booklice). Lyal (1985) determined that the Amblycera formed the basal element in the Phthiraptera and that a single psocopteran family, the Liposcelididae, were the sister group to the lice. Comparisons of a range of specimens indicated that good homologies would be easier established between the Liposcelididae and the Amblycera, than between the Amblycera and other Phthiraptera and consequently, specimens of the booklouse *Liposcelis bostrychophilus* were chosen as the outgroup taxon for this study.

2.3.2: Scanning electron microscopy

The amblyceran genera present in the NHM spirit collection were sampled for use in scanning electron micrography (SEM). Specimens were critical point dried, mounted on stubs and coated with a gold-palladium mixture. Observation was via a Philips 500 scanning electron microscope set at 6-12kv. Due to the age of the specimens in the spirit collection (commonly in excess of 75 years old) many of the images obtained were unable to be used for character development. However, as semi-transparent whole mounted material (such as lice) appear layered using light microscopy, dorsal and ventral features were initially difficult to discern and the SEMs became an invaluable aid in the primary interpretation of the external morphology.

2.3.3: Character development

Characters were developed both by extensive observation and adaptation of descriptions from a number of taxonomic and review papers. Synonyms have accumulated in the literature for a number of amblyceran morphological structures and Lakshminarayana's (1985) glossary of taxonomic characters for the study of chewing-lice was found to be an invaluable aid in highlighting many such examples. The source of the terminology for characters developed for this study is indicated where appropriate.

2.3.4: Character recording and coding

All character state data and associated notes were recorded using Nexus Data Editor (NDE) Version 0.5.0 (Page, 2001). The specimens were thoroughly sampled and 147 characters (113 binary and 34 multi-state) suitable

for phylogenetic analysis were collated. A descriptive list of characters and comments was prepared during this study and is presented as Appendix 2.

In this study a mixture of reductive and composite character-coding methods were used: both of which methods have positive and negative aspects. The reductive coding method consists of an initial delimiting character and any number of dependent characters which are scored as inapplicable where appropriate (e.g., character 17: *Dorsal head seta (DHS) 24*: (0) absent, (1) present ... character 18: *DHS 24 (where present)*: (0) macroseta, (1) microseta, (-) inapplicable). Taxa which do not possess DHS 24 are scored as inapplicable for the setal development character. This method maintains the hierarchy between the presence or absence of a morphological "part" and any variability in the "condition" of that part. It also allows separate primary homology statements and transformational independence, so each character can diagnose clades at the appropriate level in the tree (Lee & Bryant, 1999). Thus, reductive coding allows character information to be partitioned more effectively. However, this method can also be potentially problematic for computational software as the inapplicable character states (-) are treated as missing values (?) and therefore homologous to truly applicable states. Globally parsimonious trees can therefore contain local sub optimal solutions (if homoplastic gains are separated by regions of primitive absence) and clades supported exclusively by homoplasies may need to be optimised by hand (Strong & Lipscomb, 1999).
In the composite coding method the presence of a part and any variability in its condition are combined within a single character (e.g., character 35: *Preocular feature of the dorsolateral head margin*: (0) no feature, unbroken margin, (1) notch, (2) slit). In this method transformations in part and condition are not

independent, homology statements are not separate and essentially there is much less phylogenetic information (Lee & Bryant, 1999). There is also the added problem of how to construct composite characters, which contain the part and a number of related condition variables (e.g., the *number*, *position* and *development* of setae on part "X").

In this study the reductive coding method was favoured, where feasible, to maintain as much phylogenetic information as possible and avoid overly complex characters. Composite coding was only used in those situations where a confident proposal of homology was not possible. The full data matrix for the 147 characters is presented as Appendix 3.

2.3.5: Phylogenetic analysis

A heuristic search was completed using PAUP* 4.0b10 (Swofford, 2002) with stepwise addition and tree bisection reconstruction (TBR) branch swapping. All trees were held for inclusion into the branch swapping process and in this second stage of analysis multi-parsimonious trees were also held. This approach allows for the possibility that additional branch swapping on equally and even less parsimonious trees may result in obtaining the shortest tree length. 10000 random addition sequence replicates were employed to increase the probability of finding all the most parsimonious trees. All characters were treated as unordered and of equal weight. Where taxa had been coded as having multiple states, PAUP* was set to interpret these data as "variable" (the respect "( )" versus "{ }" option), in order that a distinction would be made between uncertainty and polymorphism. Branch support statistics were determined by three types of analysis: bootstrap (1000 replicates

with TBR branch swapping) (Felsenstein, 1985), parsimony Jackknife (33% character deletion, 1000 replicates with TBR branch swapping) (Farris *et al.*, 1996) and Bremer support (Bremer, 1988). Bremer support values were obtained using AutoDecay (Eriksson, 1997) and PAUP*. Character state distributions were interpreted using MacClade 4.0 (Maddison & Maddison, 2000) and unambiguous state changes mapped onto the trees using Winclada 0.9.9 (BETA) (Nixon, 1999).

2.4: Results

The analysis found 6 maximum parsimony (MP) trees (on 1 island) with a length of 650 steps (CI: 0.326; RI: 0.585). The strict consensus of these trees is presented in Fig. 2. Jackknife (bold type) and bootstrap values (regular type) above 50% are shown above their respective nodes. Bremer support values are shown below each node.

The strict consensus tree is fully resolved at all but two nodes, with disagreement only within two subgroups of the large clade containing the Menoponidae. In one unresolved group 3 of the 6 MP trees support *Cuculiphilus* Uchida as the sister taxon to the clade containing *Colpocephalum* and *Ardeiphilus* Bedford, whilst in 2 of the 6 arrangements *Ciconiphilus* Bedford has this relationship. In one tree *Cuculiphilus* and *Ciconiphilus* form a sister group to the *Ardeiphilus* clade. In the second unresolved group *Dennyus* Neumann and *Myrsidea* Waterston are always sister taxa, but there are two conflicting arrangements for the other genera. Three trees define a sister group to the *Dennyus+Myrsidea* clade where *Ancistrona* Westwood is placed basal to

Pseudomenopon Mjöberg and *Bonomiella* Conci. The remaining three trees suggest *Ancistrona* and *Pseudomenopon* are sister taxa with *Bonomiella* as the sister taxon to the *Dennyus+Myrsidea* clade.

2.4.1: Cladistic analysis

The tree presented in Fig. 2 and the support obtained, for particular clades, from the jackknife, bootstrap and Bremer support statistical analyses are discussed below. Unambiguous character state changes were plotted onto the strict consensus tree and are presented as Figs. 3-5. For each character discussed, the character number and corresponding state variable are indicated in parentheses.

In the strict consensus tree (Fig. 2), there is strong support for the deep branch relationships between the families and in many cases for supra-generic groupings within the families. Rooted on the outgroup taxon *Liposcelis*, the Boopiidae, Menoponidae and Ricinidae each form monophyletic groups (the Laemobothriidae is monogeneric).

At the base of the tree, the Ricinidae are very strongly supported by jackknife and bootstrap values of 100% and a Bremer support value of 12 (Fig. 2). *Trochiloecetes* Paine and Mann is the sister taxon to a clade containing *Ricinus* De Geer and *Trochiliphagus* Carriker. Three synapomorphies identify this small family. All ricinds have three pairs of dorsal head setae down the midline of the head (character 29:1), lack the labial palps present in other amblycerans (45:0) and have a poorly developed tergal setal row (100:0) (Fig. 3).

A clade containing the other three families (Laemobothriidae, Boopiidae and Menoponidae) also has very strong support (Fig. 2). Character state synapomorphies for this clade are all dorsal head setae (DHS): the mid dorsal head seta DHS 17 (9:1), ocular seta DHS 20 (12:1), occipital setae DHS 21 (13:1), DHS 22 (14:1) and temple setae DHS 25 (19:1), DHS 26 (21:1), DHS 27 (23:1) and DHS 29 (26:1) (Fig. 3). All genera within the Laemobothriidae, Boopiidae and Menoponidae have the transverse pronotal carina (65:1), except the menoponid *Rediella* Hopkins (65:0) (Fig. 4). The small seta at each anterior end of tergite 2 (Fig. 15C) termed "*a*" by (Clay, 1969) is usually present in these families (109:1) as is a pair of isolated subterminal setae on the distal segment of the maxillary palp (41:1) (but see Figs. 4 & 5). These three characters are not present in members of the Ricinidae.

The Laemobothriidae is a monogeneric family, which is strongly supported as the sister taxon to the clade containing the Menoponidae and Boopiidae (Fig. 2). *Laemobothrion* Nitzsch has three short setae at the anterior ventrolateral head margin (55:2) and, unusually, the setal patches on sternites 5 (134:2) and 6 (136:2) are composed of microcombs (Fig. 3) rather than regular setae, as in some Menoponidae. The clade containing the Boopiidae and Menoponidae is also very strongly supported (Fig. 2). This finding supports Clay (1970) who proposed a sister relationship for these two families. Both the Boopiidae and Menoponidae have a complete setal row across the edge of the dorsal prothorax (66:2), which is always less developed in the Ricinidae and Laemobothriidae (Fig. 3). The mesonotum and metanotum are always separate (73:0) and on each tergite the postspiracular setae are generally posterior to the

spiracle (112:0), whereas in the other families they are laterally placed (112:1) (Fig. 3). All taxa, with the exception of the menoponid *Numidicola* Ewing have the anterior mesonotal setae (69:1) usually clustered around the postnotum (Fig. 3).

The monophyly of the Boopiidae is very strongly supported (Fig. 2). Synapomorphies for this clade are: a seta on a rounded protuberance each side of the mesonotum (72:1) and gonapophyses in the female (142:1) (Fig. 3). The euplantula of the first tarsus is normally present in the Amblycera but has been lost in *Latumcephalum* Le Souëf and *Paraboopia* Werneck and Thomson. Where present in the Boopiidae, the euplantula has an unusual serrated and globular appearance (96:2) (Fig. 3). At the base of the boopiid clade, the avian infesting *Therodoxus* Clay is the sister taxon to a reasonably supported clade containing all of the marsupial parasites. The male genitalia of the marsupial lice has a bulbous, well defined mesosomal arch (145:1) and with the exception of *Paraheterodoxus* Harrison and Johnston, the abdominal spiracles open onto the lateral plates instead of the usual amblyceran site on the tergites (110:1) (Fig. 3). In *Paraheterodoxus*, the lateral plate is only partially divided (110:2) (Fig. 3). *Latumcephalum* and *Paraboopia* are very strongly supported as sister taxa within the boopiid clade and have less than the normal four segments in the maxillary palp (39:1) (Fig. 3).

The monophyly of the largest family, the Menoponidae has good support (Fig. 2). A setal comb row lining the antennal margin (56:1) is characteristic of this family and is undeveloped only in *Machaerilaemus* Harrison and *Ancistrona* (56:0) (Figs. 4 & 5). At the base of this large group, *Rediella* is the sister taxon to a clade containing the rest of the Menoponidae.

All menoponid taxa have a setal fringe around the female terminalia (140:1), except *Somaphantus* Paine (140:0) and they usually have a brush (91:2) or combs (91:3) of setae on the ventral aspect of the third femur (Figs. 4 & 5).

Within the Menoponidae, there are four main suprageneric groups (clades "A-D") (Figs. 2, 4 & 5). Within clade "A" (Figs. 2 & 4), *Chapinia* Ewing is the sister taxon to a clade containing five genera, which has only moderate support (Fig. 2). These five genera have a complete marginal border encircling the prosternal plate (85:2) (Fig. 4). Also within clade "A", there is very strong support for *Dennyus* and *Myrsidea* (Dennyinae *sensu* Eichler, 1963) as sister taxa.

Clade "B" (Figs. 2 & 4) contains three genera from the Austromenoponinae (*sensu* Eichler, 1963) and *Machaerilaemus* (Machaerilaeminae). Support for clade "B" is poor (Fig. 2). These genera share a well-developed temple seta, DHS 25 (20:0) (Fig. 4) and, with the exception of *Machaerilaemus*, all have the dorsal head sensillum "*c*" (see Fig. 6A) *sensu* Clay (1969) (32:1) and a smooth junction of the dorsolateral head and temple margins (36:0) (Fig. 4).

Suprageneric clades "C" and "D" (Figs. 2 & 5) are sister groups in this analysis, but this relationship is poorly supported (Fig. 2). Most of the genera in clades "C" and "D" have an additional submarginal row of short setae on the tibia of legs two and three (93: 1) (Fig. 5) but this trait is later lost within clade "D". The taxa in clade "C" (Figs. 2 & 5) generally represent the "*Colpocephalum* complex" (*sensu* Clay, 1969) (see Table 1) and Colpocephalidae (*sensu* Eichler, 1963) (see Table 2) but the monophyly of this clade is weakly supported (Fig. 2). Some of the internal branches in clade "C"

have good support e.g. the clade containing *Colpocephalum* and *Comatomenopon* Uchida (Colpocephalinae *sensu* Eichler, 1963). There is, however, some difficulty in resolving the position of *Ciconiphilus* and *Cuculiphilus* (Fig. 2). Only genera in clade "C" have setal combs on the third ventral sternite (St 3) (128:2) and combs are also present on the ventral aspect of the third femur (91:3) with the exception of *Eomenopon* Harrison (91:2) (Fig. 5). *Osborniella* Thompson is sister taxon to a clade containing the remainder of the *Colpocephalum*-like genera and *Eomenopon*, which groups with *Piagetiella* Neumann. The last suprageneric group, clade "D" (Figs. 2 & 5) is very poorly supported (Fig. 2). At the base of this clade *Gruimenopon* Clay and Meinertzhagen and *Hoazineus* Guimarães form a sister group to the other genera. These taxa are the only genera which have robust submarginal temporal setae (62: 2) (Fig. 5).

Clade "D" also contains the two genera (*Amyrsidea* Ewing and *Menacanthus* Neumann) included in the "*Menacanthus* complex" (*sensu* Clay, 1969) but these not sister taxa in this analysis (Figs. 2 & 5). There is some support for the grouping of *Menacanthus* and *Colimenopon* Clay and Meinertzhagen as sister taxa. These two genera have more setae on the posterior aspect of the first coxa (89:1) (Fig. 5) than the usual four or five setae commonly found in the Menoponidae. *Menopon* Nitzsch and *Numidicola* (Menoponinae *sensu* Eichler, 1963) are sister taxa in clade "D" (Figs. 2 & 5). Exclusive to these taxa, is the form of the sculpturing on the ventral submargin of the temple, which is composed of multi-tipped spikes (64:1) and in place of the usual wide female anal fringe, these genera have a small rounded protruding anal margin with a short fine fringe (141:2) (Fig. 5).

2.5: Discussion

Clay (1970) considered the Menoponidae, Boopiidae and Ricinidae to be monophyletic groups. The tree derived from the cladistic analysis (Fig. 2) corroborates this view and the stability of each family is strongly supported. Clay (1970) also believed that the Menoponidae would hold the basal position in an amblyceran phylogeny, which has not been found by this study. The placing of the Ricinidae and Laemobothriidae at the base of the tree (see Fig. 2) may, however, only be an artefact of ingroup and outgroup selection. Both these families and the chosen outgroup taxon *Liposcelis* lack a number of characters and the arrangement may change with the addition of genera of other amblyceran families or different outgroup taxa. Clay (1970) wrote that the close morphological similarity of the Menoponidae and Boopiidae was indicative of a sister taxa relationship, which has been strongly supported in this analysis.

The suprageneric groups defined here only agree in part with the classifications of both Eichler (1963) and Clay (1969). Overall, there is little support for the intricate amblyceran classification of Eichler (1963) (see Table 2). In his treatment of the Ricinidae, Eichler placed *Ricinus* and *Trochiloecetes* in two monogeneric families and in turn included the Ricinidae and *Laemobothrion* under the superfamily Laemobothrioidea, which is strongly paraphyletic in this study (Fig. 2). He regarded the Boopiidae as sharing more similarities with the Gyropidae and Trimenoponidae (rather than with the Menoponidae), placing these families under the superfamily Gyropoidea (see Table 2). The Boopiidae (*sensu* Eichler, 1963) are also strongly paraphyletic in

this analysis with respect to his monogeneric Latumcephalidae (Fig. 2). Within the largest family, the Menoponidae, some of Eichler's generic groupings are unusual and, in comparison with the tree presented in Fig. 2, most of his subdivisions are paraphyletic or polyphyletic. The tree found here supports the historical view that Eichler's groups were sometimes little more than arbitrary. Clay (1947), when discussing the preliminary classification of Eichler (1941), wrote of his groups "...that in many cases they bear little relationship to the facts". None of the seven new families he proposed within the Menoponidae are monophyletic in this current analysis. Nevertheless, a few of Eichler's subfamilies are supported. The Menoponinae (*Menopon* and *Numidicola*), Colpocephalinae (*Colpocephalum* and *Comatomenopon*) and Dennyinae (*Dennyus* and *Myrsidea*) are all monophyletic, with the last two subfamilies having high levels of branch support (Fig. 2). *Dennyus* and *Myrsidea* were also presented as sister taxa by (Cruickshank *et al.*, 2001) in a molecular study using the EF1α gene, although with a poorer level of branch support, than presented here.

Clade "C" (Figs. 2 & 5) contains the superfamily Colpocephaliformia (*sensu* Eichler, 1963) and the six taxa considered as part of the "*Colpocephalum* complex" (*sensu* Clay, 1969) (see Table 1). *Eomenopon* has combs on some of the ventral sternites, which are more robust but generally similar to the combs found in the other genera in clade "C", but both Eichler (1963) and Clay (1969) appear to have overlooked this similarity when constructing their classifications. Clade "C" is more reflective of the Eichler (1963) classification than that of Clay (1969). With the exception of *Osborniella*, all of Eichler's Colpocephalidae are included in a single clade and

his monogeneric Piagetiellidae is the sister taxon of *Eomenopon*. Eichler's Anserphilinae is paraphyletic and his monogeneric Psittacomenoponinae is the sister taxon to the Colpocephalinae. The Cuculiphilinae (*sensu* Eichler, 1963) is polyphyletic. Clay (1969) defined her "*Colpocephalum* complex" as "...all those genera with ctenidia (setal combs) on the venter of the third femur, with the exception of *Cuculiphilus sens. lat.*, *Bucerocolpocephalum*, *Piagetiella*, *Turacoeca* and *Odoriphila*", although she did consider that possibly the last genus should be included in the "*Colpocephalum* complex". The tree presented in this analysis (Fig. 2) suggests that this group should be extended to include *Odoriphila* Clay and Meinertzhagen, *Cuculiphilus* and *Piagetiella*.

Within clade "D" (Figs. 2 & 5), *Amyrsidea* and *Menacanthus*, the two genera included from the "*Menacanthus* complex" (*sensu* Clay, 1969) do not group together. There is strong branch support (Fig. 2) for the clade containing *Menacanthus* and *Colimenopon* with *Amyrsidea* grouped with *Menopon* and *Numidicola*. However, Clay (1969) did suggest that *Menopon* and *Somaphantus* should also possibly be included in the "*Menacanthus* complex". The tree presented in Fig. 2 suggests they should be included, but there is also some support for *Numidicola* and *Colimenopon* to be considered as part of the "complex".

2.5.1: Evidence for host-parasite cospeciation

Lice are considered to be very host-specific parasites and are widely assumed to be good models for co-evolutionary analyses. The extent of host-parasite cospeciation has been investigated in the gopher lice (Ischnocera: Trichodectidae) by, for example, Hafner *et al.* (1994) and Hafner and Page

(1995). These studies found that within genera, parasite and host phylogenies were almost completely congruent with one another, whilst the relationships between genera have been found to be only partially so (Page, Price & Hellenthal, 1995). Within the Amblycera (specifically within the menoponid genus *Dennyus*) there have also been tests of host-parasite cospeciation. Clayton, Price and Page (1996) compared louse phenetic trees with a molecular cytochrome *b* (cyt *b*) phylogeny for their swift and swiftlet hosts, but the topologies were mostly incongruent. In a later publication, some evidence was found for cospeciation when molecular cyt *b* phylogenies for *Dennyus* (*Collodennyus*) species and their hosts were compared (Page *et al.*, 1998).

The association between most louse species and their hosts is not necessarily an exclusive one-to-one relationship. A host may harbour more than one louse species, and a louse species may also be found on a limited number of hosts. This pattern also extends to louse genera. Some louse species are parasitic only on hosts of a particular order, sometimes even a single family, but many are distributed across multiple host orders and families, indicating a complex history of parasitism. Therefore, it would be very difficult, or even futile, in this study to investigate the extent of co-evolution, when the phylogeny presented in Fig. 2 contains only single representatives of genera.

However, where there are louse genera, which are only present on a particular closely related group of hosts some limited inference may be made. Smith (2000), for example, found that louse species which were present only on certain hosts were confined to single clades in his morphological analysis of the Goniodidae and Heptapsogasteridae (Ischnocera). In this study of

amblyceran lice, the tree presented in Fig. 2 reveals similar results. Within the Boopiidae (Fig. 2), the clade comprising *Boopia* Piaget, *Paraheterodoxus*, *Paraboopia* and *Latumcephalum* contains genera which parasitise the marsupial order Diprotodontia (Kangaroos, wombats etc). Aside from *Boopia,* which has a wider distribution, the other three genera are exclusive to this host group. Similarly, within the menoponid clade "D" (Figs. 2 & 5), *Amyrsidea*, *Menopon*, *Numidicola, Somaphantus* and *Menacanthus* are grouped with *Colimenopon*. Excepting the latter genus, these four taxa are all parasitic on the avian Galliformes (pheasants, fowl etc). Notably, the first four genera are contained within a single clade and their distribution is restricted to only two avian host families (Phasianidae and Numididae) which have been shown to be sister taxa (e.g. Sibley & Ahlquist, 1990). Such a result suggests that a co-speciation analysis of host and parasite using specific exemplars from some or all of the genera outlined above may bear interesting results. Some clades in the phylogeny presented here may enable more detailed co-evolutionary analyses of the Amblycera and thus contribute to our presently limited understanding of the potentially complicated history of parasitism in these lice.

The complete data matrix and all trees presented in this paper are accessible through TreeBASE (http://herbaria.harvard.edu.treebase) as study accession number S739.

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2.7: Bibliography

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Table 1: Suprageneric classification of the Menoponidae *sensu* Clay (1969). *Colpocephalum* and *Menacanthus* "complexes". Parentheses indicate those genera which Clay felt should possibly be included in these groups. * indicates three genera later considered to be subgenera of *Amyrsidea*. Taxa included in this study are highlighted in bold type.

Table 1: Suprageneric classification of the Menoponidae *sensu* Clay (1969). *Colpocephalum* and *Menacanthus* "complexes".

"*Colpocephalum* complex"	"*Menacanthus* complex"
Genus	Genus
Colpocephalum	*Menacanthus*
Comatomenopon	*Amyrsidea*
Ardeiphilus	*Argimenopon**
Ciconiphilus	*Cracimenopon**
Osborniella	*Desumenopon**
Psittacomenopon	
(Odoriphila)	*(Menopon)*
	(Somaphantus)
	(Clayia)
not *Cuculiphilus*	
Piagetiella	

Table 2: Suprageneric classification of the Amblycera (part) *sensu* Eichler (1963). Taxa included in this study for the four families: Menoponidae (M), Boopiidae (B), Laemobothriidae (L) and Ricinidae (R) are highlighted in bold type. The conservative familial classification of Clay (1970) is indicated on the right. *Microctenia* (in parentheses) was not included due to poor specimen quality and is presented only to illustrate the presence of the family *sensu* Eichler. Additionally, only currently recognized genera from this classification have been listed.

Table 2: Suprageneric classification of the Amblycera (part) *sensu* Eichler (1963).

Superfamily	Interfamily	Family	Subfamily	Genus	Clay-1970
Laemobothrioidea	Laemobothriformia	Laemobothriidae		*Laemobothrion*	L
	Riciniformia	Ricinidae		*Ricinus*	R
		Trochiloecetidae		*Trochiloecetes*	R
Gyropoidea	Boopiformia	Boopiidae	Heterodoxinae	*Heterodoxus*	B
				Paraheterodoxus	B
			Boopinae	*Boopia*	B
				Paraboopia	B
		Latumcephalidae		*Latumcephalum*	B
Menoponoidea	Menoponiformia	Somaphantidae	Somaphantinae	*Amyrsidea*	M
				Clayia	M
				Rediella	M
				Somaphantus	M
			Bonomiellinae	*Bonomiella*	M
		Menoponidae	Menoponinae	*Menopon*	M
				Numidicola	M
			Menacanthinae	*Hohorstiella*	M
				Menacanthus	M
				Nosopon	M
			Machaerilaeminae	*Colimenopon*	M
				Eureum	M
				Machaerilaemus	M
				Neomenopon	M
			Dennyinae	*Dennyus*	M
				Myrsidea	M
		Ancistronidae	Ancistroninae	*Ancistrona*	M
			Austromenoponinae	*Austromenopon*	M
				Eidmanniella	M
				Holomenopon	M
				Plegadiphilus	M
			Actornithophilinae	*Actornithophilus*	M
				Chapinia	M
				Gruimenopon	M
				Longimenopon	M
				Meromenopon	M
			Hoazineinae	*Eomenopon*	M
				Hoazineus	M
		Pseudomenoponidae		*Pseudomenopon*	M
		Trinotonidae		*Trinoton*	M
	Colpocephaliformia	Colpocephalidae	Colpocephalinae	*Colpocephalum*	M
				Comatomenopon	M
			Anserphilinae	*Ardeiphilus*	M
				Ciconiphilus	M
				Dicteisia	M
			Cuculiphilinae	*Cuculiphilus*	M
				Carrikeria	M
				Odoriphila	M
				Osborniella	M
			Psittacomenoponinae	*Psittacomenopon*	M
			Microcteniinae	*(Microctenia)*	M
		Piagetiellidae		*Piagetiella*	M

Fig. 1: Strict consensus of the twenty-four most parsimonious trees recovered from a cladistic analysis (1000 random addition replicates) of a morphological data matrix by Clay (1970) (Length= 24 steps, CI= 0.833, RI= 0.833, HI= 0.167). Data for an outgroup taxon *Liposcelis bostrychophilus* was added to the original matrix. Bootstrap support (>50%, 100 replicates) for two main clades of lice ("A" and "B") is shown. A representative host for each louse family is also indicated.

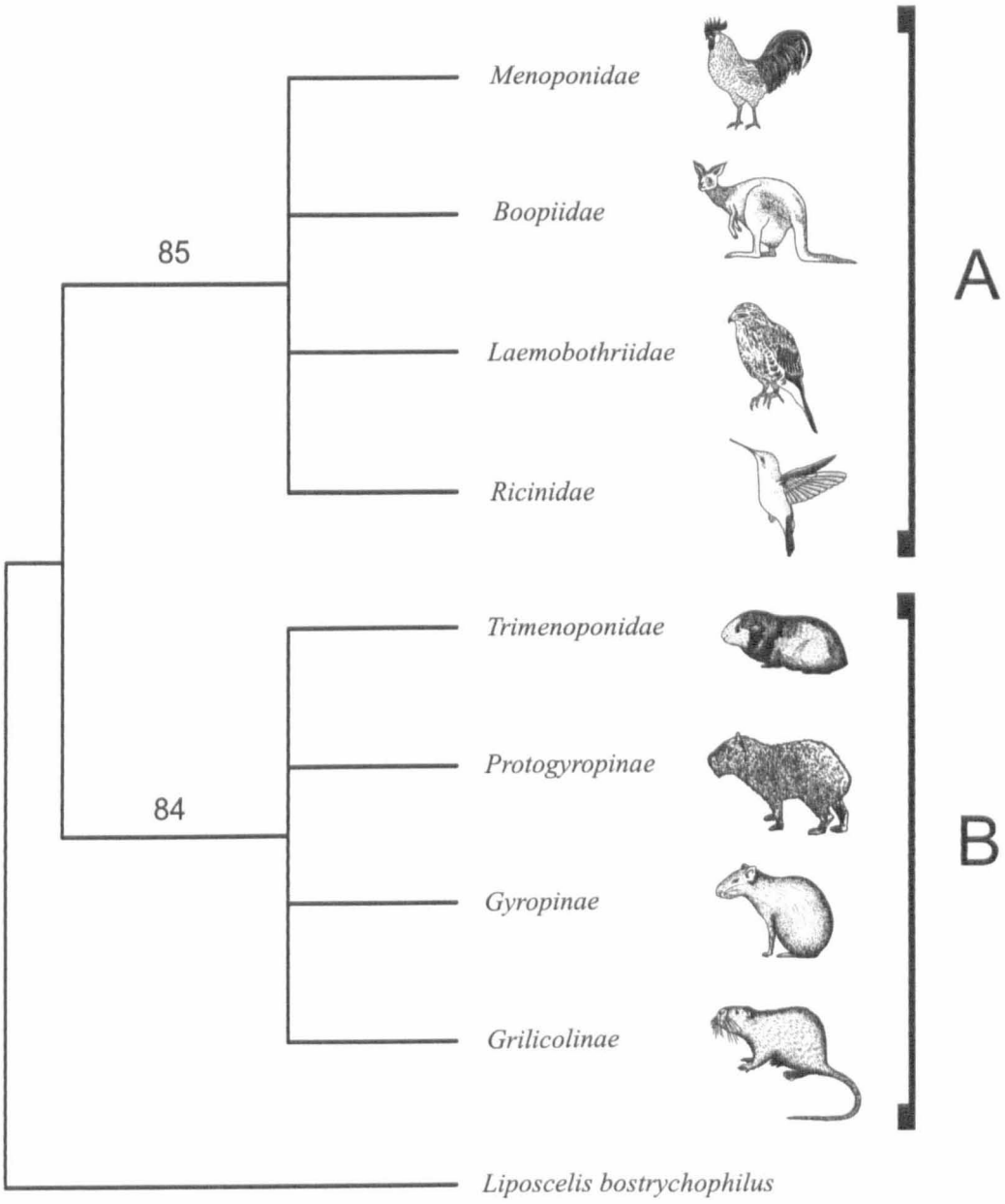

Menoponidae
Boopiidae
85
Laemobothriidae
Ricinidae
A
Trimenoponidae
Protogyropinae
84
Gyropinae
Grilicolinae
B
Liposcelis bostrychophilus

Fig. 2: Strict consensus of six equally parsimonious trees recovered from a cladistic analysis using 10000 random addition replicates (Length= 650 steps, CI= 0.326, RI= 0.585, HI= 0.683). Jackknife (33% deletion, bold type) and bootstrap values (>50%, regular type) each based on 1000 replicates are shown above the nodes, with Bremer support values (decay indices) shown below. Louse families and major clades within the Menoponidae are indicated.

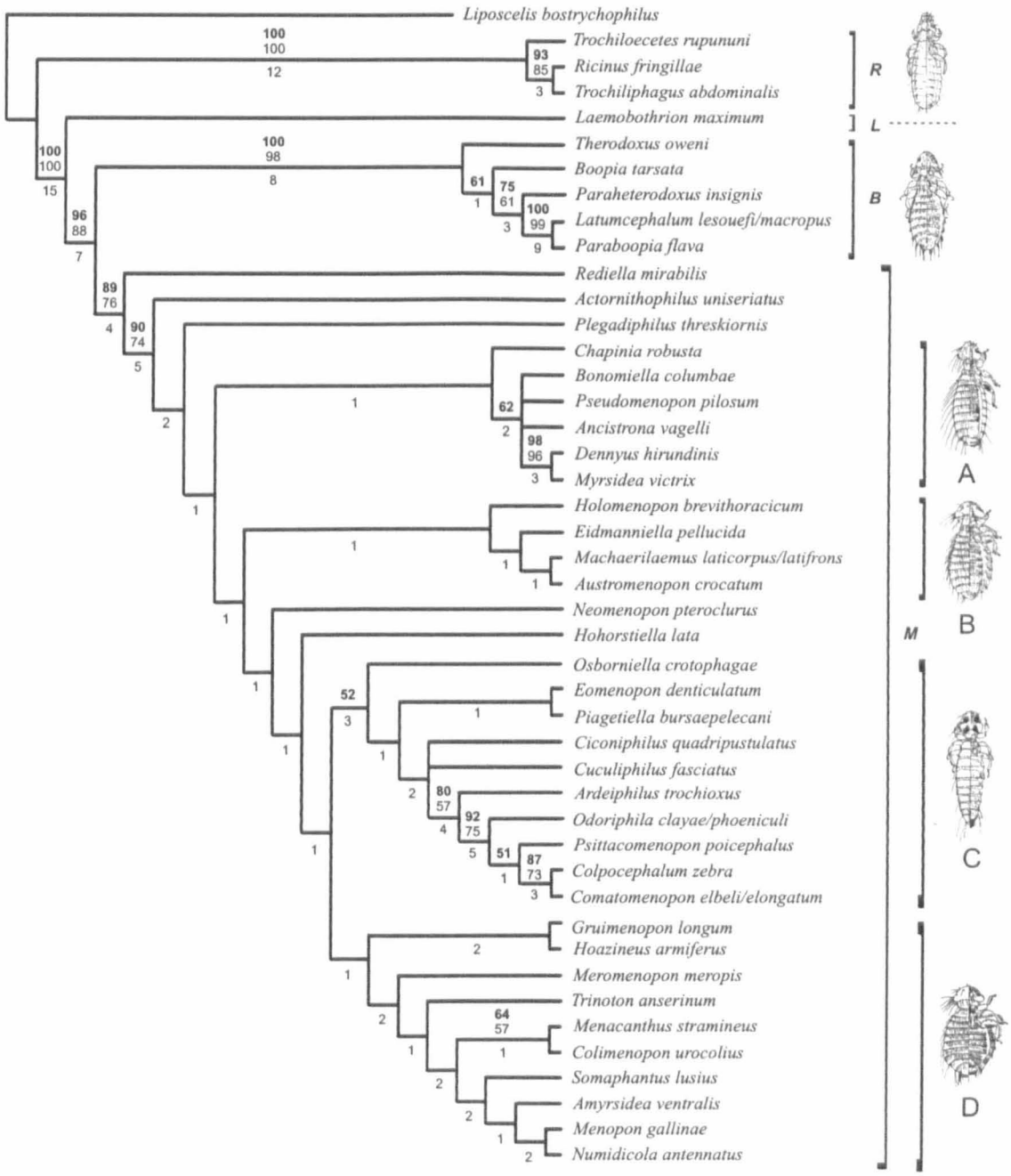

Liposcelis bostrychophilus
Trochiloecetes rupununi
Ricinus fringillae
Trochiliphagus abdominalis
Laemobothrion maximum
Therodoxus oweni
Boopia tarsata
Paraheterodoxus insignis
Latumcephalum lesouefi/macropus
Paraboopia flava
Rediella mirabilis
Actornithophilus uniseriatus
Plegadiphilus threskiornis
Chapinia robusta
Bonomiella columbae
Pseudomenopon pilosum
Ancistrona vagelli
Dennyus hirundinis
Myrsidea victrix
Holomenopon brevithoracicum
Eidmanniella pellucida
Machaerilaemus laticorpus/latifrons
Austromenopon crocatum
Neomenopon pteroclurus
Hohorstiella lata
Osborniella crotophagae
Eomenopon denticulatum
Piagetiella bursaepelecani
Ciconiphilus quadripustulatus
Cuculiphilus fasciatus
Ardeiphilus trochioxus
Odoriphila clayae/phoeniculi
Psittacomenopon poicephalus
Colpocephalum zebra
Comatomenopon elbeli/elongatum
Gruimenopon longum
Hoazineus armiferus
Meromenopon meropis
Trinoton anserinum
Menacanthus stramineus
Colimenopon urocolius
Somaphantus lusius
Amyrsidea ventralis
Menopon gallinae
Numidicola antennatus
R
L
B
M
A
B
C
D

Fig. 3: Character state evolution within the Ricinidae, Laemobothriidae & Boopiidae. Characters which change unambiguously are shown mapped onto the strict consensus tree. Unique changes = □. For character state descriptions, see Appendix 2.

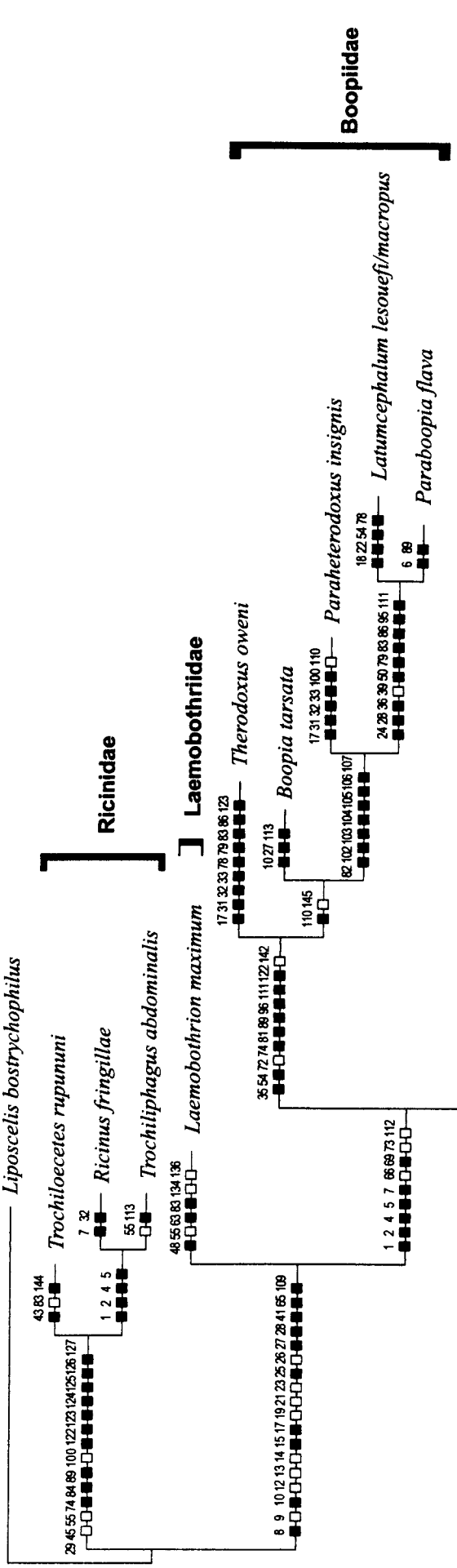

Liposcelis bostrychophilus
Trochiloecetes rupununi
Ricinus fringillae
Trochiliphagus abdominalis
Laemobothrion maximum
Therodoxus oweni
Boopia tarsata
Paraheterodoxus insignis
Latumcephalum lesouefi/macropus
Paraboopia flava
Menoponidae (Figs. 4 & 5)
Ricinidae
Laemobothriidae
Boopiidae

Fig. 4: Character state evolution within the Menoponidae (clades "A" & "B"). Characters which change unambiguously are shown mapped onto the strict consensus tree. Unique changes = □. For character state descriptions, see Appendix 2.

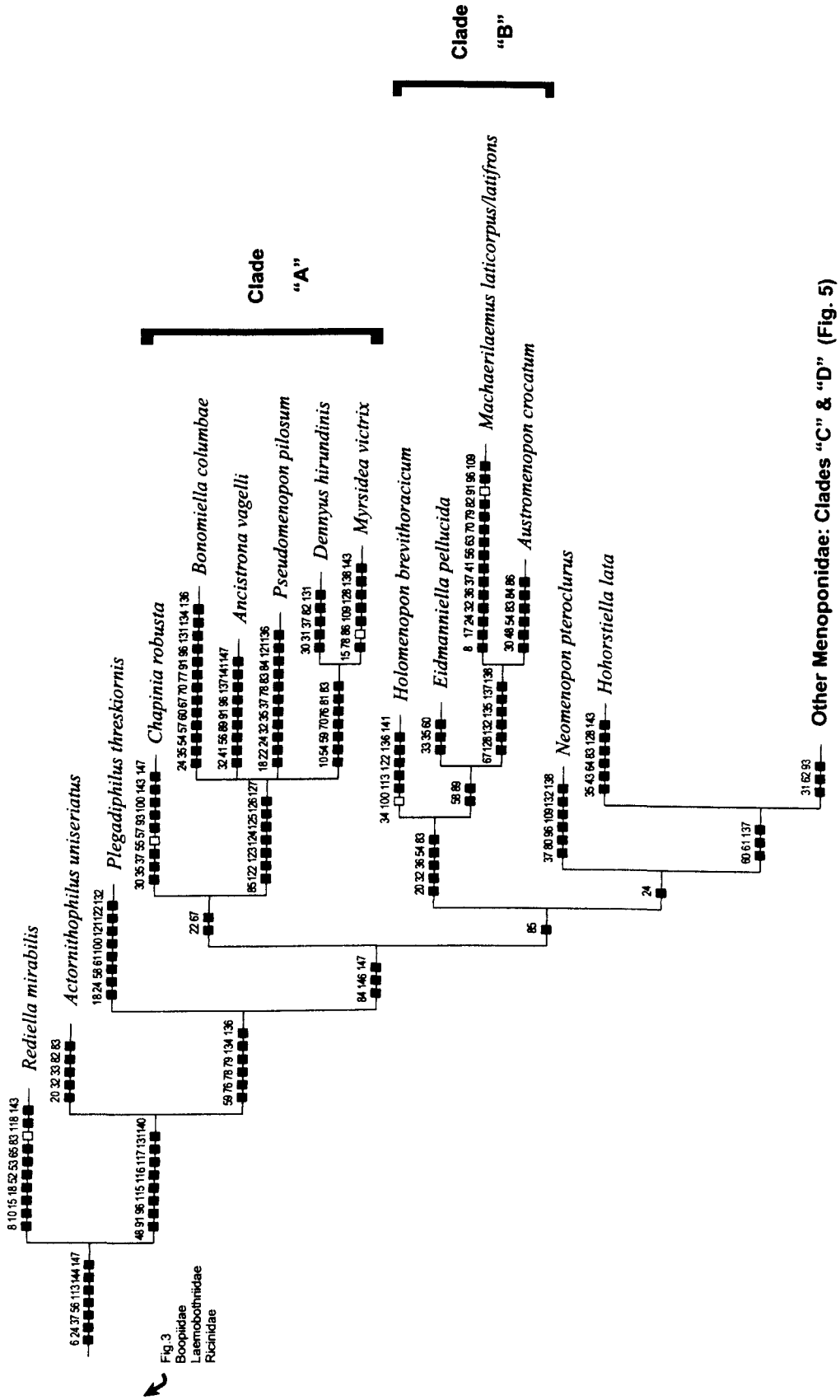

Clade "A"
Clade "B"
Rediella mirabilis
Actornithophilus uniseriatus
Plegadiphilus threskiornis
Chapinia robusta
Bonomiella columbae
Ancistrona vagelli
Pseudomenopon pilosum
Dennyus hirundinis
Myrsidea victrix
Holomenopon brevithoracicum
Eidmanniella pellucida
Machaerilaemus laticorpus/latifrons
Austromenopon crocatum
Neomenopon pteroclurus
Hohorstiella lata
Other Menoponidae: Clades "C" & "D" (Fig. 5)
Fig.3
Boopiidae
Laemobothriidae
Ricinidae

Fig. 5: Character state evolution with the Menoponidae (clades "C" & "D"). Characters which change unambiguously are shown mapped onto the strict consensus tree. Unique changes = □. For character state descriptions, see Appendix 2.

