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Phylogeny of "*Philoceanus* complex" seabird lice (Phthiraptera: Ischnocera) inferred from mitochondrial DNA sequences

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Abstract

The *Philoceanus* complex is a large assemblage of lice that parasitise procellariiform seabirds (petrels, albatrosses, and their relatives). We obtained mitochondrial 12S rRNA and cytochrome oxidase I DNA sequences from 39 species from diverse hosts and localities. Resolution of deeper relationships between genera was limited, however there is evidence for two major clades, one hosted by albatrosses, the other by petrels. Based on our results, the genera hosted by albatrosses are excellent candidates for detailed analysis of cospeciation. Our results also suggest that a previous estimate of a 5-fold difference in the relative rate of sequence evolution in lice and their avian hosts is an artefact of limited taxonomic sampling.

Keywords: Phthiraptera; Lice; Seabirds; 12S rRNA; COI; Elongation factor-1α; Cospeciation

1. Introduction

Lice hosted by procellariiform seabirds (petrels, shearwaters, albatrosses, and their relatives) have long attracted the attention of parasitologists as being an excellent group for investigating coevolution between lice and their avian hosts. Taxonomic work by Edwards (1951, 1961) and Timmermann (1965) suggested that seabird lice classification parallels that of their hosts. Ongoing taxonomic work (Palma, 1994; Palma and Pilgrim, 1983, 1984, 1988, 2002) has revealed a high degree of lineage specificity in these insects, consistent with cospeciation. However, it was not until the pioneering molecular phylogenetic studies by Paterson and Banks (2001), Paterson and Gray (1997), Paterson et al. (1993), and Paterson et al. (2000) that concrete evidence of cospeciation between seabird lice and their hosts emerged. Statistical tests using random trees showed that louse phylogenies where more similar to those of their hosts than could be expected due to chance alone (Fig. 1), and that seabird lice mitochondrial DNA evolves more rapidly than the homologous region in seabirds (Paterson and Banks, 2001; Paterson et al., 2000).

Given the importance of comprehensive taxonomic sampling for accurate estimates of the extent of host–parasite cospeciation (Page et al., 1996), it would be highly desirable to put the lice studied by Paterson et al. into a broader phylogenetic context. There are over 100 procellariiform seabird species distributed worldwide (Harrison, 1983), each of which host several louse genera (Clay and Moreby, 1967; Palma and Barker, 1996; Pilgrim and Palma, 1982; Timmermann, 1965). The bulk of these lice fall are informally referred to as the "*Philoceanus* complex" (Edwards, 1951; Ledger, 1980) and we use that term here. Edwards (1951) provided a detailed, if speculative, evolutionary scenario for the *Philoceanus* complex (Fig. 2). He divided the bulk of the procellariiform lice into two groups, the "Philoceani" and the "Pseudonirmini." The Philoceani comprised the genera *Halipeurus*, *Naubates*, and *Philoceanus*, all of

