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Phylogenetic Analysis of Partial Sequences of Elongation Factor 1α Identifies Major Groups of Lice (Insecta: Phthiraptera)

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

Key Words: **elongation factor 1α; leaf stability analysis; molecular systematics; niche specialization; phylogenetics; lice; Phthiraptera; Ischnocera.**

INTRODUCTION

Lice (order Phthiraptera) are obligate ectoparasitic insects of birds and mammals. Unlike fleas, they have no free-living stage and of all the insects are the most completely committed to parasitism (Askew, 1971). Most families of birds and mammals have their own specific lice. Notable exceptions among the mammals are the monotremes, anteaters, armadillos, bats, cetaceans, and sirenians, none of which appear to have lice. The sister group of the lice is thought to lie within the paraphyletic order "Psocoptera" (psocids, booklice, barklice), possibly within the family Liposcelidae (Lysal, 1985a). Psocopterans are free-living insects that feed upon microflora and organic debris. Some are associated with birds and mammals, dwelling in nests or found among their plumage or fur, but none are parasitic (Smithers, 1996). The Phthiraptera and Psocoptera together constitute the superorder Psocodea (Lyal, 1985a).

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

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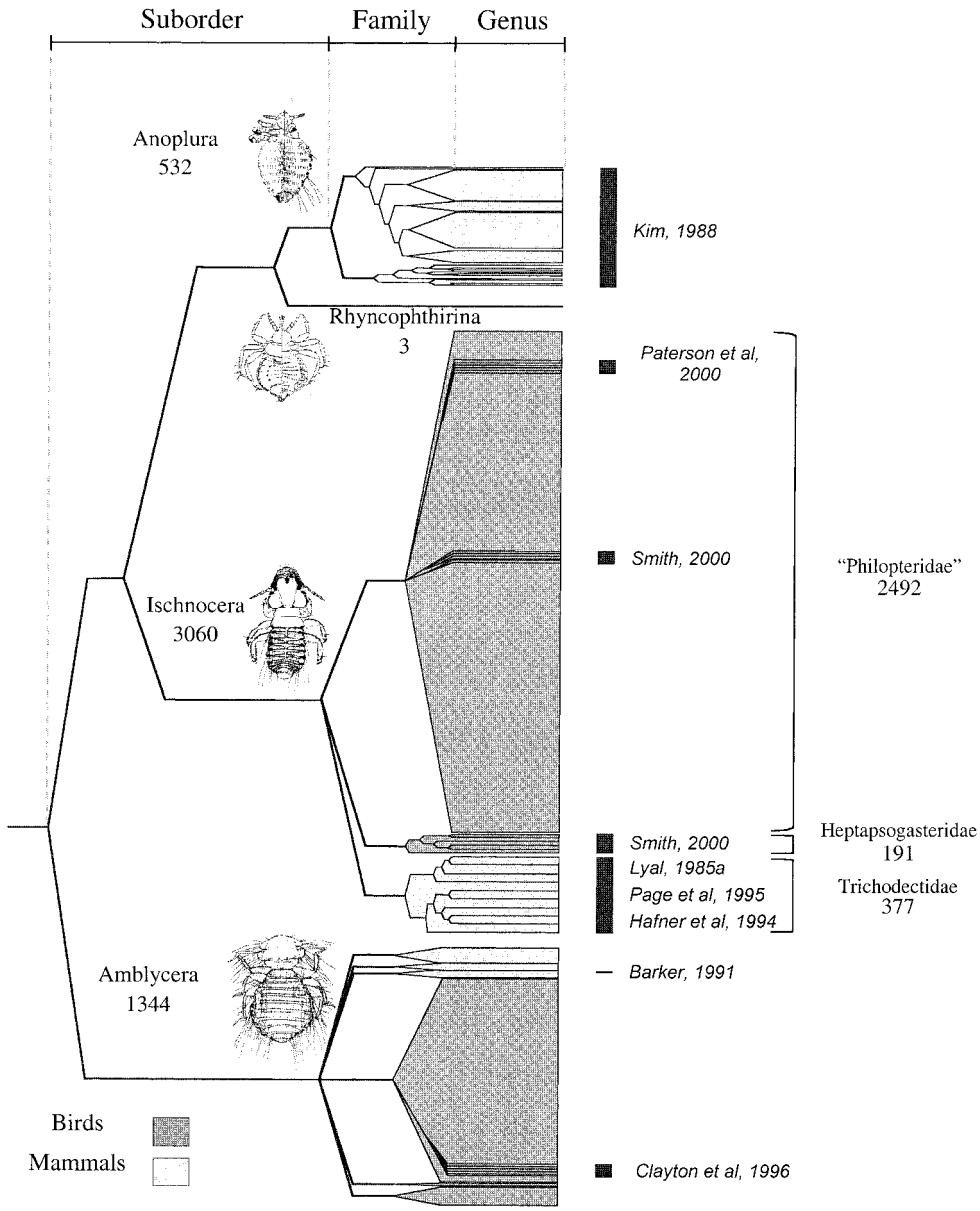

FIG. 1. Our current understanding of louse phylogeny. Cladogram illustrating phthirapteran familial relationships. Subordinal phylogeny based on Lyal (1985a), amblyceran families as diagnosed by Clay (1970) and R. Price (pers. comm.), ischnoceran families modified from Hopkins and Clay (1952) [Trichodectidae (Lyal, 1985b), Heptapsogasteridae (Smith, 2000), Goniodidae (Smith, 2000)], and anopluran families based on Kim (1988). Scale corresponds to the number of species per family (numbers supplied by R. Price, pers. comm., see text).

tivation for the first aim of our phylogenetic analysis: an investigation of the relationships between the four suborders of lice.

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

genetic analysis: an investigation of the relationships within the suborder Ischnocera.

Our Current Understanding of Louse Phylogeny

Current knowledge of louse relationships is summarized in Fig. 1. Every conceivable arrangement of the four suborders of lice has been proposed at some time. Morphological data supporting the monophyly of "Mallophaga" were proposed by Kim and Ludwig (1978b, 1982), although these results were controversial (Haub, 1980). Lyal (1985a) conducted a detailed review of the morphological data supporting the monophyly of the four suborders and their relative relationships. His study confirmed the monophyly of all four suborders, although ischnoceran monophyly was the least well supported. The subordinal phylogeny established by Lyal (1985a) is concordant with comments in Clay (1970) and Konigsmann (1960), who both considered the Amblycera sister taxon to a monophyletic group comprising the Ischnocera, Rhyncophthirina, and Anoplura.