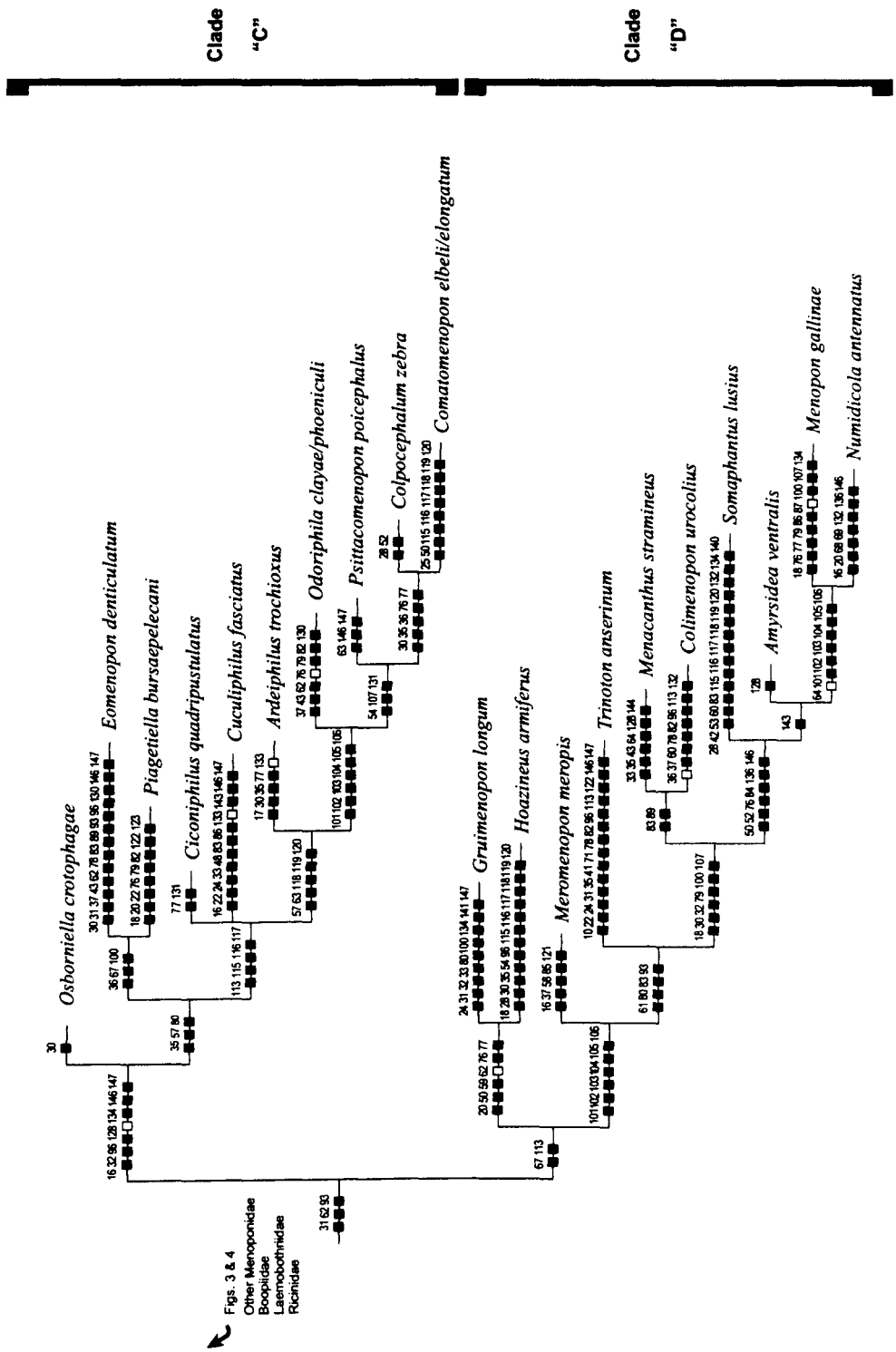

Clade "C"
Clade "D"
Osborniella crotophagae
Eomenopon denticulatum
Piagetiella bursaepelecani
Ciconiphilus quadripustulatus
Cuculiphilus fasciatus
Ardeiphilus trochioxus
Odoriphila clayae/phoeniculi
Psittacomenopon poicephalus
Colpocephalum zebra
Comatomenopon elbeli/elongatum
Gruimenopon longum
Hoazineus armiferus
Meromenopon meropis
Trinoton anserinum
Menacanthus stramineus
Colimenopon urocolius
Somaphantus lusius
Amyrsidea ventralis
Menopon gallinae
Numidicola antennatus
Figs. 3 & 4
Other Menoponidae
Boopiidae
Laemobothriidae
Ricinidae

Fig. 6: Characters of the dorsal head, the dorsal head setae (DHS 8 - 31) and sensilla (a - e) *sensu* Clay (1969) from (A) a typical menoponid head, (B) *Paraboopia*, (C) *Colpocephalum*, (D) *Colimenopon*, (E) *Austromenopon* and (F) *Trochiloecetes*. Characters illustrated are described in Appendix 2.

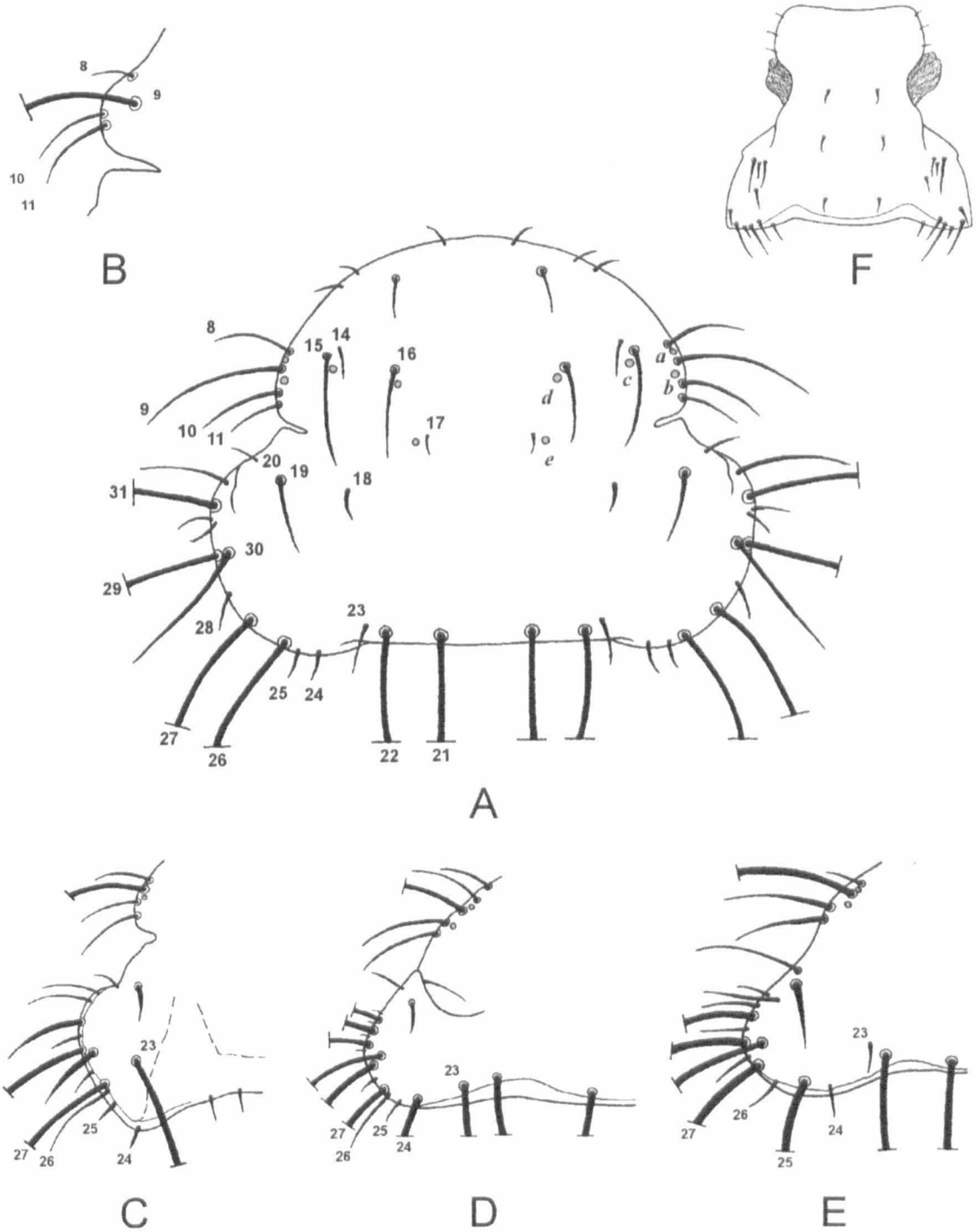

8
9
10
11
B
F
8
15
14
16
a
c
b
d
9
10
11
17
e
20
19
18
31
30
29
28
23
25
24
27
26
22
21
A
23
27
26
25
24
C
23
27
26
25
24
D
23
27
26
25
24
E

Fig. 7: Forms of the tentorial bridge in (A) *Actornithophilus*, (B) *Ricinus*, (C) *Odoriphila* and (D) *Dennyus*. Characters illustrated are described in Appendix 2.

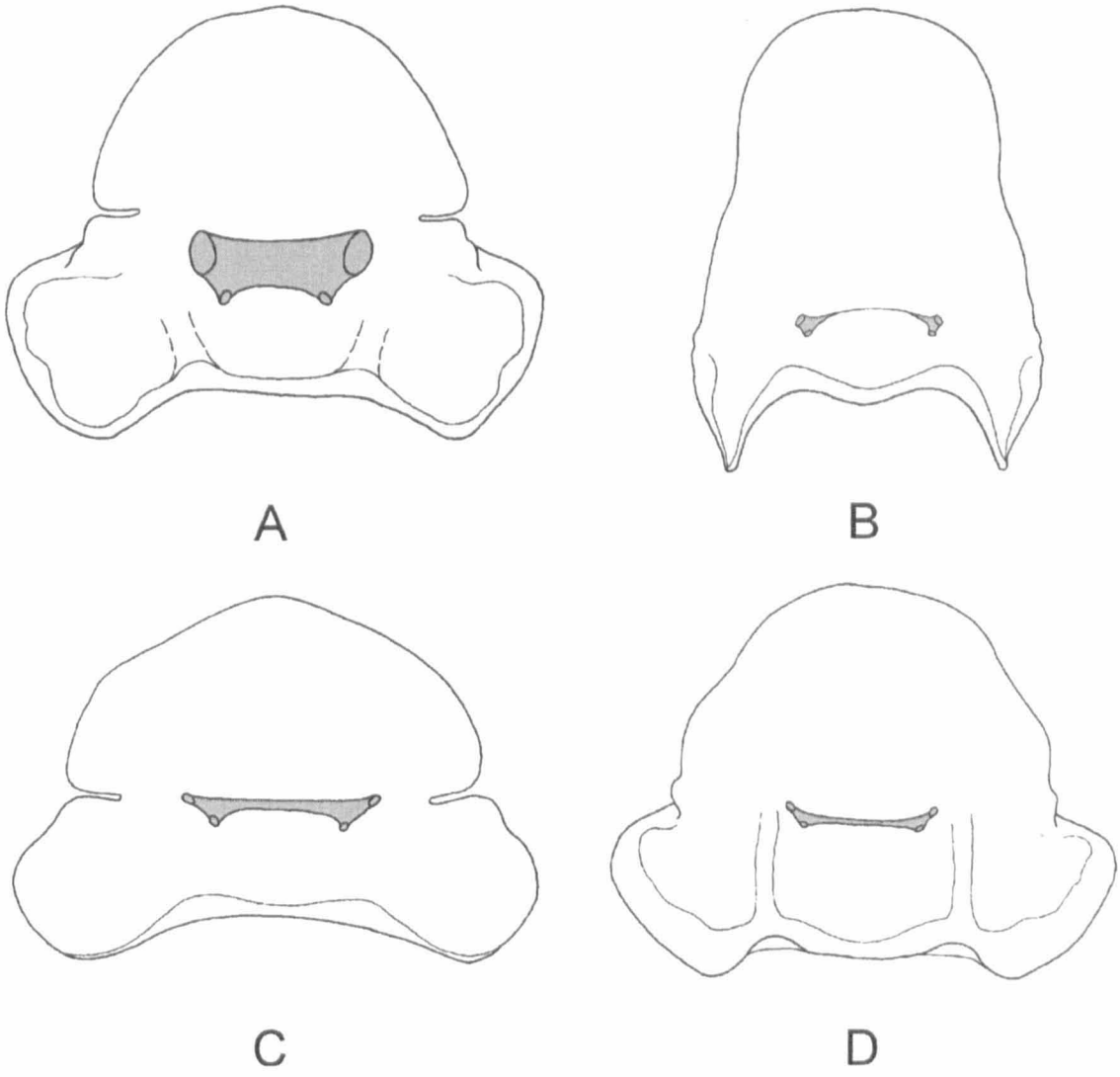

A
B
C
D

Fig. 8: Characters of the ventral head and mouthparts in (A) *Colpocephalum* with maxillary palp, labial palp, antennal fossae (darker shading) and mandibles (lighter shading), (B) outgroup taxon *Liposcelis* with maxillary palp (*mp*) and labial palp (*lp*) (shaded), (C) amblyceran labial palp (detail) and (D) *Trochiloecetes*, piercing mouthparts. (E) – (H): maxillary palps with post-palpal processes (*pp*) (shaded) in (E) *Latumcephalum* with subterminal setae (*ss*) indicated by arrow, (F) *Laemobothrion*, (G) *Menacanthus* and (H) *Odoriphila*. Characters illustrated are described in Appendix 2.

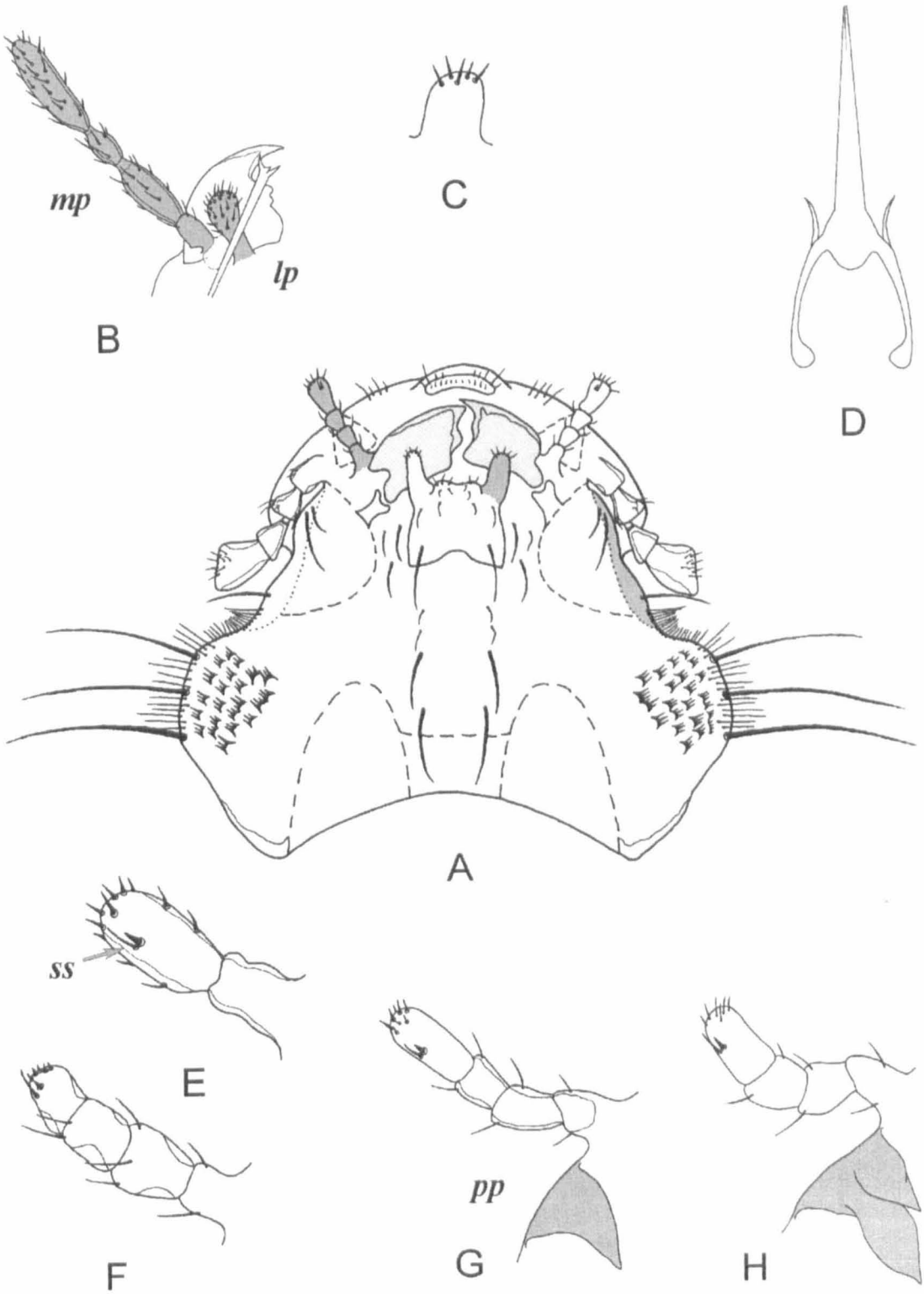

mp
lp
B
C
D
A
ss
E
F
pp
G
H

Fig. 9: Form of the antennae in (A) *Rediella* with scape (*s*), pedicel (*p*) and flagellum (*f*) components defined, (B) *Gruimenopon*, (C) *Hohorstiella*, (D) *Cuculiphilus*, (E) *Paraheterodoxus*, (F) *Laemobothrion*, (G) *Trochiliphagus* and (H) outgroup taxon *Liposcelis* with segments 1 - 4 and 12 - 13 of antenna shown. Characters illustrated are described in Appendix 2.

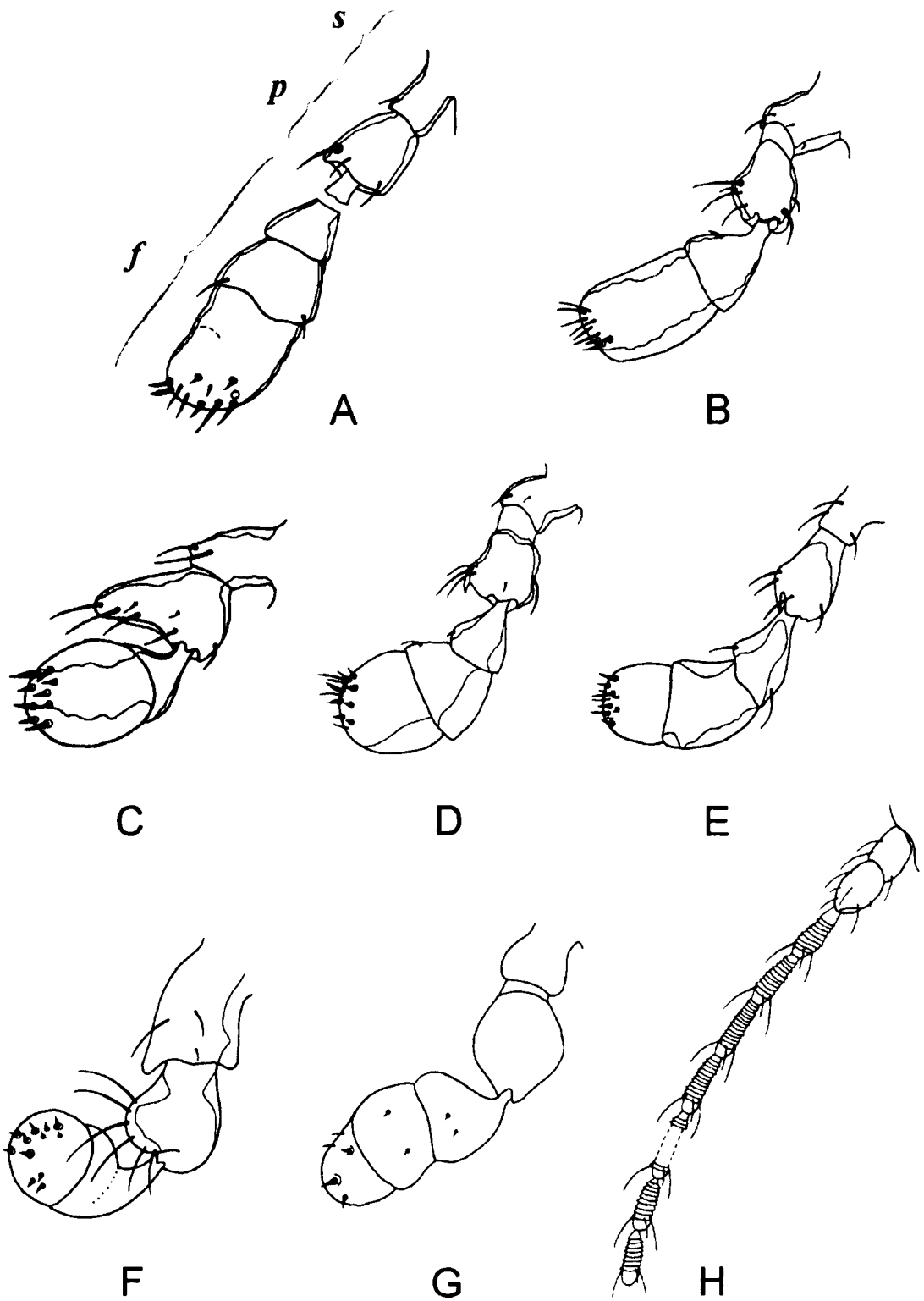

s
p
f
A
B
C
D
E
F
G
H

Fig. 10: Characters of the ventrolateral head with the antennal fossae (shaded) in (A) *Somaphantus*, (B) *Numidicola*, (C) *Colimenopon*, (D) *Hohorstiella* and (E) *Gruimenopon*. (F) *Laemobothrion* with fringe-like temple sculpturing (detail). (G) – (K): setae at the anterior termination of the ventrolateral head margin in (G) *Ricinus*, (H) *Laemobothrion*, (I) *Trochiliphagus*, (J) *Chapinia* and (K) *Plegadiphilus*. Characters illustrated are described in Appendix 2.

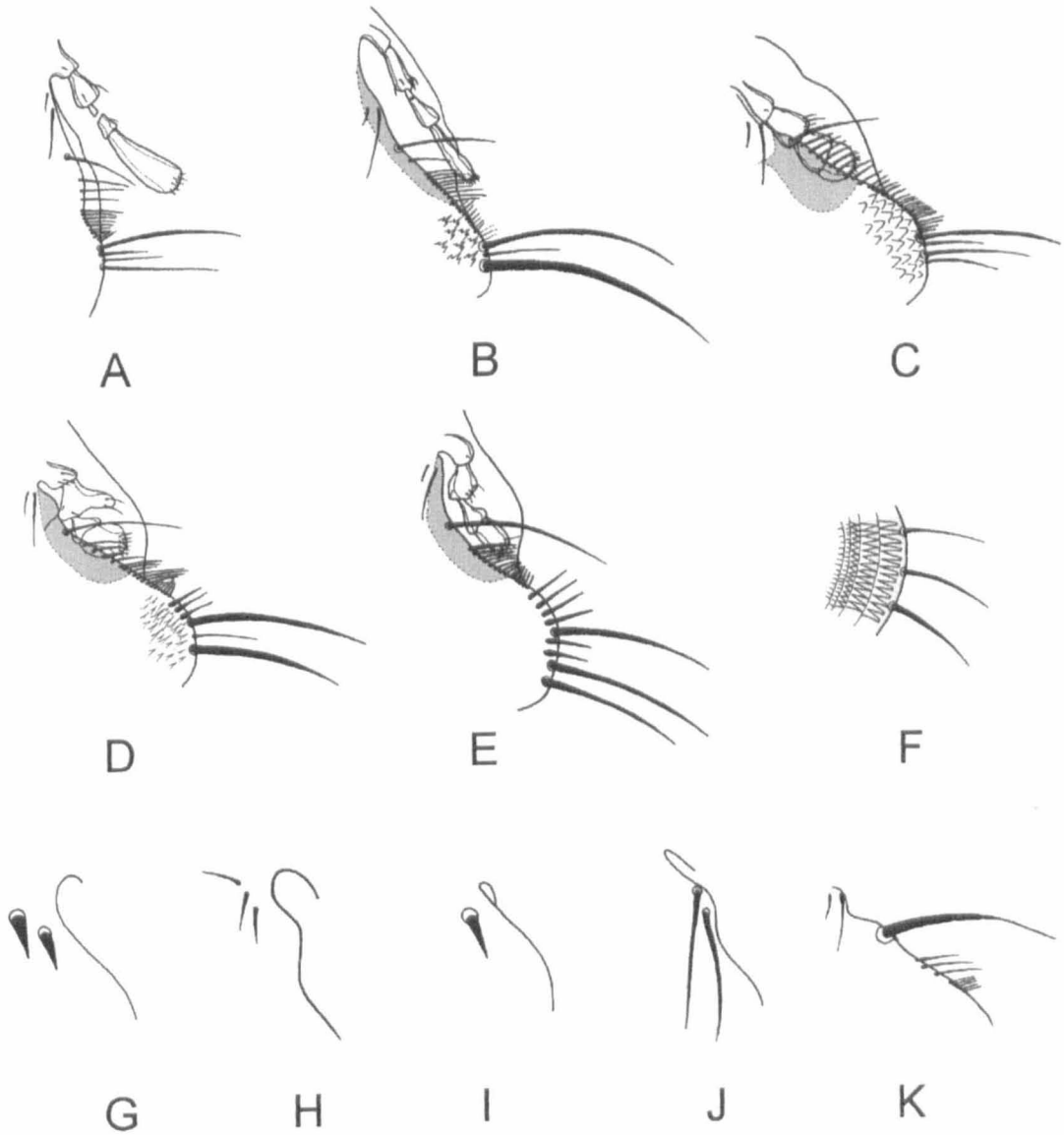

A
B
C
D
E
F
G
H
I
J
K

Fig. 11: Characters of the dorsal thorax in (A) *Trochiliphagus*, (B) *Rediella*, (C) *Dennyus* with the thoracic segments, pronotum (*p*), mesonotum (*ms*) and metanotum (*mt*) and the first abdominal tergal segment (*t 1*) indicated. Postnotum (shaded) with postnotum (detail, with four setae) shown alongside Fig 11C, (D) *Odoriphila*, (E) *Therodoxus* showing metanotum fused with t 1 indicated by dashed line and (F) *Machaerilaemus*, metanotum (detail). Characters illustrated are described in Appendix 2.

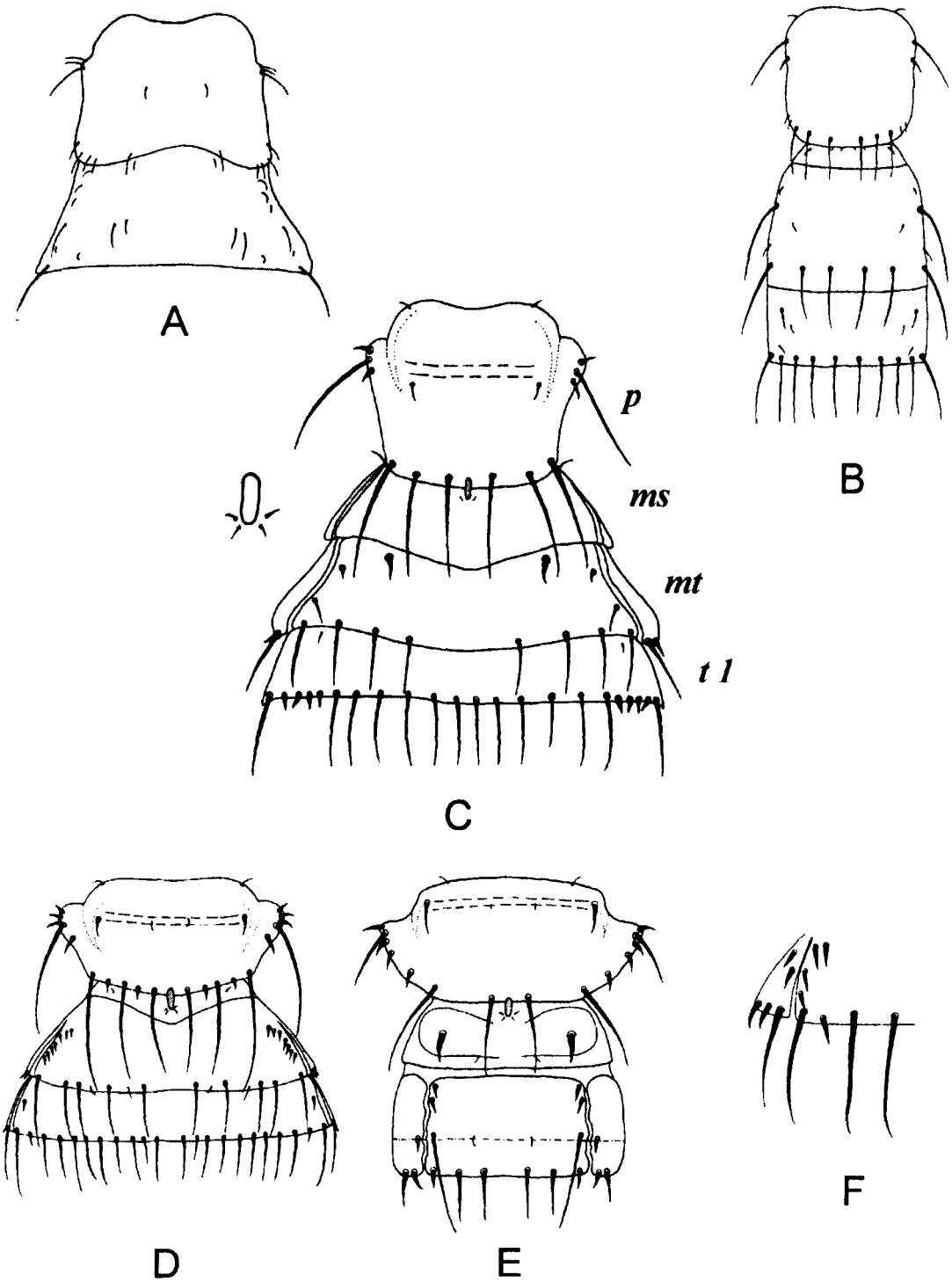

A
B
p
ms
mt
t 1
C
D
E
F

Fig. 12: Thoracic prosternal plates with marginal border (shaded) in (A) *Colpocephalum*, (B) *Dennyus*, (C) *Myrsidea,* (D) *Eomenopon*, (E) *Chapinia*, (F) *Eidmanniella*, (G) *Colimenopon*, (H) *Ricinus*, (I) *Trochiloecetes*, (J) *Laemobothrion*, (K) *Paraheterodoxus* and (L) *Therodoxus*. Characters illustrated are described in Appendix 2.

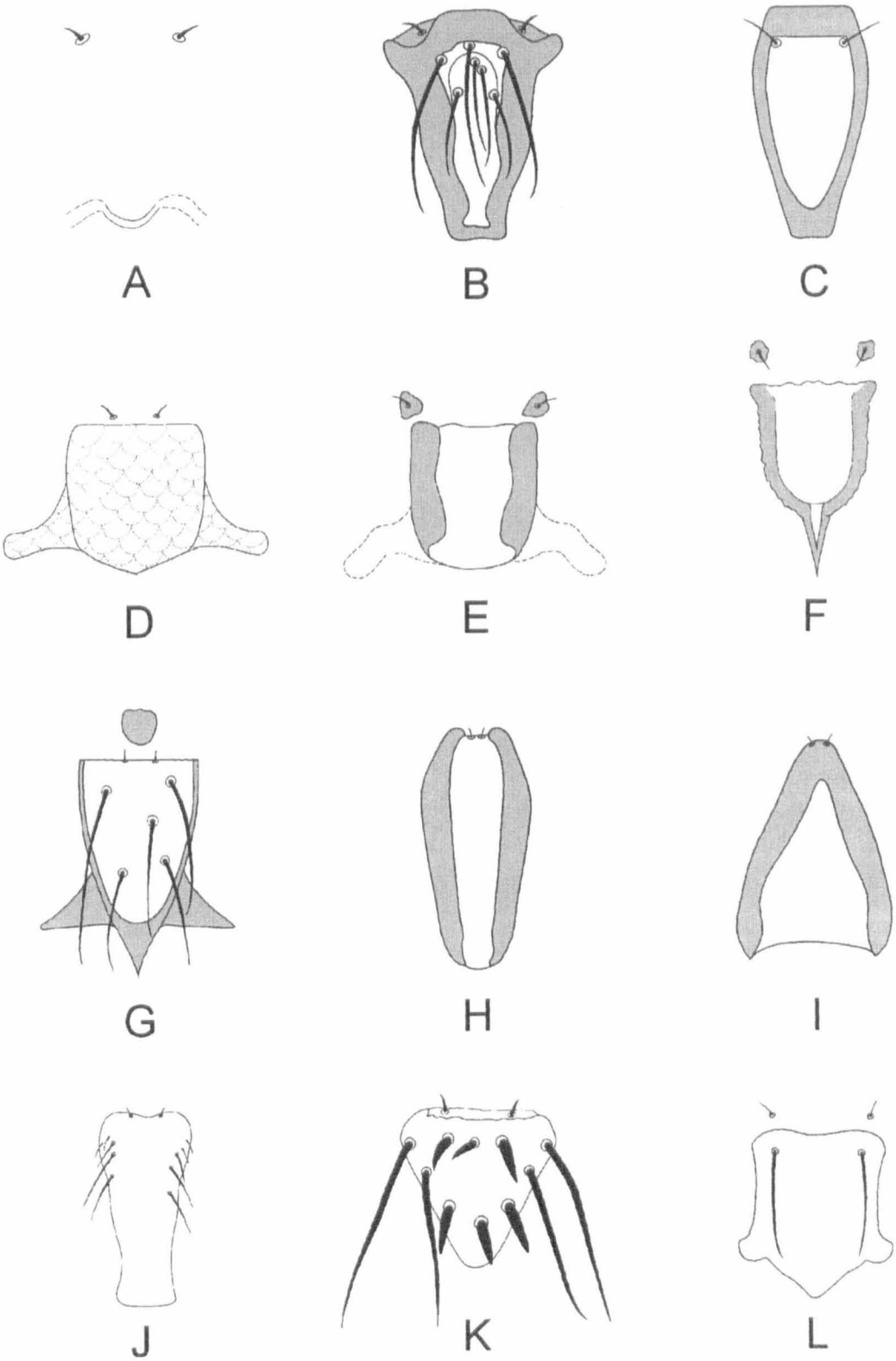

A
B
C
D
E
F
G
H
I
J
K
L

Fig. 13: Characters of the ventral thorax in (A) *Holomenopon* with mesosternal and metasternal plates, first coxa and third femur (*f 3*) (all shaded), (B) *Therodoxus* with first coxa (*c 1*), mesosternal plate (*mes p*) and metasternal plate (*met p*) (shaded) and (C) *Menacanthus*, first coxa. (D) – (G): ventral aspect of the third femur in (D) *Boopia*, (E) *Ricinus*, (F) *Machaerilaemus* and (G) *Laemobothrion* showing large patch of microtrichia (with detail). (H) – (J): metanotal legs of (H) outgroup taxon *Liposcelis*, (I) *Comatomenopon* with euplantula of first tarsus (*e 1*) and submarginal tibial setal row (*s*) indicated and (J) *Paraheterodoxus*. Characters illustrated are described in Appendix 2.

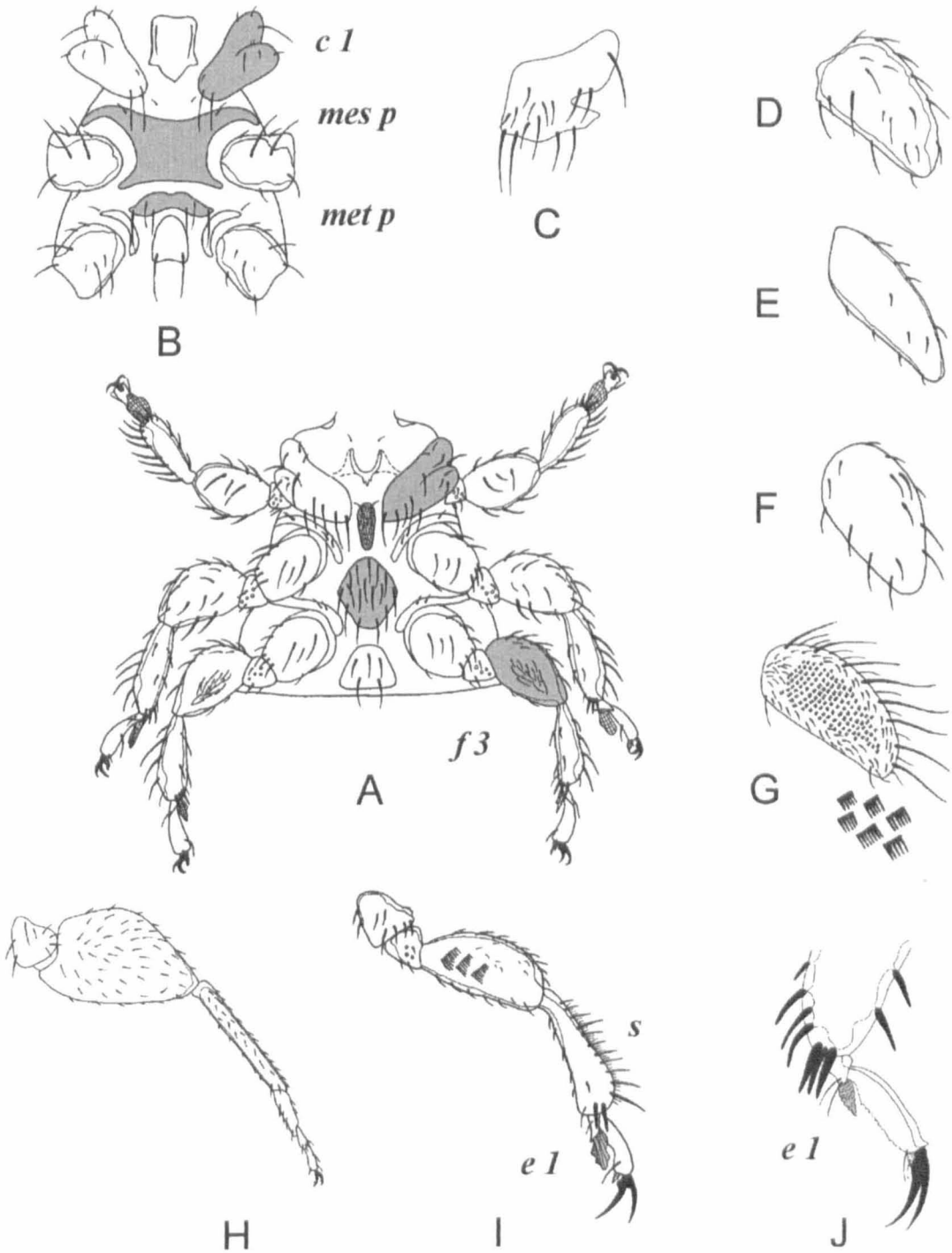

c 1
mes p
met p
B
C
D
E
F
G
f 3
A
H
s
e 1
I
e 1
J

Fig. 14: The dorsal abdomen of *Trochiliphagus* showing lateral tergal thickening (shaded). Characters illustrated are described in Appendix 2.

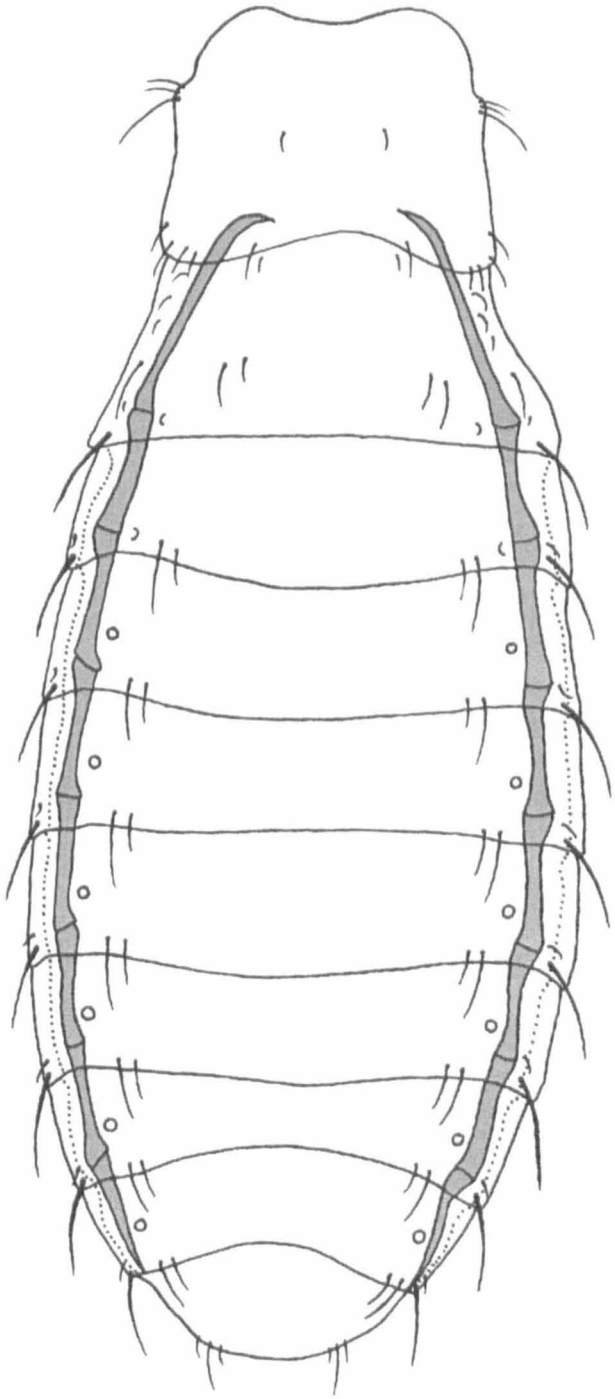


Fig. 15: Characters of the dorsal and sternal abdomen with spiracles (shaded) in (A) *Amyrsidea* with fifth sternite (*st 5*), sixth lateral plate (*lp 6*) and third tergite (*t 3*) (all shaded), (B) *Psittacomenopon*, tergites 4 - 6, (C) position of seta "a", commonly found on tergites 1 - 2, (D) relationship between post-spiracular seta "c" and lateral seta "b", (E) *Meromenopon*, fourth tergite, (F) *Latumcephalum*, fourth tergite, (G) *Paraheterodoxus*, third and fourth tergite, with *t 4* indicated, (H) *Ciconiphilus*, third sternite and lateral plate and (I) *Pseudomenopon*, lateral plates (*lp*) 2 - 4. Characters illustrated are described in Appendix 2.

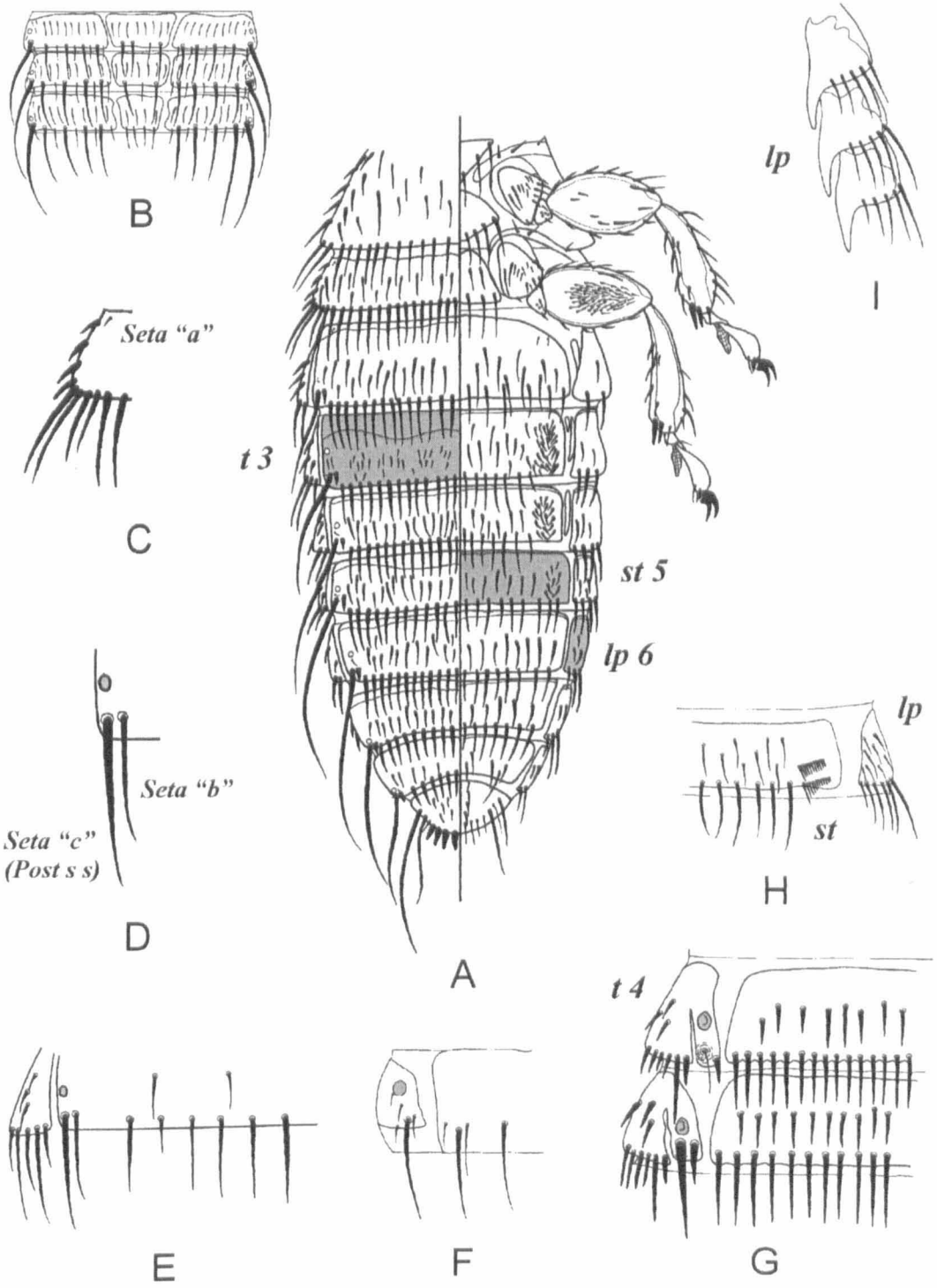

B
Seta "a"
C
t 3
st 5
lp 6
A
lp
I
lp
st
H
Seta "b"
Seta "c"
(Post s s)
D
t 4
E
F
G

Fig. 16: Female terminalia of (A) *Boopia* with gonapophyses (g), (B) *Osborniella*, (C) *Ancistrona* and (D) *Numidicola*. Characters illustrated are described in Appendix 2.