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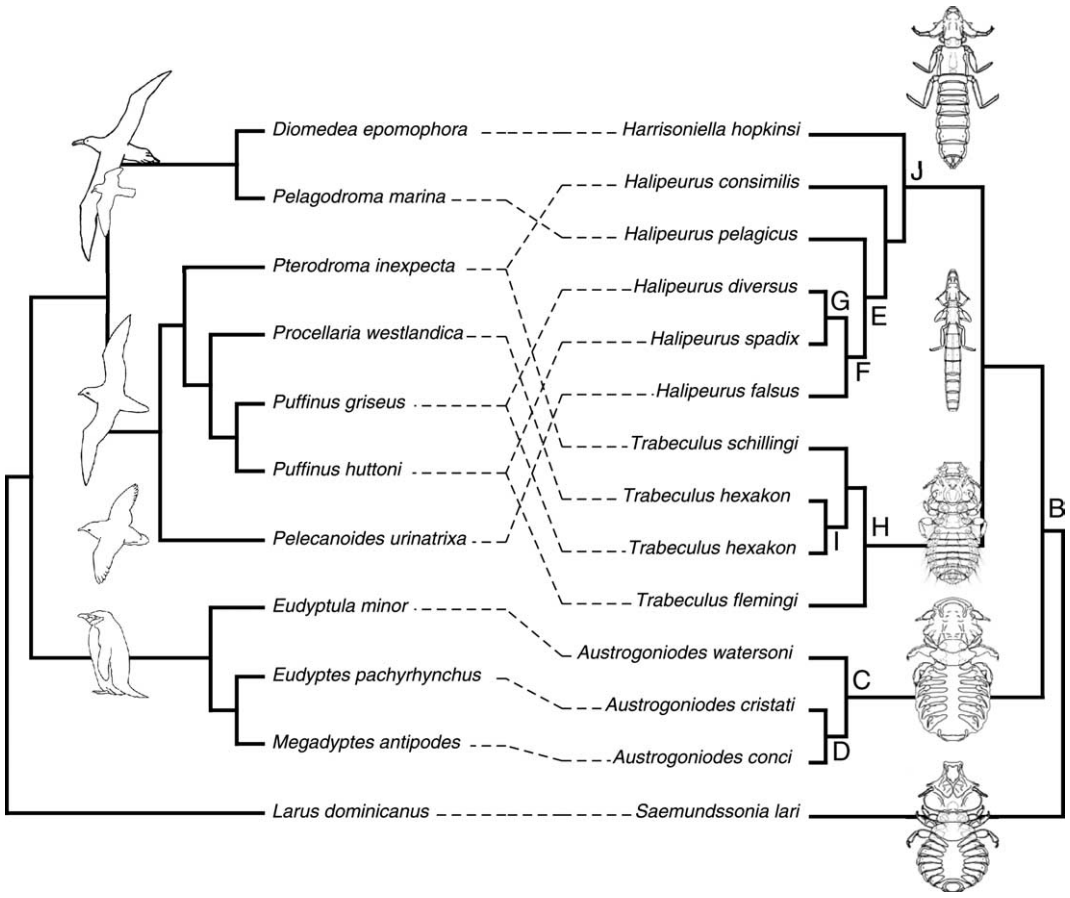

Fig. 1. Tanglegram for seabirds (albatrosses, petrels, and penguins) and their ischnoceran lice, based on 12S rRNA mitochondrial DNA sequences. Lice are linked to their corresponding host by a dashed line. The gull *Larus dominicanus* and its louse *Seamundssonia lari* are the outgroups for the bird and louse trees, respectively, redrawn from Paterson et al. (2000, Fig. 3).

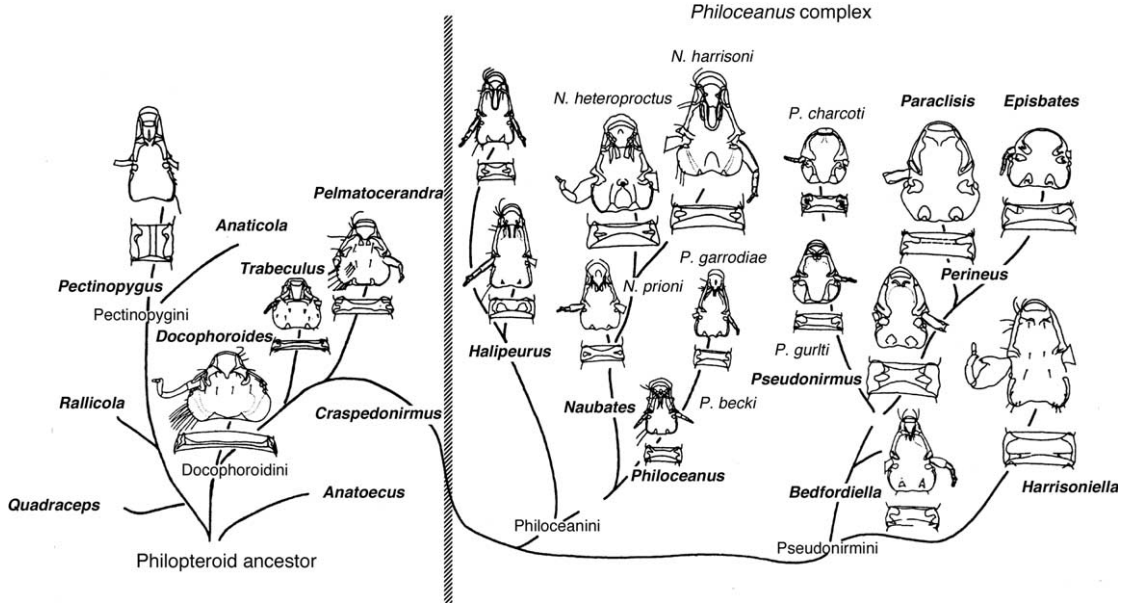

Fig. 2. An evolutionary scenario for *Philoceanus* complex lice, redrawn from Edwards (1951, Fig. 3).