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

The number of families making up the Ischnocera is a matter of some contention. Eichler (1963) recognized 21 families while Hopkins and Clay (1952) accepted just 3. This discrepancy can partly be explained by the diversity of form exhibited among the genera, as ischnoceran lice vary considerably in terms of their size and general morphology. This diversity makes even generic differences hard to define, and comparative morphological studies within this group are exceedingly difficult. No clear justification of the scheme proposed by Eichler (1963) was ever published, and it has subsequently been rejected by most authorities due the assumption that it was unduly biased toward the host classification. More recent studies on Ischnocera recognize at least three monophyletic groups (Lyal, 1985a; Mey, 1994; Smith, 2000), Trichodectidae, Heptapsogasteridae, and Goniodidae. A fourth group (the Philopteridae *sensu* Hopkins and Clay) comprises some 70% of ischnoceran species and is present on almost all families of birds. It is generally accepted that this is a miscellaneous collection of genera and is almost certainly para- or polyphyletic. However, the relationships among these taxa have never been studied. A monotypic taxon (the Trichophilopteridae) represented by a single species present on Madagascan primates (Lemuridae and Indriidae) may be related to the avian Philopteridae. This species bears a number of significant morphological characters in common with the Philopteridae and the mammalian trichodectids. Consequently, it has been variably placed among both these groups and in an independent family within Ischnocera (Emerson and Price, 1985; Ferris, 1933; Stobbe, 1913).

A Molecular Phylogeny for Lice

As a first attempt to use molecular data to resolve higher order relationships within the lice, we sequenced 347 bp of the elongation factor 1α gene (EF1α) of 127 individual lice (representing 105 species in 70 genera) as well as outgroup taxa from the order Psocoptera. This nuclear gene was chosen for an initial survey of the phylogeny of lice for a number of reasons, including its low copy number, ease of alignment (due to conservation of amino acid sequence and lack of insertions/deletions), and proven phylogenetic utility in other insect groups (Friedlander *et al.*, 1998), as well as the important technical reason that universal primers already available gave reliable PCR amplification in lice.

METHODS

Sequence Determination

Total genomic DNA was extracted from single lice using the DNAeasy Tissue Kit (Qiagen). The heads of the lice were removed prior to DNA extraction and both the head and the body were incubated in lysis buffer over 2 nights, after which the exoskeletons of the head and body were removed for slide mounting as vouchers. Three hundred forty-seven base pairs of the EF1α gene were amplified and sequenced using the primers EF1-For3 and Cho10 (Danforth and Ji, 1998). After removal of introns, redundant taxa, and 2 bp at the 5′ end of the sequence (see below), the data set consisted of 345 characters for 111 taxa, of which 158 (46%) are variable and 143 (41%) are parsimony informative. Most of the variation (73% of variable and 80% of informative

sites) was at the third codon position. Amplification products were gel purified using the QIAquick Gel Extraction Kit (Qiagen) and sequenced using the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq DNA polymerase, FS (Perkin–Elmer). Sequencing products were ethanol precipitated and run on an ABI 373 Stretch automated sequencing machine.

Taxon Sampling

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

Since we were interested in examining the relationships between both the four suborders of lice and those with the suborder Ischnocera, all four suborders were sampled with the densest sampling within the Ischnocera (80 taxa). All major groups of Ischnocera were sampled. Although only 4 anopluran taxa were included, these span the root of the tree published by Kim (1988). Unfortunately the 22 amblyceran taxa sampled come from just two families (Menoponidae and Ricinidae) and therefore a substantial portion of amblyceran diversity remains unsampled. This includes all of the families of amblyceran mammal lice (Gyropidae, Trimenoponidae, and Boopidae) as well as one family of amblyceran bird lice (Laemobothriidae).

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

Phylogenetic Analysis

An initial unweighted maximum parsimony (MP) search found 704 equally most parsimonious trees. The strict consensus of these trees (Fig. 2) represents a conservative estimate of phylogeny; however, the data may contain more resolution than this consensus reveals. For this reason we turned to other methods of analysis in an attempt to improve the resolution of the tree. A test of stationarity of base composition (Rzhetsky and Nei, 1995) was performed on a subset of the data chosen to include representatives of all major clades found in the initial MP trees. Stationarity was rejected ($P < 0.005$), i.e., the base composition differs significantly in different parts of the tree. This can be a problem for some methods of phylogenetic analyses that may group taxa together due to similarity of base composition rather than genuine shared ancestry (Galtier and Gouy, 1995). This suggests that only methods which can correct for nonstationarity of base composition (e.g., LogDet distance-based methods) should be used to analyze this data set. It is possible, however, that the loss of information due to the use of distances rather than discrete characters could adversely affect the performance of the phylogenetic analyses. This loss of information may have a greater detrimental effect than failure to correct for nonstationarity of base composition. For this reason we used a combined distance and discrete character-based approach. An initial neighbor-joining (NJ) tree was constructed using LogDet distances, which correct for nonstationarity of base composition (Lockhart *et al.*, 1994). Rate heterogeneity was assumed, with two rate classes, i.e., constant sites vs varying sites. The proportion of invariant sites (0.542) was estimated using maximum-likelihood. Inclusion of a relatively large number of taxa for a study of this sort means that rate heterogeneity parameters can be estimated very reliably (i.e., they will have very small confidence intervals) (Sullivan *et al.*, 1999). Constant sites were removed in proportion to base frequencies estimated from constant sites only, according to the suggestion of Swofford *et al.* (1996). This tree was then used as the starting tree for two different kinds of branch swapping: TBR branch swapping under the criterion of minimum evolution and NNI branch swapping under the criterion of maximum-likelihood (ML). ML was chosen as the discrete method since we considered it least likely to be adversely affected by nonstationarity of base composition and because it is more data inclusive than parsimony which does not consider parsimony-uninformative sites. Data inclusiveness may be particularly important in the analysis of this data set, which contains relatively few characters for the number of taxa involved. Parameters for ML branch swapping were estimated using the program MODELTEST (Posada, 1998) (but setting base frequencies to empirical values rather than estimating them due to a bug in PAUP* version 4.0b2a (PPC)). The model with the minimum information theoretical content is K81uf + I + γ, which has three substitution rates (A $\leftrightarrow$ C = G $\leftrightarrow$ T, A $\leftrightarrow$ G = C $\leftrightarrow$ T and A $\leftrightarrow$ T = C $\leftrightarrow$ G), unequal base frequencies, invariable sites, and gamma distributed rates at variable sites. Figure 3 shows an Adams consensus of the two branch swapping methods with nodes not found in either of the two fundamental trees removed. All phylogenetic analyses were conducted using PAUP* version 4.0b2a (PPC) (Swofford, 1999). The trees have been deposited in TreeBASE (http://herbaria.harvard.edu/treebase/) (study accession No. S570).