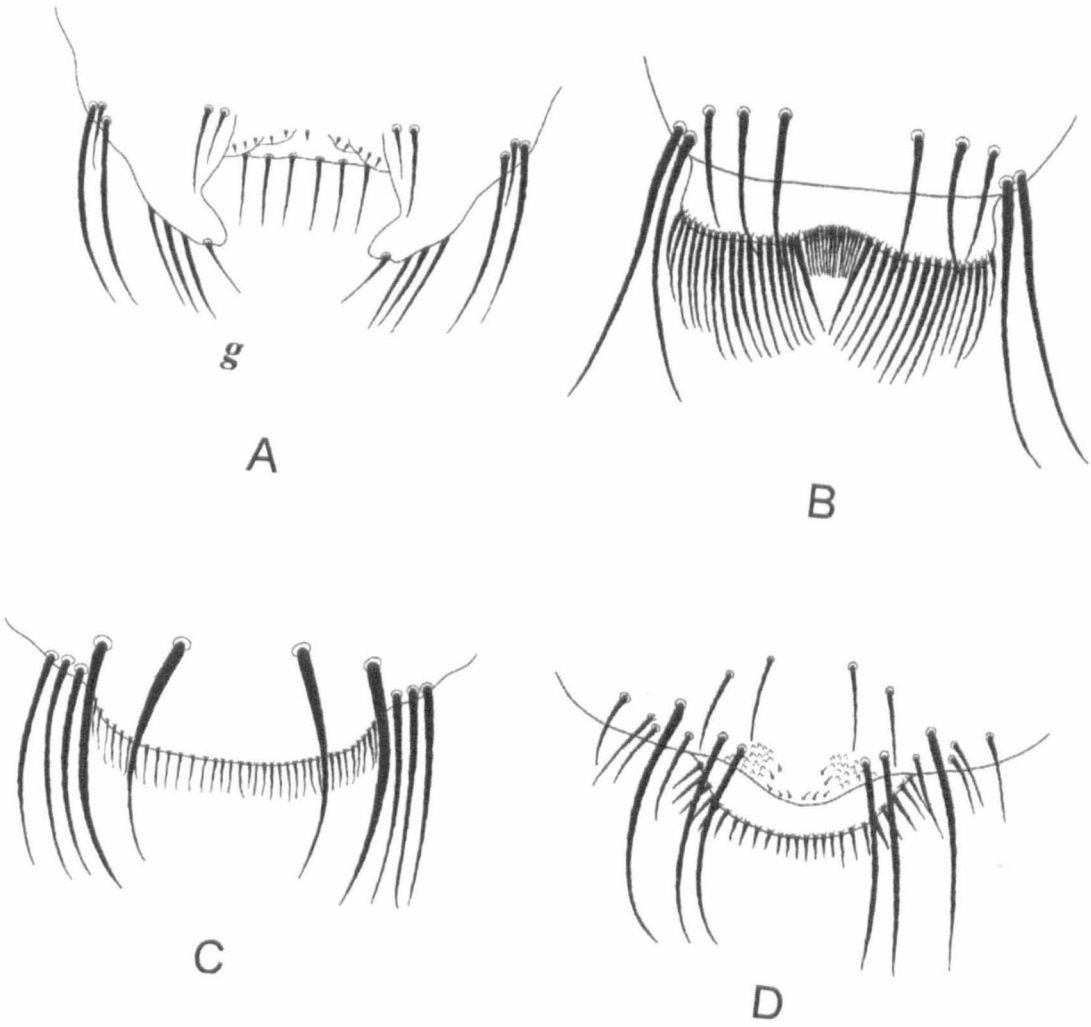
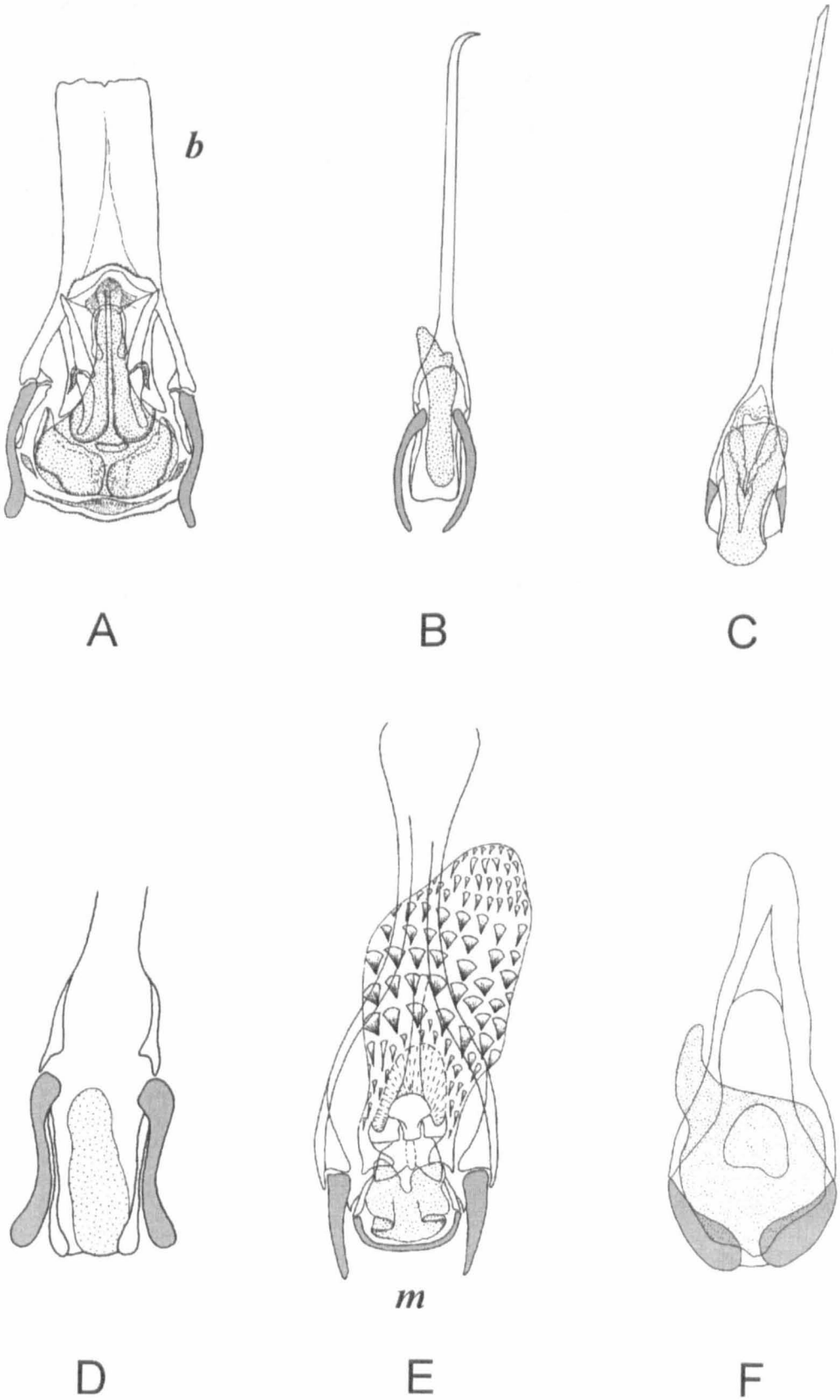

g
A
B
C
D

Fig. 17: Male genitalia with parameres (shaded) in (A) *Chapinia*, with basal apodome (*b*) indicated, (B) *Plegadiphilus*, (C) *Colpocephalum*, (D) *Menopon*, (E) *Latumcephalum* showing mesosomal arch (*m*) (shaded) and (F) *Ricinus*. Characters illustrated are described in Appendix 2.


b
A
B
C
m
D
E
F

2.8: Appendix 1: Taxa included in the cladistic analysis. Type species for the genera examined and their type host species are denoted by a superscript ^{T}, with species authority given for each taxon studied. Abbreviations: Brit. Mus. refers to the British Museum of Natural History accession number, coll. refers to collection.

Taxon	Host Taxon	Material examined
Actornithophilus uniseriatus ^{T} (Piaget, 1880)	ex- *Recurvirostra avosetta*	9 adult ♀, 2 adult ♂ (Brit. Mus. 1962 - 127 [2 slides], Meinertzhagen coll. #4391, #8024, #11011).
Amyrsidea ventralis ^{T} (Nitzsch, 1866)	ex- *Argusianus argus* ^{T}	6 adult ♀, 4 adult ♂ (Brit. Mus. #1970 - 224 [2 slides], #1964 - 163, Meinertzhagen coll. #10889).
Ancistrona vagelli ^{T} (Fabricius, 1787)	ex- *Fulmarus glacialis* ^{T} ex- *Daption capense*	7 adult ♀ (Brit. Mus. # 1959 - 419, Meinertzhagen coll. #11402). 2 adult ♀, 1 adult ♂ (Brit. Mus. #1970 - 208, #1974 -278 & 1 slide unnumbered).
Ardeiphilus trochioxus ^{T} (Burmeister, 1838)	ex- *Botaurus stellarus* ^{T}	9 adult ♀, 5 adult ♂ (Brit. Mus. #1960 - 265, Meinertzhagen coll. #3832, Hopkins coll. [unnumbered] & 1 slide unnumbered).
Austromenopon crocatum ^{T} (Nitzsch, 1866)	ex- *Numenius a. arquata* ^{T}	12 adult ♀, 2 adult ♂ (Meinertzhagen coll. #289 [2 slides], #16685, Hopkins coll. [unnumbered], Waterston coll. [BM 1930 - 232, 2 slides]).
Bonomiella columbae Emerson, 1957 Type species: *B. insolitunguicolata* (Conci, 1942)	ex- *Columba livia* ^{T}	7 adult ♀, (Brit. Mus. #1981 - 171 [7 slides], #1966 - 653).
Boopia tarsata ^{T} Piaget, 1880	ex- *Vombatus ursinus* ^{T}	4 adult ♀, 2 adult ♂ (Brit. Mus. #1971 - 470, #1976 - 469).
Chapinia robusta ^{T} Ewing, 1927	ex- *Ceratogymna atrata* ^{T}	5 adult ♀, 3 adult ♂, 1⊙ (3 slides unnumbered).
Ciconiphilus quadripustulatus ^{T} (Burmeister, 1838)	ex- *Ciconia c. ciconia* ^{T}	11 adult ♀, 8 adult ♂ (Brit. Mus. #1957 - 434, Meinertzhagen coll. #1122, #7857, #20514).

Colimenopon urocolius [T] (Bedford, 1930)	ex- *Colius indicus* [T]	2 adult ♀, 3 adult ♂, 4 ⊙ (Brit. Mus. #1954 - 474, #1958 - 76 [3 slides], Meinertzhagen coll. #3872).
Colpocephalum zebra (Burmeister, 1838) Type species: *sub judice*	ex- *Ciconia ciconia* [T]	4 adult ♀, 7 adult ♂ (Brit. Mus. 1954 - 474, Meinertzhagen coll. #14820, #20184 [BM 1953 - 225]).
Comatomenopon elbeli Emerson, 1958	ex- *Ardea p. purpurea* [T]	2 adult ♀, 2 adult ♂ (Meinertzhagen coll. #7581/7582 [2 slides - paratypes]).
Comatomenopon elongatum [T] Uchida, 1920	ex- *Egretta garzetta gularis* Type host: *Sterna sinensis*	2 adult ♀ (unnumbered).
Cuculiphilus fasciatus [T] (Scopoli, 1763)	ex- *Cuculus c. canorus* [T]	5 adult ♀, 2 adult ♂ (Brit. Mus. #1954 - 137, #1964 - 126, #1971 - 257, Hopkins coll. [unnumbered]).
Dennyus hirundinis [T] (Linnaeus, 1761)	ex- *Apus apus* [T]	4 adult ♀, 2 adult ♂ (Brit. Mus. #1955 - 735, #1957 – 571 & 2 slides unnumbered).
Eidmanniella pellucida [T] (Rudow, 1869)	ex- *Phalacrocorax carbo* [T]	5 adult ♀, 2 adult ♂ (Meinertzhagen coll. #1325, #11581, #20552, Waterston coll. [BM 1930 - 232], Morison coll. [unnumbered]).
Eomenopon denticulatum [T] Harrison, 1915	ex- *Trichoglossus haematodus* [T]	5 adult ♀, 4 adult ♂ (Brit. Mus. #1972 - 578 [2 slides], Thomson coll. [5 slides unnumbered]).
Gruimenopon longum [T] (Giebel, 1874)	ex- *Grus grus* [T]	8 adult ♀, 4 adult ♂ (Meinertzhagen coll. #1164 [4 slides - neoparatypes]).
Hoazineus armiferus [T] (Kellogg, 1909)	ex- *Opisthocomus hoazin* [T]	5 adult ♀, 6 adult ♂ (Brit. Mus. #1961 - 188 [3 slides], #1975 - 308, Meinertzhagen coll. #12612).
Hohorstiella lata [T] (Piaget, 1880)	ex- *Columba liva* [T]	5 adult ♀, 1 adult ♂ (Brit. Mus. #1968 - 384, Hopkins coll. [unnumbered]).
Holomenopon brevithoracicum (Piaget, 1880) Type species: *H. albofasciatum* (Piaget, 1880)	ex- *Cygnus melancoriphus* [T]	17 adult ♀, 9 adult ♂ (Meinertzhagen coll. #13436).

Laemobothrion maximum [T] (Scopoli, 1763)	ex- *Buteo buteo* [T]	2 adult ♀, 2 adult ♂ (Brit. Mus. #1959 - 234, Meinertzhagen coll. #19743).
Latumcephalum lesouefi Harrison & Johnston, 1916	ex- *Wallabia bicolor* [T] ex- *Macropus ualabatus*	1 adult ♀, 2 adult ♂ (Brit. Mus. #1962 - 186 [2 slides]). 1 adult ♀ [unnumbered].
Latumcephalum macropus [T] (Le Souëf, 1902)	ex- *Wallabia bicolor* Type host: *Macropus dorsalis*	1 adult ♀ (Brit. Mus. #1962 - 677).
Machaerilaemus laticorpus (Carriker, 1903)	ex- *Euphagus carolinus* Type host: *Thamnophilus doliatus*	2 adult ♀ (Brit. Mus. #1933 - 615 [2 slides]).
Machaerilaemus latifrons [T] Harrison, 1915	ex- *Poephila gouldiae* [T] ex- *Poephila mirabilis*	1 adult ♂ (Brit. Mus. #1980 - 40). 3 adult ♀ (Brit. Mus. #1980 - 40 [3 slides]).
Menacanthus stramineus (Nitzsch, 1818) Type species: *M. robustus* (Kellogg, 1896)	ex- *Gallus domesticus*[T]	5 adult ♀, 4 adult ♂ (Brit. Mus. #1955 - 351, Thomson coll. [5 slides unnumbered], & 2 slides [unnumbered]).
Menopon gallinae [T] (Linnaeus, 1758)	ex- *Gallus domesticus* [T]	5 adult ♀, 4 adult ♂ (Brit. Mus. #1956 - 117, #1958 - 660, #1967 - 739, #1980 - 40).
Meromenopon meropis [T] Clay and Meinertzhagen, 1941	ex- *Merops apiaster* [T]	5 adult ♀, 5 adult ♂ (Brit. Mus. #1950 - 389, #1966 - 241, Hopkins coll. [unnumbered]).
Myrsidea victrix [T] Waterston, 1915	ex- *Ramphastos tocard* [T] ex- *Ramphastos ambiguus*	2 adult ♀, 2 adult ♂ (Brit. Mus. #1914 - 535 [2 slides - paratypes], Hopkins coll. [unnumbered - paratype]). 1 adult ♀, 1 adult ♂ (Brit. Mus. #1968 - 86).
Neomenopon pteroclurus [T] Bedford, 1920	ex- *Pterocles alchata* Type host: *P. namaqua*	4 ⊙ (Brit. Mus. #1928 - 327 [4 slides]).
Numidicola antennatus [T] (Kellogg & Paine, 1911)	ex- *Numida meleagris* [T]	5 adult ♀, 2 adult ♂ (Brit. Mus. #1953 - 89 [2 slides], #1955 - 229 [5 slides]).
Odoriphila clayae Tendeiro, 1960	ex- *Phoeniculus purpureus* [T]	1 adult ♀(unnumbered).

Odoriphila phoeniculi [T] Clay & Meinertzhagen, 1941	ex- *Phoeniculus bollei jacksoni* [T]	13 adult ♀, 9 adult ♂ (c).
Osborniella crotophagae [T] (Stafford, 1943)	ex- *Crotophaga ani* [T]	6 adult ♀, 6 adult ♂ (Brit. Mus. #1961 - 188 [2 slides], #1975 - 308, Hopkins coll. [unnumbered]).
Paraboopia flava [T] Werneck & Thompson, 1940	ex- *Macropus robustus* [T]	2 adult ♀, 2 adult ♂ (Brit. Mus. #1962 - 677 [3 slides - paratype, lecotype], #1981 - 142).
Paraheterodoxus insignis [T] Harrison & Johnston, 1916	ex- *Aepyrymnus rufescens* [T]	2 adult ♀, 2 adult ♂ (Brit. Mus. #1962 - 186 [2 slides]).
Piagetiella bursaepelecani [T] (Perry, 1876)	ex- *Pelecanus occidentalis* [T]	8 adult ♀, 7 adult♂ (Brit. Mus. #1953 - 63, #1963 - 351, #1973 - 270, Meinertzhagen coll. #12850).
Plegadiphilus threskiornis [T] Bedford, 1939	ex- *Threskiornis aethiopicus* [T]	10 adult ♀, 12 adult ♂ (Brit. Mus. #1965 – 526, Meinertzhagen coll. #7218.7219, Hopkins coll. [unnumbered]).
Pseudomenopon pilosum [T] (Scopoli, 1763)	ex- *Fulica atra* [T]	6 adult ♀, 5 adult ♂ (Brit. Mus. #1969 - 595 [2 slides], #1980 - 40, Meinertzhagen coll. #2942, #10510 [neoparatypes]).
Psittacomenopon poicephalus [T] (Bedford, 1920)	ex- *Poicephalus meyeri* [T]	16 adult ♀, 11 adult ♂, 1 ⊙ (Brit. Mus. #1954 - 507, #1957 - 219, Hopkins coll. [unnumbered]).
Rediella mirabilis [T] Hopkins, 1948	ex- *Glareola ocularis* [T]	1 adult ♀, 2 adult ♂ (Meinertzhagen. coll. #16660, Hopkins coll. #paratype "6").
Ricinus fringillae [T] (De Geer, 1778)	ex- *Emberiza schoeniclus* Type host: *Emberiza citrinella*	2 adult ♀, 1 adult ♂ [3 slides unnumbered].
Somaphantus lusius [T] Paine, 1914	ex- *Numida meleagris* [T]	5 adult ♀, 7 adult ♂ (Brit. Mus. #1955 - 229 [2 slides], #1954 - 474, #1980 - 40, Hopkins coll. [unnumbered]).
Therodoxus oweni [T] Clay, 1971	ex- *Casuarius casuarius* [T]	2 adult ♀, 2 adult ♂ (Brit. Mus. #1972 - 222 [2 slides]).
Trinoton anserinum [T] (Fabricius, 1805)	ex- *Anser anser* [T] ex- *Cygnus olor* ex- *Plectropterus gambiensis*	2 adult ♀, 1 adult ♂ (Meinertzhagen coll. #19758 (BM #1952 - 143), #20222 (BM 1953 - 658) & 1 slide unnumbered). 1 adult ♀, 2 adult ♂ (Brit. Mus. #1965 - 223, #1972 - 221 [2 slides]). 2 adult ♀ (Brit. Mus. #1980 - 40 [2 slides]).

Trochiloecetes rupununi Carriker, 1963 Type species: *T. prominens* (Kellogg & Chapman, 1899)	ex- *Phaethornis superciliosis* [T]	2 adult ♀, 2 adult ♂ (Brit. Mus. #1970 - 726 [2 slides]).
Trochiliphagus abdominalis [T] Carriker, 1960	ex- *Anthracothorax nigricollis* [T]	2 adult ♀, 1 adult ♂, 3 ⊙ (Brit. Mus. #1961 - 606 [3 slides]).
Liposcelis bostrychophilus Badonnel, 1931	ex- *jar of rice, London* ex- *"household", Cornwall, England*	12 adult ♀ (Ref: 16/81). 9 adult ♀ (Ref: 83/83).

2.9: Appendix 2: Characters and comments

2.9.1: Characters of the Head

2.9.1.1: Dorsal head setae

The dorsal head setae (DHS) *sensu* (Clay, 1969) are paired setae of the mid and posterior dorsal head (Fig. 6). They are numbered DHS 8-31 and extend from the preocular margin, down through the midline of the head to the posterior occipital margin and around the temple. Most genera within the Menoponidae have the full complement of setae, but some setal subgroups are absent within other families. The most anterior head setae are not included in this analysis as they can be present or absent between species of the same genus and even sexually dimorphic (Clay, 1969). Setal development is also variable at different taxonomic levels and is not easily grouped into a number of developmental types. For this analysis, they can only be divided into macro and microsetae.

a) Preocular setae (sensu Clay, 1969): DHS 8 -11

There are four setae in this group, which are located on the preocular margin. These setae are absent in *Trochiloecetes* (Ricinidae) and *Laemobothrion* (Laemobothriidae). *Laemobothrion* has 8-10 very robust setae on the anterior of the preocular margin in place of DHS 8 & 9 and a patch of 4-5 setae where DHS 10 & 11 might be expected.

1. DHS 8: (0) absent (Fig. 6F); (1) present (Fig. 6A-E).

This seta is usually quite poorly developed but it is long and quite robust in *Somaphantus* and *Numidicola* (Menoponidae).

2. DHS 9: (0) absent (Fig. 6F); (1) present (Fig. 6A-E).

This is usually the most developed seta of this group and easily identified. It may sometimes be as long as some of the more developed temple setae.

3. Position of DHS 9 (where present): (0) marginal (Fig. 6A, C-E); (1) submarginal and separate from other preocular setae (Fig. 6B).

In the most of the taxa studied, this seta is marginally located (in line with the other preocular setae) but in some boopiid genera (*Boopia*, *Latumcephalum* and *Paraboopia*) it is noticeably submarginal.

4. DHS 10: (0) absent (Fig. 6F); (1) present (Fig. 6A, C-E).

5. DHS 11: (0) absent (Fig. 6F); (1) present (Fig. 6A, C-E).

Meromenopon Clay and Meinertzhagen (Menoponidae) is unusual in that it has two copies of this seta on either side of the head.

b) Dorsal setae (sensu Price & Beer, 1963), or setal complex (sensu Clay, 1969): DHS 14-16

DHS 14 & 15 are usually grouped closely together with DHS 16 lying medially to this pair. DHS 14 is usually less developed and its position in relation to DHS 15 varies from directly anterior (e.g. *Dennyus*) to medial (e.g. *Chapinia*), with most taxa somewhere in between, making this unsuitable as a character state. DHS 16 may be closely associated with DHS 14 & 15 as in *Pseudomenopon* or situated far towards the midline of the head (e.g. *Amyrsidea*). Within the Ricinidae this setal group is absent in the hummingbird

(Trochilidae) lice, *Trochiliphagus* and *Trochiloecetes*, although DHS 15 is present in the passeriform-infesting *Ricinus*.

6. DHS 14: (0) absent (Fig. 6F); (1) present (Fig. 6A).

7. DHS 15: (0) absent (Fig. 6F); (1) present (Fig. 6A).

8. DHS 16: (0) absent (Fig. 6F); (1) present (Fig. 6A).

c) Mid-dorsal head setae (sensu Clay, 1969): DHS 17-18

These setae are found in a part of the dorsal head, which tends to be over the site of the internal tentorium. DHS 18 is lateral to DHS 17, and its position usually corresponds to the width of the tentorial bridge. These setae may be widely spaced and one (or both) may be very small, which means they can be difficult to see in some genera (e.g., *Psittacomenopon* Bedford, *Gruimenopon,* and *Ancistrona*).

9. DHS 17: (0) absent (Fig. 6F); (1) present (Fig. 6A).

10. DHS 18: (0) absent (Fig. 6F); (1) present (Fig. 6A).

d) Ocular setae (sensu Clay, 1969): DHS 19 -20

DHS 19 marks the division (or former site of the division) of the two ommatidia on each side of the head (Clay, 1969). Kéler (1971) also figured this setae for the Boopiidae. There is extensive variation in the development of the amblyceran eye (Wundrig, 1936) and the condition ranges from ommatidia with well-developed biconvex lenses (e.g. *Plegadiphilus* Bedford) to those with no lens at all (e.g. *Amyrsidea*).

DHS 20 is located on the ocular margin, either marginal or slightly submarginal. It is usually much smaller than DHS 19 and may be difficult to

see, although it is quite developed in *Austromenopon* Bedford. *Trochiloecetes* (Ricinidae) has a patch of setae at this site.

11. DHS 19: (0) absent (Fig. 6F); (1) present (Fig. 6A, C-E).

12. DHS 20: (0) absent (Fig. 6F); (1) present (Fig. 6A, C-E).

e) Occipital setae (sensu Clay, 1969): DHS 21-22

These setae are normally long and well developed setae, which emanate from the posterior head margin. They are unusually small and fine in *Colpocephalum* and absent in the Ricinidae. Kéler (1971, p112: fig 100) labels the dorsal head "frontal setae" of *Boopia tarsata* Piaget as 1-2. In my opinion these setae represent DHS 21 and DHS 22, respectively, the reasons for which are discussed below.

13. DHS 21: (0) absent (Fig. 6F); (1) present (Fig. 6A, C-E).

14. DHS 22: (0) absent (Fig. 6F); (1) present (Fig. 6A, C-E).

f) Temple setae (sensu Clay, 1969): DHS 23-31

These setae continue on from the occipital setae, running towards the anterior temples. Kéler (1971, p112: fig 100) numbers some of the temple setae in *Boopia tarsata*. However, I have found that the setal pattern in the Boopiidae almost mirrors that of the Menoponidae, allowing the confident proposal of homologies using the more extensive numbering system set out by (Clay, 1969). Kéler's (1971) "frontal setae 3" therefore represents DHS 23. The identity of his remaining temple is as follows: Seta 2 = DHS 25, Seta 1 = DHS 29, Seta 3 = DHS 30. Clay (1981) has since used this numbering system in the description of new species from this family.

Absent in the Ricinidae, *Trochiloecetes* has a patch of approximately six setae around the area of the posterior lateral temple margin while *Ricinus* and *Trochiliphagus* have about three to four setae.

15. DHS 23: (0) absent (Fig. 6F); (1) present (Fig. 6A, C-E).

This seta is absent in *Myrsidea* and *Rediella* (Menoponidae).

16. Position of DHS 23 (where present): (0) near DHS 22 (Fig. 6A, D-E); (1) sited far across the temple into the parietal area (Fig. 6C).

Clay (1969) states that DHS 23 may be anterior to DHS 22 (e.g. *Gruimenopon*), lateroanterior (e.g. *Cuculiphilus*) or in a straight line (e.g. *Psittacomenopon*). However, in many of the taxa where DHS 23 is far removed from DHS 22, it is very difficult to assign such character states. This is especially the case regarding taxa where the line of the temples has the tendency to run slightly backwards. Proximity to the occipital seta is a more conservative coding for the position of DHS 23.

17. DHS 24: (0) absent (Fig. 6F); (1) present (Fig. 6A, C-E).

18. Development of DHS 24 (where present): (0) macroseta. Well developed with distinct large alveoli, usually very robust and if long becomes finer distally until a very fine point, often reaching to the transverse pronotal carina (Fig. 6D); (1) microseta. Noticeably less developed than other head seta. May appear as fine and short, small and peg-like or so small as to appear as a micro dot setae (Fig. 6A, C, E).

19. DHS 25: (0) absent (Fig. 6F); (1) present (Fig. 6A, C-E).

20. Development of DHS 25 (where present): (0) macroseta. Well developed with distinct large alveoli, usually quite robust and if long becomes finer distally until a very fine point, often reaching to the transverse pronotal carina

(Fig. 6E); (1) microseta. Noticeably less developed than other head setae. Fine and short, small and peg-like or micro dot seta (Fig. 6A, C-D).

21. DHS 26: (0) absent (Fig. 6F); (1) present (Fig. 6A, C-E).

22. Development of DHS 26 (where present): (0) macroseta. Moderately to well developed with distinct large alveoli, robust and if long becomes finer distally until a very fine point (Fig. 6A); (1) microseta. Noticeably less developed than other head setae. Fine and short, small and peg-like or micro dot seta (Fig. 6C-E).

23. DHS 27: (0) absent (Fig. 6F); (1) present (Fig. 6A, C-E).

Where present, this is always a large and well-developed setae, which is easily identified. The position of DHS 27 aids in the identification of other temple setae.

24. Alveoli of DHS 26 & 27 (where present): (0) alveoli separate (Fig. 6A, E); (1) alveoli contiguous (Fig. 6C-D).

Clay (1969) discusses the alveoli of these setae in conjunction with the condition of DHS 26 (Character 22). Commonly, when the alveoli are separate, both DHS 26 and DHS 27 setae are long and robust. When the alveoli are contiguous, DHS 26 is reduced (with varying extent) towards a fine microseta, a condition which is generally (although not always) the case. In some taxa the alveoli are separate but DHS 26 is poorly developed.

25. DHS 28: (0) absent (Fig. 6F); (1) present (Fig. 6A, C-E).

Where present this seta is always somewhat reduced in comparison with the macrosetae of the temple. It is usually a small microseta.

26. DHS 29: (0) absent (Fig. 6F); (1) present (Fig. 6A, C-E).

As in DHS 27, this seta is always well-developed setae and easily identified. Its position aids in the identification of DHS 30.

27. DHS 30: (0) absent (Fig. 6F); (1) present (Fig. 6A, C-E).

This seta is usually associated with DHS 29, either directly medially submarginal (e.g. *Somaphantus*), anteriorly submarginal (e.g. *Pseudomenopon*) or directly anterior on the temple margin (e.g. *Amyrsidea*). However, due to a high level of grading between these suggested states for setal position, I am unable to explore this character further.

28. DHS 31: (0) absent (Fig. 6F); (1) present (Fig. 6A, C-E).

Clay (1969) wrote, "...one of the setae anterior to DHS 30 (here called DHS 31) may be long and stout". As there is always at least one short setae (commonly two), between DHS 30 and the next macroseta, DHS 31 is interpreted as being the first macroseta after the DHS 29 and DHS 30 group.

29. Six setae (three pairs) down the midline of the dorsal head: (0) absent (Fig. 6A); (1) present (Fig. 6F).

These setae are peculiar to members of the Ricinidae. Nelson (1972) assigned a large number of chateotaxic labels in his revision of *Ricinus*, in a work that mirrored Clay's (1969) treatment of the Menoponidae. Nelson called these central dorsal head setae the "d series" (d1, d2 and d3) and laid down terminology later followed by workers describing species from other ricinid genera (e.g. Oniki, 1995).

2.9.1.2: Dorsal head sensilla (sensu Clay, 1969)

Previously numbered as 1-5 (Clay, 1961), the dorsal head sensilla were re-labelled when a numerical system was applied to the dorsal head setae.

30. Sensillum "a": (0) absent (Fig. 6B, F); (1) present (Fig. 6A, C-E).

This sensillum can be difficult to find as it is located between DHS 8 and DHS 9, which are often very close and can also be marginal. It is present only in some of the Menoponidae, including, in particular, all those genera that are restricted to galliform hosts.

31. Sensillum "b": (0) absent (Fig. 6B, F); (1) present (Fig. 6A, C-E).

This sensillum is usually just posterior to DHS 9, but in some cases (e.g. *Somaphantus*) it appears more associated with DHS 10.

32. Sensillum "c": (0) absent (Fig. 6F); (1) present (Fig. 6A).

Sensillum "c" is associated with DHS 14 &15. In most instances it is situated posterior, or lateroposterior, to DHS 15, although in *Menopon* and *Numidicola* it appears more associated with DHS 14.

33. Sensillum "d": (0) absent (Fig. 6F); (1) present (Fig. 6A).

Where present this sensillum is found close to DHS 16.

34. Sensillum "e": (0) absent (Fig. 6F); (1) present (Fig. 6A).

Sensillum "e" is associated with DHS 17. In this assemblage of taxa, it was present only in *Holomenopon* Eichler.

2.9.1.3: Dorsal head shape

35. Preocular feature of dorsolateral head margin: (0) no feature, unbroken margin (Fig. 6D-F); (1) notch (Fig. 6C); (2) slit (Fig. 6A-B).

This character is first described by Clay (1947), in her preliminary key for the Menoponidae, with some reservation regarding consistency within some isolated genera but later proposed as a useful generic character in the later, revised publication (Clay, 1969). Occasionally, it is difficult to discern a wide

slit from a notch, so I have followed the definitions set out by Clay (1947) and consulted the original generic alpha-taxonomic publications where appropriate. I have observed no consistency in the form of the preocular feature, within either the *Colpocephalum* or *Menacanthus* generic complexes. Notably, members of the Boopiidae, all posses a preocular slit.

36. Dorsolateral head junction with temple margin: (0) smooth line junction from dorsolateral margin through ocular margin to anterior temple margin (Fig. 6E-F); (1) ocular margin pronounced, but does not overlap anterior temple margin (Fig. 6C); (2) ocular margin overlaps anterior temple margin (Fig. 6A); (3) ocular margin and temple margin overlap dorsolateral margin (Fig. 6D).

Colimenopon is an unusual genus in that the ocular and temple margins overlap the dorsolateral margin.

2.9.1.4: Internal head

37. Form of the Tentorial bridge: (0) thick or with little reduction (Fig. 7A); (1) reduced and narrow (Fig. 7D); (2) reduced and wide (Fig. 7C); (3) reduced to a fine ligament (Fig. 7B).

The tentorium is a chitinous endoskeleton of the head, for the attachment of muscles for the mouthparts, antennae and oesophagus. In the Amblycera, it comprises of a pair of anterior arms linked by a bridge of hollow chitin. Nelson (1972) refers to the tentorial "bar" in his review of *Ricinus* (Ricinidae).

Symmons (1952) described four forms of amblyceran tentorium: a generalized robust shape similar to that in the Psocoptera and three forms of reduced bridge ranging down to a fine membranous ligament. She conceded

from her groupings, that there may be a degree of parallel reduction in bridge sclerotisation, between some menoponid genera and those of other families. Most menoponids have a thick or partially reduced bridge (Symmons' groups 1 & 2). In other menoponid genera this is reduced to a rod like shape, which is either narrow or wide relative to the width of the head. It is represented only as a fine ligament in the Boopiidae, Ricinidae (Nelson, 1972) and Laemobothriidae (Symmons' groups 3a and 4).

2.9.1.5: Mouthparts

38. *Mouthparts:* (0) developed chewing mandibles (Fig. 8A-B); (1) mandibles reduced with mouthpart structures modified into hollow stylets for piercing (Fig. 8D).

The mandibles are generally similar in the Amblycera, but within the Ricinidae genera exhibit varying degrees of modification. *Trochiloecetes* and *Trochiliphagus*, on hummingbirds (Trochilidae), show the most structural change. Although *Trochiloecetes* had been described by Paine and Mann (1913), no references to differences in the mouthparts were made until Clay (1949). The modifications consist of three structures of hypopharyngeal origin: a middle needle-like sucking tube originating from the sitophore sclerite, lying within a two-portioned sheath apparatus. The mandibles are minute and reduced to small cones Carriker (1960). *Ricinus* species parasitic on Passerines have "regular" mandibles but they are less sclerotised and more elongate than in other Amblycera. *Ricinus* species on hummingbirds show similar changes to the mandibles and hypopharynx but they are not so modified Clay (1949).

39. Maxillary palp: (0) 4 segmented (Fig. 8A-B, F-H); (1) less than 4 segments (Fig. 8E).

For a long period of time the amblyceran maxillary palp was misidentified. Kellogg (1896) stated quite clearly that he did not understand the maxillae and wrote there were no terminal free lobes, just a large basal part (labium) articulating with a conspicuous 4-segmented palpi. Kellogg repeatedly labelled amblyceran maxillary palps as labial palps, but in some psocopterans, he labelled them correctly. Snodgrass (1899) also assigned labial origin to these structures. However, in a later publication, Snodgrass (1944) re-examined the mouthparts and wrote "...because of the close connections of the maxillae with the labium some writers have regarded the palpi as labial organs, but a comparison with the Corrodentia leaves little question that the mallophagan palpi are maxillary".

There are generally four segments in the maxillary palp, although this has been reduced to two in *Latumcephalum* and three in *Paraboopia* (Boopiidae).

40. Maxillary palp segmentation: (0) alternately short and long (Fig. 8B); (1) first few segments similar in length (Fig. 8A, E-H).

All the amblyceran families presented here have segments of similar length in the maxillary palp.

41. Isolated subterminal setae on the distal segment of the maxillary palp: (0) absent (Fig. 8B); (1) present (Fig. 8A, E-H).

All taxa scored as present have a pair of subterminal setae, one of which is usually peg-like. *Cuculiphilus*, is unusual in that it has three setae in this group.

42. Alveoli of maxillary palp subterminal setae (where present): (0) margins separate (Fig. 8F); (1) margins contiguous (Fig. 8A, E, G-H).

Clay (1966; 1968) illustrated the contiguous alveoli of the subterminal setae in *Myrsidea*. Only three taxa, *Laemobothrion* (Laemobothriidae), *Therodoxus* (Boopiidae) and *Somaphantus* (Menoponidae) have separate alveoli in this assemblage of taxa.

43. Ventral post-palpal processes: (0) absent (Fig. 8A-B); (1) present (Fig. 8G-H).

These arise just posterior to the base of the maxillary palps and have the appearance of loose flaps. They were extensively figured at species level for *Menacanthus* by Zlotorzycka (1965) and termed "facial hooks". Uchida (1926) and Price (1975; 1977) referred to them as "ventral spinous head processes". Clay (1961; 1962; 1966) called them "sclerotised processes" or "oral spines".

44. Number of post-palpal processes (where present): (0) one (Fig. 8G); (1) two (Fig. 8H).

45. Labial palps: (0) absent; (1) present (Fig. 8A-B).

These are present as small lobes in the Amblycera. Notably, they are absent only in genera from the Ricinidae. Clay (1962) illustrates the labial palp of *Actornithophilus* Ferris.

46. Number of terminal setae on labial palpus (where present): (0) 5 (Fig. 8A, C); (1) more than 10 (Fig. 8B).

There are five terminal setae on the labial palps of all these amblyceran genera, although there may be four in other genera of Boopiidae (Clay, 1970) not included in this study.

2.9.1.6: Antennal characters

47. Antennal length: (0) long (Fig. 9H); (1) much reduced (Figs. 8A, 9A-G).

The amblyceran antennae are very short (4-5 segments) in comparison with those of *Liposcelis* (15 segments).

48. Number of flagellar segments (in the short antennae): (0) two segmented (Figs. 8A, 9B-C, F); (1) three segmented (Fig. 9A, D-E, G).

The amblyceran antennae comprises of a basal scape, pedicel and a flagellum of two or three segments, the terminal segment in some taxa being subdivided (Clay, 1969). The majority of the Menoponidae examined here have two flagellomeres, although *Rediella*, *Austromenopon* and *Cuculiphilus* have three. There are also three flagellomeres in the Ricinidae and Boopiidae.

49. Secondary annulation of flagellar segments: (0) absent (Figs. 8A, 9A-G); (1) present (Fig. 9H).

No annulation is present in the amblyceran taxa.

50. Flagellum shape: (0) filliform (Figs. 8A, 9A-B, E, H); (1) globular (Fig. 9C-D, F-G).

The antennae may have a long slender look, with a filiform shaped flagellum. This feature is found in some boopiid and menoponid genera and is particularly a characteristic of the galliform-infesting taxa (*Amyrsidea*, *Somaphantus*, *Menopon* and *Numidicola*).

51. Shape of the first flagellar segment: (0) cylindrical (Fig. 9H); (1) pedunculate (Figs. 8A, 9A-G).

The first flagellomere of the amblyceran antenna is always pedunculate or wine-glass shaped (Clay, 1969).

52. Sclerotisation of the first flagellar segment: (0) regular and complete sclerotisation (Fig. 9B-H); (1) irregularly sclerotised (Figs. 8A, 9A).

It has previously been suggested (Tendeiro, 1967) that, in some Menoponidae, the pedunculate first flagellar segment may be divided in two, due to a darker pigmentation of the segmental "stalk". However, this has been refuted by Clay's (1969) scanning electron micrographs which show no line of division. Character 52 is only concerned with the degree of sclerotisation down the flagellomere and does not consider any colour difference. A number of the taxa (e.g. *Chapinia*) do have a darker stalk but sclerotisation is still complete along the segment. However, in some genera this is not the case, giving the impression of a wide gap between stalk and "bowl". This is apparent in e.g. *Rediella* and *Somaphantus*, however Paine (1914) did not mention this in his description of the latter genus.

2.9.1.7: Ventrolateral head

53. Antennal fossae: (0) absent or poorly developed (Fig. 10A); (1) present (Figs. 8A, 10B-E).

The antennal fossae, where present, are located behind the ventrolateral head margin. They are absent in *Rediella* and *Somaphantus*.

54. Form of the antennal fossae (where present): (0) long and shallow (Fig. 10B); (1) short, very deep and pouch-like (Fig. 10C); (2) short and shallow (Fig. 8A); (3) short and deep, capable of containing the antennae (Fig. 10D-E).

Most commonly, the antennal fossae are short and shallow or short and deep, although the long and shallow state is found in both the Menoponidae

and Boopiidae. In a few menoponids (e.g. *Colimenopon*), it has the appearance of a deep pouch.

55. Setae at the anterior termination of the ventrolateral head margin: (0) one long, one short (Figs. 8A, 10A-E, K); (1) two short and stout (Fig. 10G); (2) three all short (Fig. 10H); (3) one short and stout (Fig. 10I); (4) two long (Fig. 10J).

The Boopiidae and Menoponidae have two setae (one long, one short) at this site (Clay, 1969), however both setae are well developed in *Chapinia*. *Laemobothrion* has three, all short. In the Ricinidae, there may be one or two very stout setae.

56. Presence of a well-developed and compact setal comb row lining the subocular head margin: (0) absent; (1) present at least posteriorly (Figs. 8A, 10A-E, K).