which are found on petrels. The Pseudonirmini included *Pseudonirmus*, found on fulmars, and the genera *Episbates*, *Perineus*, and *Harrisoniella*, predominantly parasites of albatrosses. He placed the genera *Docophoroides* (on albatrosses), *Trabeculus* (on petrels), and *Pelmatocerandra* (on diving petrels) at the base of the tree. The genus *Craspedonirmus* (on loons) is depicted as an intermediate between these lice and the *Philoceanus* complex. The monophyly of the *Philoceanus* complex has subsequently received support from morphological data (Smith, 2001) and analysis of nuclear elongation factor-1α (EF1α) gene sequences (Cruickshank et al., 2001).

Through our own collecting, the collections of the Museum of New Zealand Te Papa Tongarewa, and a network of seabird workers, we have assembled a large collection of *Philoceanus* complex lice from numerous hosts around the world. In this paper, we used mitochondrial and nuclear DNA sequences to investigate the phylogeny of this group. We then discuss the implications of this phylogeny for ongoing studies of cospeciation between seabirds and their lice.

2. Material and methods

2.1. Sampling

Where possible, lice were freshly collected into 95% ethanol. Additional material came from the collections of the Museum of New Zealand Te Papa. In most cases lice in the Te Papa collections had been obtained from live hosts, but in some instances the hosts had been dead for an unknown period of time (e.g., washed up on a beach after a storm). The oldest material successfully sequenced was collected in March 1992. Where possible all material was either identified by RLP prior to sequencing, or the specimen from which DNA was extracted was slide mounted and subsequently identified by RLP. Prior to adopting this protocol we extracted DNA from lice by grinding 1–2 individuals up. Those sequences for which we do not have vouchers and which were not determined by RLP prior to sequencing are indicated in our list of specimens used (Appendices A and B).

2.2. Sequences

Total genomic DNA was extracted from single lice using the DNeasy Tissue Kit (Qiagen). Negative controls were included with each set of extractions. The head of the each louse was separated from its body and both were incubated in lysis buffer over two nights. After extraction the exoskeletons were removed for slide mounting as vouchers. The third domain of the mitochondrial 12S rRNA gene was amplified and sequenced using the insect specific primers 12Sai and 12Sbi (Simon et al., 1994). For mitochondrial COI we used the L6625 and H7005 primers (Hafner et al., 1994).

The PCR conditions were denaturation at 94 °C for 1 min followed by 40 cycles of 92 °C for 30 s, annealing at 45 °C for 40 s, and an extension of 65 °C for 90 s, with a final extension of 72 °C for 10 min. Negative controls were included with each set of PCRs. Amplification products were gel purified using the QIAquick Gel Extraction Kit (Qiagen) and sequenced by an automated sequencer using the PCR primers.

Previously published 12S rRNA sequences for seabird lice (Paterson et al., 2000) were obtained from the alignment used in their paper (available from ftp://ag.arizona.edu/dept/systbiol/issues/49_3/paterson.wd). The corresponding sequences in GenBank are shorter than those reported in their paper, hence we used those from their published alignment. A further two sequences (Accession Nos.: Y14917 and Y14919) that are described as being from the louse *Naubates* were deposited in GenBank by Paterson et al. (2000). However, they did not use these sequences in their study, and we omitted them from our own analyses as they are misidentified (see below).

Previously published elongation factor 1α (EF1α) sequences (Cruickshank et al., 2001; Page et al., 2002) were supplemented by a small number of additional sequences obtained using the methods described in Cruickshank et al. (2001).