TABLE 1

Taxa Sampled

Louse	Host	Number sequenced	Species determination	Sequencing laboratory	GenBank accession
Actornithophilus ceruleus	*Anous Tenuirostris* (Lesser noddy)	1	RLP	G	AF320349
Actornithophilus piceus	*Larus* sp. (gull)[a]	1	RJA	U	AF320350
Amyrsidea spicula	*Ortalis vetula* (plain chachalaca)	2	RDP	G/U	AF320351/2
Anaticola crassicornis	*Anas platyrhynchos* (mallard duck)	1	RJA	U	AF320353
Anaticola crassicornis	*Somateria mollissima* (eider duck)	1	VSS	G	AF320354
Anatoecus sp.	*Anas platyrhynchos* (mallard duck)	1	RJA	U	AF320355
Anatoecus sp.	*Somateria mollissima* (eider duck)	1	VSS	G	AF320356
Ancistrona vagelli	*Fulmarus glacialis* (Northern fulmar)	1	VSS	G	AF320357
Ancistrona vagelli	*Puffinus tenuirostris* (short-tailed shearwater)	1	VSS	G	AF320358
Aquanirmus occidentalis	*Aechmophorus occidentalis* (Western grebe)	1	RJA	U	AF320359
Archolipeurus nandu	*Rhea americana* (greater rhea)	1	EM	G	AF320360
Ardeicola ardeae	*Ardea cinerea* (gray heron)	1	VSS	G	AF320361
Austrogoniodes waterstoni	*Eudyptula minor* (little penguin)	1	AMP	G	AF320362
Austromenopon echinatum	*Calonectris diomedea* (Cory's shearwater)	1	VSS	G	AF320363
Austromenopon merguli	*Alle alle* (little auk)	1	VSS	G	AF320364
Austrophilopterus subsimilis	*Ramphastos sulfuratus* (keel-billed toucan)	2	RDP	G/U	AF320365/6
Austrophilopterus sp.	*Pteroglossus torquatus* (collared aracari)	1	RDP	U	AF320367
Bedfordiella unica	*Lugensa brevirostris* (kerguelen petrel)	2	VSS/RLP	G	AF320368/9
Bovicola bovis	*Bos taurus* (domestic cattle)	1	VSS	G	AF320370
Brueelia marginella	*Momotus momota* (blue-crowned motmot)	1	RDP	G	AF320371
Brueelia sp.	*Parus niger* (black tit)	1	RJA	U	AF320372
Brueelia sp.	*Ploceus velatus* (Southern masked-weaver)	1	RJA	U	AF320373
Brueelia sp.	*Pycnonotus nigricans* (black-fronted bulbul)	1	RJA	U	AF320374
Brueelia sp.	*Sylvia subcaeruleum* (rufous-vented warbler)	1	RJA	U	AF320375
Brueelia sp.	*Trogon massena* (slaty-tailed trogon)	1	RJA	U	AF320376
Campanulotes compar	*Columbia livia* (feral pigeon)	2	VSS/RJA	G/U	AF320377/8
Chelopistes oculari	*Penelope purpurescens* (crested guan)	1	RJA	U	AF320379
Chelopistes texanus	*Ortalis vetula* (plain chachalaca)	1	RJA	U	AF320380
Colilipeurus colius	*Urocolius indicus* (red-faced mousebird)	1	RJA	U	AF320381
Colimenopon urocolius	*Urocolius indicus* (red-faced mousebird)	1	RJA	U	AF320382
Coloceras sp.	*Streptopelia capicola* (ring-necked dove)	1	RJA	U	AF320383
Columbicola baculoides	*Zenaida macroura* (mourning dove)	1	RJA	U	AF320384
Columbicola columbae	*Columba livia* (feral pigeon)	2	VSS/RJA	G/U	AF320385/6
Cuclotogaster hopkinsi	*Scleroptila africanus* (gray-winged francolin)	1	RJA	U	AF320387
Cuculicola atopus	*Piaya cayana* (squirrel cuckoo)	1	RDP	G	AF320388
Dennyus cypsiurus	*Cypsiurus parvus* (African palm-swift)	1	RJA	U	AF320389
Dennyus hirundinis	*Apus apus* (common swift)	2	VSS/DHC	G/U	AF320390/1
Discocorpus mexicanus	*Crypturellus cinnamomeus* (thicket tinamou)	2	RDP	G/U	AF320392/3
Docophoroides brevis	*Diomedea epomophora* (royal albatross)	1	AMP	G	AF320394
Docophoroides harrisoni	*Diomedea bulleri* (Buller's albatross)	1	AMP	G	AF320395
Echinophthirius horridus	*Phoca vitulina* (harbor seal)	2	VSS	G/U	AF320396/7
Felicola subrostratus	*Felis catus* (domestic cat)	1	VSS	G	AF320398
Formicaricola analoides	*Formicarius moniliger* (Mexican ant-thrush)	1	RDP	G	AF320399
Fulicoffula heliornis	*Heliornis fulica* (sungrebe)	1	RDP	U	AF3203400
Geomydoecus chapini	*Orthogeomys hispidus* (hipsid pocket gopher)	1	MSH	G	AF3203401
Geomydoecus costaricensis	*Orthogeomys heterodus* (variable pocket gopher)	1	MSH	G	AF3203402
Goniocotes sp.	*Scleroptila africanus* (gray-winged francolin)	1	RJA	U	AF3203403
Goniodes isogenos	*Scleroptila africanus* (gray-winged francolin)	1	RJA	U	AF3203404
Haematomyzus elephantis	*Elephas maximus* (Asian elephant)	1	LAD	U	AF3203405
Haffneria grandis	*Catharacta skua* (great skua)	1	VSS	G	AF3203406
Halipeurus bulweriae	*Bulweria bulwerii* (Bulwer's petrel)	1	VSS	G	AF3203407
Halipeurus pelagicus[b]	*Bulweria bulwerii* (Bulwer's petrel)	1	VSS	G	AF3203408
Halipeurus pelagicus	*Oceanodroma castro* (Madeiran storm-petrel)	1	VSS	G	AF3203409
Harrisoniella densa	*Diomedea immutabilis* (Laysan albatross)	1	VSS	G	AF3203410
Heptapsogaster minuta	*Nothura marculosa* (spotted nothura)	1	VSS	G	AF3203411
Heptapsogaster temporalis	*Crypturellus cinnamomeus* (thicket tinamou)	2	VSS	G/U	AF3203412/3
Hohorstiella lata	*Columba livia* (feral pigeon)	1	VSS	G	AF3203414
Hohorstiella passerinae	*Columbina inca* (Inca dove)	1	RJA	U	AF3203415
Hoplopleura sciuricola	*Sciurus carolinensis* (gray squirrel)	1	LAD	U	AF3203416
Kurodaia sp.	*Otus guatemalae* (Middle-American screech owl)	1	RDP	U	AF3203417
Linognathoides marmotae	*Marmota flaviventris* (yellow-bellied marmot)	1	VSS	G	AF3203418
Liposcelis sp.	None	2	RLP	G	AF3203419/20