In most of the Menoponidae, the comb row is present, running posteriorly down the subocular margin towards the junction with the ventral temple margin. In *Machaerilaemus* (Harrison, 1915) and *Ancistrona* there are just a few setae spaced out along this edge. In *Ancistrona* there is also a row of fine setae on the underside of the dorsolateral head which should not be confused with the comb row (Clay, 1969).

57. Isolated subocular setae anterior to the comb row (where present): (0) subocular seta not isolated from comb row or anterior setae (where present) (Fig. 10C); (1) present (Figs. 8A, 10 A-B, D-E, K).

The comb row setae have their alveoli very close together. Anterior to this, along the margin is the subocular seta, which is quite well developed and usually isolated. Between the subocular seta and the comb row, there may be

some additional widely spaced setae, which (Clay, 1969) termed group "s" setae (additional subocular setae) but are referred to here as anterior setae.

58. Subocular seta (where present): (0) normal seta (Figs. 8A, 10A-E); (1) flattened (Fig.10K).

The flattened condition is peculiar to four menoponids: *Eidmanniella* Kéler, *Austromenopon*, *Plegadiphilus* and *Meromenopon* (in which the seta is also flanged) (Clay, 1969).

59. Anterior setae of comb row (where present): (0) absent (Figs. 8A, 10E); (1) present (Fig. 10A-D, K).

60. Continuity between the setae of the subocular comb row and the anterior marginal temporal setae: (0) setal groups are continuous (Fig. 10A, C); (1) not continuous, distinctly separate or separated by the inclusion of a section of differing setae, unlike either the comb row or the marginal temporal setae (Figs. 8A, 10B, D-E).

61. Submarginal ventral temporal setae: (0) absent (Fig. 10A-C); (1) present (Figs. 8A, 10D-E).

On the ventral anterior temple between the posterior end of the comb row and the anterior marginal temple setae there is often a submarginal patch or line of setae of a differing type (Clay, 1969). This is very noticeable as they are usually finer and spikier than the setae of the comb row and markedly shorter and less developed than the anterior marginal temporal setae.

62. Sub-marginal temporal setae (where present): (0) patch or irregular row of setae. Much finer than comb row, usually extending halfway around temple (Fig. 8A); (1) weakly developed, short single row of fine setae, usually widely spaced and small in number. Does not extend far into the anterior marginal

temporal setae (Fig. 10D); (2) compact, single row of quite robust setae, extending halfway around the temple (Fig. 10E).

In *Odoriphila* and *Osborniella* it is not as developed: being less compact, less deep and also extending less into the anterior temple setae.

63. Area of sculpturing on ventral submargin of the temple: (0) absent (Fig. 10A, E); (1) present (Figs. 8A, 10B-D, F).

Many taxa, when viewed using phase contrast microscopy, have a soft scale-like topology over the entire the ventral temple. This character does not describe this condition, but relates only to the anterior of the ventral temple, around the point of the antennal fossae posterior margin.

64. Form of sculpturing on ventral submargin of the temple (where present): (0) single spikes (Fig. 10 D); (1) multi-tipped spikes (Fig. 10B); (2) fringe-like (Fig. 10F); (3) simple scales (Fig. 10C); (4) spike tipped scales (Fig. 8A).

The sculpturing present on the temple of *Laemobothrion* is a sort of comb-like, flat fringe. Perez, Granados and Ruiz (1995) in SEMs of *Laemobothrion maximum* (Scopoli), referred to this sculpturing as "cephalic ctenidia".

2.9.2: Characters of the Thorax

2.9.2.1: Dorsal thorax

65. Transverse pronotal carina: (0) absent (Fig. 11A-B); (1) present (Fig. 11C-E).

This feature is found running across, through the pronotum, at around the mid-point or less down the length of the segment. Harrison (1915) refers to

the "shoulders" of *Eomenopon* and *Machaerilaemus* and an "inter-scapular bar" joining the "scapular bands", which I assume represents the lobing of the prothorax, the extent of which is variable in amblyceran genera. Bedford (1920) also termed it as an "interscapular bar" running between the "scapulars". It is only absent in the Ricinidae and in *Rediella* (Menoponidae).

66. Posterior pronotal setal row: (0) absent; (1) incomplete (Fig. 11A); (2) complete, across the posterior prothorax (Fig. 11B-E).

In the Menoponidae and Boopiidae there is a posterior row of setae on the dorsal prothorax. *Sensu* (Clay, 1962) these setae are included in the "marginal prothoracic setae" (mps) which she labelled 1...2... etc. starting from the most anterior humeral seta. There are usually three humeral setae located at the lateroanterior angles of the segment, then a small gap followed by an evenly spaced posterior row. The Ricinidae have small patches of setae in the lateroposterior region, but the row is incomplete. In *Laemobothrion* the condition is very similar.

67. Setae medial to the lateral seta of the dorsal prothorax: (0) short (Fig. 11B, D); (1) well developed (Fig. 11C-E).

The lateral seta is a large well-developed seta roughly at the lateroposterior angles of the prothorax and is easily identified. In most genera, the lateral seta mps 4 (*sensu* Clay, 1962) is the first seta after the humeral setae. Clay's numbering system cannot be used here for two reasons: some genera have more than three humeral setae present and in a few cases the long lateral seta need not be the first seta after the humeral group. Nelson (1972) labelled the two seta at the posterolateral corners of *Ricinus* as L8 and L9. In *Comatomenopon* the lateral seta appears to be absent.

68. Postnotum at the posterior pronotum: (0) absent (Fig. 11A-B); (1) present (Fig. 11C-E).

This small usually rectangular sclerite is found behind the pronotum, projecting over the mesonotum. It was previously termed "median button" by Cope (1941) who assumed it to be the vestiges of a reduced mesonotum. In the Menoponidae, it is absent only in *Rediella* and *Numidicola* (Clay, 1969).

69. Anterior mesonotal setae: (0) absent (Fig. 11A); (1) present (Fig. 11C-E).

These are a small group of microsetae on the anterior mesonotum, around the base of the postnotum (where present).

70. Number of anterior mesonotal setae (where present): (0) 2 (Fig. 11C); (1) 4 (Fig. 11B, D-E).

There are normally four setae at this position. However, it should be noted that in some cases the setae may be very close to each other, giving a false appearance of only two setae on first observation. This is the case in *Odoriphila* (Clay, 1969).

71. Position of anterior mesonotal setae: (0) clustered around the postnotum (Fig. 11 C-E); (1) widely spaced (Fig. 11B).

Commonly the setae are arranged in a tight cluster formation on either side of the sclerite. They are widely spaced out in *Trinoton* Nitzsch, *Actornithophilus* and *Rediella.*

72. Setae borne on a rounded protuberance each side of the mesonotum: (0) absent (Fig. 11A-D); (1) present (Fig. 11E).

This character is exclusive to the Boopiidae. Omitted from the original, unillustrated description of *Heterodoxus* (Boopiidae) by Le Souëf and Bullen

(1902), this feature was later figured by Paine (1912). They were also termed "elevated warts" by Kéler (1971) and "mesonotal warts" by Clay (1981).

73. Fusion of mesonotum and metanotum: (0) independent (Fig. 11B-E); (1) fused to metanotum (Fig. 11A).

In the Boopiidae and Menoponidae the mesonotum and metanotum are independent, although the former may be much reduced (Ferris, 1916). In the Laemobothriidae and Ricinidae they are fused (Clay & Price, 1970). Nelson (1972) also describes the abdominal lateral thickening which extends up to the mesothorax in *Ricinus* (and all Ricinidae) as pleural nodi.

74. Fusion of metanotum and tergum 1: (0) independent (Fig. 11B-D); (1) metanotum fused to tergum 1 (Fig. 11A, E).

In the Ricinidae, a pterothorax exists of fused mesothorax, metathorax and first abdominal segment (Clay & Price, 1970; Nelson, 1972). Members of the Boopiidae have a free mesonotum but the metanotum is always fused to the first abdominal tergite (Clay, 1970).

75. Terminal metanotal row: (0) absent (Fig. 11A, E); (1) present (Fig. 11B-D, F).

Clay (1962) termed these setae the "marginal metanotal setae" (mms) and numbered them 1...2...etc. inwards from the lateral margin, but this system cannot be applied to all of the taxa in this study. Although the metanotum and tergum 1 are fused in the Boopiidae and Ricinidae, there are a few isolated setae (but not a row) around the area where the terminal metanotal setae might be expected. Nelson (1972) refers to the sparse ricinid setae as C3 and C4.

76. Second seta of the metanotal row (where present): (0) much shorter than outer metanotal seta, often peg-like (Fig. 11F); (1) as developed as outer metanotal seta (Fig. 11B-C); (2) absent (Fig. 11D).

This is the seta next to the outer metanotal seta. It is probably absent in *Odoriphila* as here there is a gap in the row.

2.9.2.2: Ventral thorax

a) prosternal plate

77. Development of the prosternal plate: (0) absent or too undeveloped to figure (Fig. 12A); (1) present at least posteriorly (Figs. 12B-L, 13A).

78. Marginal position of anterior setae on prosternal plate: (0) absent (Fig. 12C); (1) at or near the most anterior point of the lateral margins (Figs. 12A-B, E-F, L, 13A); (2) at or near the mid point of the anterior margin (Fig. 12D, G-K).

These are very small setae found in either of two sites on the prosternal plate. They may be sited at the lateroanterior angles or close together on the anterior margin near the midline of the plate. They are absent only in *Myrsidea*.

79. Anterior setae on prosternal plate: (0) on main body of plate (Fig. 12B, G-K); (1) detached and anterior to main body of plate (Figs. 12A, D-F, L, 13A).

Always found on the main plate in within the Laemobothriidae and Ricinidae, but in the other families the condition varies.

80. Anterior setae on prosternal plate (if detached): (0) situated on small islands of sclerotisation (Fig. 12E-F); (1) on unsclerotised areas of sternal prothorax (Figs. 12A, D, L, 13A).

In the Boopiidae the detached anterior setae are always on unsclerotised areas. Within the Menoponidae both conditions are found.

81. Anterolateral setae on prosternal plate: (0) absent (Figs. 12A, D-J, 13A); (1) present (Fig. 12B-C, K-L).

In addition to the small anterior setae there is often a pair of well-developed setae present. These are situated on the main body of the plate submarginal to the anterolateral angles. They are always present in the Boopiidae and are also found in *Dennyus* and *Myrsidea* (Menoponidae).

82. Additional setae on prosternal plate aside from the anterior setae and the anterolateral setae (where present): (0) absent (Fig. 12A, C-F, H-I, L, 13A); (1) present (Fig. 12B, G, J-K).

83. Posterior margin of prosternal plate: (0) rounded (Fig. 12E, H, K); (1) angular (Fig. 12D, L); (2) long pointed spine (Figs. 12F-G, 13A); (3) pedestal (Fig. 12B-C, J); (4) flat and square; (5) concave (Fig. 12I); (6) posterior margin absent.

Clay (1969) described state two as a "posterior process of the prosternal plate" for *Eidmanniella*. *Rediella* is unusual in that the posterior margin of the plate appears absent.

84. Well defined marginal border of prosternal plate: (0) absent (Fig. 12D, J-L); (1) present (Figs. 12B-C, E-I, 13A).

A sclerotised border around the prosternal plate is found in all the Ricinidae and the majority of the Menoponidae, which possess a defined plate.

85. Marginal border of prosternal plate (where present): (0) only lateral or lateral and anterior (Fig. 12 E, H-I); (1) lateral and posterior but not anterior (Figs. 12 F-G, 13A); (2) complete, encircling the plate (Fig. 12B-C).

The marginal border is only lateral or lateral and anterior in the Ricinidae. It is termed "lateral nodi" by Nelson (1972) in his review of *Ricinus*. Where present in the Menoponidae, the border is usually just lateral and posterior but in *Dennyus*, *Myrsidea* and *Ancistrona* the border is complete.

b) mesosternum and metasternum

86. Mesosternum type: (0) articulation of leg separated from the other side by an area without a plate (Figs. 13A, 15A); (1) articulation of leg separated from the other side by a plate; (2) mesonotum, pleura and sternum fused in a sclerotised ring around the body (Fig. 13B).

Clay (1969) highlights three forms of mesosternum. There may be a distinct mesosternal plate or an area without a plate separating the points where the legs articulate, or the mesosternum, pleura and mesonotum can be fused, forming a ring of sclerotisation around the body. *Cuculiphilus* and *Myrsidea* (Clay, 1966) have the sclerotised ring and although there has been a degree of fusion between the meso and metasternal plates of *Trinoton*, the legs are still separated.

87. Metasternal plate: (0) absent; (1) present (Figs. 13A-B, 15A).

A metasternal plate is normally present but appears to be absent in *Menopon*.

c) legs

88. Shape of the first coxa: (0) roughly spherical; (1) anteroposteriorly extended (Fig. 13A-C).

Mayer (1954) describes the antero-posteriorly extended coxa as an "elongate bladder" lying flat on the body. The first coxa is almost "v-shaped" with a rounded posterior margin and the medial superior lobe lying a bit to the right over the lateral inferior one. Although *Trinoton*, shows some antero-posterior extension to the coxa, extent of modification is noticeably less than for other genera.

89. Posterior setae of first coxa: (0) four or five setae (Fig. 13A); (1) more than five (Fig. 13C); (2) two or three (Fig. 13B).

Within the Menoponidae, there are usually four or five setae around the posterior of the first coxa. In some isolated groups of genera there may be more (Clay, 1969). This is apparent in, e.g. *Ancistrona*, *Austromenopon*, and *Eidmanniella*.

90. Shape of the third femur: (0) hugely inflated compared to femora 1 & 2 (Fig. 13H); (1) femora 3 not inflated (Figs. 13A-B, 15A).

91. Pattern of setae on the venter of the third femur: (0) many small setae dotted all over (Fig. 13H); (1) many setae above and below, but absent from the venter of the third femur (Fig. 13F); (2) many setae arranged into a central discrete patch (Figs. 13A, 15A); (3) many setae arranged into central discrete combs (Fig. 13I); (4) large patch of microtrichia (Fig. 13G); (5) fewer setae but with no evident pattern (Fig. 13D); (6) femur almost devoid of setae (Fig. 13E).

In the outgroup taxon *Liposcelis* there are many small setae evenly distributed over the ventral aspect of the third femur. All amblyceran taxa show some form of setal aggregation. The setal patch of the Menoponidae is usually quite well developed e.g. in *Dennyus* (Emerson, 1956) and *Austromenopon*

(Price & Clay, 1972) but in some taxa e.g. *Holomenopon* the setae are quite loosely packed. *Machaerilaemus* is unusual in that setae are absent from the area in question (Bedford, 1920). Setal combs are interpreted as described by Clay (1947) as a "row of short, stout setae, with the alveoli lying close together and approximately in a straight line". *Laemobothrion* has a patch but it is not composed of regular setae. It is a patch of small combs (microtrichia), which under SEM photography (Perez *et al.*, 1995) bears little resemblance to the condition found in the menoponid comb-bearing genera.

92. Number of combs on venter of the third femur: (0) two; (1) three (Fig. 13I); (2) four.

93. Dorsal tibial setae. Additional submarginal row of short setae on legs two and three: (0) absent (Figs. 13A, H, 15A); (1) present (Fig. 13I).

Some genera within the Menoponidae have a developed submarginal row of setae on the dorsal aspect, whilst the legs others are quite bare (Clay, 1969).

94. Number of tarsal segments: (0) three (Fig. 13H); (1) two (Figs. 13A, I-J, 15A).

The tarsus is always two-segmented in the Amblycera.

95. Euplantula 1: (0) absent (Fig. 13H); (1) present (Figs. 13A, I-J, 15A).

The smaller first tarsus is identified due to the possession of a pair of setae close to its distal margin. Distal to this is a pad-like lobe called the euplantula (Clay, 1969). Mjöberg (1910), described the first joint of the tarsus as short and bearing a large flap-like appendage on the inner side, whilst the second tarsus was longer with a small finger-like process, which collapsed in

balsam mounts (euplantula 2). The variable condition of this latter structure excludes its inclusion in this analysis.

96. Form of Euplantula 1: (0) horizontal and vertical banding (Figs. 13A, 15A); (1) vertical banding only (Fig. 13I); (2) serrated and globular (Fig. 13J).

Mjöberg (1910) observed the distinct horizontal and vertical banding in *Holomenopon albofasciatum* (Piaget) and the serrated sculpturing on *Boopia grandis* Piaget.

97. Claw shape: (0) claws have a protuberance proximally and are not serrated (Figs. 13A, I-J, 15A); (1) claws have one sharp tooth distally and are serrated proximally (Fig. 13H).

2.9.3: Characters of the Abdomen

2.9.3.1: Dorsal abdomen

98. Lateral tergal thickening: (0) absent (Fig. 15A-B, E-G); (1) present (Fig. 14).

Exclusive to the Ricinidae and Laemobothriidae, are two conspicuous lateral bands of sclerotisation running posteriorly through the abdomen. These may vary in degree of pigmentation but are generally darker than the regular colour of the abdomen. They are composed of segmental parts separated by diagonal sutures (Kellogg, 1896; Nelson, 1972) and are sited midway between the lateral aspect of the tergites and the lateral plates. This feature has been variously described by a number of authors. Paine and Mann (1913) refer to two pale "submarginal bands" in *Trochiloecetes*, whilst Nelson (1972) and Oniki (1995) term them "pleural nodi". Clay (1969) describes the condition as

a "continuous lateral buttress of internal tergal thickening each side". Kellogg (1896) also described two new species of menoponids as having "angular lateral bands on segment 3-8" (*Colpocephalum*) and "broad lateral bands projecting inwards"(*Menopon*). I have found no later works, (neither review papers nor alpha taxonomic descriptions) to corroborate his observations. It seems he may have been referring to the increased sclerotisation seen around the spiracle in some menoponid genera.

99. Female tergites: (0) composed of one plate (Figs. 14, 15A, E-G); (1) tripartite with narrow central plate (Fig. 15B).

The rare condition of tripartite tergites is exclusive to the Menoponidae and is present only in *Colpocephalum* (Bedford, 1940; Mjöberg, 1910; Price & Beer, 1965a) and *Psittacomenopon* where it is apparent in tergites 4 - 8 (Price & Beer, 1966).

100. Tergal posterior setal rows: (0) absent or very sparse (Fig. 14); (1) regular row of setae reaches across the tergite (*Fig. 15* E-F); (2) very well developed and compact row (Fig. 15A-B, G).

The term *posterior* is adopted here rather than marginal, as the setal rows in the Amblycera are not always on the posterior margin of the tergite (e.g. *Latumcephalum* Fig. 15F).

101. Additional anterior setae on tergite 2 (T2), at least in females (sensu Clay, 1962; Price & Beer, 1966): (0) absent (Fig. 14); (1) present (Fig. 15A).

In many of the Menoponidae and Boopiidae there are additional rows or clusters of setae anterior to the posterior tergal row. In some genera, this is more apparent in females and there appears to be some clearly distinct patterns of anterior setal distribution. Condensing these patterns into one character for

the whole abdomen would result in a loss of phylogenetic information and thus I have chosen the more conservative approach of scoring each tergite separately.

102. Additional anterior setae on T3, at least in females: (0) absent (Fig. 14); (1) present (Fig. 15A).

103. Additional anterior setae on T4, at least in females: (0) absent (Fig. 14); (1) present (Fig. 15A-B, E, G).

104. Additional anterior setae on T5, at least in females: (0) absent (Fig. 14); (1) present (Fig. 15A-B, G).

105. Additional anterior setae on T6, at least in females: (0) absent (Fig. 14); (1) present (Fig. 15A-B).

106. Additional anterior setae on T7, at least in females: (0) absent (Fig. 14); (1) present (Fig. 15A).

107. Additional anterior setae on T8, at least in females: (0) absent (Fig. 14); (1) present (Fig. 15A).

108. Tergite 1, seta "a": (0) absent; (1) present (Fig. 15A, C).

At each end of tergite 1 and 2 in the Menoponidae there is a small anterolateral setae (Clay, 1969).

109. Tergite 2, seta "a": (0) absent (Fig. 14); (1) present (Fig. 15A).

110. Spiracle position: (0) open onto tergites (Figs. 14, 15A-B, E); (1) open onto lateral plates (Fig. 15F); (2) on the middle part of a partially divided lateral plate (Fig. 15G).

In these amblyceran families, the abdominal spiracles are present on T3 - 8 (Clay, 1969). In the marsupial- infesting Boopiidae they are normally

present on the lateral plate, but *Paraheterodoxus* is unusual in that they are on a partially divided lateral plate.

111. Distribution of post-spiracular seta "c" (where present): (0) absent; (1) present on T2 - 8 (Figs. 14, 15A-B, E-F); (2) modified as trichobothria on T2 - 4 and present as normal setae on T5 - 8 (Fig. 15G).

The post-spiracular setae, labelled "c" (see Fig. 15D) *sensu* Clay (1970) are found near the lateral margins of T1 - 8. On T2 - 8 they are easily identified due to the presence of two small associated setae, the alveoli of which are contiguous with that of the well-developed post-spiracular seta (Clay, 1954).

112. Position of post-spiracular seta "c" on T3 - 8 (where present): (0) generally posterior to spiracle, sometimes slightly lateral or medial (Fig. 15A-B, D-E, G); (1) extremely lateroposterior to spiracle (Fig. 14).

The post-spiracular seta is usually found behind the spiracles but in the Laemobothriidae and Ricinidae they are laterally displaced. Nelson (1972) wrote in his review of *Ricinus* that the postspiracular setae were on the dorsal halves of the pleurites, somewhat removed from the spiracles".

113. Position of post-spiracular setae "c" to the posterior tergal setae on T2 - 8: (0) marginal (Fig. 14); (1) submarginal (Fig. 15A-B, E-G).

In some taxa the post-spiracular seta may merge with the posterior tergal row, but in others e.g. *Somaphantus* it is found between the spiracle and posterior tergal setae (Clay, 1954).

114. Abdominal tergal seta "b": (0) absent; (1) present (Figs. 14, 15A-G).

Medial to the post-spiracular seta and the two small setae, is a small seta called the associated post-spiracular seta (Clay, 1966), or seta "b" (see Fig.

15D) *sensu* Clay (1969). Together these four setae make up the post-spiracular setal complex. Seta "b" is absent only in *Laemobothrion*.

115. Position of seta "b" to post-spiracular setae "c" (T2): (0) directly anterior (Fig. 14); (1) submarginal; (2) marginal (Fig. 15A); (3) posterior.

There appears to be some clearly distinct patterns in the changing position of seta "b" down the abdomen. However, as in character 101, condensing these patterns into one would result in a loss of information and each tergite is scored separately. T8 is not scored due to the difficulty in seeing this small setae in all the specimens.

116. Position of seta "b" to post-spiracular setae "c" (T3): (0) directly anterior (Fig. 14); (1) submarginal; (2) marginal (Fig. 15A); (3) posterior.

117. Position of lateral seta "b" to post-spiracular setae "c" (T4): (0) directly anterior (Fig. 14); (1) submarginal (Fig. 15F); (2) marginal (Fig. 15A, D-E); (3) posterior (Fig. 15B, G).

118. Position of seta "b" to post-spiracular setae "c" (T5): (0) directly anterior (Fig. 14); (1) submarginal; (2) marginal (Fig. 15A, G); (3) posterior (Fig. 15B).

119. Position of seta "b" to post-spiracular setae "c" (T6): (0) directly anterior (Fig. 14); (1) submarginal; (2) marginal (Fig. 15A); (3) posterior (Fig. 15B).

120. Position of seta "b" to post-spiracular setae "c" (T7): (0) directly anterior (Fig. 14); (1) submarginal; (2) marginal (Fig. 15A); (3) posterior.

2.9.3.2: Ventral abdomen

121. Lateral plate shape: (0) normal and squared off (Fig. 15A, F-H); (1) ventral posterior margin developed into a medially posterior running

protuberance (Fig. 15I).

Normally, the inner posterior angle of the lateral plate looks quite square when viewed from the ventral aspect but in some taxa the plate is more developed at this site. This unusual condition is present in only a few menoponids: *Gruimenopon*, *Pseudomenopon* (Mjöberg, 1910; Price, 1974) and *Plegadiphilus* (Bedford, 1940).

122. Additional setae on the anterior of the second lateral plate (LP2), at least in females: (0) absent (Fig. 15I); (1) present (Fig. 15A).

For reasons outlined above in characters 101 and 115, the presence of these setae are scored separately for each abdominal segment. Again, LP8 is not scored due to the difficulty of seeing the plate properly in all the mounts.

123. Additional setae on the anterior of LP3, at least in females: (0) absent (Fig. 15I); (1) present (Fig. 15A, H).

124. Additional setae on the anterior of LP4, at least in females: (0) absent (Fig. 15I); (1) present (Fig. 15A, E-G).

125. Additional setae on the anterior of LP5, at least in females: (0) absent; (1) present (Fig. 15A, G).

126. Additional setae on the anterior of LP6, at least in females: (0) absent; (1) present (Fig. 15A).

127. Additional setae on the anterior of LP7, at least in females: (0) absent; (1) present (Fig. 15A).

128. Pattern of setae on sternite 3 (St 3): (0) regularly spaced, non-aggregated; (1) setal patch (Fig. 15A); (2) setal combs (Fig. 15H).

In some genera there is a distinct aggregation of setae on the lateral aspects of the sternal plates. Both patches and combs of setae (ctenidia) are

found and these usually mirror the condition seen on the venter of the third femur. There are complex patterns, down the length of the abdomen, of presence and degree of development, so for the reasons outlined above (characters 101, 115, 122) each sternite is treated independently. The sternal patches and combs are conspicuous and are usually fully described and by previous authors (e.g., Clay & Meinertzhagen, 1941; Harrison, 1915; Price & Beer, 1965b). Clay (1962) photographed the sternal patches in *Actornithophilus*.

129. Development of St 3 patch: (0) well developed (Fig. 15A); (1) weakly developed.

130. Number of combs on St 3: (0) one; (1) two (Fig. 15H).

131. Pattern of setae on St 4: (0) regularly spaced, non-aggregated; (1) setal patch (Fig. 15A); (2) setal combs.

132. Development of St 4 patch: (0) well developed (Fig. 15A); (1) weakly developed.

133. Number of combs on St 4: (0) one; (1) two; (2) three or more.

134. Pattern of setae on St 5: (0) regularly spaced, non-aggregated; (1) setal patch (Fig. 15A); (2) patch of microcombs.

On close observation the apparent setal patch of *Laemobothrion* is markedly different from that present in other taxa. Perez *et al* (1995) presented a photograph of this area using scanning electron microscopy and demonstrated that the "patch" was not composed of regular setae but small combs (microtrichia) which, as described above in character 91, are quite different from the combs found in some menoponid genera.

135. Development of St 5 patch: (0) well developed; (1) weakly developed (Fig. 15A).

136. Pattern of setae on St 6: (0) regularly spaced, non-aggregated (Fig. 15A); (1) setal patch; (2) patch of microcombs.

137. Development of St 6 patch: (0) well developed; (1) weakly developed.

138. Pattern of setae on St 7: (0) regularly spaced, non-aggregated (Fig. 15A); (1) setal patch.

139. Development of St 7 patch: (0) well developed; (1) weakly developed.

2.9.3.3: Female terminalia

140. Presence of a setal fringe around the female anal margin: (0) absent (Fig. 16A); (1) present (Figs. 15A, 16B-D).

Rediella and *Somaphantus* are unusual in the Menoponidae in that the typical anal corona of setae is absent.

141. Form of the female anal corona (where present): (0) wide anal margin with, a usually obvious, thick fringe of setae (Fig. 16B); (1) as above, but fringe very short and fine (Fig. 16C); (2) small rounded protruding anal margin with short fine fringe (Fig. 16D); (3) anal fringe composed of short stout spine-like setae (Fig. 15A).

142. Presence of gonapophyses in the female: (0) absent (Figs. 15A, 16B-D); (1) present (Fig. 16A).

The gonapophyses are characteristic of the Boopiidae and are described by Kéler (1971) as "sickle-shaped bluntly or sharply pointed appendages". They are found on each side of the postgenital sternum, behind the vulval margin. There is usually a single fine seta on the tip of each one. Clay (1970)

observed some structure in *Chapinia* (Menoponidae) that she believed may be homologous with gonapophyses of the Boopiidae, however, no such structure was viewed in these specimens of *Chapinia*.

2.9.3.4: Male genitalia

The components of the male genitalia are perhaps the most difficult structures to confidently identify in the Amblycera. Clay (1956) wrote that the sclerites of the male genitalia "may be fused in such a way as to make their homologies obscure and it is not always possible to homologize the parts even between species of the same genus". Both Harrison (1915) and Carriker (1963) wrote that they were not confident in their observations of menoponid genitalia. Others have avoided the issue either providing figures with no descriptions or making statements akin to "male genitalia as in *Fig. 4*", whilst Ewing (1927) gave some description but with no illustration.

There has also been some variation in the descriptive terminology ascribed to some structures. Snodgrass (1899) described and illustrated the lateral parameres as "processes" and "lateral prongs", whilst Price (1967) and Price & Beer (1965a) termed them "lateroposterior projections" and "points". More recently most authors have been consistent in following the terminology originally laid out by Clay (1956) and Blagoveshtchensky (1964) and a few have provided quite comprehensive and detailed accounts. The male genitalia are described with labelled illustrations for the Boopiidae (Kéler, 1971), Ricinidae (Nelson, 1972) and *Amyrsidea* (Menoponidae) (Scharf & Price, 1977).

I was able to use these key papers and other illustrated publications (e.g., Price, 1975; Price & Beer, 1965a; 1972; Waterston, 1915) to confirm the identification of the parameres, basal plate, endomeral plate, genital sclerite, mesosomal arch and in some cases endomeres and epimeres. In *Eomenopon*, however, the identification of component structures is difficult (Price,1966). I believe this is because the genitalia appears to be turned on its side, so what is viewed is actually the lateral aspect.

143. Paramere shape: (0) outwardly curved (Fig. 17A, D); (1) straight or inwardly curved (Fig. 17 B-C, E-F).

144. Paramere position: (0) parameres arise from around half way down the body of the aedeagus (Fig. 17A-D); (1) parameres arise near the posterior of the aedeagus (Fig. 17E-F).

145. Mesosomal arch: (0) indistinct (Fig. 17A-D, F); (1) bulbous well-defined arch (Fig. 17E).

Kéler (1971) describes the boopiid mesosome as "membranous, stiffened dorsally by a chitinous arch".

146. Basal apodome: (0) unsclerotised, largely absent (Fig. 17D); (1) very thin, stick-like rod (Fig. 17B-C); (2) medium to wide tapering rod (Fig. 17A, F); (3) bulbous, paddle-like rod (Fig. 17E).

147. Basal apodome apex shape: (0) unsclerotised (Fig. 17E); (1) rounded tip (Fig. 17F); (2) hooked tip (Fig. 17B); (3) pointed tip (Fig. 17C); (4) wide squared apex (Fig. 17A).

2.10: Appendix 3: Data matrix for 147 morphological characters.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Liposcelis bostrychophilus	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	-	0
Laemobothrion maximum	0	0	-	0	0	0	0	1	1	1	1	1	1	1	1	1	1
Ricinus fringillae	1	1	0	1	1	0	1	0	0	0	1	0	0	0	0	-	0
Trochiloecetes rupununi	0	0	-	0	0	0	0	0	0	0	0	0	0	0	0	-	0
Trochiliphagus abdominalis	1	1	0	1	1	0	0	0	0	0	1	0	0	0	0	-	0
Therodoxus oweni	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	0	0
Paraheterodoxus insignis	1	1	0	1	1	0	1	1	1	1	1	1	1	1	1	0	0
Boopia tarsata	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	0	1
Latumcephalum lesouefi/ macropus	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	0	1
Paraboopia flava	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Amyrsidea ventralis	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Rediella mirabilis	1	1	0	1	1	1	1	0	1	0	1	1	1	1	0	-	1
Somaphantus lusius	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Bonomiella columbae	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Menopon gallinae	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Numidicola antennatus	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Hohorstiella lata	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Menacanthus stramineus	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Colimenopon urocolius	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Machaerilaemus laticorpus/ latifrons	1	1	0	1	1	1	1	0	1	1	1	1	1	1	1	0	0
Neomenopon pteroclurus	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Dennyus hirundinis	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	0	1
Myrsidea victrix	1	1	0	1	1	1	1	1	1	0	1	1	1	1	0	-	1
Ancistrona vagelli	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Austromenopon crocatum	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Eidmanniella pellucida	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Holomenopon brevithoracicum	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Plegadiphilus threskiornis	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Actornithophilus uniseriatus	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Chapinia robusta	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Gruimenopon longum	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Meromenopon meropis	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Eomenopon denticulatum	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Hoazineus armiferus	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Pseudomenopon pilosum	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Trinoton anserinum	1	1	0	1	1	1	1	1	1	0	1	1	1	1	1	0	1
Colpocephalum zebra	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Comatomenopon elbeli/ elongatum	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Ardeiphilus trochioxus	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0
Ciconiphilus quadripustulatus	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Cuculiphilus fasciatus	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	0	1
Odoriphila clayae/ phoeniculi	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Osborniella crotophagae	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Psittacomenopon poicephalus	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Piagetiella bursaepelecani	1	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1

	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
Liposcelis bostrychophilus	-	0	-	0	-	0	-	0	0	0	0	0	0	0	0	0	0
Laemobothrion maximum	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
Ricinus fringillae	-	0	-	0	-	0	-	0	0	0	0	1	0	0	1	0	0
Trochiloecetes rupununi	-	0	-	0	-	0	-	0	0	0	0	1	0	0	0	0	0
Trochiliphagus abdominalis	-	0	-	0	-	0	-	0	0	0	0	1	0	0	0	0	0
Therodoxus oweni	-	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	0
Paraheterodoxus insignis	-	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	0
Boopia tarsata	1	1	1	1	1	1	1	1	1	0	1	0	0	0	0	0	0
Latumcephalum lesouefi/ macropus	0	1	1	1	0	1	0	1	1	1	0	0	0	0	0	0	0
Paraboopia flava	1	1	1	1	1	1	0	1	1	1	0	0	0	0	0	0	0
Amyrsidea ventralis	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0
Rediella mirabilis	0	1	1	1	1	1	0	1	1	1	1	0	0	0	0	0	0
Somaphantus lusius	0	1	1	1	1	1	1	1	1	1	0	0	1	1	1	0	0
Bonomiella columbae	1	1	1	1	0/1	1	1	1	1	1	1	0	0	0	0	0	0
Menopon gallinae	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0
Numidicola antennatus	0	1	0	1	1	1	1	1	1	1	1	0	1	1	1	0	0
Hohorstiella lata	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
Menacanthus stramineus	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	0
Colimenopon urocolius	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0
Machaerilaemus laticorpus/ latifrons	-	1	0	1	1	1	1	1	1	1	1	0	0	0	0	0	0
Neomenopon pteroclurus	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0
Dennyus hirundinis	1	1	1	1	0	1	0	1	1	1	1	0	1	1	0	0	0
Myrsidea victrix	1	1	1	1	0	1	0	1	1	1	1	0	0	0	0	0	0
Ancistrona vagelli	0/1	1	0/1	1	0	1	0	1	1	1	1	0	0	0	1	0	0
Austromenopon crocatum	1	1	0	1	1	1	0	1	1	1	1	0	1	1	1	0	0
Eidmanniella pellucida	1	1	0	1	1	1	0	1	1	1	1	0	0	1	1	1	0
Holomenopon brevithoracicum	1	1	0	1	1	1	0	1	1	1	1	0	0	0	1	0	1
Plegadiphilus threskiornis	0	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0
Actornithophilus uniseriatus	1	1	0	1	1	1	0	1	1	1	1	0	0	1	1	1	0
Chapinia robusta	1	1	1	1	0	1	0	1	1	1	1	0	1	0	0	0	0
Gruimenopon longum	1	1	0	1	1	1	0	1	1	1	1	0	0	0	1	1	0
Meromenopon meropis	1	1	1	1	1	1	1	1	1	1	1	0	0	1	0	0	0
Eomenopon denticulatum	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0	0
Hoazineus armiferus	0	1	0	1	1	1	1	1	1	1	0	0	1	1	0	0	0
Pseudomenopon pilosum	0	1	1	1	1	1	1	1	1	1	1	0	0	0	1	0	0
Trinoton anserinum	1	1	1	1	0	1	0	1	1	1	1	0	0	0	0	0	0
Colpocephalum zebra	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	0	0
Comatomenopon elbeli/ elongatum	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	0	0
Ardeiphilus trochioxus	-	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0
Ciconiphilus quadripustulatus	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0
Cuculiphilus fasciatus	1	1	1	1	0	1	0	1	1	1	1	0	0	1	1	1	0
Odoriphila clayae/ phoeniculi	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0
Osborniella crotophagae	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	0
Psittacomenopon poicephalus	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0
Piagetiella bursaepelecani	0	1	0	1	0	1	1	1	1	1	1	0	0	1	1	0	0

	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51
Liposcelis bostrychophilus	0	0	0	0	0	0	0	-	0	-	1	1	0	-	1	0	0
Laemobothrion maximum	0	1	3	0	0	1	1	0	0	-	1	0	1	0	0	1	1
Ricinus fringillae	0	0	3	0	0	1	0	-	0	-	0	-	1	1	0	1	1
Trochiloecetes rupununi	0	0	?	1	0	1	0	-	1	0	0	-	1	1	0	1	1
Trochiliphagus abdominalis	0	0	3	1	0	1	0	-	0	-	0	-	1	1	0	1	1
Therodoxus oweni	2	1	3	0	0	1	1	0	1	0	1	0	1	1	0	0	1
Paraheterodoxus insignis	2	1	3	0	0	1	1	1	0	-	1	0	1	1	0	0	1
Boopia tarsata	2	1	3	0	0	1	1	1	1	0	1	0	1	1	0	0	1
Latumcephalum lesouefi/ macropus	2	2	3	0	1	1	1	1	1	1	1	0	1	1	0	1	1
Paraboopia flava	2	2	3	0	1	1	1	1	0	-	1	0	1	1	0	1	1
Amyrsidea ventralis	2	0	0	0	0	1	1	1	0	-	1	0	1	0	0	0	1
Rediella mirabilis	0	0	0	0	0	1	1	1	0	-	1	0	1	1	0	0	1
Somaphantus lusius	0	1	0	0	0	1	1	0	0	-	1	0	1	0	0	0	1
Bonomiella columbae	1	2	0	0	0	1	1	1	0	-	1	0	1	0	0	1	1
Menopon gallinae	2	0	0	0	0	1	1	1	0	-	1	0	1	0	0	0	1
Numidicola antennatus	0	1	0	0	0	1	1	1	0	-	1	0	1	0	0	0	1
Hohorstiella lata	2	2	0	0	0	1	1	1	1	0	1	0	1	0	0	1	1
Menacanthus stramineus	2	2	0	0	0	1	1	1	1	0	1	0	1	0	0	1	1
Colimenopon urocolius	0	3	2	0	0	1	1	1	0	-	1	0	1	0	0	1	1
Machaerilaemus laticorpus/ latifrons	0	2	2	0	0	1	0	-	0	-	1	0	1	0	0	1	1
Neomenopon pteroclurus	0	2	2	0	0	1	1	1	0	-	1	0	1	0	0	1	1
Dennyus hirundinis	0	2	1	0	0	1	1	1	0	-	1	0	1	0	0	1	1
Myrsidea victrix	0	2	0	0	0	1	1	1	0	-	1	0	1	0	0	1	1
Ancistrona vagelli	0	2	0	0	0	1	0	-	0	-	1	0	1	0	0	1	1
Austromenopon crocatum	0	0	0	0	0	1	1	1	0	-	1	0	1	1	0	1	1
Eidmanniella pellucida	2	0	0	0	0	1	1	1	0	-	1	0	1	0	0	1	1
Holomenopon brevithoracicum	0	0	0	0	0	1	1	1	0	-	1	0	1	0	0	1	1
Plegadiphilus threskiornis	1	2	0	0	0	1	1	1	0	-	1	0	1	0	0	1	1
Actornithophilus uniseriatus	1	2	0	0	0	1	1	1	0	-	1	0	1	0	0	1	1
Chapinia robusta	2	2	1	0	0	1	1	1	0	-	1	0	1	0	0	1	1
Gruimenopon longum	0	2	0	0	0	1	1	1	0	-	1	0	1	0	0	0	1
Meromenopon meropis	0	2	1	0	0	1	1	1	0	-	1	0	1	0	0	1	1
Eomenopon denticulatum	2	0	2	0	0	1	1	1	1	0	1	0	1	0	0	1	1
Hoazineus armiferus	2	2	0	0	0	1	1	1	0	-	1	0	1	0	0	0	1
Pseudomenopon pilosum	2	2	2	0	0	1	1	1	0	-	1	0	1	0	0	1	1
Trinoton anserinum	1	2	0	0	0	1	0	-	0	-	1	0	1	0	0	1	1
Colpocephalum zebra	1	1	0	0	0	1	1	1	0	-	1	0	1	0	0	1	1
Comatomenopon elbeli/ elongatum	1	1	0	0	0	1	1	1	0	-	1	0	1	0	0	0	1
Ardeiphilus trochioxus	1	2	0	0	0	1	1	1	0	-	1	0	1	0	0	1	1
Ciconiphilus quadripustulatus	2	2	0	0	0	1	1	1	0	-	1	0	1	0	0	1	1
Cuculiphilus fasciatus	2	2	0	0	0	1	?	1	0	-	1	0	1	1	0	1	1
Odoriphila clayae/ phoeniculi	2	2	2	0	0	1	1	1	1	1	1	0	1	0	0	1	1
Osborniella crotophagae	0	2	0	0	0	1	1	1	0	-	1	0	1	0	0	1	1
Psittacomenopon poicephalus	2	2	0	0	0	1	1	1	0	-	1	0	1	0	0	1	1
Piagetiella bursaepelecani	2	0	0	0	0	1	1	1	0	-	1	0	1	0	0	1	1