2.3. Sequence alignment and analysis

COI sequences were aligned using ClustalX (Thompson et al., 1997). EF1α sequences were aligned by eye. Louse 12S rRNA sequences show considerable length variation, more so than in all other insect groups combined (Page et al., 2002). Consequently, it is very difficult to align some regions with any confidence, even across relatively closely related taxa. Using the louse secondary structure model developed by Page et al. (2002) as a guide, we identified the core stem regions 33–36, 38, 38′, 36′–34′, and 33′ and deleted from the alignment those portions that could not be confidently aligned across all louse taxa. These deleted regions comprised bases between stem 36 and 38, between 38 and 38′ (including stems 39 and 42), and between 34′ and 33′ (including stem 47).

2.4. Phylogenetic analysis

We performed a range of phylogenetic analyses using the programs PAUP* version 4b10 (Swofford, 2001) and MrBayes 2.01 (Huelsenbeck and Ronquist, 2001). Parsimony trees were built using equal weights for all sites and character changes. For the mitochondrial genes we used 10 random addition sequences. Bootstrap support values were computed using standard heuristic searches with 1000 bootstrap replicates. The nuclear gene dataset was analysed using a branch and bound search. Model

parameters for maximum likelihood analyses were obtained using the Akaike criterion in ModelTest 3.06 (Posada and Crandall, 1998). Neighbour joining trees were computed using maximum likelihood distances. Bayesian analysis was performed using MrBayes with the following settings. The maximum likelihood model employed 6 substitution types ("nst = 6"), with base frequencies set to the empirically observed values ("basefreq = empirical"). Rate variation across sites was modelled using a gamma distribution ("rates = gamma"). The Markov chain Monte Carlo search was run with 4 chains for 1,000,000 generations, with trees being sampled every 100 generations (the first 1000 trees were discarded as "burnin"). All analyses were performed on a Sun Ultra 10 workstation.

We used the genera *Docophoroides* and *Trabeculus* as outgroups to locate the root of the *Philoceanus* complex, based on their proximity to members of this complex (Smith, 2001).

2.5. Host nomenclature and phylogeny

For bird names we follow Sibley and Monroe (1990), with some modifications. For albatrosses we follow Nunn and Stanley (1998). Olson (2000) has argued that the Kerguelen Petrel, usually called either *Pterodroma brevirostris* or *Lugensa brevirostris* should be referred to as *Aphrodroma brevirostris*, which we do here. We also recognise some subspecies of *Puffinus ilherminieri* and *Puffinus assimilis*, following Jouanin and Mougin (1979).

To generate a host phylogeny we used the cytochrome *b* dataset assembled by Kennedy and Page (2002) as our starting point. To this dataset we added a sequence for the Great Skua *Catharacta skua* (GenBank Accession No.: U76807, Cohen et al., 1997) and an unpublished sequence for the Band-rumped Storm-petrel *Oceanodroma castro* (GenBank Accession No.: AJ004204). We constructed a tree for procellariiform birds using MrBayes as described above.

2.6. Cospeciation analysis

We visualised the coevolutionary history of bird and louse associations using the jungles algorithm (Charleston, 1998; Charleston and Perkins, 2002) implemented in TreeMap 2.02β (available from http://taxonomy.zoology.gla.ac.uk/~mac/treemap/). TreeMap requires fully resolved trees, so we used the consensus of the Bayesian trees for hosts and lice. Because of the size of the dataset we broke the Bayesian louse tree (Fig. 5) into manageable subtrees for analysis, and compared each with a subtree for the hosts obtained from the host phylogeny constructed above. Because the number of possible reconstructions for the history of a host–parasite assemblage can be very large (Charleston, 1998), finding all possible solutions can be computationally prohibitive in terms of both time and memory. Hence we constrained the set of possible solutions to those with no more than three hosts switches. We set the event costs to the defaults (codivergence = 0; duplication = host switch = sorting event = 1). Detailed cospeciation analysis is beyond the scope of this paper, so in this study we restrict ourselves to a simple test of whether there is significant evidence for cospeciation in each clade that we examined. Using TreeMap we found the maximum number of codivergence events for each pair of host and parasite trees. The significance of this value was determined by generating 100 random parasite trees and determining how many of those supported solutions had as many codivergence events as the observed parasite tree (Charleston and Robertson, 2002).

2.7. Electronic availability of data

Datasets of aligned sequences and TreeMap data files are available from our website (http://taxonomy.zoology.gla.ac.uk/rod/data/Philoceanus).