TABLE 1—*Continued*

Louse	Host	Number sequenced	Species determination	Sequencing laboratory	GenBank accession
Machaerilaemus sp.	*Hirundo abyssinica* (lesser striped-swallow)	1	RJA	U	AF3203421
Megapeostus asymmetricus	*Crypturellus cinnamomeus* (thicket tinamou)	2	RDP	G/U	AF3203422/3
Menacanthus eurysternus	*Lybius torquatus* (black-collared barbet)	1	RJA	U	AF3203424
Menacanthus sp.	*Atilla spadiceus* (bright-rumped attila)	1	RDP	U	AF3203425
Menacanthus sp.	*Penelope purpurescens* (crested guan)	1	RDP	U	AF3203426
Mulcticola sp.	*Nyctiphrynus yucatanicus* (Yucatan poorwill)	1	RDP	U	AF3203427
Myrsidea eisentrauti	*Sporopipes squamifrons* (scaly weaver)	1	RJA	U	AF3203428
Myrsidea ledgeri	*Philetarius socius* (social weaver)	1	RJA	U	AF3203429
Myrsidea sp.	*Psilorhinus morio* (brown jay)	2	RDP	G/U	AF3203430/1
Naubates harrisoni	*Puffinus assimilis* (little shearwater)	1	VSS	G	AF3203432
Neohaematopinus sciuri	*Sciurus carolinensis* (gray squirrel)	1	LAD	U	AF3203433
Nyctibicola longirostris	*Nyctibius jamaicensis* (Northern potoo)	1	RDP	U	AF3203434
Otidoecus houbarae	*Chlamydotis undulata* (Houbara bustard)	1	VSS	G	AF3203435
Oxylipeurus chiniri	*Ortalis vetula* (plain chachalaca)	2	RDP	G/U	AF3203436/7
Paragonicotes microgaster	*Amazona albifrons* (white-fronted parrot)	1	RDP	U	AF3203438
Paragonicotes sp.	*Aratinga astec* (Aztec parakeet)	1	RDP	U	AF3203439
Pectenosoma verrucosa	*Crypturellus cinnamomeus* (thicket tinamou)	2	VSS	G/U	AF3203440/1
Pectinopygus brevicornis	*Phalacrocorax aristotelis* (European shag)	1	VSS	G	AF3203442
Pectinopygus bassani	*Sula bassana* (Northern gannet)	1	VSS	G	AF3203443
Pectinopygus sp.	*Sula sula* (red-footed booby)	1	VSS	G	AF3203444
Penenirmus zumpti	*Lybius torquatus* (black-collared barbet)	1	RJA	U	AF3203445
Penenirmus sp.	*Myrmecocichla formicivora* (Southern anteater-chat)	1	RJA	U	AF3203446
Penenirmus sp.	*Serinus atrogularis* (black-throated canary)	1	RJA	U	AF3203447
Perineus nigrolimbatus	*Fulmarus glacialis* (Northern fulmar)	1	VSS	G	AF3203448
Philopterus sp.	*Batis pririt* (Pririt batis)	1	RJA	U	AF3203449
Philopterus sp.	*Habia* sp. (ant-tanager)	1	RDP	G	AF3203450
Philopterus sp.	*Momotus momota* (blue-crowned motmot)	1	RDP	U	AF3203451
Physconelloides cubanus	*Geotrygon montana* (ruddy quail-dove)	1	RDP	G	AF3203452
Physconelloides eurysema	*Columbina passerina* (common ground-dove)	1	RJA	U	AF3203453
Picicola capitatus	*Dendropicos fuscescens* (cardinal woodpecker)	1	RJA	U	AF3203454
Pseudolipeurus similis	*Crypturellus cinnamomeus* (thicket tinamou)	1	RDP	G	AF3203455
Pseudomenopon carrikeri	*Heliornis fulica* (sungrebe)	1	RDP	G	AF3203456
Quadraceps punctatus	*Larus cirrocephalus* (gray-headed gull)	1	RJA	U	AF3203457
Quadraceps sp.	*Uria aalge* (guillemot)	1	VSS	G	AF3203458
Rallicola columbiana	*Dendrocolaptes certhia* (barred woodcreeper)	1	RDP	G	AF3203459
Rallicola fuliginosa	*Dendrocincla anabatina* (tawny-winged woodcreeper)	1	RDP	U	AF3203460
Rallicola sp.	*Aramides cajanea* (gray-necked wood-rail)	1	RDP	U	AF3203461
Ricinus sp.	*Cyanocompsa parellina* (blue bunting)	2	RDP	G/U	AF3203462/3
Saemundssonia lari	*Larus argentatus* (herring gull)	1	VSS	G	AF3203464
Saemundssonia peusi	*Calonectris diamedea* (Cory's shearwater)	1	VSS	G	AF3203465
Saemundssonia stresemanni	*Catharacta skua* (great skua)	1	VSS	G	AF3203466
Strigiphilus crucigerus	*Otus guatemalae* (Middle-American screech owl)	2	RDP	G/U	AF3203467/8
Striphilus rostratus	*Tyto alba* (barn owl)	1	VSS	G	AF3203469
Struthiolipeurus struthionis	*Struthio camelus* (common ostrich)	6	EM	G	AF3203470-5
Trichophilopterus babakotophilus	*Propithecus verreauxi* (Verreaux's sifaka)	1	VSS	G	AF3203476
Trogoninirmus sp.	*Trogon melanocephalus* (black-headed trogon)	1	RDP	G	AF3203477