	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68
Liposcelis bostrychophilus	0	0	-	0	0	-	-	-	-	0	-	0	-	0	0	-	0
Laemobothrion maximum	0	1	2	2	0	-	-	-	-	0	-	1	2	1	1	-	0
Ricinus fringillae	0	1	3	1	0	-	-	-	-	0	-	0	-	0	1	-	0
Trochiloecetes rupununi	0	1	3	1	0	-	-	-	-	0	-	0	-	0	1	-	0
Trochiliphagus abdominalis	0	1	3	3	0	-	-	-	-	0	-	0	-	0	1	-	0
Therodoxus oweni	0	1	0	0	0	-	-	-	-	0	-	0	-	1	2	1	1
Paraheterodoxus insignis	0	1	0	0	0	-	-	-	-	0	-	0	-	1	2	1	1
Boopia tarsata	0	1	0	0	0	-	-	-	-	0	-	0	-	1	2	1	1
Latumcephalum lesouefi/ macropus	0	1	2	0	0	-	-	-	-	0	-	0	-	1	2	1	1
Paraboopia flava	0	1	0	0	0	-	-	-	-	0	-	0	-	1	2	1	1
Amyrsidea ventralis	1	1	0	0	1	1	0	1	1	0	-	1	3	1	2	1	1
Rediella mirabilis	1	0	-	0	1	1	0	0	0	0	-	0	-	0	2	0	0
Somaphantus lusius	1	0	-	0	1	1	0	1	0	0	-	0	-	1	2	1	1
Bonomiella columbae	0	1	2	0	1	0	0	1	1	0	-	0	-	1	2	0	1
Menopon gallinae	1	1	0	0	1	1	0	1	1	0	-	1	1	1	2	1	1
Numidicola antennatus	1	1	0	0	1	1	0	1	1	0	-	1	1	1	2	1	0
Hohorstiella lata	0	1	3	0	1	1	0	1	1	1	1	1	0	1	2	0	1
Menacanthus stramineus	0	1	3	0	1	0	0	1	1	0	-	1	0	1	2	1	1
Colimenopon urocolius	0	1	1	0	1	0	0	1	0	0	-	1	3	1	2	1	1
Machaerilaemus laticorpus/ latifrons	0	1	1	0	0	-	-	-	-	0	-	1	4	1	2	1	1
Neomenopon pteroclurus	0	1	3	0	1	1	0	1	0	0	-	1	4	1	2	0	1
Dennyus hirundinis	0	1	2	0	1	1	0	0	0	0	-	0	-	1	2	1	1
Myrsidea victrix	0	1	2	0	1	1	0	0	0	0	-	0	-	1	2	1	1
Ancistrona vagelli	0	1	3	0	0	-	-	-	-	0	-	0	-	1	2	1	1
Austromenopon crocatum	0	1	3	0	1	1	1	1	0	0	-	0	-	1	2	1	1
Eidmanniella pellucida	0	1	1	0	1	1	1	1	1	0	-	0	-	1	2	0	1
Holomenopon brevithoracicum	0	1	1	0	1	1	0	1	0	0	-	0	-	1	2	0	1
Plegadiphilus threskiornis	0	1	3	0	1	1	1	1	0	1	1	0	-	1	2	0	1
Actornithophilus uniseriatus	0	1	2	0	1	1	0	0	0	0	-	0	-	1	2	0	1
Chapinia robusta	0	1	3	4	1	0	0	1	0	0	-	0	-	1	2	1	1
Gruimenopon longum	0	1	3	0	1	1	0	0	1	1	2	0	-	1	2	1	1
Meromenopon meropis	0	1	2	0	1	1	1	1	1	1	0	0	-	1	2	1	1
Eomenopon denticulatum	0	1	3	0	1	0	0	1	1	1	1	0	-	1	2	1	1
Hoazineus armiferus	0	1	0	0	1	1	0	0	1	1	2	0	-	1	2	1	1
Pseudomenopon pilosum	0	1	3	0	1	1	0	1	0	0	-	0	-	1	2	1	1
Trinoton anserinum	0	1	1	0	1	0	0	1	1	0	-	0	-	1	2	1	1
Colpocephalum zebra	1	1	2	0	1	1	0	0	1	1	0	1	4	1	2	0	1
Comatomenopon elbeli/ elongatum	0	1	2	0	1	1	0	0	1	1	0	1	4	1	2	0	1
Ardeiphilus trochioxus	0	1	3	0	1	1	0	1	1	1	0	1	4	1	2	0	1
Ciconiphilus quadripustulatus	0	1	3	0	1	0	0	1	1	1	0	0	-	1	2	0	1
Cuculiphilus fasciatus	0	1	3	0	1	0	0	1	1	1	0	0	-	1	2	0	1
Odoriphila clayae/ phoeniculi	0	1	3	0	1	1	0	0	1	1	1	1	4	1	2	0	1
Osborniella crotophagae	0	1	3	0	1	1	0	1	1	1	0	0	-	1	2	0	1
Psittacomenopon poicephalus	0	1	2	0	1	1	0	1	1	1	0	0	-	1	2	0	1
Piagetiella bursaepelecani	0	1	3	0	1	0	0	1	1	1	0	0	-	1	2	1	1

	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85
Liposcelis bostrychophilus	0	-	-	0	1	0	0	-	1	1	0	-	1	1	4	0	-
Laemobothrion maximum	0	-	-	0	1	0	1	1	1	2	0	-	0	1	3	0	-
Ricinus fringillae	0	-	-	0	1	1	0	-	1	2	0	-	0	0	0	1	0
Trochiloecetes rupununi	0	-	-	0	1	1	0	-	1	2	0	-	0	0	5	1	0
Trochiliphagus abdominalis	0	-	-	0	1	1	0	-	1	2	0	-	0	0	0	1	0
Therodoxus oweni	1	1	0	1	0	1	0	-	1	1	1	1	1	0	1	0	-
Paraheterodoxus insignis	1	1	0	1	0	1	0	-	1	2	0	-	1	1	0	0	-
Boopia tarsata	1	1	0	1	0	1	0	-	1	2	0	-	1	0	0	0	-
Latumcephalum lesouefi/ macropus	1	1	0	1	0	1	0	-	1	1	1	1	1	1	1	0	-
Paraboopia flava	1	1	0	1	0	1	0	-	1	2	1	1	1	1	1	0	-
Amyrsidea ventralis	1	1	0	0	0	0	1	1	1	1	0	-	0	0	1	0	-
Rediella mirabilis	1	1	1	0	0	0	1	1	1	2	0	-	0	0	6	0	-
Somaphantus lusius	1	1	0	0	0	0	1	1	1	1	0	-	0	0	4	0	-
Bonomiella columbae	1	0	0	0	0	0	1	0	0	1	1	1	0	0	-	-	-
Menopon gallinae	1	1	0	0	0	0	1	0	0	1	1	1	0	0	-	-	-
Numidicola antennatus	0	-	-	0	0	0	1	1	1	1	0	-	0	0	1	0	-
Hohorstiella lata	1	1	0	0	0	0	1	0	1	1	1	0	0	0	2	1	1
Menacanthus stramineus	1	1	0	0	0	0	1	0	1	1	0	-	0	0	2	1	1
Colimenopon urocolius	1	1	0	0	0	0	1	0	1	2	0	-	0	1	2	1	1
Machaerilaemus laticorpus/ latifrons	1	0	0	0	0	0	1	0	1	1	0	-	0	1	2	1	1
Neomenopon pteroclurus	1	1	0	0	0	0	1	0	1	1	1	1	0	0	0	1	1
Dennyus hirundinis	1	0	0	0	0	0	1	1	1	1	0	-	1	1	3	1	2
Myrsidea victrix	1	0	0	0	0	0	1	1	1	0	-	-	1	0	3	1	2
Ancistrona vagelli	1	1	0	0	0	0	1	0	1	1	1	0	0	0	0	1	2
Austromenopon crocatum	1	1	0	0	0	0	1	0	1	1	1	0	0	0	1	0	-
Eidmanniella pellucida	1	1	0	0	0	0	1	0	1	1	1	0	0	0	2	1	1
Holomenopon brevithoracicum	1	1	0	0	0	0	1	0	1	1	1	0	0	0	2	1	1
Plegadiphilus threskiornis	1	1	0	0	0	0	1	0	1	1	1	0	0	0	0	0	-
Actornithophilus uniseriatus	1	1	1	0	0	0	1	1	1	2	0	-	0	1	3	0	-
Chapinia robusta	1	1	0	0	0	0	1	0	1	1	1	0	0	0	0	1	0
Gruimenopon longum	1	1	0	0	0	0	1	1	0	1	1	1	0	0	-	-	-
Meromenopon meropis	1	1	0	0	0	0	1	0	1	1	1	0	0	0	0	1	0
Eomenopon denticulatum	1	1	0	0	0	0	1	0	1	2	1	1	0	0	1	0	-
Hoazineus armiferus	1	1	0	0	0	0	1	1	0	1	1	0	0	0	-	-	-
Pseudomenopon pilosum	1	1	0	0	0	0	1	0	1	2	1	1	0	0	1	0	-
Trinoton anserinum	1	1	1	0	0	0	1	0	1	2	1	1	0	1	1	1	1
Colpocephalum zebra	1	1	0	0	0	0	1	1	0	1	1	1	0	0	-	-	-
Comatomenopon elbeli/ elongatum	1	1	0	0	0	0	1	1	0	1	1	1	0	0	-	-	-
Ardeiphilus trochioxus	1	1	0	0	0	0	1	0	0	1	1	1	0	0	-	-	-
Ciconiphilus quadripustulatus	1	1	0	0	0	0	1	0	0	1	1	1	0	0	-	-	-
Cuculiphilus fasciatus	1	1	0	0	0	0	1	0	1	1	1	1	0	0	1	0	-
Odoriphila clayae/ phoeniculi	1	1	0	0	0	0	1	2	1	1	0	-	0	1	0	0	-
Osborniella crotophagae	1	1	0	0	0	0	1	0	1	1	1	0	0	0	0	1	1
Psittacomenopon poicephalus	1	1	0	0	0	0	1	0	1	1	1	1	0	0	0	1	1
Piagetiella bursaepelecani	1	1	0	0	0	0	1	1	1	1	0	-	0	1	0	1	1

	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100	101
Liposcelis bostrychophilus	1	1	0	0	0	0	-	0	0	0	-	1	0	0	1	1
Laemobothrion maximum	1	1	1	0	1	4	-	0	1	1	1	0	1	0	1	0
Ricinus fringillae	1	1	1	2	1	6	-	0	1	1	1	0	1	0	0	0
Trochiloecetes rupununi	1	1	1	2	1	6	-	0	1	1	1	0	1	0	0	0
Trochiliphagus abdominalis	1	1	1	2	1	6	-	0	1	1	1	0	1	0	0	0
Therodoxus oweni	2	1	1	2	1	5	-	0	1	1	2	0	0	0	1	0
Paraheterodoxus insignis	1	1	1	2	1	5	-	0	1	1	2	0	0	0	2	0
Boopia tarsata	1	1	1	2	1	5	-	0	1	1	2	0	0	0	1	0
Latumcephalum lesouefi/ macropus	2	1	1	2	1	5	-	0	1	0	-	0	0	0	1	0
Paraboopia flava	2	1	1	0	1	5	-	0	1	0	-	0	0	0	1	0
Amyrsidea ventralis	1	1	1	0	1	2	-	0	1	1	0	0	0	0	2	1
Rediella mirabilis	1	1	1	0	1	5	-	0	1	1	1	0	0	0	1	0
Somaphantus lusius	1	1	1	0	1	2	-	0	1	1	0	0	0	0	2	1
Bonomiella columbae	1	1	1	0	1	5	-	0	1	1	1	0	0	0	1	0
Menopon gallinae	0	0	1	0	1	2	-	0	1	1	0	0	0	0	1	0
Numidicola antennatus	1	1	1	0	1	2	-	0	1	1	0	0	0	0	2	0
Hohorstiella lata	1	1	1	0	1	2	-	0	1	1	0	0	0	0	1	0
Menacanthus stramineus	1	1	1	1	1	2	-	0	1	1	0	0	0	0	2	1
Colimenopon urocolius	1	1	1	1	1	2	-	0	1	1	1	0	0	0	2	1
Machaerilaemus laticorpus/ latifrons	1	1	1	1	1	1	-	0	1	1	1	0	0	0	1	0
Neomenopon pteroclurus	1	1	1	0	1	2	-	0	1	1	1	0	0	0	1	0
Dennyus hirundinis	1	1	1	0	1	2	-	0	1	1	0	0	0	0	1	0
Myrsidea victrix	2	1	1	0	1	2	-	0	1	1	0	0	0	0	1	0
Ancistrona vagelli	1	1	1	1	1	5	-	0	1	1	1	0	0	0	1	0
Austromenopon crocatum	0	1	1	1	1	2	-	0	1	1	0	0	0	0	1	0
Eidmanniella pellucida	1	1	1	1	1	2	-	0	1	1	0	0	0	0	1	0
Holomenopon brevithoracicum	1	1	1	0	1	2	-	0	1	1	0	0	0	0	2	0
Plegadiphilus threskiornis	1	1	1	0	1	2	-	0	1	1	0	0	0	0	2	0
Actornithophilus uniseriatus	1	1	1	0	1	2	-	0	1	1	0	0	0	0	1	0
Chapinia robusta	1	1	1	0	1	2	-	1	1	1	0	0	0	0	2	0
Gruimenopon longum	1	1	1	0	1	2	-	1	1	1	0	0	0	0	2	0
Meromenopon meropis	1	1	1	0	1	2	-	1	1	1	0	0	0	0	1	1
Eomenopon denticulatum	1	1	1	1	1	2	-	0	1	1	0	0	0	0	2	0
Hoazineus armiferus	1	1	1	0	1	2	-	1	1	1	1	0	0	0	1	0
Pseudomenopon pilosum	1	1	1	0	1	2	-	0	1	1	0	0	0	0	1	0
Trinoton anserinum	1	1	1	0	1	2	-	0	1	1	1	0	0	0	1	1
Colpocephalum zebra	1	1	1	0	1	2&3	1	1	1	1	1	0	0	1	1	1
Comatomenopon elbeli/ elongatum	1	1	1	0	1	3	1	1	1	1	1	0	0	0	1	1
Ardeiphilus trochioxus	1	1	1	0	1	2&3	2	1	1	1	1	0	0	0	1	0
Ciconiphilus quadripustulatus	1	1	1	0	1	2&3	1	1	1	1	1	0	0	0	1	0
Cuculiphilus fasciatus	2	1	1	0	1	2&3	2	1	1	1	1	0	0	0	1	0
Odoriphila clayae/ phoeniculi	1	1	1	0	1	3	0	1	1	1	1	0	0	0	2	1
Osborniella crotophagae	1	1	1	0	1	2&3	1	1	1	1	1	0	0	0	1	0
Psittacomenopon poicephalus	1	1	1	0	1	3	1	1	1	1	1	0	0	1	2	1
Piagetiella bursaepelecani	1	1	1	0	1	2&3	2	1	1	1	1	0	0	0	2	0

	102	103	104	105	106	107	108	109	110	111	112	113
Liposcelis bostrychophilus	1	1	1	1	1	1	0	0	0	0	-	-
Laemobothrion maximum	0	0	0	0	0	0	1	1	0	1	1	1
Ricinus fringillae	0	0	0	0	0	0	-	0	0	1	1	1
Trochiloecetes rupununi	0	0	0	0	0	0	-	0	0	1	1	1
Trochiliphagus abdominalis	0	0	0	0	0	0	-	0	0	1	1	0
Therodoxus oweni	0	0	0	0	0	0	-	1	0	2	0	1
Paraheterodoxus insignis	1	1	1	1	1	1	-	1	2	2	0	1
Boopia tarsata	0	0	0	0	0	0	-	1	1	2	0	0
Latumcephalum lesouefi/ macropus	1	1	1	1	1	1	-	1	1	1	0	1
Paraboopia flava	1	1	1	1	1	1	-	1	1	1	0	1
Amyrsidea ventralis	1	1	1	1	1	1	1	1	0	1	0	1
Rediella mirabilis	0	0	0	0	0	0	1	1	0	1	0	0
Somaphantus lusius	1	1	1	1	1	1	1	1	0	1	0	1
Bonomiella columbae	0	0	0	0	0	0	1	1	0	1	0	0
Menopon gallinae	0	0	0	0	0	0	1	1	0	1	0	1
Numidicola antennatus	0	0	0	0	0	1	1	1	0	1	0	1
Hohorstiella lata	0	0	0	0	0	0	1	1	0	1	0	0
Menacanthus stramineus	1	1	1	1	1	1	1	1	0	1	0	1
Colimenopon urocolius	1	1	1	1	1	1	1	1	0	1	0	0
Machaerilaemus laticorpus/ latifrons	0	0	0	0	0	0	1	0	0	1	0	0
Neomenopon pteroclurus	0	0	0	0	0	0	1	0	0	1	0	0
Dennyus hirundinis	0	0	0	0	0	0	1	1	0	1	0	0
Myrsidea victrix	0	0	0	0	0	0	1	0	0	1	0	0
Ancistrona vagelli	0	0	0	0	0	0	1	1	0	1	0	0
Austromenopon crocatum	0	0	0	0	0	0	1	1	0	1	0	0
Eidmanniella pellucida	0	0	0	0	0	0	1	1	0	1	0	0
Holomenopon brevithoracicum	0	0	0	0	0	0	1	1	0	1	0	1
Plegadiphilus threskiornis	0	0	0	0	0	0	1	1	0	1	0	0
Actornithophilus uniseriatus	0	0	0	0	0	0	1	1	0	1	0	0
Chapinia robusta	0	0	0	0	0	0	1	1	0	1	0	0
Gruimenopon longum	0	0	0	0	0	0	1	1	0	1	0	1
Meromenopon meropis	1	1	1	1	1	0	1	1	0	1	0	1
Eomenopon denticulatum	0	0	0	0	0	0	1	1	0	1	0	0
Hoazineus armiferus	0	0	0	0	0	0	1	1	0	1	0	1
Pseudomenopon pilosum	0	0	0	0	0	0	1	1	0	1	0	0
Trinoton anserinum	1	1	1	1	1	0	1	1	0	1	0	0
Colpocephalum zebra	1	1	1	1	1	1	1	1	0	1	0	1
Comatomenopon elbeli/ elongatum	1	1	1	1	1	1	1	1	0	1	0	1
Ardeiphilus trochioxus	0	0	0	0	0	0	1	1	0	1	0	1
Ciconiphilus quadripustulatus	0	0	0	0	0	0	1	1	0	1	0	1
Cuculiphilus fasciatus	0	0	0	0	0	0	1	1	0	1	0	1
Odoriphila clayae/ phoeniculi	1	1	1	1	1	0	1	1	0	1	0	1
Osborniella crotophagae	0	0	0	0	0	0	1	1	0	1	0	0
Psittacomenopon poicephalus	1	1	1	1	1	1	1	1	0	1	0	1
Piagetiella bursaepelecani	0	0	0	0	0	0	1	1	0	1	0	0

	114	115	116	117	118	119	120	121	122	123	124	125
Liposcelis bostrychophilus	0	-	-	-	-	-	-	0	1	1	1	1
Laemobothrion maximum	0	-	-	-	-	-	-	0	1	1	1	1
Ricinus fringillae	1	0	0	0	0	0	0	0	0	0	0	0
Trochiloecetes rupununi	1	0	0	0	0	0	0	0	0	0	0	0
Trochiliphagus abdominalis	1	0	0	0	0	0	0	0	0	0	0	0
Therodoxus oweni	1	1	1	1	2	2	2	0	0	0	1	1
Paraheterodoxus insignis	1	3	3	3	2	2	2	0	0	1	1	1
Boopia tarsata	1	3	3	3	2	2	2	0	0	1	1	1
Latumcephalum lesouefi/ macropus	1	1	1	1	2	2	2	0	0	1	1	1
Paraboopia flava	1	1	1	1	2	2	2	0	0	1	1	1
Amyrsidea ventralis	1	2	2	2	2	2	2	0	1	1	1	1
Rediella mirabilis	1	1	1	1	1	2	2	0	1	1	1	1
Somaphantus lusius	1	1	1	1	1	1	1	0	1	1	1	1
Bonomiella columbae	1	2	2	2	2	2	2	0	0	0	0	0
Menopon gallinae	1	2	2	2	2	2	2	0	1	1	1	1
Numidicola antennatus	1	2	2	2	2	2	2	0	1	1	1	1
Hohorstiella lata	1	2	2	2	2	2	2	0	1	1	1	1
Menacanthus stramineus	1	2	2	2	2	2	2	0	1	1	1	1
Colimenopon urocolius	1	2	2	2	2	2	2	0	1	1	1	1
Machaerilaemus laticorpus/ latifrons	1	2	2	2	2	2	2	0	1	1	1	1
Neomenopon pteroclurus	1	2	2	2	2	2	2	0	1	1	1	1
Dennyus hirundinis	1	2	2	2	2	2	2	0	0	0	0	0
Myrsidea victrix	1	2	2	2	2	2	2	0	0	0	0	0
Ancistrona vagelli	1	2	2	2	2	2	2	0	0	0	0	0
Austromenopon crocatum	1	2	2	2	2	2	2	0	1	1	1	1
Eidmanniella pellucida	1	2	2	2	2	2	2	0	1	1	1	1
Holomenopon brevithoracicum	1	2	2	2	2	2	2	0	0	1	1	1
Plegadiphilus threskiornis	1	2	2	2	2	2	2	1	0	1	1	1
Actornithophilus uniseriatus	1	2	2	2	2	2	2	0	1	1	1	1
Chapinia robusta	1	2	2	2	2	2	2	0	1	1	1	1
Gruimenopon longum	1	2	2	2	2	2	2	0	1	1	1	1
Meromenopon meropis	1	2	2	2	2	2	2	1	1	1	1	1
Eomenopon denticulatum	1	2	2	2	2	2	2	0	1	1	1	1
Hoazineus armiferus	1	1	1	1	1	1	1	0	1	1	1	1
Pseudomenopon pilosum	1	2	2	2	2	2	2	1	0	0	0	0
Trinoton anserinum	1	2	2	2	2	2	2	0	0	1	1	1
Colpocephalum zebra	1	3	3	3	3	3	3	0	1	1	1	1
Comatomenopon elbeli/ elongatum	1	2	2	2	2	2	2	0	1	1	1	1
Ardeiphilus trochioxus	1	3	3	3	3	3	3	0	1	1	1	1
Ciconiphilus quadripustulatus	1	3	3	3	2	2	2	0	1	1	1	1
Cuculiphilus fasciatus	1	3	3	3	2	2	2	0	1	1	1	1
Odoriphila clayae/ phoeniculi	1	3	3	3	3	3	3	0	1	1	1	1
Osborniella crotophagae	1	2	2	2	2	2	2	0	1	1	1	1
Psittacomenopon poicephalus	1	3	3	3	3	3	3	0	1	1	1	1
Piagetiella bursaepelecani	1	2	2	2	2	2	2	0	0	0	1	1

	126	127	128	129	130	131	132	133	134	135	136	137
Liposcelis bostrychophilus	1	1	0	-	-	0	-	-	0	-	0	-
Laemobothrion maximum	1	1	0	-	-	0	-	-	2	0	2	0
Ricinus fringillae	0	0	0	-	-	0	-	-	0	-	0	-
Trochiloecetes rupununi	0	0	0	-	-	0	-	-	0	-	0	-
Trochiliphagus abdominalis	0	0	0	-	-	0	-	-	0	-	0	-
Therodoxus oweni	1	1	0	-	-	0	-	-	0	-	0	-
Paraheterodoxus insignis	1	1	0	-	-	0	-	-	0	-	0	-
Boopia tarsata	1	1	0	-	-	0	-	-	0	-	0	-
Latumcephalum lesouefi/ macropus	1	1	0	-	-	0	-	-	0	-	0	-
Paraboopia flava	1	1	0	-	-	0	-	-	0	-	0	-
Amyrsidea ventralis	1	1	1	0	-	1	0	-	1	1	0	-
Rediella mirabilis	1	1	0	-	-	0	-	-	0	-	0	-
Somaphantus lusius	1	1	0	-	-	1	1	-	0	-	0	-
Bonomiella columbae	0	0	0	-	-	0	-	-	0	-	0	-
Menopon gallinae	1	1	0	-	-	1	0	-	0	-	0	-
Numidicola antennatus	1	1	0	-	-	1	1	-	1	1	1	1
Hohorstiella lata	1	1	1	1	-	1	0	-	1	0	1	1
Menacanthus stramineus	1	1	1	0	-	1	0	-	1	0	1	1
Colimenopon urocolius	1	1	0	-	-	1	1	-	1	1	1	1
Machaerilaemus laticorpus/ latifrons	1	1	1	1	-	1	1	-	1	1	1	1
Neomenopon pteroclurus	1	1	0	-	-	1	1	-	1	0	1	0
Dennyus hirundinis	0	0	0	-	-	0	-	-	1	0	1	0
Myrsidea victrix	0	0	1	1	-	1	0	-	1	0	1	0
Ancistrona vagelli	0	0	0	-	-	1	1	-	1	1	1	1
Austromenopon crocatum	1	1	1	1	-	1	1	-	1	1	1	1
Eidmanniella pellucida	1	1	0	-	-	1	0	-	1	0	1	0
Holomenopon brevithoracicum	1	1	0	-	-	1	0	-	1	0	0	-
Plegadiphilus threskiornis	1	1	0	-	-	1	1	-	1	0	1	0
Actornithophilus uniseriatus	1	1	0	-	-	1	0	-	0	-	0	-
Chapinia robusta	1	1	0	-	-	1	0	-	1	0	1	0
Gruimenopon longum	1	1	0	-	-	1	0	-	0	-	0	-
Meromenopon meropis	1	1	0	-	-	1	0	-	1	0	1	1
Eomenopon denticulatum	1	1	2	-	0	2	-	0	0	-	0	-
Hoazineus armiferus	1	1	0	-	-	1	0	-	1	0	0	-
Pseudomenopon pilosum	0	0	0	-	-	1	1	-	1	1	0	-
Trinoton anserinum	1	1	0	-	-	1	0	-	1	0	1	1
Colpocephalum zebra	1	1	2	-	1	0	-	-	0	-	0	-
Comatomenopon elbeli/ elongatum	1	1	2	-	1	0	-	-	0	-	0	-
Ardeiphilus trochioxus	1	1	2	-	1	2	-	1	0	-	0	-
Ciconiphilus quadripustulatus	1	1	2	-	1	0	-	-	0	-	0	-
Cuculiphilus fasciatus	1	1	2	-	1	2	-	2	0	-	0	-
Odoriphila clayae/ phoeniculi	1	1	2	-	0	2	-	0	0	-	0	-
Osborniella crotophagae	1	1	2	-	1	0	-	-	0	-	0	-
Psittacomenopon poicephalus	1	1	2	-	1	0	-	-	0	-	0	-
Piagetiella bursaepelecani	1	1	2	-	1	2	-	0	0	-	0	-

	138	139	140	141	142	143	144	145	146	147
Liposcelis bostrychophilus	0	-	0	-	0	-	-	-	-	-
Laemobothrion maximum	0	-	0	-	0	1	1	0	2	0
Ricinus fringillae	0	-	0	-	0	1	1	0	2	1
Trochiloecetes rupununi	0	-	0	-	0	1	0	0	2	1
Trochiliphagus abdominalis	0	-	0	-	0	1	1	0	2	1
Therodoxus oweni	0	-	0	-	1	1	1	0	3	0
Paraheterodoxus insignis	0	-	0	-	1	1	1	1	3	0
Boopia tarsata	0	-	0	-	1	1	1	1	3	0
Latumcephalum lesouefi/ macropus	0	-	0	-	1	1	1	1	3	0
Paraboopia flava	0	-	0	-	1	1	1	1	3	0
Amyrsidea ventralis	0	-	1	3	0	0	0	0	0	-
Rediella mirabilis	0	-	0	-	0	0	0	0	1	2
Somaphantus lusius	0	-	0	-	0	1	0	0	0	-
Bonomiella columbae	0	-	1	0	0	-	-	-	-	-
Menopon gallinae	0	-	1	2	0	0	0	0	0	-
Numidicola antennatus	0	-	1	2	0	0	0	0	2	1
Hohorstiella lata	0	-	1	0	0	0	0	0	2	1
Menacanthus stramineus	0	-	1	0	0	1	1	0	2	1
Colimenopon urocolius	0	-	1	0	0	1	0	0	2	1
Machaerilaemus laticorpus/ latifrons	1	1	1	0	0	0	0	0	2	0
Neomenopon pteroclurus	1	0	?	?	0	-	-	-	-	-
Dennyus hirundinis	0	-	1	0	0	1	0	0	2	1
Myrsidea victrix	1	0	1	0	0	0	0	0	2	1
Ancistrona vagelli	0	-	1	1	0	1	0	0	2	4
Austromenopon crocatum	1	1	1	0	0	1	0	0	2	1
Eidmanniella pellucida	0	-	1	0	0	0	0	0	2	0
Holomenopon brevithoracicum	0	-	1	1	0	1	0	0	2	1
Plegadiphilus threskiornis	0	-	1	0	0	1	0	0	1	2
Actornithophilus uniseriatus	0	-	1	1	0	1	0	0	1	2
Chapinia robusta	0	-	1	0	0	0	0	0	2	4
Gruimenopon longum	0	-	1	1	0	1	0	0	2	2
Meromenopon meropis	0	-	1	0	0	1	0	0	2	1
Eomenopon denticulatum	0	-	1	0	0	1	0	0	2	1
Hoazineus armiferus	0	-	1	0	0	1	0	0	2	1
Pseudomenopon pilosum	0	-	1	0	0	1	0	0	2	1
Trinoton anserinum	0	-	1	0	0	1	0	0	1	3
Colpocephalum zebra	0	-	1	0	0	1	0	0	1	3
Comatomenopon elbeli/ elongatum	0	-	1	0	0	1	0	0	1	3
Ardeiphilus trochioxus	0	-	1	0	0	1	0	0	1	3
Ciconiphilus quadripustulatus	0	-	1	0	0	1	0	0	1	3
Cuculiphilus fasciatus	0	-	1	0	0	0	0	0	2	1
Odoriphila clayae/ phoeniculi	0	-	1	0	0	1	0	0	1	3
Osborniella crotophagae	0	-	1	0	0	1	0	0	1	3
Psittacomenopon poicephalus	0	-	1	0	0	1	0	0	2	1
Piagetiella bursaepelecani	0	-	1	0	0	1	0	0	1	3

CHAPTER 3

Competing Phylogenies in lice (Phthiraptera: Amblycera). Conflict between EF1α, COI and morphology.

3.1: Abstract

I compare the ability of different molecular and morphological datasets to estimate the phylogenetic relationships of a suborder of lice (Phthiraptera: Amblycera). Twenty-two genera from three of the seven families of amblyceran lice (Menoponidae, Laemobothriidae and Ricinidae) and an outgroup taxon, the booklouse, *Liposcelis bostrychophilus* (Liposcelididae) were included in the analyses. The molecular datasets comprised 348 bp DNA from the nuclear encoding elongation factor 1 alpha (EF1α) and 384 bp DNA from the mitochondrial gene cytochrome oxidase subunit 1 (COI). The morphological data was extracted from the dataset compiled in chapter two.

Results of a partition homogeneity test suggested that each of the datasets were emitting different phylogenetic signals. The molecular datasets were analysed both individually and in combination using both equally weighted maximum parsimony and maximum likelihood reconstruction methods. The morphological data were analysed using equally weighted maximum parsimony. For the same reconstruction method, the analyses of the different datasets produced trees which were incongruent. In some cases the

phylogenies also differed between different methods of reconstruction for the same dataset. These findings suggest, that at present, we still lack an appropriate molecular marker for amblyceran louse phylogeny.

3.2: Introduction

The phylogenetic relationships of lice (Phthiraptera) have proven difficult to resolve. Studies investigating a range of groups at different levels have found that molecular markers do not necessarily provide much phylogenetic information (e.g., Cruickshank *et al.*, 2001; Johnson & Whiting, 2002). Here, the relative utility of a nuclear gene, a mitochondrial gene, and morphological data for estimating the phylogeny of three families of amblyceran lice is investigated.

There are four suborders within the Phthiraptera: the Ischnocera, Amblycera, Rhyncophthirina and Anoplura (Königsmann, 1960). Within the Amblycera there are seven recognised families of lice, which parasitise most orders of birds and a small selection of mammals (Fig. 1). The three families of lice examined in this study (Menoponidae, Laemobothriidae and Ricinidae) are all avian parasites (see Fig. 1A-C). The Menoponidae is by far the largest family in the Amblycera, comprising approximately 70 genera, and are hosted by almost every bird order. The Laemobothriidae contains only the genus *Laemobothrion*, members of which mainly parasitise the Falconiformes and Gruiformes (e.g., vultures, hawks, rails and bustards). Species of *Laemobothrion* are also present, albeit rarely, on members of the Ciconiiformes, Cuculiformes, Podicipediformes and Strigiformes (e.g., on some herons and storks, the Hoatzin, the Black-necked Grebe and the Eurasian Tawny Owl). The third family in this study, the Ricinidae, contains only three genera: *Trochiloecetes* and *Trochiliphagus* are confined to the hummingbirds (Apodiformes: Trochilidae), whilst *Ricinus* is a widespread parasite of the Passeriformes (Price *et al.*, in press).

There have been many previous studies focused on amblyceran morphology but the majority of these have been alpha-taxonomic descriptions (e.g., Price, 1965, 1969; Clay, 1971) or review papers, often with keys for the identification of genera or species (e.g., Clay, 1969; Clayton, Price & Page, 1996; Ledger, 1971). Thus, there now exists a huge number of descriptive works in the literature, but very few which have sought to resolve relationships within these lice. The phylogenetic analysis presented in chapter two strongly supported the monophyly of the Menoponidae, Ricinidae and Boopiidae (the Laemobothriidae is a monogeneric family). Additionally that study also proposed four major clades of lice within the largest family, the Menoponidae.

Advances in the field of molecular biology have recently allowed the evolutionary relationships of lice to be investigated using sequence data. Molecular data collection allows the potential acquisition of much greater numbers of characters for phylogenetic analyses than morphology can possibly provide. There have, however, been only a small number of molecular phylogenetic analyses of the Phthiraptera which have included amblyceran taxa. These studies have either focused on recovering a species phylogeny within a particular genus (e.g., Barker, Briscoe & Close, 1992; Page *et al.*, 1998) or have included only a small number of amblyceran genera as part of a larger investigation into the phylogeny of another louse suborder (e.g. Johnson & Whiting, 2002) or the Phthiraptera as a whole (e.g., Barker *et al.*, submitted; Cruickshank *et al.*, 2001).

This study provides the first opportunity to compare a morphologically based phylogeny for amblyceran genera (see chapter two) with trees generated from molecular datasets. The aims of this study are to compare the results from

three datasets (one morphological and two molecular) for amblyceran genera, and test whether they could be combined in a single phylogenetic analysis by examining the phylogenetic signal in the data. If significant conflict exists between the datasets, the reasons for this will be investigated. For example, could any differences between the phylogenies generated be explained by the presence of just a few floating taxa (i.e., difficult to place taxa) and thus provide independent support for the phylogenetic positions of the remaining lice? Alternatively, is there a fundamental incongruence between the competing topologies?

3.3: Materials and Methods

3.3.1: Selection of taxa

The selection of taxa for this study was determined by the availability of both morphological and sequence data. The different datasets were compared and the genera common to all three were used in the subsequent analyses. A morphological dataset for 44 amblyceran genera and an outgroup taxon *Liposcelis bostrychophilus* was available from a previous study using 147 morphological characters (chapter two). A molecular dataset of 348 base pairs (bp) DNA from the nuclear gene EF1α from 44 amblyceran species (plus *Liposcelis*) was available from Cruickshank *et al.* (2001). A second molecular dataset of 384 bp from the mitochondrial gene COI for 41 amblyceran species (plus *Liposcelis*) was provided by Kevin P. Johnson.

Of the maximum possible 44 taxa, there were 23 genera for which I had all three types of data. In 7 cases the morphological and molecular data were

for the same species. For the remaining 16 genera, the species differed, and a closest match was chosen based on host taxon relationships. The taxa selected for each of the final datasets (morphology, EF1α, COI, and EF1α+COI) are shown in Table 1.

3.3.2: Comparison of signal

The partition homogenity test in PAUP* (Swofford, 2002; the ILD of Farris *et al.*, 1994; 1995) was used to compare the phylogenetic signal in the data (1000 replicates). Stepwise addition sequence replicates were used with tree bisection reconstruction (TBR) branch swapping on the best trees only. Multi-parsimonious trees (MULPARS option) were held during the branch-swapping process. Comparisons between data partitions were made as follows: morph/EF1α, morph/COI, morph/EF1α+COI, morph/EF1α/COI and EF1α/COI.

3.3.3: Parsimony analyses

Individual phylogenetic reconstructions for each of the datasets (morphology, EF1α, COI, and EF1α+COI) were obtained using equally weighted maximum parsimony in PAUP* (unless otherwise stated all analyses were conducted in PAUP*). Heuristic searches using 1000 random addition sequence replicates with stepwise addition and TBR branch swapping were completed. All equally parsimonious trees were held for inclusion into the branch swapping and all multi-parsimonious trees were held during this process. All characters were treated as unordered and of equal weight. Branch

support values were determined via the bootstrap method (Felsenstein, 1985) in a heuristic search using 100 replicates with TBR branch swapping.

3.3.4: Maximum likelihood analyses

The parameters for the maximum likelihood search were obtained from Akaike Information Criterion output scores produced using Modeltest 3.06 (Posada & Crandall, 1998).

Maximum likelihood trees for each of the four datasets outlined above, were obtained in a heuristic search using the as-is addition sequence with TBR branch swapping on best trees only and multi parsimonious trees were also held. Branch support values for the clades recovered in the likelihood analyses were determined via the fast bootstrap method.

3.3.5: Split decomposition

Data sets may contain different and conflicting phylogenetic signal. Split decomposition analysis, as implemented in SplitsTree (Huson, 1998), analyses how tree-like a dataset is. This method does not force data to produce a tree, but data are instead transformed into a sum of "weakly compatible splits" and presented as a splits graph. For perfect data (i.e. with no conflict) this is a tree, whereas for a less than perfect dataset this is more of a tree-like network which displays the conflict in the data (Huson, 1998). A dataset with no signal would therefore be represented as a star formation. Splits were calculated from distance matrices. For the molecular data, Hamming distances were produced in SplitsTree, and for the morphological dataset pairwise distances were obtained via PAUP* using mean character distance.

3.3.6: Tree comparison

In a final assessment the trees obtained from the morphology, EF1α, and COI datasets were compared using the partition metric in Component 2.0 (Page, 1993). The strict consensus cladograms for morphology (maximum parsimony), EF1α (maximum likelihood) and COI (maximum likelihood) were compared. The partition metric (Day, 1985; Penny & Hendy, 1985) counts the total of number of nodes that differ in the two trees. A majority rule consensus tree was produced to summarise all three trees.

3.4: Results

3.4.1: Partition homogeneity test

The comparisons of the data partitions all failed the partition homogeneity test (i.e. had P values ≤ 0.05). For the partitions morph/Ef1α/COI, morph/EF1α, morph/COI and morph/EF1α+COI, P= 0.001, whilst for the partition EF1α+COI, P= 0.005. Given that all five possible comparisons failed this test, this suggests that the datasets each contain a different underlying phylogenetic signal.

3.4.2: Parsimony analyses

The results of the equally weighted maximum parsimony analyses of the four datasets (Fig. 2A-D) clearly differ with regard to their degree of resolution, position of the three families and branch support statistics. From the parsimony analyses the tree produced from analysis of the morphological data (Fig. 2A) has the greatest level of bootstrap support.

The parsimony analysis of the morphological dataset recovered 4 trees (length: 400 steps, CI: 0.470, RI: 0.548). For this set of taxa 118 of the 147 characters were parsimony informative, 19 were variable but non-parsimony informative and 10 characters were constant. The strict consensus tree for the morphological dataset (Fig. 2A) is relatively well resolved. The three amblyceran families comprise separate, strongly supported clades, with *Laemobothrion* as the sister taxon to the Menoponidae. The Ricinidae (*Ricinus* and *Trochiloecetes*) have a strongly supported position at the base of the tree. The disagreement between the four most parsimonious trees recovered from the morphological dataset is restricted just to the positions of suprageneric groups within the Menoponidae.

Parsimony analysis of the EF1α dataset recovered 11 trees (length: 674 steps, CI: 0.384, RI: 0.326). 119 of the sites were parsimony informative, 32 variable but parsimony uninformative and 197 characters were constant. Most nodes in the strict consensus tree (Fig. 2B) are unresolved and branch support values are relatively weak, except for the grouping of *Hohorstiella* and *Meromenopon*. In contrast with the morphological analysis, the Menoponidae are not monophyletic in this tree. The Ricinidae (*Trochiloecetes* and *Ricinus*) have much weaker support as sister taxa than was found in the morphological analysis and are nested within a large clade containing most of the Menoponidae.

The analysis of the COI dataset (Fig. 2C) recovered a single most parsimonious tree (length: 1377 steps, CI: 0.360, RI: 0.265). 201 of the characters were parsimony informative, 47 variable but parsimony uninformative and 136 characters were constant. The COI tree has no bootstrap

support at the >50% level. As was found in the analysis of the EF1α dataset, the Menoponidae are not monophyletic. In addition to this finding, the analysis of the COI gene would also suggest that the Ricinidae are not a monophyletic group.

A single tree was also produced from the analysis of the combined EF1α+COI molecular dataset, (length: 2106 steps, CI: 0.358, RI: 0.255). As in the analysis of the EF1α data, the tree for the combined molecular data (Fig. 2D) also suggests the monophyly of the Ricinidae, although with no bootstrap support. *Trochiloecetes* and *Ricinus* are supported as sister taxa in a clade with *Ancistrona*. Combining the molecular data also failed to produce a tree supporting a monophyletic Menoponidae. However, analysing the molecular data together does produce a more resolved tree than that obtained from analysis of the EF1α data alone. The combined tree contains a lesser number of supported nodes than the Ef1α tree, though more nodes are supported than are found for the analysis of the COI data.

3.4.3: Maximum likelihood analyses

The results of the maximum likelihood analyses of the three molecular datasets (EF1α, COI and EF1α+COI) are shown in Fig. 3 (A-C). The models of evolution used for each of the analyses are presented in Table 2. As was found from the parsimony analyses of the three molecular datasets, the trees found by maximum likelihood analyses of the data are also quite different.

Maximum likelihood analysis of the EF1α sequences produced 3 trees with the ln-likelihood of -3152.0572. The strict consensus of these trees is presented in Fig. 3A. The EF1α tree is almost fully resolved but only 4 nodes

have bootstrap support at the >50% level. The Ricinidae are reasonably well supported as a monophyletic group, but again the Menoponidae are not monophyletic. Interestingly, all nodes present in the parsimony analysis of EF1α (Fig. 2B) are also present in the maximum likelihood analysis (Fig. 3A). Aside from a more resolved tree produced from the likelihood analysis, the only difference in the EF1α trees found by these two methods of reconstruction is in their bootstrap support values: notably, the Ricinidae and the grouping of *Dennyus+Myrsidea* have much better support in the maximum likelihood EF1α tree (Fig. 3A).

Maximum likelihood analysis of the COI sequences produced 3 trees with the ln-likelihood of -5348.9182. The strict consensus of these trees are presented in Fig. 3B. The tree is almost fully resolved, but bootstrap analysis revealed no branch support >50% for this topology. The parsimony (Fig. 2C) and maximum likelihood (Fig. 3B) trees for the COI dataset are completely different. Aside from the rooting of these trees, they do not appear to have a single node in common. Both trees (Figs. 2C, 3B) produced from the COI dataset also have no bootstrap support.

The analysis of the combined EF1α+COI sequences produced 3 trees with the ln-likelihood of - 8721.9522. The strict consensus of these trees is presented in Fig. 3C. Bootstrap analysis of the data revealed only poor support for the monophyly of the Ricinidae.

Contrary to the findings of the parsimony analyses (see Fig. 2B & D), the maximum likelihood analysis of the combined molecular data (Fig. 3C) does not produce a tree similar to that produced from the likelihood analysis of the EF1α dataset (Fig. 3A). Under maximum likelihood, combining the

molecular data produces a topology with an unresolved position near the base of the tree for the Ricinidae with poor bootstrap support for its monophyly (Fig. 3C), whereas the analysis using only the EF1α gene results in a better supported Ricinidae placed near the tip of the tree (Fig. 3A).

3.4.4: SplitsTree analyses

A splits decomposition graph produced from the SplitsTree analysis of the morphological dataset is presented in Fig. 4. This graph summarises the raw phylogenetic signal in the data, which ideally would produce an image resembling a tree. The star-like part of the graph in Fig. 4 shows that there are many taxa for which there is no definite phylogenetic signal. There is also some conflict of signal present in the dataset, which can be identified by the box-like networks between some genera: this is especially apparent near the outgroup taxon *Liposcelis*.

All SplitsTree graphs (Figs. 4-7) are presented using equal edges (rather than to actual scale) for clarity of presentation. Equal edges diagrams can allow the easy identification of groups of taxa and also allow one to easily compare the degree of signal and conflict across a number of different datasets. The SplitsTree analysis of the morphological dataset (Fig. 4) identified 3 groups: *Dennyus+Myrsidea*, *Menacanthus+Colimenopon*, and *Colpocephalum+Ciconiphilus+Cuculiphilus*, although regarding the latter group there appears to be some signal conflict between these three genera.

The drawback from using the equal edges viewing method is that the actual extent of the conflicts between taxa are not represented. For example, in a 'to scale' Splits graph for the morphological dataset (not shown), the split

between *Ricinus* and *Trochiloecetes* was relatively small; what you may expect for two genera accepted as belonging to the same family (Ricinidae). The ricinids and *Liposcelis* were quite far removed from the other taxa, and these genera with the addition of *Laemobothrion* were, in turn, quite distant from the rest of the genera (all Menoponidae) in the dataset.

The splits decomposition graph for EF1α (Fig. 5) shows that although there is not a lot of signal present in this dataset, 5 groups of taxa are clearly defined. However, regarding the *Chapinia+Laemobothrion+Liposcelis* group there is just not enough signal present in the data to resolve these 3 taxa further. Three of the groups found (*Ricinus+Trochiloecetes*, *Dennyus+Myrsidea* and *Colpocephalum+Ciconiphilus*) are also present in the morphology splits graph (Fig. 4). However, the majority of the taxa in Fig. 5 are represented as part of a central star-like network.