3. Results

3.1. Sequences and alignments

The mitochondrial dataset comprises 12S rRNA sequences from 84 lice, and COI from 75 lice (Appendix A). For 74 samples we sequenced both genes. However, we were unable to obtain COI from 9 lice, and could not get 12S rRNA from one outgroup species (*Docophoroides levequei*). We analysed the two mitochondrial genes both separately and together. For the combined parsimony analyses we included all 84 taxa, but for the combined maximum likelihood and Bayesian analyses we included only the 74 taxa for which we had both genes. The 12S rRNA alignment had a total of 474 positions, from which we excluded 270 positions due to difficulties in alignment. Hence the final 12S rRNA dataset had 204 characters (of which 138 were parsimony informative), and the COI dataset comprised 379 characters (183 being parsimony informative). The EF1α dataset (Appendix B) comprised 10 previously published sequences from Cruickshank et al. (2001) and 6 sequences obtained for this study. The alignment had a length of 347 characters, 58 of which were parsimony informative.

GenBank contains two short (185–188 bp) sequences of 12S rRNA from *Naubates fuliginosus* and *Naubates pterodromi* (Accession Nos.: Y14917 and Y14919, respectively). These sequences show >30% sequence difference from our sequences from these same taxa, but are very similar (3–4%) to the *Trabeculus flemingi* 12S rRNA sequence Paterson et al. obtained from lice hosted by *Puffinus huttoni*. When we added these two putative "*Naubates*" sequences to the 12S rRNA dataset

and built a neighbour joining tree, sequences Y14917 and Y14919 indeed grouped with *T. flemingi*. Hence, these two sequences are clearly not from *Naubates*, but are likely mislabelled individuals of *T. flemingi*. For this reason, we have not included them in our analysis.

3.2. Nuclear sequences

Due to difficulties in amplifying EF1α from *Philoceanus* complex lice, our dataset is limited to 16 sequences. The branch and bound parsimony search yielded 30 equally parsimonious trees of 130 steps (CI = 0.777, RI = 0.819) whose strict consensus appears in Fig. 3A. This consensus tree shows little resolution. Bayesian analysis yields a more resolved tree (Fig. 3B), but most groups receive little support. Both trees identify a clade of petrel lice (*Bedfordiella*, *Halipeurus*, and *Naubates*), below which occur the albatross lice *Harrisoniella*, *Paraclisis*, and *Perineus*, and the skua louse *Haffneria*.

3.3. Mitochondrial sequences

Parsimony analysis of the 84 mtDNA sequences (12S rRNA and COI combined) yielded 1796 equally parsimonious trees of 2700 steps in length (CI = 0.248, RI = 0.687). The strict consensus of these trees is shown in Fig. 4. Most nodes that are not resolved comprise sets of nearly identical sequences from conspecific lice on different hosts (e.g., *Docophoroides brevis*). The parsimony tree shows a basal split between the largely albatross-hosted genera *Episbates*, *Haffneria*, *Harrisoniella*, and *Perineus*, and the remaining genera, which are hosted by petrels. The *Naubates* species *N. fuliginosus* and *N. harrisoni* are embedded in a larger clade of the petrel louse genus *Halipeurus*, the remaining *Naubates* species are grouped with the smaller genera *Bedfordiella*, *Philoceanus*, and *Pseudonirmus*. The genera *Paraclisis* and *Pelmatocerandra* are sister taxa.

The explicitly model-based methods yielded trees similar to that found by parsimony. The Bayesian analysis (Fig. 5) provides weak support (posterior probability of 68%) for a clade of albatross lice. The relationships of the smaller genera *Bedfordiella*, *Pelmatocerandra*, *Philoceanus*, and *Pseudonirmus* differ greatly between the two trees. Within genera there is strong support for resolution within the outgroup genera *Docophoroides* and *Trabeculus*, and the albatross genus *Paraclisis*. Some groupings within *Halipeurus* also received good support. The neighbour joining and maximum likelihood trees (not shown) showed broadly similar topologies to the