Note. Species determination: RLP, Ricardo Palma; RJA, Richard Adams; RDP, Roger Price; VSS, Vince Smith; EM, Eberhard Mey; AMP, Adrian Paterson; DHC, Dale Clayton; MSH, Mark Hafner; LAD, Lance Durden. Sequencing laboratory: G, Glasgow; U, Utah.

[a] *Larus glaucescens* (glaucous-winged gull) × *Larus occidentalis* (Western gull) hybrid.

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

RESULTS

Introns

Both the fruit fly and the honeybee are known to have two copies of EF1α (F1 and F2) (Danforth and Ji, 1998; Hovemann *et al.*, 1988). In both cases the F2 copies of the gene contain an intron (intron 5) which is not present in the F1 copy. Although we have found only a single copy of EF1α in both lice and booklice, the booklouse (*Liposcelis*) sequences contain an intron not present in any of the louse sequences. This was removed from the data set prior to phylogenetic analysis. The position of this intron is identical to that of intron 5 in the F2 copies of EF1α in the fruit fly and the honeybee. This could mean that the EF1α sequences from *Liposcelis* are paralogous with those from lice.

These sequences would still represent the closest available outgroup unless the gene duplication event which gave rise to them predates the last common ancestor of the Psocodea and any alternative outgroup taxon for which a truly homologous sequence is available.

In common with our sequences from lice, the F1 copies of EF1α in the fruit fly and the honeybee (GenBank Accession Nos. X06869 and X52884, respectively) lack intron 5. If these sequences are genuine homologs of the louse sequences, while the booklouse sequences are paralogs, then the honeybee and fruit fly sequences may represent closer outgroups. However, this seems unlikely since the genetic distances between the lice and the honeybee and fruit fly (24.8 and 22.5%, respectively) are both greater than the distance between the lice and the booklice (19.4%). If the *Liposcelis* sequences are indeed paralogous to the sequences from the lice, it seems likely that the gene duplication event which gave rise to them is independent of that/those which gave rise to the duplicate copies in the honeybee and fruit fly and occurred closer to the root of the Psocodea. For this reason we consider the *Liposcelis* sequences to be the closest available outgroup at this time.

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

Polymorphism

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

Phylogenetic Relationships

Figures 2 and 3 show trees derived from a simple unweighted parsimony search and a more sophisticated combined distance and likelihood approach. Neither tree is very well resolved, particularly toward the base, but this is to be expected for a data set with such a high taxon/character ratio. Nevertheless, the two trees do agree on a number of points, which include the following. (1) The anopluran *Echinophthirius horridus* and the ischnoceran *Heptapsogaster temporalis* form a group. We consider this group likely to be an artifact of character sampling (see below). (2) The Trichodectidae are monophyletic. The relationships within the Trichodectidae are identical in the two trees. (3) *Mulcticola* is consistently found away from the rest of the Quadraceptinae, which are otherwise monophyletic in both trees. The relationships within the Quadraceptinae are also the same in both trees. (4) *Rallicola fuliginosa* is the sister taxon of the Quadraceptinae, but the other two members of the genus *Rallicola* are found elsewhere in the tree. (5) The Degeeriellidae are monophyletic and a sister taxon to *Cuclotogaster*. The relationships within the degeeriellids are the same in both trees. (6) *Oxylipeurus* and *Chelopistes* appear together in a group of cracid lice away from their traditional taxonomic placements. Clay (1976) suggested an association between *Chelopistes* and *Oxylipeurus*. Smith (2000) also cites karyological evidence that *Chelopistes* does not belong in the Goniodidae. This is based on the observation that *Chelopistes* has the typical philopterid chromosome number of 12 (Perrot, 1934), rather than the typical goniodid chromosome number of 11 (Kettle, 1977) (although since lice have holokinetic chromosomes the karyotype may be particularly plastic in lice). *Chelopistes* is a short fat louse (Kéler, 1939) which appears to be adapted to living among the downy feathers of the head and neck where it can avoid preening since the host cannot reach these areas. *Oxylipeurus*, on the other hand, is a fairly long, thin louse (Carriker, 1944) which may be adapted to living on the wing feathers where it can conceal itself between the feather shafts and avoid being dislodged during preening or flight. This clade represents an interesting case in which lice adapted to different microhabitats on the same host, previously thought to be unrelated, in fact form a clade. This is in contrast to the head and wing lice of procellariiform seabirds, for example, which are indeed unrelated. (The head lice belong to the Quadraceptinae and the wing lice to the Pseudonirminae.) Another group of birds in which the head and wing lice seem to be related are the ducks. Both head and wing