The splits decomposition graph for COI (Fig. 6) reveals that there is no clear phylogenetic signal present for almost all of the taxa in this dataset. The analysis identified only 2 groups, neither of which are present in the graphs for the morphological or EF1α datasets (Figs. 4 & 5).

A splits decomposition graph for the combined molecular data (EF1α+ COI) is presented in Fig. 7. The graph shows that when these data are analysed together there is less signal in the data than was found for EF1α (Fig. 5), but more than was present for the COI data (Fig. 6). Two of the three groups identified by the analysis of the combined dataset (Fig. 7, *Ricinus+Trochiloecetes, Trinoton+Liposcelis*) are also found in the separate analyses of EF1α and COI (Figs. 5 & 6, one from each). The grouping of

Myrsidea and *Ciconiphilus* for EF1α+ COI (Fig. 7) was not found in any of the other SplitsTree analyses.

3.4.5: Tree comparison

All four analyses so far (partition homogeneity test, parsimony, maximum likelihood and SplitsTree) have each indicated that the three original datasets (morphology, EF1α and COI) are quite different from each other.

These differences are summarised by a comparison of the strict consensus trees for morphology (maximum parsimony), EF1α (maximum likelihood) and COI (maximum likelihood). The maximum likelihood tree was chosen for EF1α due to the better resolution of the data using this method. The likelihood tree for COI was then selected over the parsimony tree, only to preserve a degree of consistency between the molecular datasets, since neither reconstruction method for this gene resulted in a better supported tree. The comparison of the three strict consensus trees (Fig. 8A) reveals that they are almost equally distant from each other: morphology-COI = 34, COI-EF1α = 38, EF1α-morphology = 28). The EF1α dataset appears slightly closer to the morphological dataset than any of these two are to COI.

The median tree (which is also the majority rule consensus tree) is the tree with the smallest total difference to each of the three strict consensus trees (represented in Fig. 8A by a red dot). The median tree illustrates, in tree space, the conflict between the trees recovered from the three separate datasets, and is presented as the cladogram in Fig. 8B, rooted on the outgroup taxon

Liposcelis. This tree shows that when all three trees are considered, the result is an almost unresolved topology.

3.5: Discussion

3.5.1: 'Conditional combination' or 'total evidence'?

The results from the partition homogeneity tests between all five comparisons of the data were significant, indicating that the datasets differ in their underlying phylogenetic signal. Some authors (e.g., Bull *et al.*, 1993; de Queiroz, 1993) have suggested that this would make these data (in any partition combination) non-compatible for the purposes of combined analyses and thus they would argue that the three datasets should only be analysed separately: in other words they advocate only the 'conditional combination' of data. However, the EF1α and COI datasets *were* combined in some of the analyses in this study after two considerations. Firstly, it has been shown (Dolphin *et al.*, 2000), that the partition homogeneity test may return significant results (such as those found here) if, for example, two molecular data sets are evolving at very different rates. EF1α is a nuclear gene and therefore relatively slowly evolving compared with mitochondrial genes. It was noted that these sequences were quite conserved amongst the taxa in this study, whilst the mitochondrial COI dataset appeared to show a high degree of variation. Recent work by Johnson *et al.* (submitted) has estimated that COI is evolving about 100 fold faster than EF1α in lice. This value is far greater than has been found for other insects, e.g. *Drosophila* (Moriyama & Powell, 1997) and suggests that with a history of multiple substitutions COI may be a very

noisy dataset. Therefore rate heterogeneity between molecular datasets must be at least considered as a possibility for the result obtained in 2 of the 5 comparisons using the partition homogeneity test. A second reason for analysing the combined molecular data stems from the results of Johnson and Whiting (2002). In analyses testing the monophyly of the suborder Ischnocera within a representative sample of the Phthiraptera, they did *not* find a significant difference between EF1α and COI datasets using the partition homogeneity test.

3.5.2: Are the families monophyletic and what are their relationships?

The morphology tree (Fig. 2) has high bootstrap support for both the monophyly of the Ricinidae and Menoponidae and is generally congruent with the full morphological phylogeny of the amblyceran taxa (see chapter two). The positions of some previously poorly supported taxa in certain clades are, however, now unresolved in the current analysis. The Ricinidae are monophyletic in all trees except those produced from the analysis of COI alone (Figs. 2C & 3B). However, given that the COI data could be mostly 'noise' and that all the nodes in the COI trees received no bootstrap support (whilst support for monophyly of the group was 62-99% in other trees) the COI dataset's lack of signal should be taken into consideration.

Both the parsimony and maximum likelihood analyses using either separate or combined gene regions (Figs. 2B-D, 3A-C) put the monophyly of the Menoponidae in question. However, there is either no, or poor, bootstrap support at these nodes. Johnson and Whiting (2002) also failed to recover a monophyletic Menoponidae in a similar study of the Phthiraptera (which

included 7 amblyceran genera) where they compared parsimony and likelihood analyses of three genes (EF1α, COI, and 18S). In a similar result to that presented here Johnson and Whiting (2002) also found that they recovered quite different trees for each of the genes they used.

There is no real consensus about the relationships of the families in this study. Only the morphological tree (Fig. 2A) places the Ricinidae outside a clade containing the Menoponidae and *Laemobothrion*. The likelihood analysis of the combined molecular data (Fig. 3C) similarly places a monophyletic Ricinidae nearer the base of the tree. Notably, *Laemobothrion* is also placed near the base of all trees, except the COI parsimony tree (Fig. 2A) and the combined EF1α+COI maximum likelihood tree (Fig. 3C).

3.5.3: Parsimony versus maximum likelihood?

Both the parsimony (Fig. 2B) and likelihood trees (Fig. 3A) for the EF1α dataset supported the following groups of taxa: *Dennyus +Myrsidea*, *Hohorstiella+Meromenopon*, *Ricinus+Trochiloecetes* and *Ciconiphilus+Colpocephalum*. Maximum likelihood provided a more resolved phylogeny for this dataset than maximum parsimony. For COI, however, both methods of reconstruction produced trees which were entirely incongruent with each other.

3.5.4: Does branch support increase when molecular data are combined?

Although larger datasets may be expected to receive increased bootstrap support, the number of nodes supported for the trees actually fell when the molecular data was combined. This was true of both the parsimony

and the likelihood analyses. If, however, the two molecular datasets are quite heterogeneous and differ in levels of homoplasy, then this result may be expected (Johnson & Clayton, 2000). In this case, the lack of signal and large amount of noise in the COI data may have negated some weak signal in the EF1α dataset.

3.5.5: What does the raw signal in the data indicate?

The SplitsTree analyses (Figs. 4-7) showed some strong but sometimes conflicting signal in the morphological dataset (Fig. 4), whereas there appeared to be very little signal present in both of the molecular datasets. The shape of the EF1α graph (Fig. 5) is similar to COI (Fig. 6). As EF1α is a very slowly evolving gene (with many constant sites for the taxa presented here) the most probable explanation for the EF1α splits graph (Fig. 5) would be that the pattern observed is due to a lack of phylogenetic signal. Conversely, the shape of the COI graph (Fig. 6) could be mainly due to the presence of high but very strongly conflicting signal (noise). If we consider the uncommonly elevated rate of mitochondrial substitution against rates of nuclear substitution in lice (> 100 times, Johnson *et al.*, submitted), then it may be that the COI gene is quite saturated in the Amblycera, and is therefore probably evolving far too fast to be of any use in generic level analyses such as those presented here. COI may be more useful at resolving species relationships within genera instead.

3.5.6: Future prospects

Molecular analyses of louse phylogeny may encounter a number of problems. Differences in rates of evolution mean that certain genes could

sometimes be more suitable than others for reconstructing relationships, depending on the particular taxonomic level under investigation. This study has suggested that EF1α is either too slow to unravel an amblyceran generic level phylogeny, or is too conserved for the relatively small sample of taxa available for this analysis. The mitochondrial COI gene on the other hand may be evolving too quickly to resolve these relationships. There is an elevated rate of mitochondrial substitution in lice (see Page, Cruickshank & Johnson, 2002; Johnson *et al.*, submitted). COI has been shown to be evolving 2-3 times faster in lice than their gopher hosts (Page, 1996), whilst a similar result was found for cytochrome *b* in *Dennyus* lice (Menoponidae) and their swiftlet hosts (Page *et al.*, 1998). Page *et al.* (1998) also noted that this gene had higher rate of replacement in the Phthiraptera compared to other insects.

Both EF1α and COI have relatively high rates of substitution compared with other genes such as 18S. In a study spanning the four suborders of the Phthiraptera, Johnson and Whiting (2002) found that a likelihood analysis of EF1α, COI and 18S combined gave support for regions not supported by 18S alone. Thus, genes which have higher substitution rates are not likely to retain strong enough signal for deep branch relationships, but may have the ability to resolve relationships nearer the tips of the tree (Johnson & Whiting, 2002).

An additional problem for molecular analyses is that some sequences can be difficult to align. Mitochondrial 12S rRNA has been shown to have considerable differences in secondary structure as well as length variation among the 25 amblyceran, ischnoceran and anopluran lice compared by Page *et al.* (2002).

Caterino, Soowan and Sperling (2000) outlined four molecular markers

(COI, 16S [both mitochondrial], EF1α and 18S [both nuclear]) as being well-surveyed and informative across a range of divergence levels. Two of these genes COI and EF1α have proved to be relatively uninformative for the genera studied in the analysis presented here. In order to resolve relationships within the Amblycera, and lice in general, the reality of the current situation is that we need to sample more genes. The "golden gene" or combination of genes may still be out there, we just have to find it.

3.6: Acknowledgements

I would like to thank the following people for their help during the course of this work: Rob Cruickshank, Kevin P. Johnson and Dale Clayton for supplying most of the sequence data. Rod Page, Martyn Kennedy and Vince Smith helped with using the computing software and provided helpful comments on the manuscript.

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Table 1: Taxa included in the analyses.

Table 1: Taxa included in the analyses.

Genus	morphology	molecules
Actornithophilus, Ferris, 1916	*uniseriatus*	*ceruleus*
Amyrsidea, Ewing, 1927	*ventralis*	*spicula*
Ancistrona, Westwood, 1874	*vagelli*	*vagelli*
Austromenopon, Bedford, 1940	*crocatum*	*echinatum*
Chapinia, Ewing, 1927	*robusta*	*lophocerus*
Ciconiphilus, Bedford, 1940	*quadripustulatus*	sp. ex *Cygnus olor*
Colimenopon, Clay, 1941	*urocolius*	*urocolius*
Colpocephalum, Nitzsch, 1818	*zebra*	sp.
Cuculiphilus, Uchida, 1926	*fasciatus*	sp. ex *Chrysococcyx*
Dennyus, Neumann, 1906	*hirundinis*	*hirundinis*
Hoazineus, Gruimaraes, 1940	*armiferus*	*armiferus*
Hohorstiella, Eichler, 1940	*lata*	*lata*
Laemobothrion, Nitzsch, 1818	*maximum*	*atrum*
Liposcelis, Badonnel, 1931	*bostrychophilus*	*bostrychophilus*
Machaerilaemus, Harrison,1915	*laticorpus & latifrons*	sp. ex *Hirundo abyssinica*
Menacanthus, Neumann, 1912	*stramineus*	sp. ex *Penelope*
Meromenopon, Clay, 1941	*meropis*	sp.
Myrsidea, Waterston,1915	*victrix*	*laciniaesternata*
Piagetiella, Neumann, 1906	*bursaepelecani*	*bursaepelecani*
Pseudomenopon, Mjöberg, 1910	*pilosum*	*carrikeri*
Ricinus, De Geer, 1778	*fringillae*	sp. ex *Cyanocompsa*
Trinoton, Nitzsch, 1818	*anserinum*	*querquedulae*
Trochiloecetes, Paine, 1913	*rupununi*	sp.

Table 2: Models of evolution selected by ModelTest for the maximum likelihood analyses. The estimated model parameters are shown.

Table 2: Models of evolution selected by ModelTest for the maximum likelihood analyses.

Gene region	Model	Nucleotide base frequencies				Rate matrix values							
		A	C	G	T	[A-C]	[A-G]	[A-T]	[C-G]	[C-T]	[G-T]	I	α
EF1α	TIMef+I+Γ	0.25	0.25	0.25	0.25	1.0000	0.7202	2.4731	2.4731	10.4837	1.0000	0.5245	1.2497
COI	TVM +I +Γ	0.3371	0.1416	0.1393	0.3820	1.2875	10.5489	1.0965	5.9835	10.5489	1.0000	0.2484	0.4374
EF1α+COI	K81uf +I+Γ	0.2868	0.2011	0.1896	0.3226	1.0000	8.9540	3.6018	3.6018	8.9540	1.0000	0.876	0.7527

Fig. 1: The seven families of amblyceran lice: A) Menoponidae, B) Ricinidae, C) Laemobothriidae, D) Boopiidae, E) Trimenoponidae, F) Gyropidae and G) Abrocomophagidae. All amblyceran taxa in this study are members of A, B and C.

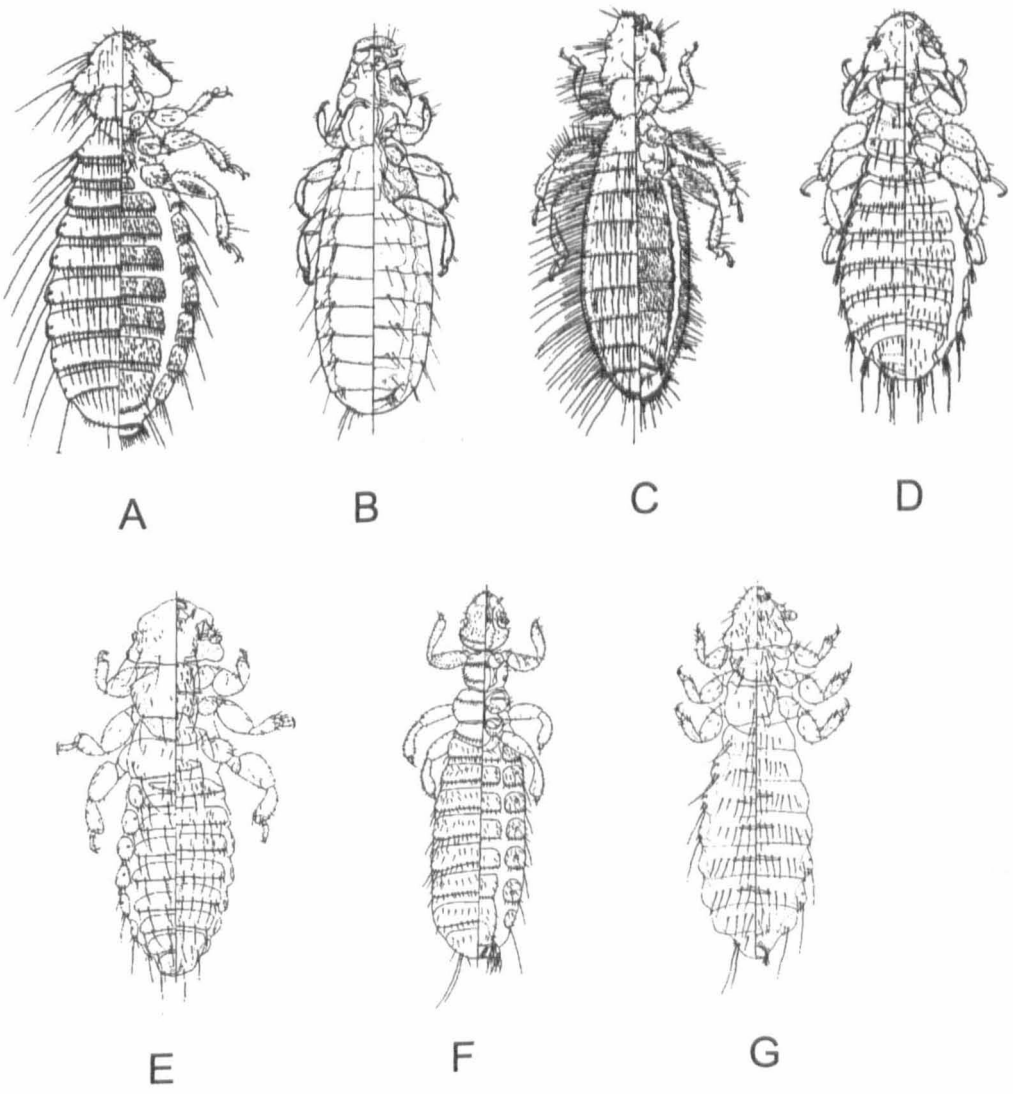

A
B
C
D
E
F
G

Fig. 2: Strict consensus trees obtained from maximum parsimony analyses of A) morphology (4 trees, length 400 steps, CI = 0.470, RI = 0.548), B) EF1α (11 trees, length 674, CI = 0.384, RI = 0.326), C) COI (1 tree, length 1377 steps, CI = 0.360, RI = 0.265) and D) EF1α+COI (1 tree, length 2106, CI = 0.358, RI = 0.255). Branch support values >50% from a heuristic bootstrap analysis (100 replicates) are shown above their respective nodes. The tree is rooted on the outgroup taxon *Liposcelis*. Key for identification of amblyceran families: Ricinidae (R) = □, Laemobothriidae (L) = ★, Menoponidae (M) = blank.

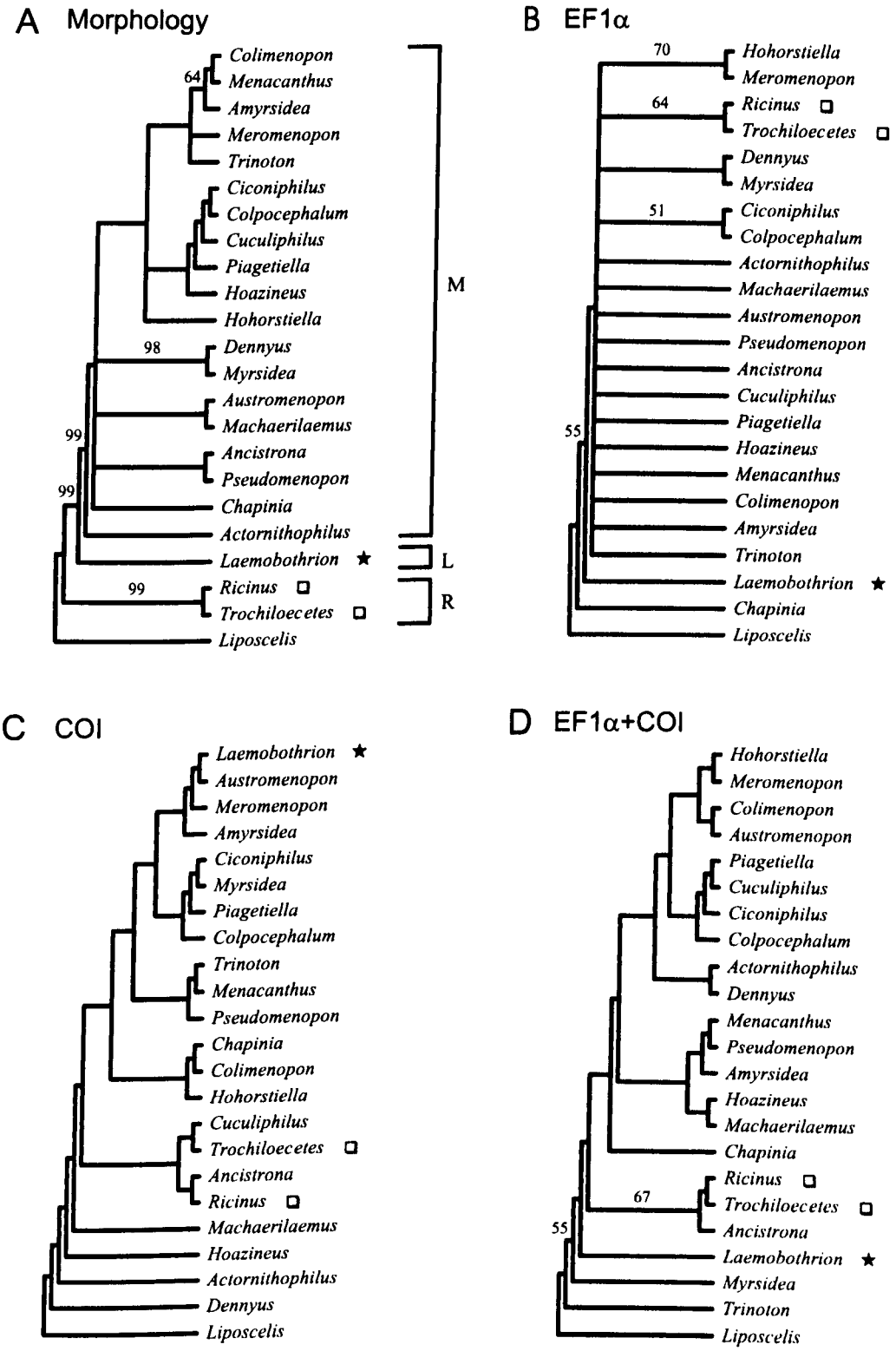
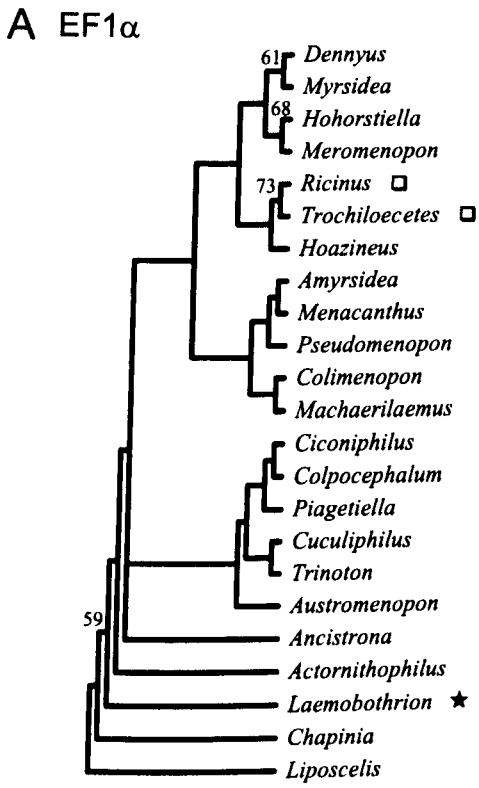

A Morphology
Colimenopon
Menacanthus
Amyrsidea
Meromenopon
Trinoton
Ciconiphilus
Colpocephalum
Cuculiphilus
Piagetiella
Hoazineus
Hohorstiella
Dennyus
Myrsidea
Austromenopon
Machaerilaemus
Ancistrona
Pseudomenopon
Chapinia
Actornithophilus
Laemobothrion
Ricinus
Trochiloecetes
Liposcelis
64
98
99
99
99
M
L
R
B EF1α
Hohorstiella
Meromenopon
Ricinus
Trochiloecetes
Dennyus
Myrsidea
Ciconiphilus
Colpocephalum
Actornithophilus
Machaerilaemus
Austromenopon
Pseudomenopon
Ancistrona
Cuculiphilus
Piagetiella
Hoazineus
Menacanthus
Colimenopon
Amyrsidea
Trinoton
Laemobothrion
Chapinia
Liposcelis
70
64
51
55
C COI
Laemobothrion
Austromenopon
Meromenopon
Amyrsidea
Ciconiphilus
Myrsidea
Piagetiella
Colpocephalum
Trinoton
Menacanthus
Pseudomenopon
Chapinia
Colimenopon
Hohorstiella
Cuculiphilus
Trochiloecetes
Ancistrona
Ricinus
Machaerilaemus
Hoazineus
Actornithophilus
Dennyus
Liposcelis
D EF1α+COI
Hohorstiella
Meromenopon
Colimenopon
Austromenopon
Piagetiella
Cuculiphilus
Ciconiphilus
Colpocephalum
Actornithophilus
Dennyus
Menacanthus
Pseudomenopon
Amyrsidea
Hoazineus
Machaerilaemus
Chapinia
Ricinus
Trochiloecetes
Ancistrona
Laemobothrion
Myrsidea
Trinoton
Liposcelis
67
55

Fig. 3: Strict consensus trees obtained from maximum likelihood analyses of the EF1α, COI and EF1α+COI datasets. Branch support, rooting and identification key as in Fig. 2.


A EF1α
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68
73
59
Dennyus
Myrsidea
Hohorstiella
Meromenopon
Ricinus
Trochiloecetes
Hoazineus
Amyrsidea
Menacanthus
Pseudomenopon
Colimenopon
Machaerilaemus
Ciconiphilus
Colpocephalum
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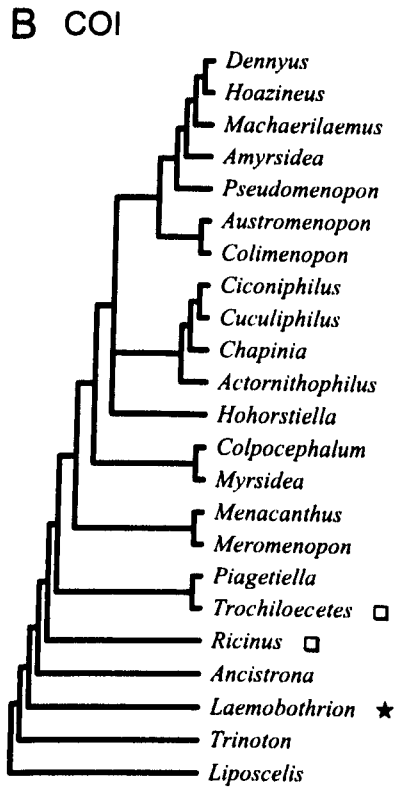
Chapinia
Liposcelis


B COI
Dennyus
Hoazineus
Machaerilaemus
Amyrsidea
Pseudomenopon
Austromenopon
Colimenopon
Ciconiphilus
Cuculiphilus
Chapinia
Actornithophilus
Hohorstiella
Colpocephalum
Myrsidea
Menacanthus
Meromenopon
Piagetiella
Trochiloecetes
Ricinus
Ancistrona
Laemobothrion
Trinoton
Liposcelis

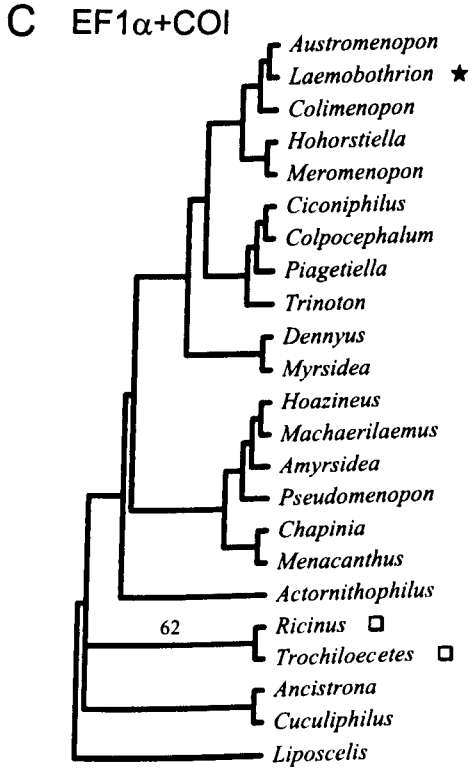
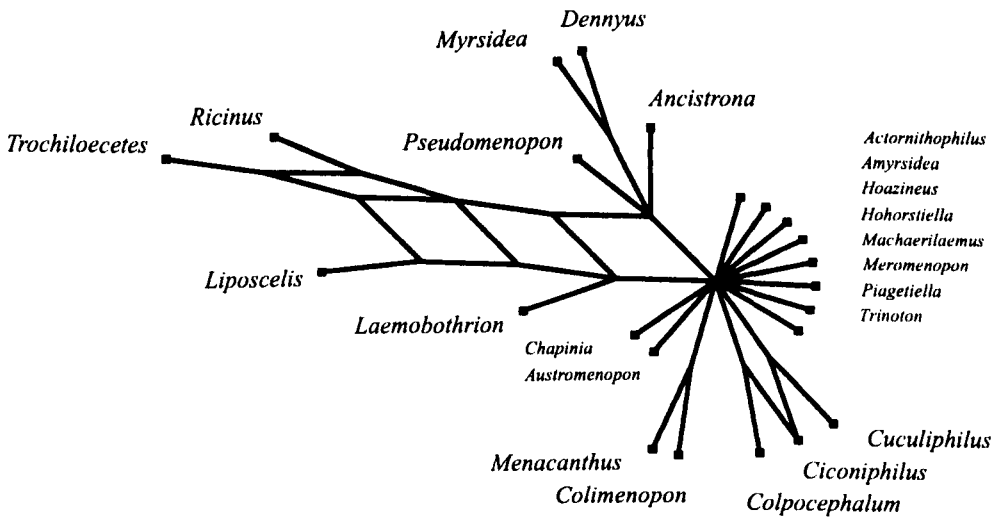

C EF1α+COI
Austromenopon
Laemobothrion
Colimenopon
Hohorstiella
Meromenopon
Ciconiphilus
Colpocephalum
Piagetiella
Trinoton
Dennyus
Myrsidea
Hoazineus
Machaerilaemus
Amyrsidea
Pseudomenopon
Chapinia
Menacanthus
Actornithophilus
62
Ricinus
Trochiloecetes
Ancistrona
Cuculiphilus
Liposcelis

Fig. 4: Results of a SplitsTree analysis of the morphological dataset. Taxa in larger type have definite phylogenetic signal. Box-like networks represent signal conflict within the dataset

Morphology


Dennyus
Myrsidea
Ancistrona
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Pseudomenopon
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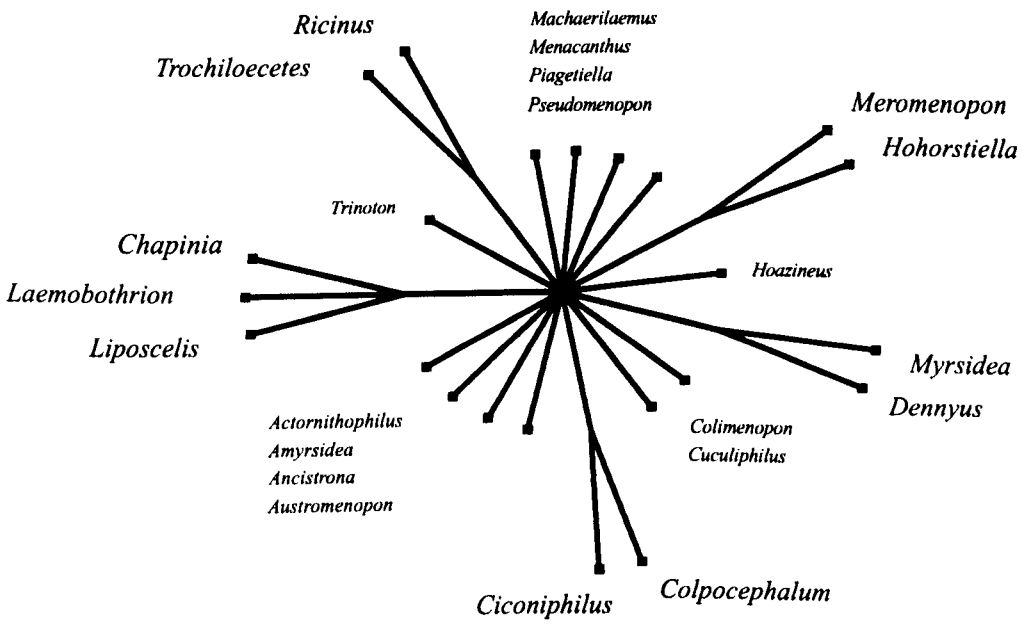
Machaerilaemus
Meromenopon
Piagetiella
Trinoton
Liposcelis
Laemobothrion
Chapinia
Austromenopon
Cuculiphilus
Menacanthus
Ciconiphilus
Colimenopon
Colpocephalum

Fig. 5: SplitsTree analysis of the EF1α dataset. Taxa in larger type have definite phylogenetic signal.

EF1α


Ricinus
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Machaerilaemus
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Hohorstiella
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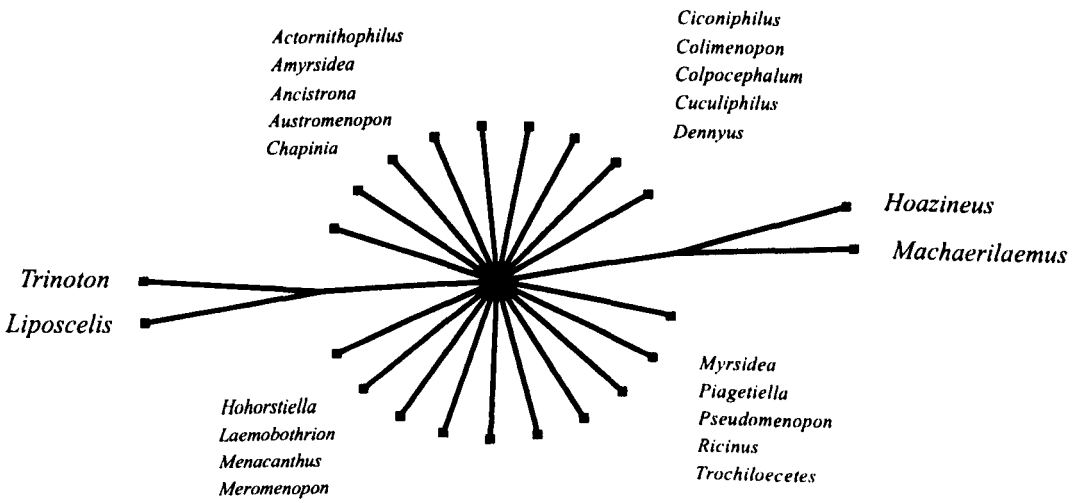
Liposcelis
Hoazineus
Myrsidea
Dennyus
Actornithophilus
Amyrsidea
Ancistrona
Austromenopon
Colimenopon
Cuculiphilus
Ciconiphilus
Colpocephalum

Fig. 6: SplitsTree analysis of the COI dataset. Taxa in larger type have definite phylogenetic signal.

COI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Ancistrona
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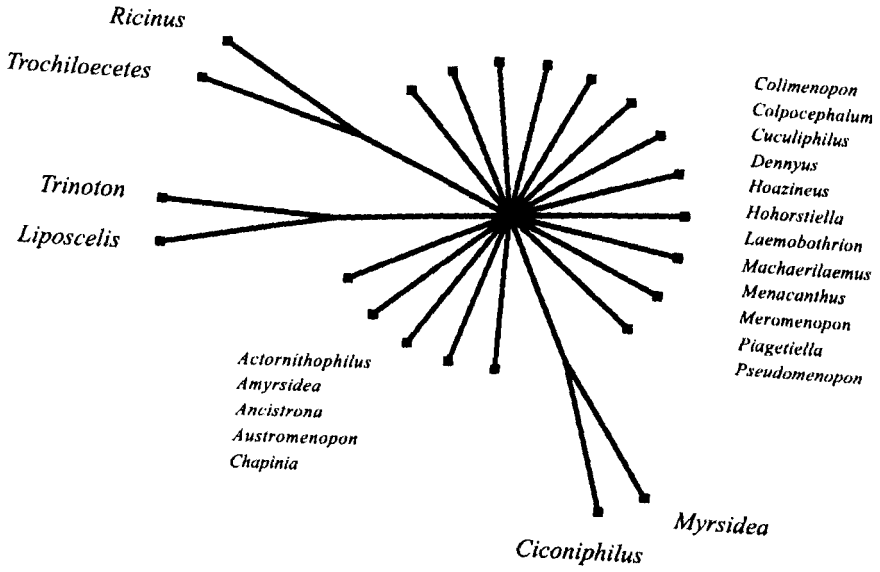
Machaerilaemus
Trinoton
Liposcelis
Hohorstiella
Laemobothrion
Menacanthus
Meromenopon
Myrsidea
Piagetiella
Pseudomenopon
Ricinus
Trochiloecetes

Fig. 7: SplitsTree analysis of the EF1α+ COI dataset. Taxa in larger type have definite phylogenetic signal.

EF1α+COI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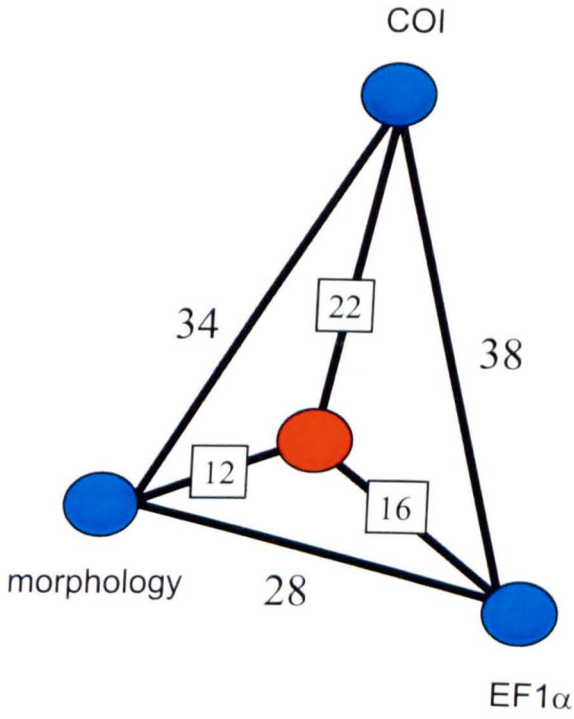
Hoazineus
Hohorstiella
Laemobothrion
Machaerilaemus
Menacanthus
Meromenopon
Piagetiella
Pseudomenopon
Myrsidea
Ciconiphilus

Fig. 8: Summary of the distance between the three datasets (morphology, EF1α and COI). A) Relative distances between datasets. Values on the lines linking the datasets are the partition metric distances between the corresponding trees. The median tree (i.e. that with the smallest total difference to each of the three strict consensus trees) is represented in the centre of the diagram by a red dot). B) The median tree rooted on the outgroup taxon *Liposcelis*.

A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morphology
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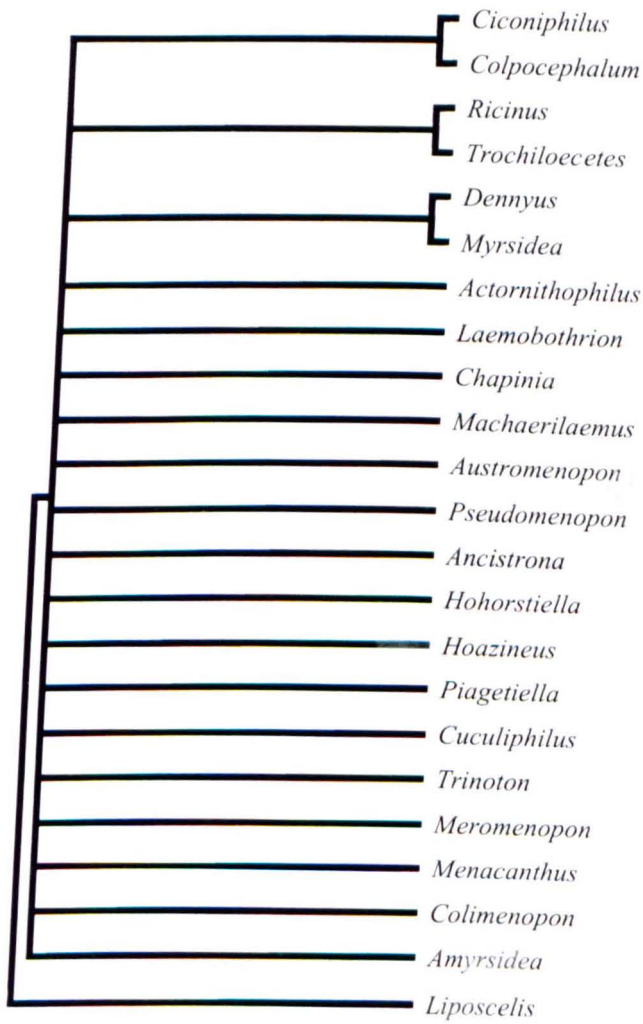
EF1α

B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Colpocephalum
Ricinus
Trochiloecetes
Dennyus
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Actornithophilus
Laemobothrion
Chapinia
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Austromenopon
Pseudomenopon
Ancistrona
Hohorstiella
Hoazineus
Piagetiella
Cuculiphilus
Trinoton
Meromenopon
Menacanthus
Colimenopon
Amyrsidea
Liposcelis

CHAPTER 4

Phylogenetic analyses of the genus *Austromenopon* (Phthiraptera: Amblycera: Menoponidae). Evaluating the coevolutionary relationships between parasites and their avian hosts.

4.1: Abstract

The relationships between 3 genera of lice *Austromenopon*, *Eidmanniella*, *Piagetiella* (Phthiraptera: Amblycera: Menoponidae) were investigated. These amblyceran relationships were compared with those of their seabird hosts (Aves: Charadriidae, Laridae, Phaethontidae, Phalacrocoracidae, Procellariidae, Scolopacidae and Sulidae). Maximum likelihood trees were produced for the lice based on 12S ribosomal RNA and COI data alone and the combined 12S+COI data. *Ancistrona* (Menoponidae) was used to root the phylogeny. Branch support for these analyses was provided by both bootstrap and Bayesian methods. *Austromenopon* was not supported as monophyletic in any of these analyses (as the three *Eidmanniella* and the single *Piagetiella* all fall within *Austromenopon*), whereas the monophyly of *Eidmanniella* did receive strong support. Although some groups of lice were repeatedly supported in the separate and combined analyses, the support for deep branches was generally poor. The tree based on the combined louse dataset was compared with a phylogeny for their hosts. The host

topology was found to be a significantly poorer fit to the louse data than the optimal louse topology. This incongruence suggests a complex shared history, rather than a simple story of pure cospeciation. To investigate the cophylogenetic history of this host-parasite system, a Jungles analysis was conducted of the host and parasite trees. This analysis returned 2 optimal reconstructions. The first proposed a total of 8 cospeciation events, 1 parasite duplication, 5 host switches and 9 parasite sorting events, whereas the second reconstruction had 8 cospeciation events, 0 parasite duplications, 6 host switches and 8 parasite sorting events. Both reconstructions contained significantly more cospeciation events than would have been expected due to chance alone. In comparison to ischnoceran lice on the same type of hosts *Austromenopon* shows a higher degree of host-switching.

4.2: Introduction

Coevolution has often been assumed to occur in host-parasite systems, where there has been a long history of close ecological association and a high dependency on the host (Humphrey-Smith, 1989). Long associations may lead to the evolution of reciprocal adaptations (coadaptation), whilst on a grander scale, there may also be parallel cladogenesis between lineages (cospeciation) (Hafner & Page, 1995; Page & Holmes, 1998).

Lice (Phthiraptera) are wingless, permanent ectoparasites of birds and mammals. They are considered highly host-specific insects and spend their entire life cycle, egg to adult, on their hosts (Clay, 1949). There are four recognised suborders of lice: Amblycera, Ischnocera, Rynchophthirina (all forms of chewing lice) and Anoplura (the sucking lice) (Königsmann, 1960). The Amblycera and Ischnocera are parasites of birds and mammals, whilst the Rynchophthirina and Anoplura are confined to placental mammals. Askew (1971) considered lice to be the insects most committed to their parasitic lifestyle. They are therefore a good choice of parasite for studies of cospeciation.

4.2.1: Factors affecting coevolutionary studies

Farenholz's rule states that the phylogenies of cospeciating hosts and parasites should be, as a consequence, topologically identical (Farenholz, 1913; Eichler, 1948). Adherence to this rule meant that for much of the last century the classification of lice was strongly influenced by that of their hosts and vice versa (Lyal, 1986). In Farenholz's rule non-cospeciating associations

are indicated by incongruence between the compared host and parasite phylogenies. In practise, this very precise rule is easily falsified (e.g. uneven numbers of host and parasite taxa would falsify the rule). This point aside, topological incongruence could result from a number of possible factors including: host-switching (e.g. Clayton, Price & Page, 1996), extinction, lineage sorting (including "missing the boat") (e.g., Clay, 1949; Page, Clayton & Paterson, 1996) and failure to speciate (e.g. Page, 1994a) (Fig. 1). Rather than simply accept or reject Farenholz's rule, modern cospeciation analysis seeks to quantify the relative roles of these processes in a given host-parasite assemblage.