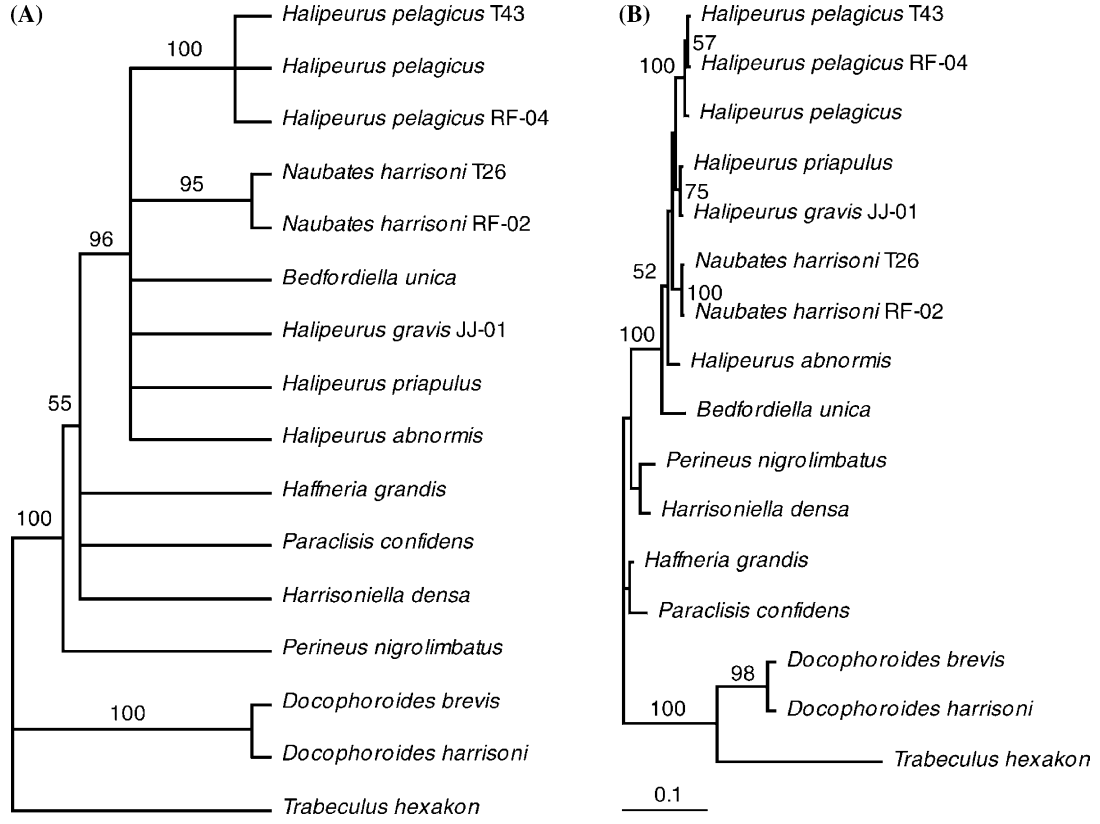

Fig. 3. Trees for EF1α sequences for *Philoceanus* lice. (A) Strict consensus of 30 equally parsimonious trees from a branch and bound analysis. Numbers on branches are bootstrap support values (where greater than 50%). (B) Consensus of Bayesian analysis with support values indicated (where greater than 50%) Sequences from the same louse species are distinguished by specimen code (see Appendix B). Scale bar represents 0.1 substitutions per site.