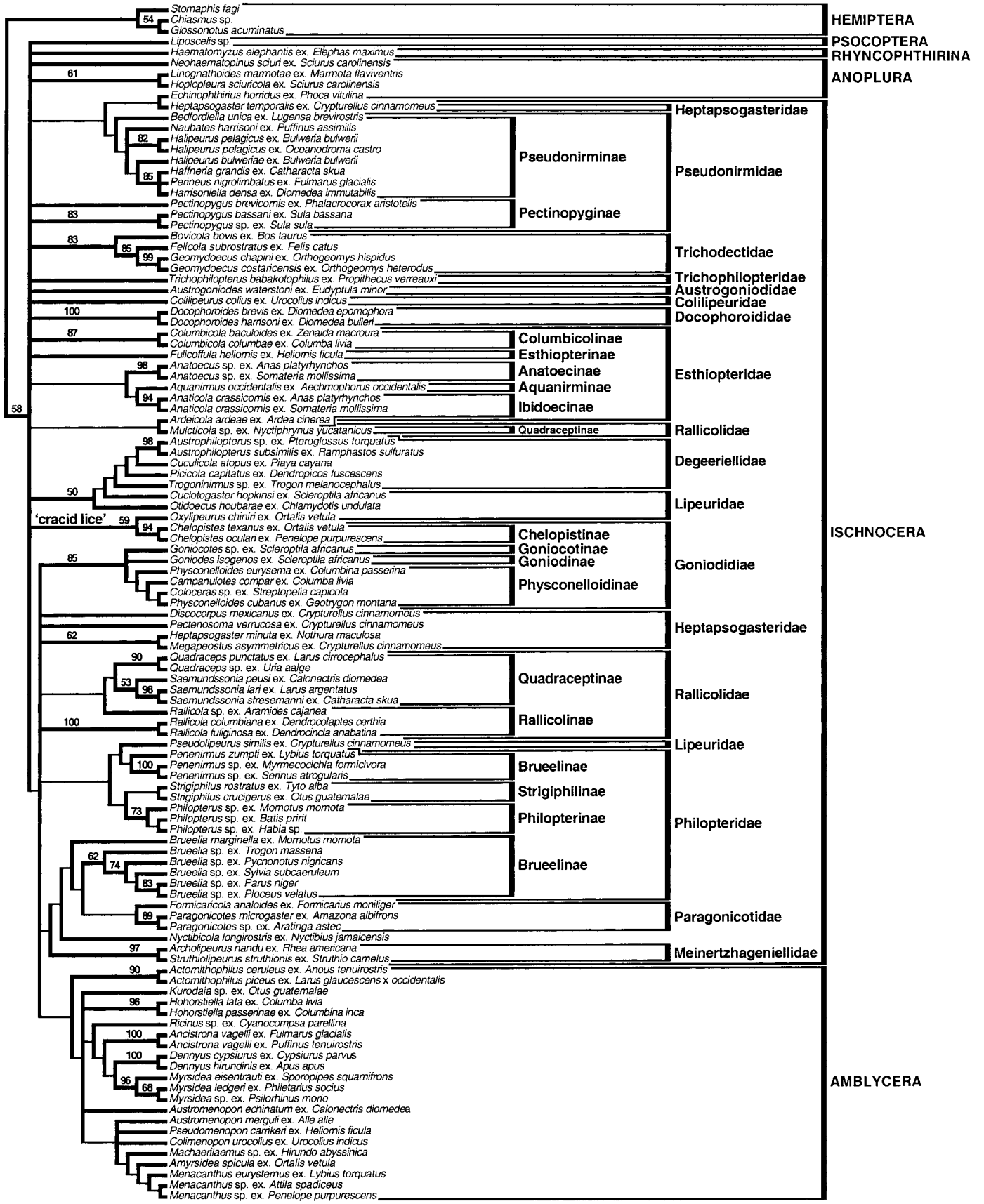

FIG. 2. Strict consensus of 704 maximum parsimony trees. Numbers above the nodes represent bootstrap proportions (10,000 replicates using stepwise addition under the criterion of maximum parsimony with no branch swapping). Internal branches drawn with thin lines occur in the optimal tree but have bootstrap support below 50%. Names of families and subfamilies of Ischnocera are from Eichler (1963). Since *Nyctibicola* was not included in Eichler's phylogeny it does not have a family label. *Archolipeurus* was recently split from *Struthiolipeurus* (Mey, 1998) and is therefore labeled as belonging to the Meinertzhageniellidae, although it did not appear in Eichler's phylogeny.