4.2.2: Previous studies of cospeciation involving lice

There have been only a small number of studies testing for cospeciation between lice and their hosts. A study by Lyal (1987) compared a phylogeny for 350 lice of the Trichodectidae (Ischnocera) with available phylogenies for their mammalian hosts. His results were consistent with a predominance of cospeciation between the two groups. Of 198 speciation events, only fifty-one (25.7%) were not compatible with a hypothesis of cospeciation: forty-one events (20.7%) were explained by host-switching and ten (5%) by independent louse speciation (duplication). Lyal (1987) believed that these values were minimum incompatibilities, which would increase with better resolved and more robust phylogenies for both parasite and host. For a recent reinterpretation of Lyal's data see Taylor and Purvis (2002).

Hafner and Nadler (1988,1990) investigated cospeciation in two genera of lice, *Geomydoecus* and *Thomomydoecus* (Ischnocera), and their pocket

gopher hosts (Rodentia: Geomyidae). The host and parasite phylogenies, based on electrophoretic data, had a topological similarity greater than that expected by chance, supporting cospeciation. Page (1990) found that Hafner and Nadler's data required only 2 host-switches out of 9 speciation events. In a later study, Hafner *et al.* (1994) sequenced 379 base pairs of the mitochondrial cytochrome c oxidase subunit I (COI) for both parasites (17 taxa) and hosts (15 taxa). Analysis using Component 2.0 (Page, 1993) showed that the fit between the parasite tree and random host trees was statistically significant, suggesting again that the gopher lice have typically cospeciated with their hosts. Having been examined now from a variety of perspectives e.g. morphology (Timm, 1983), allozymes (Hafner & Nadler, 1988) and nucleotide sequences (Hafner *et al.*, 1994), gophers and their lice have since been described as a model system (Hafner & Page, 1995) and have become the "text book example" of host-parasite cospeciation (Page & Holmes, 1998; Page & Hafner, 1996; Ridley 1993).

Conversely, Barker (1991) compared phylogenies inferred from allozymes from 11 species of the *Heterodoxus octoseriatus* group (Amblycera: Boopiidae) with their rock-wallaby hosts (Marsupialia: *Petrogale*) and found a very different pattern. He concluded that there was much evidence of host-switching, but little evidence for cospeciation or independent speciation of lice. Barker (1994) then reviewed 3 previous studies of cospeciation (Lyal 1987; Hafner & Nadler, 1988; Barker, 1991) between lice and their hosts and concluded that cospeciation was "not the prevailing pattern in the Phthiraptera", a conclusion challenged by Page *et al.* (1996).

The studies outlined above tested for cospeciation between the mammalian-infesting Phthiraptera and their hosts. Studies of the avian-infesting Phthiraptera are even fewer. Page *et al.* (1998) compared phylogenies for *Dennyus* (*Collodennyus*) lice (Amblycera: Menoponidae) and their swiftlet hosts (Aves: Collocalliinae) based on mitochondrial cytochrome *b* data. They found a high degree of cospeciation, with some evidence of host-switching and parasite duplication. As part of their louse tree was unresolved, they compared divergence rates in a subset of cospeciating taxa to reveal that the lice appeared to evolve 2-3 times faster than their hosts (Page *et al.*, 1998). This is similar to the difference in rate of divergence estimated for lice and gophers using COI (Page, 1996; Huelsenbeck, Rannala and Yang, 1997).

Paterson *et al.* (2000) investigated the extent of cospeciation between 6 genera (14 species) of ischnoceran lice and their 11 seabird hosts (Procellariiformes, Sphenisciformes and Charadriiformes) found in the New Zealand region, using mitochondrial 12S ribosomal RNA data. From reconciliation analyses (Page, 1994b) between the three most parsimonious louse trees and the host tree (Paterson, Wallis & Gray, 1995) they found that the coevolutionary history of these lice and their hosts could be explained by around 26 to 27 evolutionary events. They found evidence for cospeciation (9 events) and intra-host speciation (duplication, 3-4 events) with many subsequent sorting events (11-14). However, they found little evidence for host-switching (0-1 events), confirming a finding of an earlier study based on a morphological tree for the lice (Paterson, Gray & Wallis, 1993). Paterson *et al.* (2000) point out that host-switching could be expected to be rare in these lice given the lifestyle of their hosts. There are few interspecific seabird

interactions for these hosts (e.g. they don't share the same nest burrows), resulting in a physical barrier to the horizontal transfer of lice. Even intraspecific host interactions can be limited. For example, most Procellariiformes have little contact with others of their species, except during copulation and nesting (Paterson *et al.*, 2000). Given that lice are dependant on, and unable to survive away from, their hosts for any considerable length of time (Clay, 1949) then this would present a severe hindrance to the colonisation of a new host. Likewise, sorting events such as "missing the boat", where lice are absent on a descendant host due to their uneven distribution within the ancestral host population, may be quite common (Paterson *et al.*, 2000).

The unusually small amount of host-switching found by Paterson *et al.* (2000) inspired us to question whether a different result would be found for amblyceran genera parasitising a similar set of seabird hosts. The avian Ischnocera have a different ecology to that of the avian Amblycera. The Ischnocera are well-adapted to microhabitats on their host, which is reflected in their specialised morphology: short, rounded lice found on the head and neck region and a long thin type found on the wings and back of the host. Amblyceran lice are thought to be less closely adapted to their hosts and generally move freely through the plumage, although there are a few exceptions to this rule: *Piagetiella* is found in the throat pouches of pelicans and cormorants, whilst *Actornithophilus patellatus* spends part of its life-history inside the shaft of curlew flight feathers (Rothschild & Clay, 1952). The Amblycera are considered to have retained more of the ancestral phthirapteran characteristics than the Ischnocera (Rothschild & Clay, 1952).

Their antennae are contained within deep fossae on the ventrolateral surface of the head, which Rothschild & Clay (1952) suggest protect the antennae when the louse runs through the feathers of the host.

If the Amblycera are less adapted to microhabitats on their hosts than the Ischnocera, does that indicate that they may be more likely to switch hosts? Would there be more host-switching than was found for the ischnoceran lice in Paterson *et al.*'s (2000) study, or does the lifestyle of the seabird hosts limit the chances of horizontal transmission in both these groups of lice? The amblyceran genus *Austromenopon* (Menoponidae) is widely distributed across a variety of seabird hosts (Charadriiformes, Procellariiformes and Pelecaniformes). The phylogeny of the genus is currently unknown. Only one previous study has included *Austromenopon* in a comparison of host-parasite relationships (Eveleigh & Amano, 1977), finding that the phenetic relationships of *Austromenopon* implied different host relationships (within alcids) to that generally indicated by ischnoceran genera (*Cummingsiella* and *Saemundssonia*). In addition to *Austromenopon*, examples of other seabird-infesting amblyceran genera were also included in the analyses: *Eidmanniella*, *Piagetiella* (ex Pelecaniformes) and *Ancistrona* (ex Procellariiformes).

To address these issues this study constructed a phylogeny for these amblyceran genera using two mitochondrial genes (12S rRNA and COI). The louse tree was compared with a host phylogeny using the recently implemented Jungles algorithm to test the hypothesis of cospeciation between amblyceran parasites and their hosts. The results were compared with those previously obtained for the Ischnocera.

4.3: Materials and Methods

4.3.1: DNA extraction

The DNeasy Tissue Kit (Qiagen) was used to isolate total genomic DNA. From samples in cool storage, large adult individuals were selected from each of four amblyceran genera (*Ancistrona*, *Austromenopon*, *Eidmanniella* and *Piagetiella*) available for seabird hosts. I chose adult specimens over juveniles to maximise the quantity of DNA, which could be obtained in a single extraction. The lice were decapitated and placed individually into tubes each containing a solution of 180µl ATL (lysis buffer) and 20µl of proteinase K. After mixing the contents thoroughly, samples were incubated in a water bath at 55^0C (the optimal temperature for proteinase K) for 48 hours. A negative was included with each set of extractions.

Post lysis the liquid was transferred from the samples into fresh tubes, leaving the louse exoskeletons to which I then added 50µl of distilled H_20. The lice were then stored for future slide mounting as voucher specimens. 200µl of AL buffer was added to the liquid samples, which were then thoroughly mixed and incubated at 70^0C for 10 mins. AL buffer increases the salt concentration so that DNA will bind to the filters in the following stages. After incubation 200µl of absolute ethanol was added to each of the samples and they were mixed thoroughly. The samples were then transferred into the DNeasy minicolumns with collection tubes and centrifuged at 8000 rpm for 1 min. The flow-through and collection tubes were discarded. With the minicolumns transferred to a new set of collection tubes, 500µl of AW1 buffer was added and samples centrifuged at 8000 rpm for 1 min before discarding the waste

tubes as before. Once the minicolumns were transferred to a third set of tubes, 500µl of a second buffer (AW2) was added to the minicolumns and they were centrifuged for 3 mins at maximum speed (13000 rpm) to dry the column membranes. The high speed ensures that no ethanol is carried over into the elution process. Again, the flow-through and collection tubes were discarded. For the elution of DNA the columns were placed into new 1.5ml eppendorph tubes and 50µl of H_2O was added directly onto the DNeasy column membrane. The samples were left to incubate at room temperature for around 20-30 mins and then centrifuged at 8000 rpm for 1 min. The eluted DNA collected in the eppendorphs was stored at -80^0C for later amplification via the polymearase chain reaction (PCR).

4.3.2: PCR amplification of DNA

The DNA was amplified via PCR using the primers H7005 and L6225 (Hafner *et al.*, 1994) for COI and 12Sai and 12Sbi (Simon *et al.*, 1994) for 12S rRNA. A separate, but similar PCR "master mix" was prepared for amplifying each of the target sequences. The COI mix differed from the 12S rRNA mix only in the specific primers added. The constituents proportional to one sample of PCR mix (total 25µl) were: 2.5µl of Mg free buffer, 3.5µl $MgCl_2$ (25mmol), 2.5µl dNTPs, 0.125µl Taq, 2.0µl primer 1, 2.0µl primer 2 and 10.375µl of H_20. For each PCR sample 2.0µl of specimen DNA was added to 23µl of PCR mix. As well as PCRing the extraction negative, I also included PCR negatives with each PCR reaction, in which the DNA template was replaced with 2.0µl of H_20.

4.3.3: Gel electrophoresis

Post PCR products were run on 2% agarose gels (containing ethidium bromide, in TAE buffer). I mixed the 25µl PCR product with 5µl of 6x loading buffer and ran the samples against 5µl of a 100 bp ladder. Gels were viewed under ultra-violet (UV) light, the DNA in the fluorescent gel bands excised and products stored in tubes at $+4^0$C. I also prepared a tube with gel, which contained no DNA, to use as negative control in the following gel extraction process.

4.3.4: Gel extraction

The products were purified using the QIAquick Gel Extraction Kit (Qiagen). Gel bands were weighed in their tubes and 3 volumes of QG buffer added to 1 volume of gel. After vortexing, they were incubated in a 50^0C waterbath for around 10 mins, until the agarose had completely dissolved. 1 gel volume of Isopropanol was then added to each tube, to precipitate the DNA, and samples were vortexed briefly. Each sample was passed through a QIAquick spin column by centrifuging at 13000 rpm for 1 min. The DNA is bound to the filters in the columns, whilst the dissolved agarose and other liquids flow through and can be discarded. 0.5ml of QG buffer was added to the column filters to remove any traces of agarose and samples were centrifuged at 13000 rpm for 1 min, discarding any flow-through. 0.7ml of PE buffer was then added to the column filters to wash the DNA and they were left to incubate at room temperature for 2 mins, before spinning again at 13000 rpm and discarding the flow-through. The spin step was repeated to ensure the removal of any residual ethanol in the samples from the PE buffer. To elute the

DNA from the filters the sample columns were placed in new, fully labelled, eppendorph tubes either 25µl or 45µl of H_20 (depending on the expected amount of DNA) was pipetted directly onto the filters. The samples were allowed to incubate at room temperature for 2 mins and then centrifuged at 13000 rpm for 1 min. The spin columns were then discarded.

To quantify the amount of cleaned DNA, 5µl of each sample (mixed with 1µl of 6x loading buffer) was run on a test agarose gel. As with the PCR products, the samples were loaded into the gel and ran against 5µl of the 100 bp ladder of molecular weight markers and photographed under UV light. The molecular weight marker has a known quantity of DNA (50ng) of the 500 bp fragment.

4.3.5: DNA sequencing

DNA was sequenced using the ABI Prism Dye terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA polymerase, FS (Perkin-Elmer), ethanol precipitated and run on an ABI 373 Stretch or ABI 377 DNA automated sequencing machine. The forward and reverse sequences for each specimen were combined and checked using Sequence Navigator 1.0 (Applied Biosystems). The COI data was aligned by eye using Se-Al (Rambaut, 1995). The 12S rRNA data was aligned with reference to the secondary structure model of Page (2000) as described by Page, Cruickshank and Johnson (2002).

4.3.6: Taxon sampling

I obtained sequences for 11 representatives of the genus *Austromenopon* (12S rRNA [9 sequences] and COI), 1 for *Piagetiella* (12S

rRNA and COI) and 3 for *Eidmanniella* (COI only). It was not possible to amplify, and thus sequence, 12S for some taxa. I also sequenced 3 representatives of *Ancistrona vagelli* (12S rRNA and COI), each from a different host. *Ancistrona* is a morphologically distinctive, but monospecific genus found on the Procellariiformes. *Ancistrona* was chosen as the outgroup taxon after consideration of a morphological phylogeny of amblyceran genera (see Chapter two). The taxa sampled in this study and their respective hosts are presented in Table 2.

4.3.7: Phylogenetic analyses

The COI dataset contained 382 characters for 18 taxa, of which 213 were variable and 187 were parsimony informative. The 12S rRNA dataset contained 561 characters for 13 taxa. 338 characters were difficult to align and since the homology could not be assigned with any certainty, these were deleted from the analyses. Of the 223 remaining characters, 135 were variable and 108 parsimony informative. The combined 12S+COI dataset contained 943 characters for the 18 taxa.

Maximum likelihood was used for the phylogenetic reconstructions of the three versions of the dataset: 12S rRNA, COI and 12S+COI combined. The hierarchical likelihood ratio test (hLRT) in ModelTest (Posada & Crandall, 1998) was used to select the appropriate model of evolution for each of the analyses. The hLRTs selected the models TVM+I+G for the COI and combined versions of the dataset and HKY+G for the 12S rRNA dataset.

Phylogenetic analyses were conducted using PAUP* 4.0b10 (Swofford, 2002) unless otherwise stated. To save computational time, I used the first tree

recovered from a parsimony search as a starting topology for the respective maximum likelihood analysis. Likelihood analyses used TBR branch swapping on best trees only and multi parsimonious trees were also held during the search (MulPars option). Maximum likelihood was allowed to estimate the submodels parameters for each of the models where appropriate: nucleotide base frequencies, proportion of invariable sites (I), the shape of the gamma distribution (α) and the ratio of transitions to transversions.

4.3.8: Branch Support

A full heuristic bootstrap analysis (Felsenstein, 1985) of 100 random addition sequence replicates with TBR branch swapping on best trees only was conducted using the substitution model used to estimate the ML topologies.

Bayesian methods were also used to estimate support for the trees using MrBayes 2.01 (Huelsenbeck and Ronquist, 2001). The MrBayes block form (http://r6page.zoology.gla.ac.uk/mrbayes/mc.cfm) constructed by Rod Page was used to create MrBayes blocks to input into the programme (the block is appended to the data matrix and run through MrBayes). In this block, the appropriate likelihood model for each dataset and the MCMC search parameters were set. Four MCMC chains were used in the searches. Each of the Bayesian analyses ran for 500,000 generations, with the first 1000 trees (100,000 generations) discarded as "burnin".

4.3.9: Host phylogeny

It is important to avoid the circular argument contained within Farenholz's rule: that host and parasite phylogenies should reflect each other

(Lyal, 1986). Therefore in tests of cospeciation, it is necessary that host and parasite phylogenies are independently inferred (Hafner & Nadler, 1990). The host tree was largely derived from the DNA-DNA hybridisation tree of Sibley and Ahlquist (1990). Where taxa were not present in this tree, their position was resolved using a recent supertree for the Procellariiformes (Kennedy and Page, 2002).

4.3.10: Shimodaira-Hasegawa test

Given the parasite dataset, I asked if it could reject the topology of the host tree (if not, then there is no need to assume any process apart from cospeciation). The SH test was used to compare the parasite tree and the host tree, using the parasite dataset, in a search using RELL distribution with 1000 bootstrap replicates. If the host topology could not be rejected, then it could be argued that these parasites and their hosts have congruent trees, and that their cophylogenetic history could be explained by complete cospeciation. However, if the host topology was found to be significantly different from the louse tree, the incongruence between the parasite and host topologies would need further explanation.

4.3.11: Cospeciation analyses

The host tree and the parasite tree from the combined analysis were visually compared using TreeMap 2.0β (Charleston and Page, 2002). A tanglegram was constructed to reflect the relationships between the lice and their hosts. For this comparison, only the ingroup taxa were included. TreeMap 2.0β (Charleston and Page, 2002) was also used to evaluate the coevolutionary

history of the parasite and host trees via a Jungle analysis (Charleston, 1998). Given a tree for the hosts, a tree for the parasites and a mapping of extant parasites onto extant hosts, this analysis exhaustively searches to find all possible solutions for the relationship between the two trees within a bound "jungle" of potential solutions (Charleston, 1998). The jungle for a given host tree and associated tree is a graph which contains all the potentially optimal solutions interwoven. For a more detailed definition see Charleston (1998). TreeMap then presents the solution(s) that fall within those bounds. After extensive preliminary investigations to find the appropriate bounds for each parameter, the jungle was bound with the following parameters: ≤30 non-codivergence events (non-cospeciation), ≤30 lineage duplications (independent parasite speciation), ≤12 lineage losses (parasite extinctions), ≤12 host-switches and 0 minimum codivergences (cospeciation events). Hosts were allowed a maximum parasite load of 4 taxa at any point in the reconstructions. Events (duplications, lineage losses and host-switches) were equally weighted. Of the solutions found within these bounds, the optimal solutions minimise the overall cost. The significance of the level of cospeciation in the optimal solutions was tested by comparing the amount of cospeciation in each solution with that found between the host tree and 100 randomised parasite trees. These searches were bound with the appropriate parameters for each optimal reconstruction under test.

4.4: Results

4.4.1: Maximum likelihood analyses

The model parameters estimated during the maximum likelihood analyses of the three datasets are presented in Table 2.

The tree produced from the maximum likelihood analysis of the 12S rRNA dataset is presented in Fig. 2. *Austromenopon* is not monophyletic in this tree, since *Piagetiella* is nestled within this genus. Almost all of the branch support for this topology is found nearer the tips of the tree, with those branches reflecting the deeper relationships among taxa being weakly supported. Within the ingroup taxa, three main groups of lice are supported by both the bootstrap and Bayesian analyses. There is strong support from both measures for grouping the two *Austromenopon* from the shearwaters (*Calonectris*), with the species from the Bonin Petrel (*Pterodroma*) receiving poorer bootstrap and Bayesian support as sister taxon to these lice. There is also good bootstrap and strong Bayesian support for the pairing of *Austromenopon* from the Common Redshank (*Tringa*) with the single representative of *Piagetiella* from the cormorant (*Phalacrocorax*). These two clades are sister groups in this tree, but this relationship has no bootstrap support and only a low Bayesian probability value. A third clade contains *A. brevifimbriatum* from the Northern Fulmar (*Fulmarus*) and *A. paululum* from the Great Shearwater (*Puffinus*), which has only moderate bootstrap and Bayesian support.

The COI tree (Fig. 3) contains the same taxa as the 12S rRNA tree, but with the addition of *A. merguli*, *A. stammeri* and three representatives of the genus *Eidmanniella*. Again, most of the branch support is located nearer the

tips of the tree. Within the ingroup taxa, three main groups of lice have both bootstrap and Bayesian support. *Austromenopon* is polyphyletic in this topology. There is strong support from both bootstrap and Bayesian analyses for the grouping of the two *Austromenopon* from the *Calonectris* hosts. The monophyly of *Eidmanniella* is also strongly supported by both analyses, but there is only weak support for relationships within this genus. A clade containing seven taxa, including *Eidmanniella* and the *Calonectris* lice receives reasonable Bayesian support. There is moderate bootstrap and strong Bayesian support for the grouping of four *Austromenopon* taxa (*A. brevifimbriamtum*, *A. paululum*, *A. stammeri* and *Austromenopon* ex *Pterodroma*), all from procellariiform hosts. *Piagetiella* has reasonable Bayesian support as the sister taxon to the *Austromenopon* species from the tropicbird (*Phaethon*).

The 12S+COI combined tree (Fig. 4) is similar to the trees for each dataset analysed separately, in that most of the branch support is found near the tips of the tree and *Austromenopon* is not monophyletic. Again, within the ingroup taxa, three main groups have both bootstrap and Bayesian support. Similar to the COI tree (Fig. 3), there is good bootstrap and strong Bayesian support for a clade containing *A. brevifimbriamtum*, *A. paululum*, *A. stammeri* and the *Austromenopon* species from the Bonin Petrel (*Pterodroma*). Within this group, there is also a high Bayesian probability score for *A. brevifimbriamtum* and *A. paululum* as sister taxa. There is strong support from both measures for the *Austromenopon* from the shearwaters (*Calonectris*) as sister taxa. There is also very strong bootstrap and Bayesian support for the

monophyly of *Eidmanniella*. As in the COI tree, the relationships within *Eidmanniella* are poorly supported.

All three trees are similar in that none of them have any branch support for the main divisions and most of the support is located nearer the tips of the tree. Thus, we can have little confidence in the deep branch relationships for these lice. The tree for the 12S+COI combined dataset was used for the subsequent analyses as this offered a phylogeny based on more information, and was potentially a better estimate of the phylogeny than that suggested by the separate analysis either of the COI or 12S rRNA trees.

4.4.2: Tree comparison

The tanglegram (Fig. 5) allows us to visualise the relationships between the lice and their hosts. The tree for the lice (with the outgroup excluded), based on the 12S+COI combined data was used for this comparison. It is clear that the two trees do not "mirror" each other, and thus we do not see complete cospeciation of host and parasite. In fact, on close examination the picture is one of a potentially complicated coevolutionary history, which requires further investigation and explanation.

4.4.3: Shimodaira-Hasegawa test

The SH test found that the host topology was a significantly worse fit to the louse data than the optimal louse topology (P= 0.000). Given that the louse dataset could reject the host topology as being incongruent with the louse tree, the host and parasite relationships cannot be one of simple cospeciation.

4.4.4: Cospeciation analyses

Jungles analysis was used to infer which cophylogenetic processes (in addition to cospeciation) may have occurred in the coevolutionary history of these groups. The Jungles analysis of the host and parasite trees recovered 145 total solutions, of which 26 were found to be potentially optimal. Of these 26 solutions, 2 solutions had the least cost (total cost of non-codivergent events = 26).

The first of these two solutions is presented as a stacked reconstruction in Fig. 6. This solution proposes that the history of these lice and their hosts can be explained by a total of 8 cospeciation events and 1 parasite duplication, 5 host switches and 9 parasite sorting events. A test of the level of cospeciation in this reconstruction, by comparing the host tree against 100 random parasite trees was significant, finding only 2 trees with 8 or more cospeciation events ($p = 0.02$). The reconstruction (Fig. 6) suggests that the parasitism of these hosts by *Austromenopon* and its relatives was established on the ancestor of the Scolopacidae, Laridae and Charadriidae (*Tringa*, *Alle*, and *Haematopus*). The parasitism of the remaining host families Procellariidae, Sulidae, Phalacrocoracidae and Phaethontidae appears to have resulted from a host-switch from lice on the ancestor of the Scolopacidae (Fig.6: event A). Cospeciation event 2 (Fig. 6: event 2) results in a lineage on the Procellariiformes and a lineage on the Sulidae and Phalacrocoracidae, with the parasitism of the Phaethontidae explained by a host switch from this second lineage (Fig. 6: event B). After cospeciation event 2, an independent louse speciation on the ancestor of the Procellariiformes (Fig. 6: yellow square) leaves two lineages of *Austromenopon* on these hosts. One of these lineages is

punctuated with a number of sorting events, resulting in the only representatives being those parasites on the *Calonectris* hosts (Fig. 6: event 8). The other lineage on the Procellariiformes appears to have had a more complex history, with 3 cospeciations (Fig. 6: events 5,6 and 7), 2 sorting events and 2 host switches (Fig. 6: events C and D). One of these switches (event C) explains the presence of *A. haematopi* on the charadriiform host. Cospeciation event 3 results in one lineage of *Eidmanniella* on the Sulidae and another on the Phalacrocoracidae, with the presence of *Piagetiella* on *Phalacrocorax bougainvilli* the result of a major host switch at event E.

The second solution (Fig. 7) is almost identical to that proposed in Fig. 6. This reconstruction suggests a shared history explained by a total of 8 cospeciation events and no parasite duplications, 6 host switches and 8 parasite sorting events. There is one more host switch and one less sorting event in this reconstruction, than in Fig. 6. The amount of cospeciation in this second reconstruction was also found to be significant ($p<0.01$). The only difference between the two reconstructions concerns the parasites of the Procellariidae. Instead of an independent louse speciation event (duplication) on the ancestor of the Procellariidae (Fig. 6) resulting in two lineages of closely related lice on these hosts (one of which has a sorting event at the next node), a cospeciation event (Fig. 7: event 1) is followed by a host switch onto the alternative host lineage (Fig. 7: event A).

4.5: Discussion

4.5.1: Louse phylogeny

Although we can have little confidence in the branching patterns of any of the trees found by the maximum likelihood analyses (Figs. 2-4), they do agree about some of the relationships. The genus *Austromenopon* was not found to be monophyletic. It may be possible that this genus just does not constitute a good group. Previously, Eveleigh and Amano (1977) had difficulty with the level of clustering in this genus in their phenetic analyses, whilst they found that the clustering patterns within the ischnoceran genera they studied were clearer. Both the COI and the 12S+COI combined trees support the grouping of the *Austromenopon* taxa: *A. brevifimbriamtum*, *A. paululum*, *A. stammeri* and the *Austromenopon* species ex *Pterodroma*. In the 12S rRNA tree, where only two of these taxa are present (*A. brevifimbriamtum*, and *A. paululum*), they have good support as sister taxa. The grouping of the two *Austromenopon* taxa from the *Calonectris* hosts has a 100% support in all three analyses (Figs. 2-4). None of the data shows any genetic differentiation between these two lice. Given this lack of differentiation, and since *Calonectris edwardsii* is sometimes considered a subspecies of *Calonectris diomedea* (e.g. Sibley & Alquist, 1990), it is likely that both taxa are *A. echinatum*.

The genus *Eidmanniella* is strongly supported as a monophyletic group with the two lice from the gannet hosts (*Morus*) as sister taxa, nestled within *Austromenopon*. The sister taxon to *Eidmanniella* differs between the COI and combined trees, although neither relationship receives any support. According

to host records the unidentified *Eidmanniella* species ex *Morus bassanus* (Northern Gannet) is expected to also be *E. pustulosa*. However, there is definite genetic differentiation present in the data for the two *Eidmanniella* sp from *Morus* hosts (18.2% sequence divergence). These two lice are almost as divergent as *E. pustulosa* is with *E. pellucida* (19.3%). If the sample *Eidmanniella* sp *ex Morus bassanus* is truly the representative louse off a correctly identified host, then this suggests "*E. pustulosa*" is more than one species.

The lack of genetic differentiation in both the 12rRNA data (Fig. 2) and the COI dataset (Fig. 3) would suggest that the single species *Ancistrona vagelli* is valid for this genus, even over such a wide host and geographical distribution. There is almost no difference between these three samples in both the datasets (COI <2.4% and 12S rRNA <1.8% sequence divergence).

4.5.2: Have these lice cospeciated with their hosts?

The most basic test of host-parasite cospeciation is whether two topologies are more similar than would be expected due to chance alone. Both of the stacked reconstructions produced from the Jungles analysis (Figs. 6-7) contained amounts of cospeciation that were found to be significant, against randomly generated parasite trees. These findings support the conclusion that the general pattern of association between these amblycerans and their hosts has been one of descent, rather than association by colonisation. Paterson *et al.* (2000) obtained the same result for ischnoceran lice and their procellariiform and sphenisciform hosts. Dickens (2002) studied the cophylogenetic histories of albatrosses (Procellariiformes: Diomedeidae) and four genera of

ischnoceran lice, finding highly significant cospeciation between two of these genera and their hosts. Considered together, this study and the work of others (Paterson *et al.*, 2000; Dickens, 2002) would suggest a tendency for both amblyceran and ischnoceran lice to cospeciate with seabird hosts.

4.5.3: Do the Amblycera and the Ischnocera show similar coevolutionary histories with their hosts?

Although this study found a significant amount of cospeciation between the Amblycera and their hosts, the SH test rejected the complete cospeciation of host and parasite, indicating the need for a more detailed explanation of their coevolutionary history. The reconstructions from the Jungles analysis (Figs. 6 & 7) were very similar and suggest a parasite history of 8 cospeciations, 0-1 duplications, 8-9 sorting events and 5-6 host-switches. It is interesting to compare these results with those of Paterson *et al.* (2000), who, for a similar sized dataset (this study compared 15 hosts and parasites, they tested 11 hosts and 14 parasites), found 9 cospeciations, 3-4 duplications, 11-14 sorting events and 0-1 host-switches. The number of cospeciation events found by both studies is similar, but the numbers of instances of the three other types of event are notably different.

4.5.4: Isolation, microhabitat adaptation and independent louse speciation

Paterson *et al.* (2000) reported more intrahost speciation events (duplications) for the Ischnocera (3-4) than was found here for amblyceran lice (0-1). Why might this be so? The idea that isolation favours the acquisition of new characters, or "speciation by isolation", was discussed by Rothschild and

Clay (1952). If hosts are asocial then their parasites may speciate and radiate to fill smaller niches. Paterson *et al.* (2000) suggest such events might be expected on hosts with more than one type of feather and point out that penguins (which have only one feather type) host only one louse. However, this theory does not explain the low level of duplication events found for the Amblycera in this study. Amblyceran lice are considered to have retained more ancestral characters than the ischnocerans, are less adapted and thus not specialised for particular feathers (Rothschild & Clay, 1952). The prevalence of parasite duplication events in a host-parasite system may therefore be influenced not only by host social behaviour and morphology, but also by the ability of the parasite to exploit new microhabitats.

4.5.5: Sorting events: x-events, missing the boat and drowning on arrival

Paterson, Palma and Gray (2002) describe three possible explanations for parasite sorting events. A louse species may be present on a host, but remain uncollected due to a low parasite load (x-event). Both the total numbers of lice and parasite species richness may vary between and within host species. Young or sick birds may host more parasites than adult healthy birds (Rothschild & Clay, 1952). However, Paterson *et al.* (2002) found that above a threshold number of host individuals (>7, sometimes >5 individuals) there is no longer a significant relationship between sampling effort and number of louse species collected. At least one of the sorting events found in this study (Figs. 6 & 7, *Eidmanniella*) is due to an x-event artefact, since *E. pellucida* is also found on *P. bougainvilli*, but not present in our collections.

If the parasite has a patchy distribution, then this will increase the chances of being absent on descendant hosts when new lineages are founded by ancestral host populations: in effect the parasite will have "missed the boat". This may be an explanation for many of the sorting events found, since the majority of the hosts concerned are thought to harbour only a single species of *Austromenopon*. Paterson *et al.* (2000) found more sorting events (11-14) for the Ischnocera, than found here (8-9) for the Amblycera, and consider missing the boat to be an important factor. Paterson, Palma and Gray (1999) suggest that the reduced number of parasite species found on New Zealand birds is a consequence of many parasites missing the boat when hosts went through founder effects.

The final type of sorting event is the extinction of the parasite on the descendant host after a successful cospeciation event ("drowning on arrival"). This may be due either to the inability of the parasite to adapt to host changes, or via its displacement through competition from other newly established parasites (see below).

4.5.6: Host-switching and survival

A successful host-switch requires two elements: the opportunity to do so and the adaptive traits to become established and survive on the foreign host. Clayton, Al-Tamimi and Johnson (2002) list potential ways lice may switch hosts. The switch may be facilitated via parasites 'marooned' on detached feathers (although they point out that this has not been tested), from the use of communal dust baths (as suggested by Clay, 1949) and shared nest holes, or via phoresis on hippoboscid flies. Only two genera of ischnoceran

lice (*Philopterus* and *Brüelia*) have been found attached to these flies (e.g. Thomson, 1937). Rothschild and Clay (1952) note that skuas (Charadriiformes) host a louse peculiar to petrels (Procellariiformes) and point out that in the UK skuas are known to feed on the carcasses of at least one type of petrel (Manx Shearwater). My own observations have shown that *Columbicola* (Ischnocera) lice can survive for more than five days away from their dead host.

The successful establishment on the new host may involve adaptation to a number of potentially different environmental conditions such as the physical structure of feathers, chemical differences in blood or feathers, and host body temperature (Rothschild & Clay, 1952). These sorts of changes might affect the newly acquired lice in a variety of different ways (e.g., movement through the host plumage, feeding, or egg-laying and development) (Rothschild & Clay, 1952). The lice must also be able to successfully compete for resources against other already established parasites and there is evidence that this does happen. Louse crops have been found to contain pieces of mites and the cast larval skins and bits of other lice, and conversely mites are also found inside the empty eggshells of lice (Rothschild & Clay, 1952). Rozsa (1993) suggests that small numbers of lice found on unusual hosts, and often dismissed as "stragglers", may actually be attempting to establish themselves on a new host and that we are witnessing the initial stage in the process.

This study found a high number of host-switches for amblyceran lice (5-6) compared to the ischnocerans (0-1) studied by Paterson *et al.* (2000). They suggest that host-switching may be expected to be rare in the Procellariiformes given that there are few interspecific interactions and thus

little physical contact (Paterson, *et al.*, 1993; Paterson *et al.*, 2000). However seabirds that breed in large interspecific colonies still manage to retain their own lice, despite the opportunity for host-switching.

Taking all of this evidence together, the higher rate of successful host-switching shown here is more likely to be a consequence of the more generalist nature of amblyceran lice, compared to the Ischnocera. This view has some support from the result of a previous study discussed by Clayton *et al.* (2002), which showed that ischnoceran body lice could out-compete the more specialised ischnoceran wing lice. Thus the higher level of host-specificity the more detrimental this may be to a successful host-switch. The Ischnocera (and especially ischnoceran wing lice) may have "burnt their bridges" with respect to switching hosts, in exchange for their successful adaptation to current host microhabitats.

4.5.7: Limitations of the present study

It would have been interesting to compare the relative timing of evolutionary events between the host and parasite trees. This would have allowed the testing of the coevolutionary reconstructions and possibly to have distinguished between competing explanations of host-switching and parasite extinction. However, this was not possible as the phylogenies are not based on comparable genes (Page *et al.*, 1996).

This study offered little confidence in the deep branch relationships of the louse phylogenies and this poor resolution may have influenced the inferences made in the cospeciation analysis. Many *Austromenopon* taxa were missing from the dataset due to a lack of availability for sequencing. Given

that exhaustive sampling of clades and accurate phylogenies for the host and parasite are desirable properties for a rigorous study of cospeciation (Page *et al.*, 1996), it would be highly desirable to obtain more samples of *Austromenopon* from a wider range of hosts. However, this study has provided evidence for some groups within these taxa and has suggested that there may be differences in the coevolutionary histories of amblyceran and ischnoceran lice on the same hosts.

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Table 1: Taxa included in the analyses. LouseBASE specimen numbers for 12S and COI data are given on the right of the table. * denotes specimen data available for COI only. Key: Host families: Charadriidae (CHAR), Laridae (LAR), Phaethontidae (PHAE), Phalacrocoracidae (PHAL), Procellariidae (PRO), Scolopacidae (SCO), Sulidae (SUL). Host orders: Charadriiformes (CHAR), Pelecaniformes (PEL), Procellariiformes (PRO).
LouseBASE searchable database is provided by Page Lab, University of Glasgow: http://r6-page.zoology.gla.ac.uk/LouseBase/2/

Table 1: Taxa included in the analyses.

Louse	Host	Locale	Host Family	Host Order	LouseBASE specimen no.
Ancistrona vagelli	*Fulmarus glacialis* (Northern Fulmar)	Foula, Shetland, UK	PRO	PRO	0003b
Ancistrona vagelli	*Puffinus gravis* (Great Shearwater)	unknown	PRO	PRO	GLA523
Ancistrona vagelli	*Puffinus tenuirostris* (Short-tailed Shearwater)	Bering Sea	PRO	PRO	NI-10
Austromenopon brevifimbriatum	*Fulmarus glacialis* (Northern Fulmar)	unknown	PRO	PRO	GLA522
Austromenopon echinatum	*Calonectris diomedea* (Cory's Shearwater)	Portugal	PRO	PRO	T54
Austromenopon haematopi	*Haematopus ostralegus* (South Island Oystercatcher)	Unknown (NZ?)	CHAR	CHAR	GLA520
Austromenopon merguli	*Alle alle* (Little Auk)	Glasgow, UK	LAR	CHAR	V.9 *
Austromenopon paululum	*Puffinus gravis* (Great Shearwater)	unknown	PRO	PRO	GLA521
Austromenopon stammeri	*Pachyptila salvini* (Medium-billed Prion)	Adelaide, Australia	PRO	PRO	MP-42 *
Austromenopon sp	*Calonectris edwardsii* (Cape Verde Shearwater)	Cape Verde	PRO	PRO	RF-20
Austromenopon sp	*Phaethon rubricauda* (Red-tailed Tropicbird)	Cape Verde	PHAE	PEL	RF-08
Austromenopon sp	*Pterodroma hypoleuca* (Bonin Petrel)	Hawaii, USA	PRO	PRO	NH-08
Austromenopon sp	*Thalassarche chlororhynchos* (Atlantic Yellow-nosed Albatross)	Gough Island, UK	PRO	PRO	GLA654
Austromenopon sp	*Tringa totanus* (Common Redshank)	Foula, Shetland, UK	SCO	CHAR	RF-31
Eidmanniella pellucida	*Phalacrocorax punctatus* (Spotted Shag)	Canterbury, New Zealand	PHAL	PEL	GLA598 *
Eidmanniella pustulosa	*Morus serrator* (Australasian Gannet)	Hawkes Bay, New Zealand	SUL	PEL	GLA645 *
Eidmanniella sp	*Morus bassanus* (Northern Gannet)	Isle of Bute, UK	SUL	PEL	V.17 *
Piagetiella transitans	*Phalacrocorax bougainvillii* (Guanay Cormorant)	Punta San Juan, Peru	PHAL	PEL	GLA600

Table 2: Model parameters as estimated during the maximum likelihood analyses.

Table 2: Model parameters.

Gene region	Model	Nucleotide base frequencies				Rate matrix values							
		A	C	G	T	[A-C]	[A-G]	[A-T]	[C-G]	[C-T]	[G-T]	I	α
12S	**HKY+G**	0.325307	0.164848	0.190560	0.319285	1.00	1.568645	1.00	1.00	1.568645	1.00	0.00	0.592090
COI	**TVM+I+G**	0.314074	0.161157	0.148136	0.376633	0.79786237	11.41195	1.1586198	1.6307371	11.41195	1.00	0.335807	0.496807
12S+COI	**TVM+I+G**	0.315568	0.161842	0.160927	0.361664	0.990110	7.031800	1.293750	1.430780	7.031800	1.00	0.314298	0.800084

Fig. 1: Processes in a host-parasite association. Host and parasite cospeciate (A), or the parasite may speciate independently of its host (B, C). One or more of the descendant parasites may colonise a new host (B), or the parasite may remain on the original host (C). Absence of a parasite from a host where it would be expected to occur may be due to extinction of that parasite (D), or the ancestors of the host lineage may have not inherited the ancestral parasite (E). Hosts may speciate independently of their parasites, so that the two hosts share the same parasite (F). Figure adapted from Page (2002).

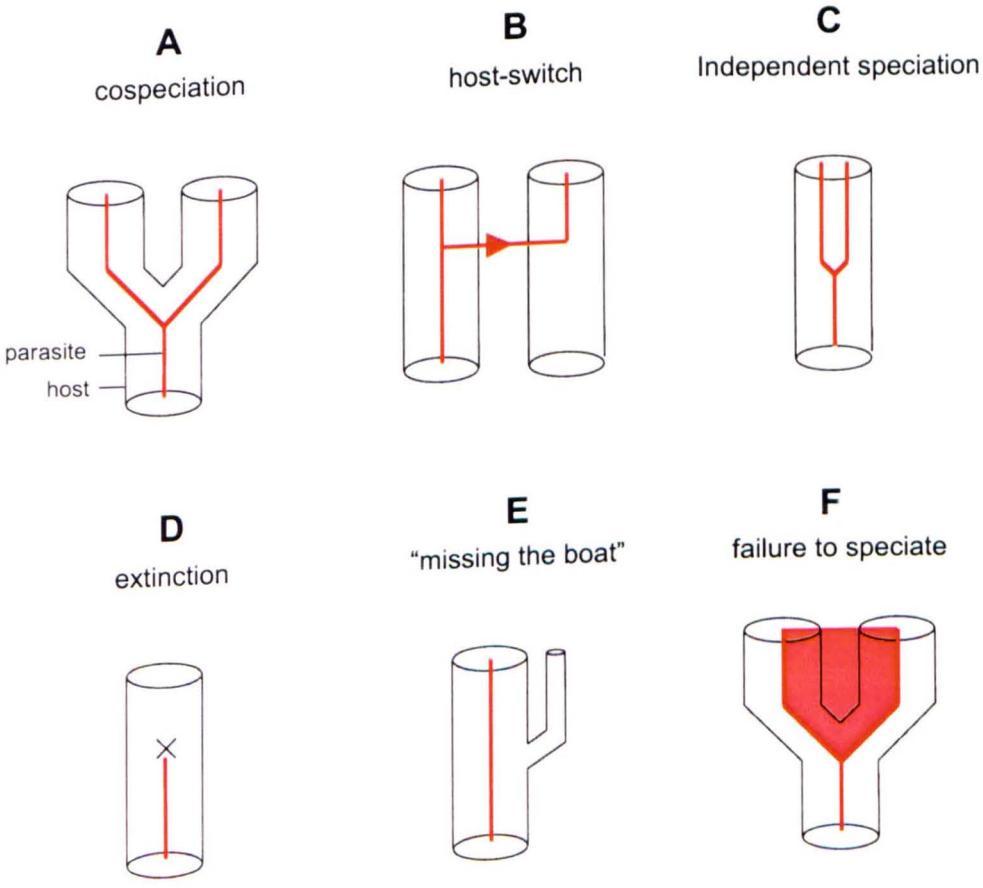

A
cospeciation
B
host-switch
C
Independent speciation
parasite
host
D
extinction
E
"missing the boat"
F
failure to speciate

Fig. 2: Maximum likelihood tree for the genus *Austromenopon* and related taxa based on 12S rRNA sequence data. Bootstrap support values >50% (100 replicates) are shown above the nodes (bold type), with posterior probability scores obtained from a Bayesian analysis (500,000 generations) shown below (regular type). Scale bar = number of substitutions per site.