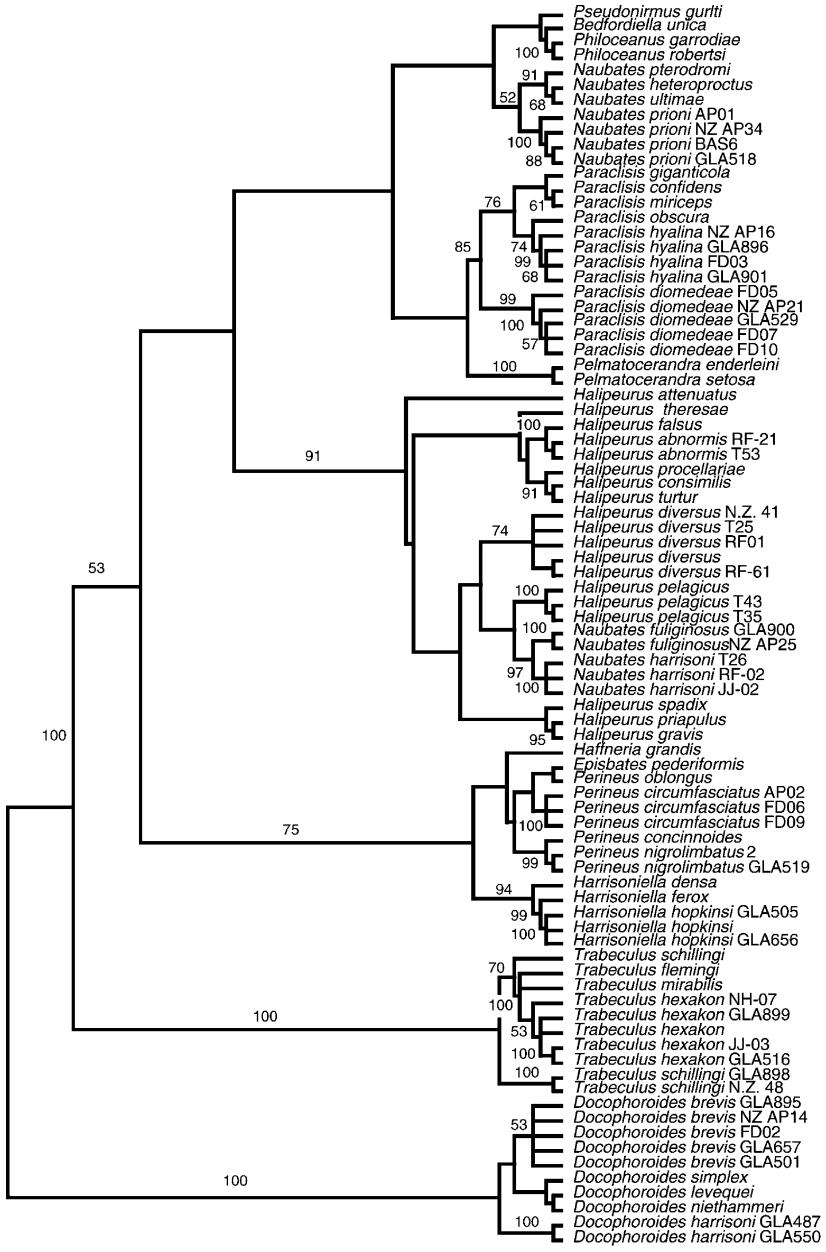

Fig. 4. Strict consensus of 1796 equally parsimonious trees for combined 12S rRNA and COI sequences for *Philoceanus* complex lice. Louse species that occur on more than one host are distinguished by specimen code (see Appendix A).

parsimony and Bayesian trees, with much of the differences involving placement of the genera *Bedfordiella*, *Pelmatocerandra*, *Philoceanus*, and *Pseudonirmus*.

3.4. Combined nuclear and mitochondrial data

We constructed a combined nuclear and mitochondrial DNA matrix by concatenating the EF1α sequences with mitochondrial sequences for the same taxa. After deleting *Halipeurus priapulus* from *Puffinus carnipes* (specimen N.Z. 43) for which no combining COI was obtained, the resulting 15 taxon matrix had 930 characters of which 305 were parsimony informative. Branch and bound parsimony analysis found 6 equally parsimonious trees of 1055 steps (CI = 0.569, RI = 0.567) whose strict consensus appears in Fig. 6. Bayesian analysis yielded a more resolved tree, with moderate support for a group comprising all albatross lice.

3.5. Cospeciation analysis

We broke the louse tree into four subtrees to investigate whether cospeciation had occurred between *Philoceanus* complex lice and their hosts. In each case, we compared the trees from the Bayesian analysis of the bird cytochrome *b* data with the Bayesian tree for the