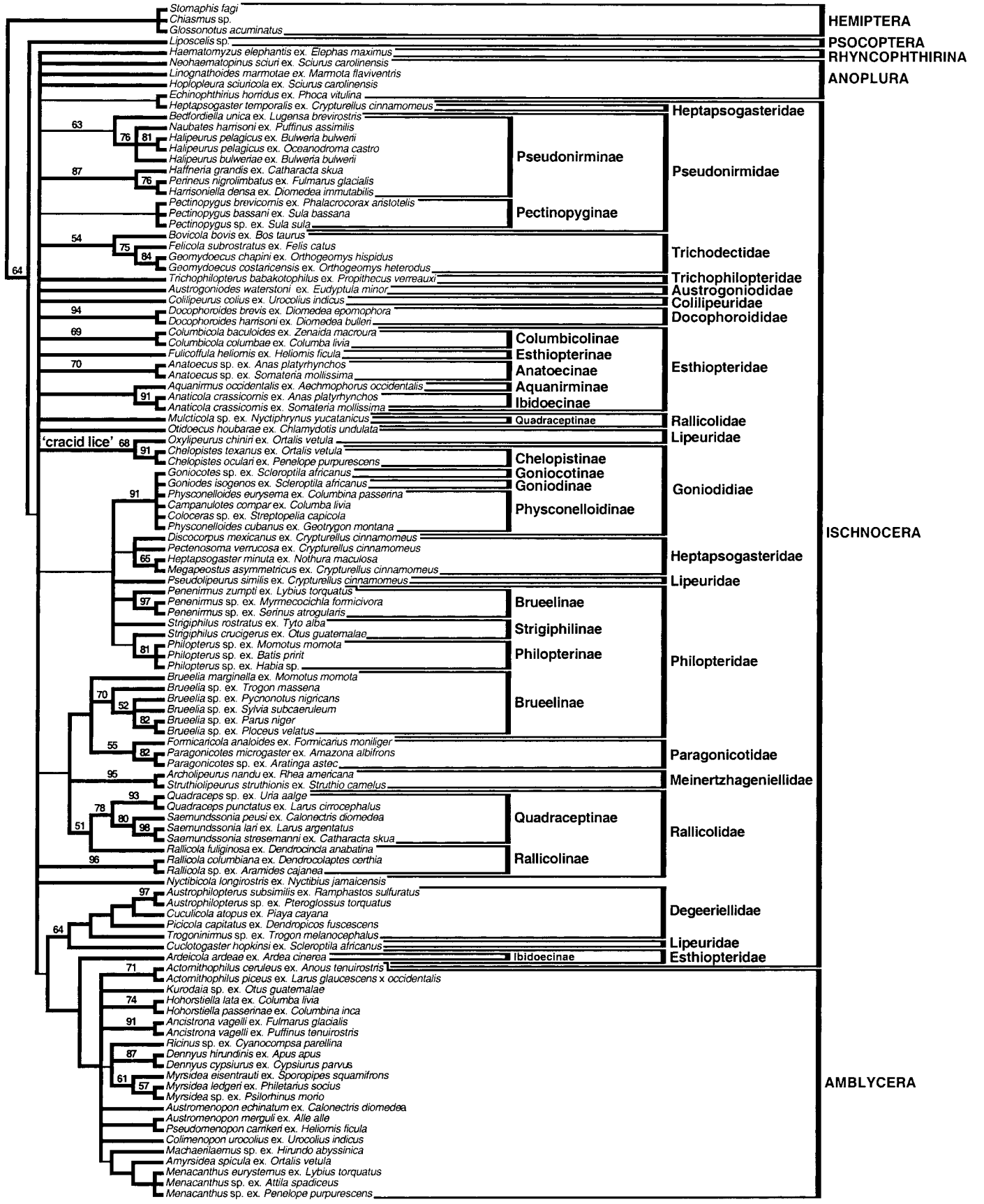

FIG. 3. Adams consensus of the minimum evolution and maximum likelihood trees with branches that do not appear in either tree collapsed. Numbers above the nodes represent bootstrap proportions (10,000 replicates using neighbor-joining with LogDet distances incorporating a correction for rate heterogeneity). Internal branches drawn with thin lines represent nodes found in either the minimum evolution tree or the likelihood tree but not both. Branches drawn with thick lines represent nodes found in both trees, i.e., in the strict consensus of the two trees.

lice of ducks are found in the family Esthiopteridae, head lice in the subfamily Anatoecinae and wing lice in the subfamily Ibidoecinae. (7) With the exception of *Chelopistes* the Goniodidae are monophyletic in both trees. (8) The genus *Brueelia* is sister taxon to a monophyletic Paragonicotinae rather than grouping with the rest of the Philopteridae (*sensu* Eichler). This group appears close to Meinertzhageniellidae in both trees. (9) Pseudolipeurus consistently appears near the Philopteridae (*sensu* Eichler) rather than the Lipeuridae. (10) The Amblycera are monophyletic and the two trees are compatible with respect to the relationships within the Amblycera, although the parsimony tree is more resolved.

DISCUSSION

Are the Suborders Monophyletic?

Of the four suborders of lice, only the Amblycera are unequivocally monophyletic. The Ischnocera appear to be paraphyletic with respect to the Amblycera, although this may be an artifact of character sampling. Since the node that places the Amblycera within the Ischnocera is not supported by bootstrap support above 50% for either maximum parsimony or minimum evolution, no confidence should be placed in this placement. The anopluran seal louse *Echinophthirius* also appears within the Ischnocera, and again this is likely to be an artifact of character sampling. Analysis using split decomposition (see below) indicates that there is conflict in the data resulting in a spurious association of *Echinophthirius* with the heptapsogasterid *Heptapsogaster temporalis*. Moving *H. temporalis* to its traditional place in the tree, as sister taxon to *H. minuta*, increases the length of the MP tree from 1750 to 1753, a jump of only three steps.

What Are the Relationships among the Suborders?

Since the position of the Amblycera remains in question, EF1α is unable to resolve this issue unequivocally. However, the gene does appear to favor the old "Mallophaga" scheme that places Amblycera and Ischnocera as sister groups with Anoplura at the base. This scheme has been discredited by more recent taxonomists who have preferred to place Anoplura and Ischnocera together, with Amblycera at the base (Lyal, 1985a).

Are the Currently Recognized Families of Ischnocera Monophyletic?

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

Which Philopterid Groups Could Be Elevated to Familial Status?

The Philopteridae *sensu* Hopkins and Clay represents a large paraphyletic assemblage from which the Goniodidae and Heptapsogasteridae have been raised to the rank of family. While we do not advocate altering the present classification of the lice on the basis of a single gene phylogeny it is interesting to speculate on the extent to which our EF1α phylogeny can be used to identify further groups of philopterids which could be elevated to familial status. Such groups could include the Pseudonirmidae (although the inclusion of *Pectinopygus* in this family remains unresolved), the "cracid lice", Austrogoniodidae, Trichophilopteridae, Docophoroididae, Esthiopteridae (perhaps excluding *Fulicoffula*, *Ardeicola*, and *Columbicola*), Colilipeuridae, Degeeriellidae (perhaps including *Cuclotogaster*), Rallicolidae (excluding *Mulcticola*) (although some taxa may have to removed from the genus *Rallicola*), Meinertzhageniellidae, and Paragonicotidae (perhaps including *Brueelia*). Elevation of these groups to the rank of family would mean that what remains of the Philopteridae (*Penenirmus*, *Philopterus*, and *Strigiphilus*) would no longer be paraphyletic. It is unclear from the EF1α data alone where taxa such as *Ardeicola*, *Mulcticola*, *Otidoecus*, and *Nyctibicola* belong.

What Is the Relationship of the Trichodectidae to the Avian Ischnocera?

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

Do the Lemur Lice, Trichophilopterus, Belong in the Trichodectidae?

It has been suggested that the lemur lice, *Trichophilopterus*, do not belong in the Trichodectidae with the other ischnoceran lice of mammals, but have more affinities with the avian Ischnocera. To the extent that EF1α says anything at all about this issue, it suggests that *Trichophilopterus* belongs in a family of its own, but this may be an artifact of the poor resolution at the base of the tree. Although the exact placement of this genus remains in question we can be confident that it does not belong in the Trichodectidae.