12S

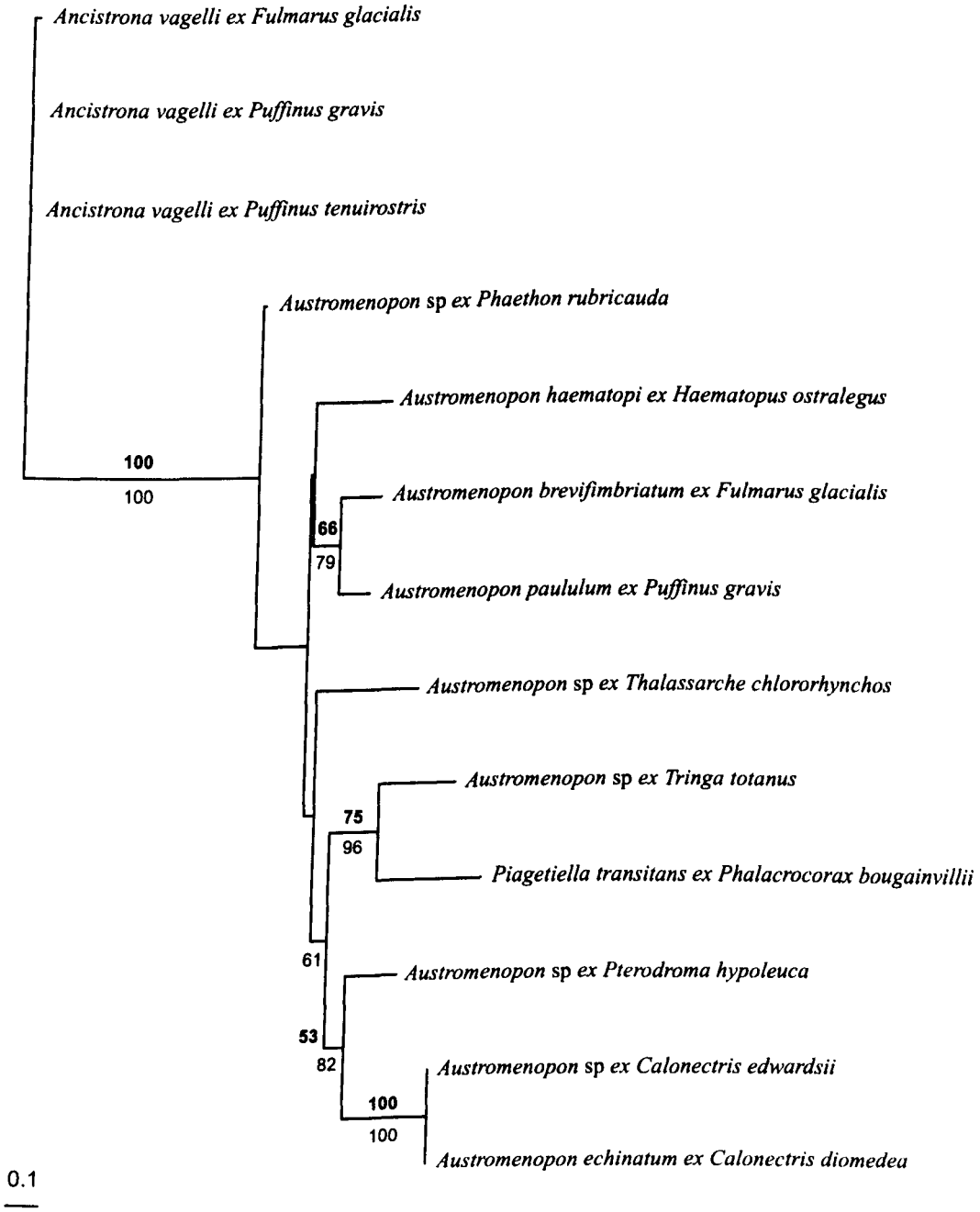

Ancistrona vagelli ex Fulmarus glacialis
Ancistrona vagelli ex Puffinus gravis
Ancistrona vagelli ex Puffinus tenuirostris
Austromenopon sp *ex Phaethon rubricauda*
Austromenopon haematopi ex Haematopus ostralegus
100
100
Austromenopon brevifimbriatum ex Fulmarus glacialis
66
79
Austromenopon paululum ex Puffinus gravis
Austromenopon sp *ex Thalassarche chlororhynchos*
Austromenopon sp *ex Tringa totanus*
75
96
Piagetiella transitans ex Phalacrocorax bougainvillii
61
Austromenopon sp *ex Pterodroma hypoleuca*
53
82
Austromenopon sp *ex Calonectris edwardsii*
100
100
Austromenopon echinatum ex Calonectris diomedea
0.1

Fig. 3: Maximum likelihood tree for the genus *Austromenopon* and related taxa based on COI sequence data. Bootstrap support values >50% (100 replicates) are shown above the nodes (bold type), with posterior probability scores obtained from a Bayesian analysis (500,000 generations) shown below (regular type). Scale bar = number of substitutions per site.

COI

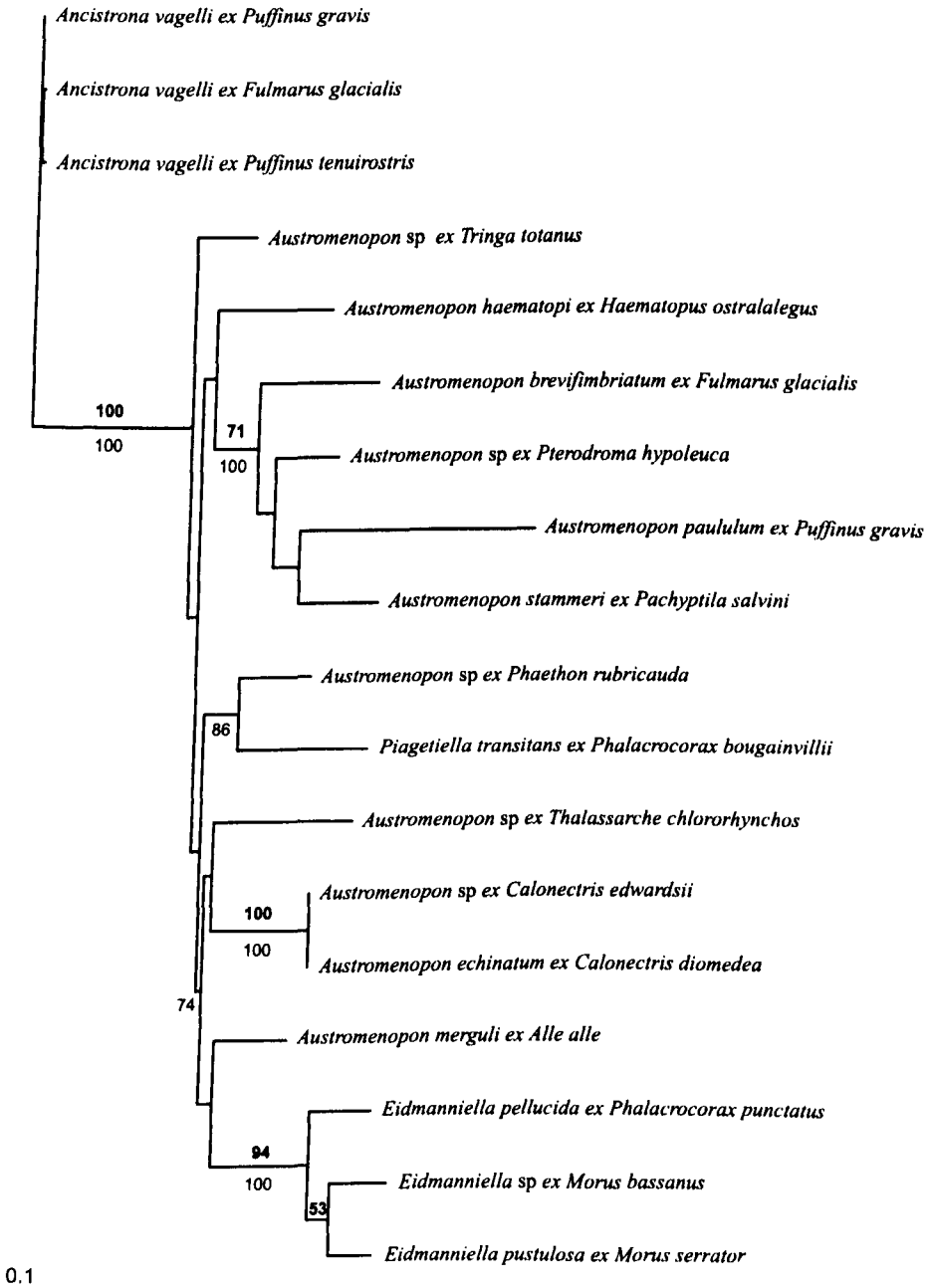

Ancistrona vagelli ex Puffinus gravis
Ancistrona vagelli ex Fulmarus glacialis
Ancistrona vagelli ex Puffinus tenuirostris
Austromenopon sp ex Tringa totanus
Austromenopon haematopi ex Haematopus ostralalegus
Austromenopon brevifimbriatum ex Fulmarus glacialis
100
100
71
100
Austromenopon sp ex Pterodroma hypoleuca
Austromenopon paululum ex Puffinus gravis
Austromenopon stammeri ex Pachyptila salvini
Austromenopon sp ex Phaethon rubricauda
86
Piagetiella transitans ex Phalacrocorax bougainvillii
Austromenopon sp ex Thalassarche chlororhynchos
Austromenopon sp ex Calonectris edwardsii
100
100
Austromenopon echinatum ex Calonectris diomedea
74
Austromenopon merguli ex Alle alle
Eidmanniella pellucida ex Phalacrocorax punctatus
94
100
Eidmanniella sp ex Morus bassanus
53
Eidmanniella pustulosa ex Morus serrator
0.1

Fig. 4: Maximum likelihood tree for the genus *Austromenopon* and related taxa based on the combined 12S+COI dataset. Bootstrap support values >50% (100 replicates) are shown above the nodes (bold type), with posterior probability scores obtained from a Bayesian analysis (500,000 generations) shown below (regular type). Scale bar = number of substitutions per site.

12S+COI

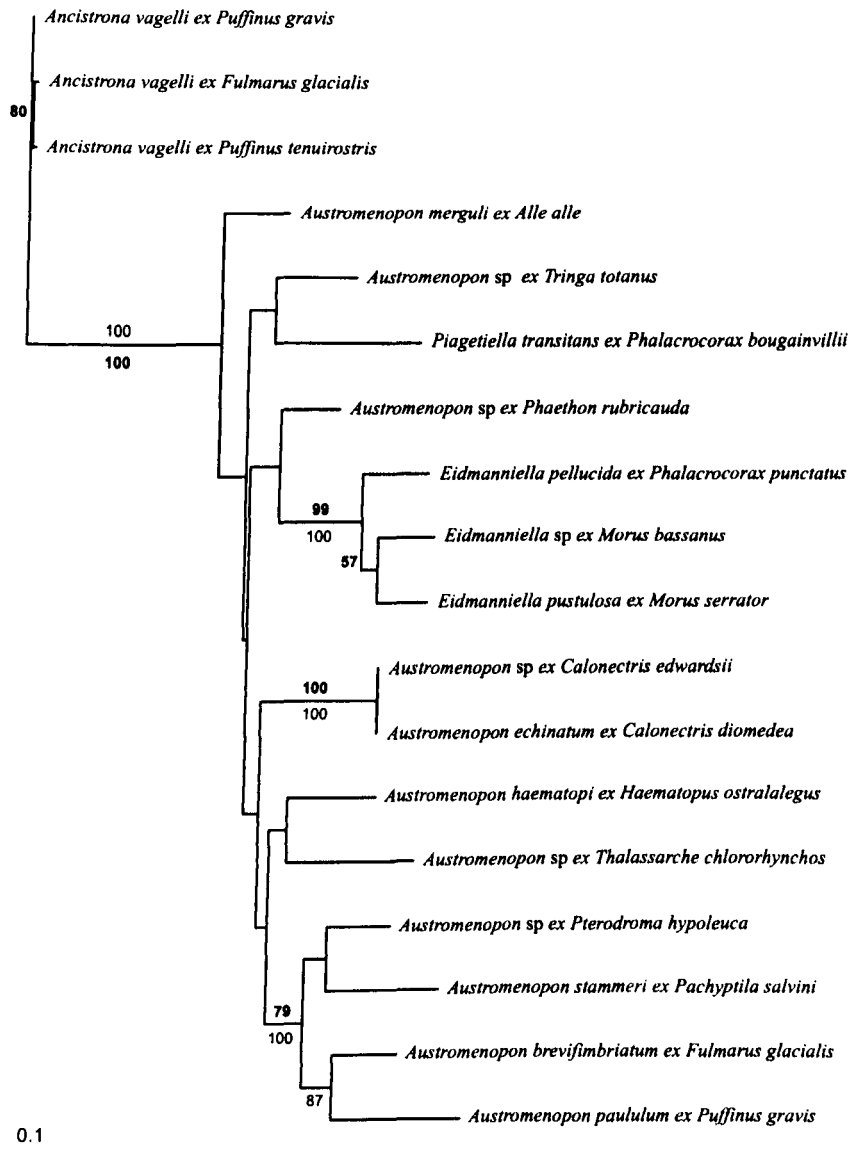

Ancistrona vagelli ex Puffinus gravis
Ancistrona vagelli ex Fulmarus glacialis
Ancistrona vagelli ex Puffinus tenuirostris
80
Austromenopon merguli ex Alle alle
Austromenopon sp ex Tringa totanus
Piagetiella transitans ex Phalacrocorax bougainvillii
100
100
Austromenopon sp ex Phaethon rubricauda
Eidmanniella pellucida ex Phalacrocorax punctatus
99
100
Eidmanniella sp ex Morus bassanus
57
Eidmanniella pustulosa ex Morus serrator
Austromenopon sp ex Calonectris edwardsii
100
100
Austromenopon echinatum ex Calonectris diomedea
Austromenopon haematopi ex Haematopus ostralalegus
Austromenopon sp ex Thalassarche chlororhynchos
Austromenopon sp ex Pterodroma hypoleuca
Austromenopon stammeri ex Pachyptila salvini
79
100
Austromenopon brevifimbriatum ex Fulmarus glacialis
87
Austromenopon paululum ex Puffinus gravis
0.1

Fig. 5: Tanglegram for the genus *Austromenopon* and related taxa and their avian hosts. The louse tree is taken from the maximum likelihood analysis of the combined 12S+COI dataset. A representative host tree was compiled using Sibley & Alquist (1990) and Kennedy & Page (2002). Parasites are connected to their hosts by thin red lines. For reasons of clarity, two of the associations are shown in blue. Scale bar on louse tree = number of substitutions per site.

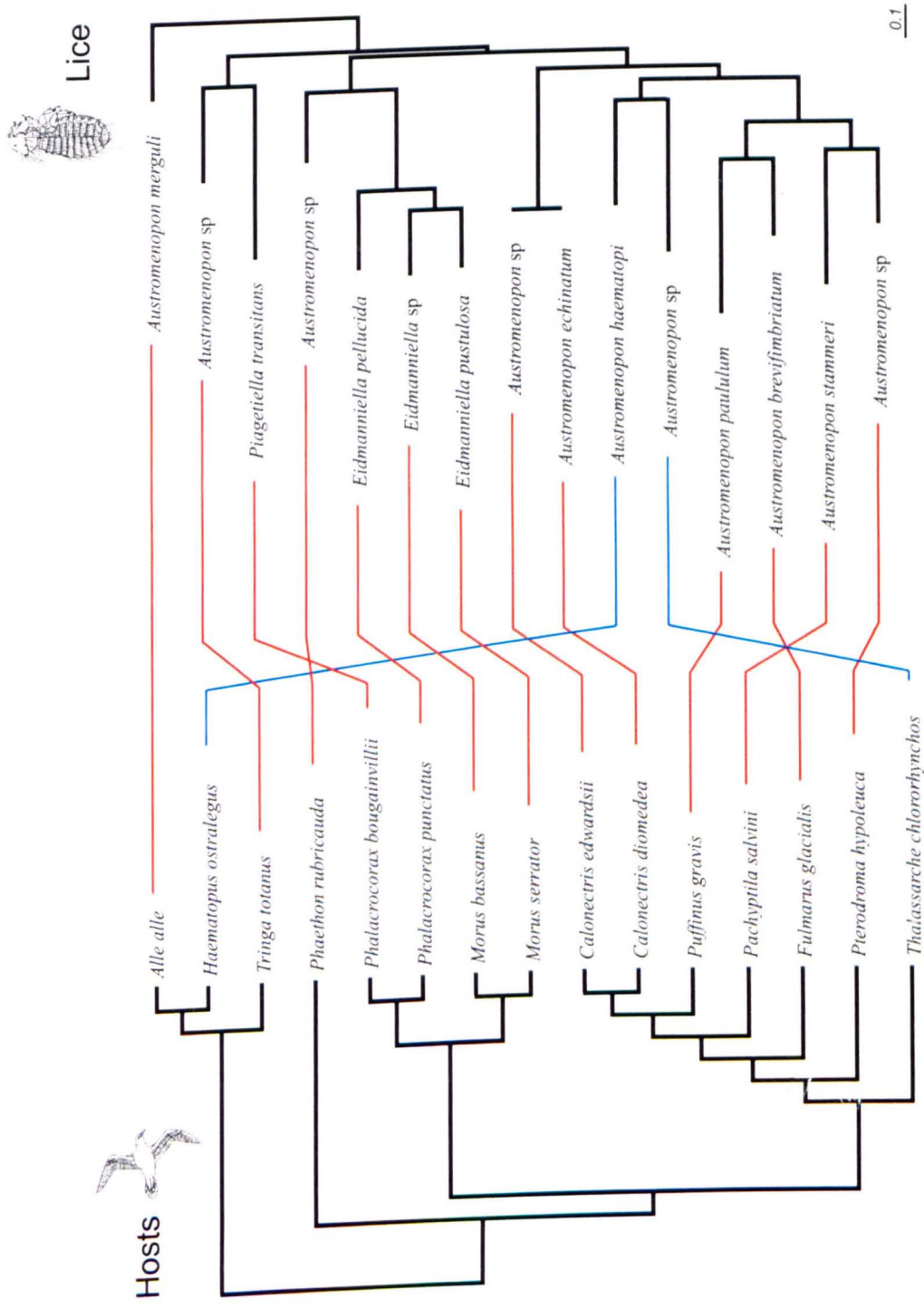

Lice
Hosts
0.1
Austromenopon merguli
Austromenopon sp
Piagetiella transitans
Austromenopon sp
Eidmanniella pellucida
Eidmanniella sp
Eidmanniella pustulosa
Austromenopon sp
Austromenopon echinatum
Austromenopon haematopi
Austromenopon sp
Austromenopon paululum
Austromenopon brevifimbriatum
Austromenopon stammeri
Austromenopon sp
Alle alle
Haematopus ostralegus
Tringa totanus
Phaethon rubricauda
Phalacrocorax bougainvillii
Phalacrocorax punctatus
Morus bassanus
Morus serrator
Calonectris edwardsii
Calonectris diomedea
Puffinus gravis
Pachyptila salvini
Fulmarus glacialis
Pterodroma hypoleuca
Thalassarche chlororhynchos

Fig. 6: Stacked reconstruction of the first most optimal solution from the Jungles analysis. The host tree is shown in blue with the proposed history of their associated parasites drawn as thin black lines. Key: black circles = cospeciations, open circles and directional arrows = host switches, red circles = sorting events, yellow square = independent louse speciation. Host families: Charadriidae (CHAR), Laridae (LAR), Phaethontidae (PHAE), Phalacrocoracidae (PHAL), Procellariidae (PRO), Scolopacidae (SCO), Sulidae (SUL).

solution 1

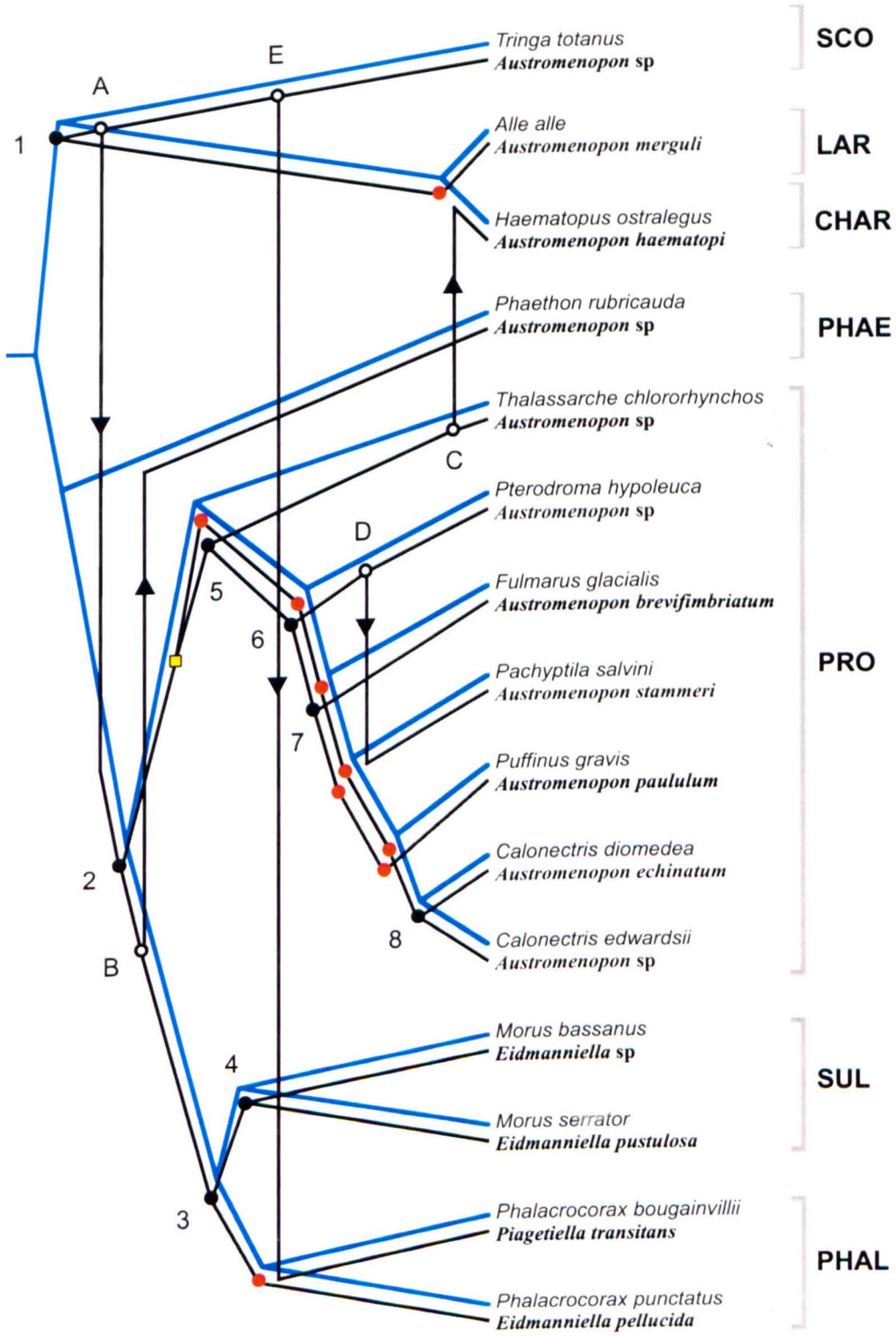
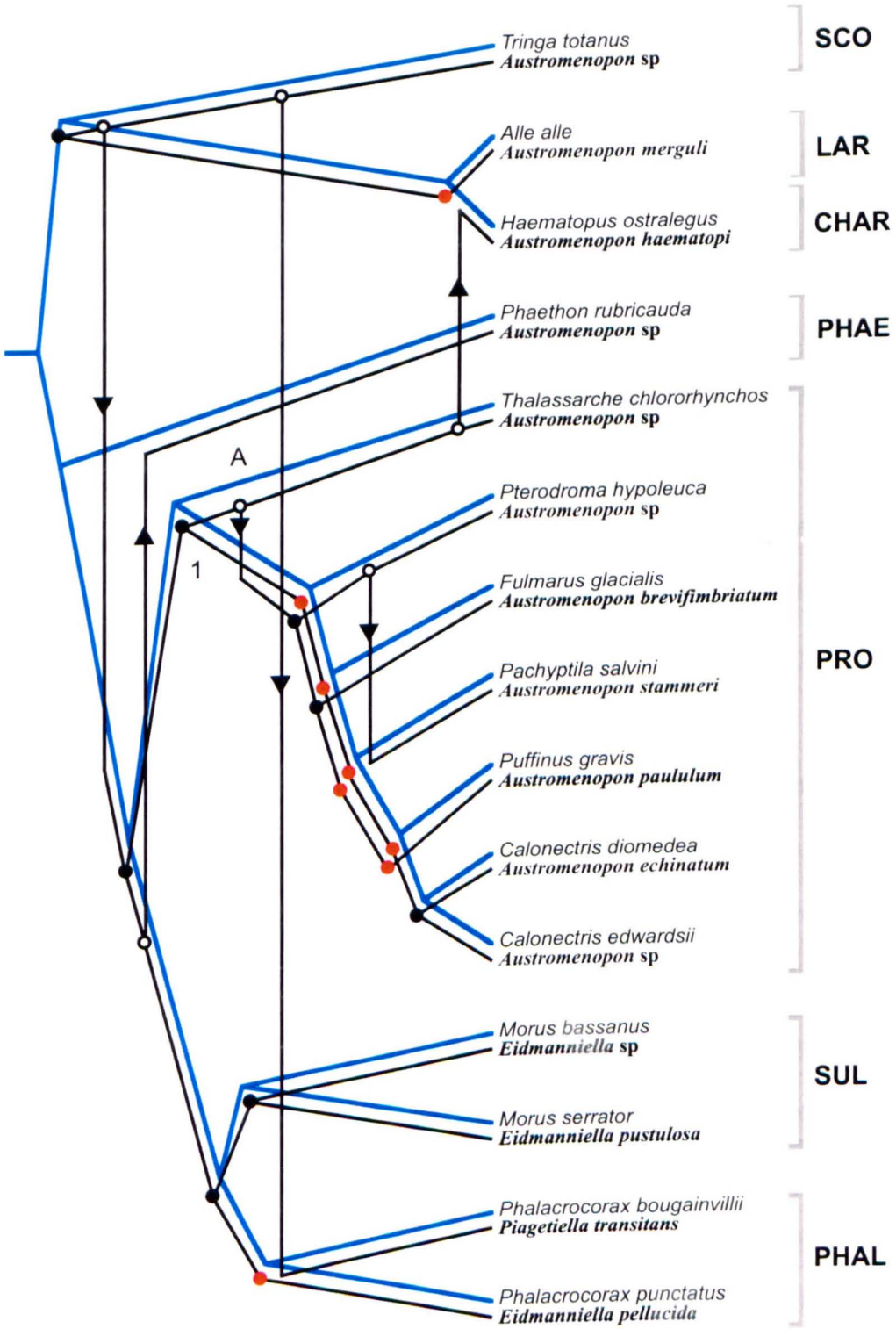

Tringa totanus
Austromenopon sp
Alle alle
Austromenopon merguli
Haematopus ostralegus
Austromenopon haematopi
Phaethon rubricauda
Austromenopon sp
Thalassarche chlororhynchos
Austromenopon sp
Pterodroma hypoleuca
Austromenopon sp
Fulmarus glacialis
Austromenopon brevifimbriatum
Pachyptila salvini
Austromenopon stammeri
Puffinus gravis
Austromenopon paululum
Calonectris diomedea
Austromenopon echinatum
Calonectris edwardsii
Austromenopon sp
Morus bassanus
Eidmanniella sp
Morus serrator
Eidmanniella pustulosa
Phalacrocorax bougainvillii
Piagetiella transitans
Phalacrocorax punctatus
Eidmanniella pellucida
SCO
LAR
CHAR
PHAE
PRO
SUL
PHAL
A
B
C
D
E
1
2
3
4
5
6
7
8

Fig. 7: Stacked reconstruction of the second most optimal solution from the Jungles analysis. The host tree is shown in blue with the proposed history of their associated parasites drawn as thin black lines. Key: black circles = cospeciations, open circles and directional arrows = host switches, red circles = sorting events. Host families: Charadriidae (CHAR), Laridae (LAR), Phaethontidae (PHAE), Phalacrocoracidae (PHAL), Procellariidae (PRO), Scolopacidae (SCO), Sulidae (SUL).

solution 2


Tringa totanus
Austromenopon sp
SCO
Alle alle
Austromenopon merguli
LAR
Haematopus ostralegus
Austromenopon haematopi
CHAR
Phaethon rubricauda
Austromenopon sp
PHAE
Thalassarche chlororhynchos
Austromenopon sp
A
Pterodroma hypoleuca
Austromenopon sp
1
Fulmarus glacialis
Austromenopon brevifimbriatum
Pachyptila salvini
Austromenopon stammeri
Puffinus gravis
Austromenopon paululum
Calonectris diomedea
Austromenopon echinatum
Calonectris edwardsii
Austromenopon sp
PRO
Morus bassanus
Eidmanniella sp
Morus serrator
Eidmanniella pustulosa
SUL
Phalacrocorax bougainvillii
Piagetiella transitans
Phalacrocorax punctatus
Eidmanniella pellucida
PHAL

CHAPTER 5

Conclusions

Because their life histories are relatively tightly coupled with that of their hosts, lice are excellent model organisms for studies of cospeciation. Their species richness and wide distribution across birds and mammals (>6000 species, Price *et al.*, in press), coupled with a high degree of host-specificity and an obligate ectoparasitic life history (Clay, 1949) mean that lice are believed to present the ideal system for investigating the associations between parasite and host. This has been empirically shown by an increasing number of authors (e.g., Hafner & Nadler, 1988; Page *et al.*, 1998; Paterson, Gray & Wallis, 1993). The ideal nature of lice as model organisms for studying cospeciation is further exemplified by the use of louse datasets as the standard examples in the recent development of analytical tools for reconstructing histories between host parasite phylogenies (e.g., Charleston, 1998; Ronquist, 1995).

There are many questions about host-parasite associations that a large and varied group like lice can be used to answer. Hypotheses concerning modes of parasite transmission, prevalence of parasite duplication or loss, the age of lineages, and even questions about modes of speciation can potentially be investigated (Page, 2002). For studies of cospeciation to be meaningful there are a number of fundamental requirements. Page, Clayton and Paterson

(1996) outlined the basics for a rigorous study of host-parasite cospeciation: adequate alpha-taxonomy of both hosts and parasites; accurate phylogenies of host and parasite; exhaustive sampling of clades of lice; molecular phylogenies based on comparable genes; and a quantitative comparison of host and parasite phylogenies. Whilst many of these seem obvious prerequisites, the fact of the matter is that some of these basics can be difficult to achieve in practice. For most of the cospeciation analyses we may conceive of we may have adequate alpha taxonomy, but it is uncommon to have a pre-existing louse phylogeny (even host phylogenies are not always known). As has already been discussed (see chapter one), our knowledge of louse relationships is generally poor. In an effort to alleviate this lack of knowledge, the work contained in this thesis aims to produce new phylogenies for amblyceran lice. The quality of these phylogenies (i.e. likely accuracy) is assessed in relation to the data types used. A phylogeny is then used to evaluate the level of host-parasite cospeciation in the Amblycera, even though the data do not meet all the prerequisites outlined by Page *et al.* (1996).

I now turn to the three core chapters of this thesis to discuss their findings in a wider context, and to suggest the best direction for future study, building on the work presented here.

5.1: Morphology

The extensive morphological study presented as chapter two is the cornerstone of this thesis. This study had four main aims: (i) to construct a phylogeny for amblyceran genera, (ii) evaluate the monophyly of the Menoponidae, Boopiidae and Ricinidae (Clay, 1970), (iii) investigate the

hypothesis of a sister group relationship between the Menoponidae and Boopiidae (Clay, 1970), and (iv) test the suprageneric classification of Clay (1969) and the more extensive amblyceran classification of Eichler (1963).

The phylogeny presented in chapter two contains good branch support for the monophyly of the Menoponidae, Boopiidae, and Ricinidae and also shows good support for the Menoponidae and Boopiidae as sister taxa. The results of this study are therefore concordant with Clay's (1970) views on the broader relationships between these lice. The analysis also shows that Eichler's (1963) classification of the Amblycera received little support, with most of his groups being either paraphyletic or polyphyletic. However, the study does strongly support a small number of Eichler's subfamilies. For example, one of the clades diagnosed within the Menoponidae in this analysis (clade "C", of chapter two) resembles Eichler's (1963) Colpocephalidae family more than the "Colpocephalum complex" of Clay (1969).

Chapter two provides the first phylogeny solely for amblyceran genera and is the most intensive study of their morphology to date. Previous studies by Clay (1969; 1970) and Symmons (1952) have examined and discussed morphology at the level of genera and above, providing keys, classifications, and some proposals of amblyceran phylogeny along the way, but none of these studies has actually attempted an explicit analysis of evolutionary relationships. The 44 taxa included in the morphological analysis probably represent almost 50% of the recognised amblyceran genera. Our current estimate is that there are 93 genera in the Amblycera, the great majority of which are contained within the Menoponidae. It is possible, however, that the number of amblyceran genera may alter slightly with the publication of the

long-awaited new checklist for chewing lice by Price *et al.* (in press), the first published documentation of amblyceran taxa since Hopkins and Clay's (1952) *Checklist of the Genera and Species of Mallophaga.*

My analysis of amblyceran morphology required the comprehensive study of the alpha taxonomic literature (spanning over 200 years) to assess the suitability of existing characters for phylogenetic analysis. This search also aided in the development of new morphological characters. The extensive discussion and illustration of the large number of characters in the morphological study will hopefully be as useful to the new student of amblyceran morphology as the publications of Clay (1969; 1970) were to myself in the initial stages of this investigation.

Although this study has met the aims outlined above, it also raises a number of further questions. The cladistic relationships of the four families presented in chapter two are not exactly as Clay (1970) envisaged. She considered the Menoponidae to be at the base of the Amblycera and wrote of "proto-menoponid" and "proto-amblyceran" characters she used. Clay (1970) polarised these characters throughout the Amblycera relative to a basal Menoponidae.

My results show that the Ricinidae occupy the basal position within a clade containing the four families studied. The Menoponidae and Boopiidae are sister taxa, but they are the terminal taxa, and are the sister group to the Laemobothriidae. Is the relationship found for the four families (see chapter two, Fig. 2) the true phylogeny for these taxa? One way to test this would be to extend the analysis to include genera from other families of Amblycera. The problem with such a study is that many of the morphological characters would

be inapplicable for these additional taxa. Characters in morphological analyses are thus, to varying extents, bounded by the group in question. A complete morphological study of all seven amblyceran families might require the development of a number of new characters. Another way in which to evaluate the relationships found in chapter two would be to include more outgroup taxa, as the absence of some characters in the outgroup (in this case the booklouse, *Liposcelis*) may have influenced the resulting phylogeny. However, a study like this would also undoubtedly require the proposal of a new set of homologies.

Homoplasy is an important factor in phylogenetic analyses and morphological estimates of phylogeny are no exception. Perhaps in a morphological analysis of all amblyceran families the addition of instar data could be used to assess whether potential characters are homoplasious or not. The utility of instar data has been extensively investigated in a recent morphological phylogeny for the avian-infesting Ischnocera (Smith, 2001). In considering the ontogeny of the characters we could search for taxa which have the same character state as adults, but differ in their development of that state. Characters states which are arrived at through different transformation series suggest convergence, whilst taxa with the same transformation series would suggest a shared ancestry. Morphology can provide a good number of robust characters for unravelling the phylogeny of the Amblycera given a good working knowledge of their anatomy and a consideration of the points outlined above. In practise it is less time consuming to collect molecular data than it is to spend the months and years required to obtain the knowledge to produce morphologically derived phylogenies.

5.2: Molecular markers

With the morphological phylogeny reconstructed for four of the amblyceran families, this thesis thus took the next logical step and asked how the morphological phylogeny compared with phylogenies reconstructed from molecular data. The analysis presented in chapter three had two main aims: (i) to construct phylogenies for amblyceran genera based on morphology and molecular data and (ii) compare the level of incongruence which may exist among competing phylogenies. If the morphological or molecular phylogenies are in any sense better than one another, the results may suggest the most appropriate way forward to estimate amblyceran phylogeny.

I found that that the phylogenies recovered by morphology and both of the genes are almost equally incongruent. There is no real consensus between the datasets on the phylogenetic relationships of the three families (Menoponidae, Laemobothriidae and Ricinidae) and neither separate nor combined gene regions recovered a monophyletic Menoponidae. Although the topologies recovered by the genes and morphology are incongruent, there is little bootstrap support for the topologies found from the sequence datasets. The amino acid sequence of both genes is highly conserved (Palumbi, 1996). with few changes at first and second codon positions, thus giving relatively little phylogenetic signal. Whilst these positions are quite conserved, third positions are comparatively free to vary and, because of the probable age of the splits between these taxa, they may be saturated. If substitutions have occurred multiple times at the third codon positions, they are likely to contain little phylogenetic signal and a large amount of noise (see, Cruickshank *et al.*, 2001). To be able to use sequence data to resolve the relationships within such

a divergent group as the Amblycera, it will be necessary to sample a range of different genes to find one or more that evolve at an appropriate rate. Non-coding genes can be difficult to align, e.g. 12S rRNA is very hard to align, but appears to evolve at a suitable rate for generic level analyses. On the other hand, COI is relatively easy to align, but is evolving quite fast.

5.3: Cospeciation analysis

Chapter four provides an example of how lice can be used to answer questions in studies of cospeciation. This study is the first to quantitatively compare cospeciation in amblyceran and ischnoceran lice. It shows that two groups of relatively closely related parasites can have quite different historical associations with the same group of hosts. Because of the lack of support for the molecular phylogenies for the higher-level amblyceran relationships, this chapter focuses on a single genus and its possible allies.

The aims of this chapter were: (i) to construct a phylogeny for the genus *Austromenopon* and their close allies using the mitochondrial genes 12S rRNA and COI, (ii) compare the level of congruence between competing topologies, (iii) assess the level of cospeciation for these lice and their seabird hosts, and (iv) investigate whether the amblyceran taxa have had a similar coevolutionary history to ischnoceran taxa on similar hosts.

One of the findings from the comparison of the phylogenetic reconstructions presented (see chapter four, Figs. 2-4) is that the topologies are generally incongruent. None of the analyses recovered a monophyletic *Austromenopon*, but overall there is good support for a subset of *Austromenopon* taxa, which repeatedly group together. The branch support for

these phylogenies is mostly located nearer the tips of the trees, with the deeper relationships between these taxa either poorly supported or completely unsupported. Thus, the apparent incongruence between the topologies is probably more related to a lack of certainty about the relationships, rather than real incongruence between the two genes. Other cospeciation studies have come across the same problem with their louse phylogenies. Paterson *et al.* (2000) also used the 12s rRNA gene in their study of the Ischnocera. They obtained trees with poor bootstrap support for deep branch relationships, which they attributed to the relatively short amount of sequence left after regions of ambiguity had been removed from the dataset. 12S rRNA is known for being highly variable in length and being particularly difficult to align in lice (Page, Cruickshank & Johnson, 2002). Similarly, Hafner *et al.*'s (1994) phylogeny using COI for the gopher lice (Ischnocera) also found most of the uncertainty near the base of their trees, and consequently they only compared the terminal and subterminal branches of host and parasite phylogenies.

This study also suggests that the genus *Austromenopon* may not be a monophyletic group. Although the deeper relationships are poorly supported, the molecular data suggests that *Austromenopon* may be either para- or polyphyletic. This is not unexpected, given earlier work on the genus. Eveleigh and Amano (1977) used morphometric data in a study which included *Austromenopon* species from alcids (Charadriiformes). Using principal axis factor analysis, they found that species of the two ischnoceran genera they studied produced far clearer cluster patterns than the analysis of *Austromenopon*. Palma (1994) noted much morphological variation between samples of *A. navigans* from different hosts, on the same host and even within

a single sample, and considered *A. bulleri* (as described by Price and Clay, 1972) to represent one end of a continuum of variation within this species. Palma (1994) consequently placed *A. bulleri* as a junior synonym of *A. navigans*. There are a large number of species contained within this genus which are widely distributed across a great number of hosts (Price *et al.*, in press). The phylogeny of *Austromenopon* would certainly benefit from a further analysis using a larger sample size of recognised species. Acquiring these samples, however, presents its own set of problems. As discussed above, these lice are widely distributed on many hosts, which tends to mean that they are also widely distributed geographically. I would have liked to have included many more species, but the size of the dataset is simply constrained by the availability of specimens that provide usable DNA. In the ideal situation those investigating louse systematics would go out into the field and collect their own lice, thus avoiding any doubts about host determination and post collection contamination, but this approach to data collection would be extremely costly. In a sense those who study lice at Glasgow are themselves the parasites of those who study birds, in that the ornithologists are the main source of specimens for our molecular analyses.

The main point of chapter four was to attempt to assess the level of cospeciation for amblyceran lice and compare them with the patterns of association published for the Ischnocera. The analysis finds that there has been significant cospeciation between these lice and their seabird hosts, and from the perspective of the few other studies of host-parasite cospeciation in lice and birds, this result is not unusual (Page *et al.*, 1998; Paterson, *et al.*, 2000). What is interesting from this study is that the amblyceran host-parasite system has

less duplication and more host switching events than was found for the Ischnocera by Paterson *et al.* (2000). These results for the Amblycera may be a consequence of their general inability to become specialised to host micro-habitats. Support for this view can be found in a recent host transfer study by Tompkins and Clayton (1999). Using *Dennyus* (Menoponidae), these authors showed that where transferred lice do survive on a 'foreign' host, they favour feather barbs of the same size to that found on the original host. Thus, the *Dennyus* lice used in this experiment were probably just attempting to find a similar habitat on a new host.

It would be interesting to investigate whether the observed amounts of duplication and host switching found here for *Austromenopon* are consistent across other genera of amblyceran lice and avian hosts (perhaps also incorporating a comparison between colonial and non-colonial birds). In addition to this, studies involving the mammalian Amblycera may reveal whether the pattern observed here constitutes a general trend for these lice, or if there is an effect of host environment.

The studies presented here have built on the works of previous authors (e.g., Clay 1969,1970; Symmons 1952) by examining a broad sample of amblyceran taxa at a range of taxonomic levels, using a variety of methods and analytical techniques. Chapters two and three represent the first steps towards unravelling the relationships among the Amblycera and have investigated the phylogeny of just under half of all genera in this suborder (Price *et al.*, in press). But these studies have (with the exception of a sample of genera extracted from the Boopiidae) largely concentrated on avian lice. The phylogeny for the mammalian-infesting Amblycera still remains largely

unknown. The reconstruction of the phylogeny for *Austromenopon* (chapter four) brings the total of amblyceran genera in which species relationships have been investigated to four (see chapter four; Barker, Briscoe & Close, 1992; Clayton, Price & Page, 1996; Lonc, 1990; Page *et al.*, 1998). Unfortunately this lack of knowledge about phylogeny is paralleled across the Phthiraptera (see chapter one, Fig. 2).

5.4: Prospects

Phylogenetic analyses need both an accurate alpha taxonomy and the data by which to evaluate evolutionary relationships. For amblyceran lice there is a dearth of both. Although a recent checklist for the Anoplura is available (Durden & Musser, 1994), the current checklist for chewing lice (Hopkins & Clay, 1952) is 50 years old. Many new taxa have been described and others placed into synonymy since that time. For the student of the Phthiraptera, and especially for the student of louse-host cospeciation, keeping up with the changes in the louse species record is demanding to say the least. This problem is soon to be rectified, however, with the forthcoming checklist for the chewing lice (Price *et al.*, in press). This publication has taken over 15 years to compile and will supersede all previous lists. The authors indicate that it will contain 252 genera of chewing lice (with pictorial keys), treat over 1700 synonyms, contain more than 11,000 host records and provide an extensive bibliography of louse references. This long-awaited revision of taxa will be, without doubt, of great benefit to louse systematists.

Data for reconstructing phylogenies are becoming more accessible and available. The advent of computer databases and the development of the

Internet have the potential to accelerate the generation of louse phylogenies. Molecular and morphological datasets and trees are now available on-line for the sharing of data (e.g., GenBank, TreeBASE: http://www.treebase.org). In fact, there is now a molecular database specifically for lice (LouseBase: http://r6-page.zoology.gla.ac.uk/LouseBase/2/), hosted by the Universities of Glasgow and Utah, which is steadily increasing in size. These electronic resources are supplemented by the largest frozen louse tissue collection of 6000 specimens from more than 200 hosts held at the Price Institute for Phthirapteran Research, University of Utah.

If we are to continue to use lice as models for studies of cospeciation, then we clearly need to obtain more phylogenies. A number of analyses have now been completed on the gopher louse data of Hafner *et al.* (1994) and they are the "text book" example of cospeciation. Studies of cospeciation are being restricted by our lack of knowledge on the evolutionary relationships between lice. New phylogenies will allow comparisons between taxa and confirm whether the results already obtained regarding processes in a host-parasite system are replicated throughout major groups of lice. A complete phylogeny for lice is an ambitious thought. Although, with the existence of tools for combining phylogenies, such as supertrees (e.g., Kennedy & Page, 2002; Sanderson, Purvis & Henze, 1998), and the emergence of even better tools which will undoubtedly be developed in the future, then perhaps large cospeciation analyses are not too far away.

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