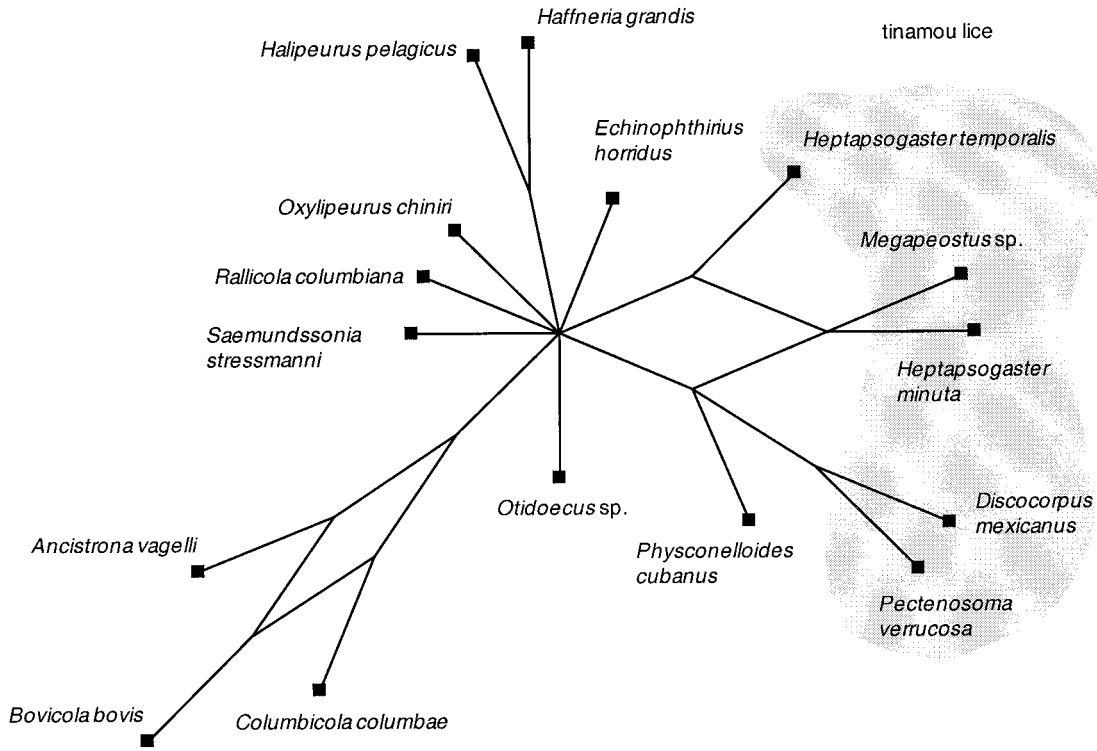

FIG. 4. A splits graph of a subset of lice EF1α sequences. Morphological data (Smith, 2000) suggest that *Heptapsogaster temporalis* belongs with the other tinamou lice, rather than the seal louse *Echinophthirius horridus*. Hence the signal linking *H. temporalis* with the seal louse is probably spurious, even though this signal predominates in the phylogenetic analyses that yielded the trees in Figs. 2 and 3.

Do the Penguin Lice, Austrogoniodes, Belong in the Goniodidae?

The penguin lice, *Austrogoniodes,* have often been considered to have affinities with the Goniodidae; however, Meg (1994) and Smith (2000) consider that the morphological similarities on which this assumption has been based are due to convergence. EF1α does indeed place *Austrogoniodes* well outside the Goniodidae; however, since the base of the tree is completely unresolved the true position of this genus remains in question. Although we cannot place *Austrogoniodes* precisely in the tree, we can be confident that it does not belong in the Goniodidae.

How Can We Account for the Spurious Positions of Echinophthirius and Heptapsogaster temporalis?

Data from the mitochondrial gene COI (Johnson, unpublished) place *Echinophthirius* near other anoplurans, suggesting that the relationship will break down once more data are added. Smith (2000) is in no doubt as to the monophyly of the Heptapsogasteridae, let alone the monophyly of the genus *Heptapsogaster,* and Lyal (1985a) seems almost as confident about the monophyly of the Anoplura, so why do these two taxa consistently group together? Worse still, if such a spurious grouping can occur between two taxa that we are almost certain belong in other parts of the tree, how can we be sure about the relationships of the taxa for which we have no prior hypothesis? Is there an independent method which would identify these two taxa as particularly prone to appearing in the wrong part of the tree, or must we call the entire phylogeny into question?

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

In the case of *H. temporalis,* we had *a priori* expectations of its correct relationships (Smith, 2000) and hence when confronted with its strange placement in our EF1α trees we were able to investigate this further,

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

Are Host Assemblages Monophyletic?

The ischnoceran lice from some host groups appear to form monophyletic groups. In addition to these ischnoceran lice, many host groups also have one or more clades of amblyceran lice. However, due to the paucity of amblyceran taxa sampled, this discussion will consider only ischnoceran lice. The classic example of a monophyletic host assemblage are the tinamou lice of the family Heptapsogasteridae. Other host groups which appear to have monophyletic assemblages of Ischnocera according to the EF1α trees include the Cracidae (*Oxylipeurus* and *Chelopistes*), ratites (Meinertzhageniellidae), and pelecaniform seabirds (Pectinopyginae). There is also some evidence for a clade of duck lice.

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

Are There Distinct Clades of Ischnoceran Wing and Body Lice?

Many groups of birds harbor two distinct groups of ischnoceran lice: wing lice, which tend to be long and thin, and body lice, which tend to be short and fat. Are the wing lice from different birds all related to each other, forming a wing louse clade? Is the same true of the body lice? For some groups of birds this seems to be true; for example, the wing lice of procellariiform and charadriiform seabirds (Pseudonirminae) and the wing lice of columbiform birds (*Columbicola*) are all found in the basal polytomy. There is some evidence, particularly in the parsimony tree, to suggest that the body lice of these groups (Quadraceptinae and Physconelloidinae, respectively) are more closely related to each other than they are to the wing lice of their own hosts. However, this relies on nodes that are not supported by bootstrap proportions above 50% and should therefore be treated with caution. In other host groups, however, there is more solid evidence that body and wing lice form a clade. For example, the ischnoceran wing and body lice of birds in the family Cracidae (*Oxylipeurus* and *Chelopistes*, respectively) are closely related, although even in this case the bootstrap support is not high. In this case though, there is other evidence in support of this clade (see above) despite the fact that it disagrees with the traditional taxonomy. Similarly, wing and body lice of ducks (Ibidoecinae and Anatoecinae, respectively) appear to be closely related. It is not clear whether these cases represent independent origins of wing lice from an ancestral stock of body lice or vice versa. More data will be required to resolve this issue. These two cases, in which head and body lice from related hosts are themselves related, suggest that

transformation between these body forms is relatively easily achieved. This further suggests that overall body shape as well as other morphological features likely to be associated with adaptation to life on different parts of the host anatomy are likely to be poor phylogenetic characters due to excessive homoplasy.

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