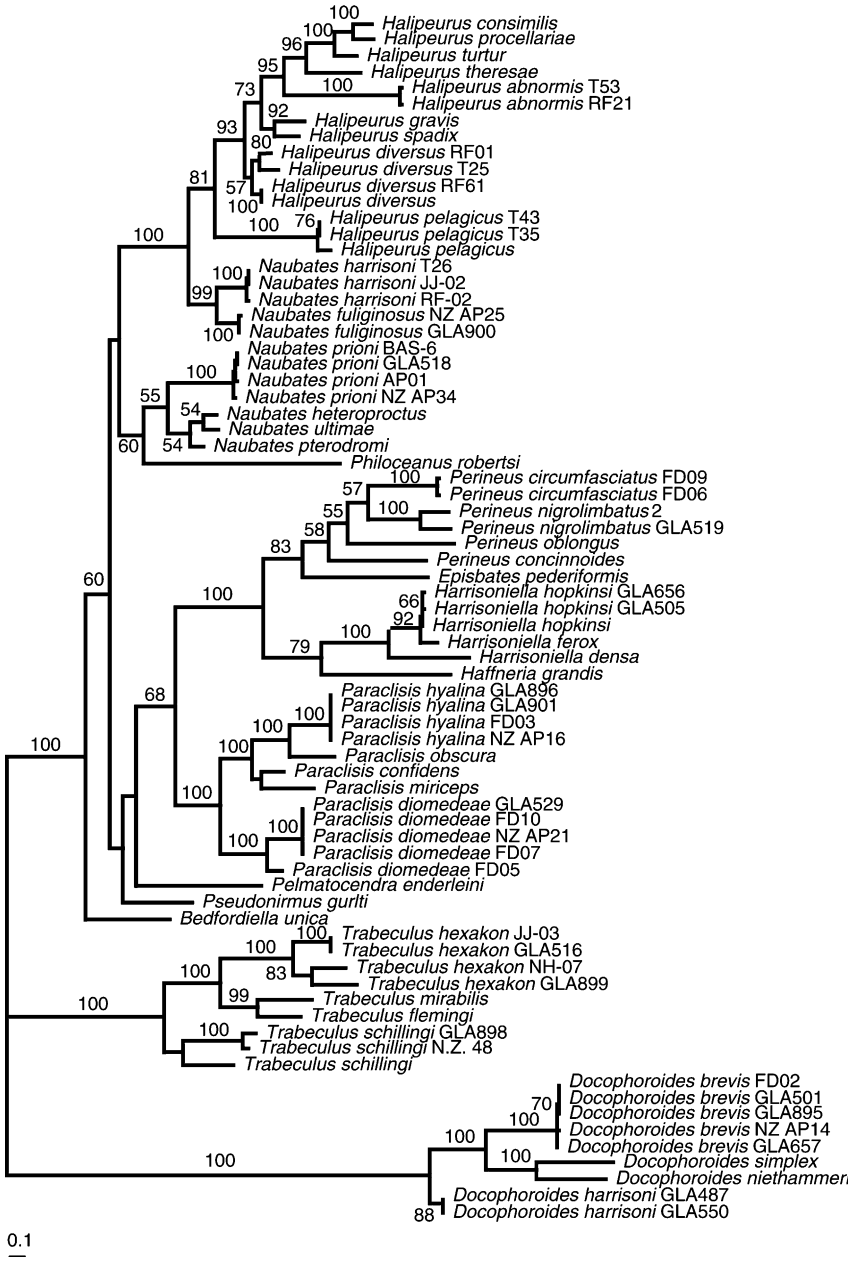

Fig. 5. Tree for combined 12S rRNA and COI sequences obtained by Bayesian analysis. Clade support values >50% are shown by each node. Branch lengths are proportional to inferred number of substitutions per site. Louse species that occur on more than one host are distinguished by specimen code (see Appendix A).

combined louse mitochondrial data (Fig. 5). Tanglegrams for four sets of lice and their hosts are presented in Figs. 7–10. Note that the bird and louse trees are not drawn to the same scale as the louse sequences tend to be much more divergent than those of their hosts.

Fig. 7A shows the tanglegram for *Paraclisis* lice, for which we have material from albatrosses and the giant petrel. The louse tree shows a striking similarity to the host tree—with the notable exception that *Paraclisis obscura* from *Macronectes* is sister to the *Paraclisis* clade on *Diomedea*. Using TreeMap we found a reconstruction that postulated 18 codivergence events (=9 instances of cospeciation), which is shown in Fig. 7B and is significant ($P = 0.001 \pm 0.001$). This reconstruction postulates two hosts switches, one being the colonisation of *Macronectes* by *P. obscura*, the other postulates that *Thalassarche melanophris* obtained its *Paraclisis diomedeae* by a host switch from *T. cauta*.

The genera *Episbates*, *Perineus*, and *Harrisoniella* comprise the other clade of *Philoceanus* complex lice on albatrosses (Fig. 8). This clade shows a more complex relationship to their hosts. The large-bodied

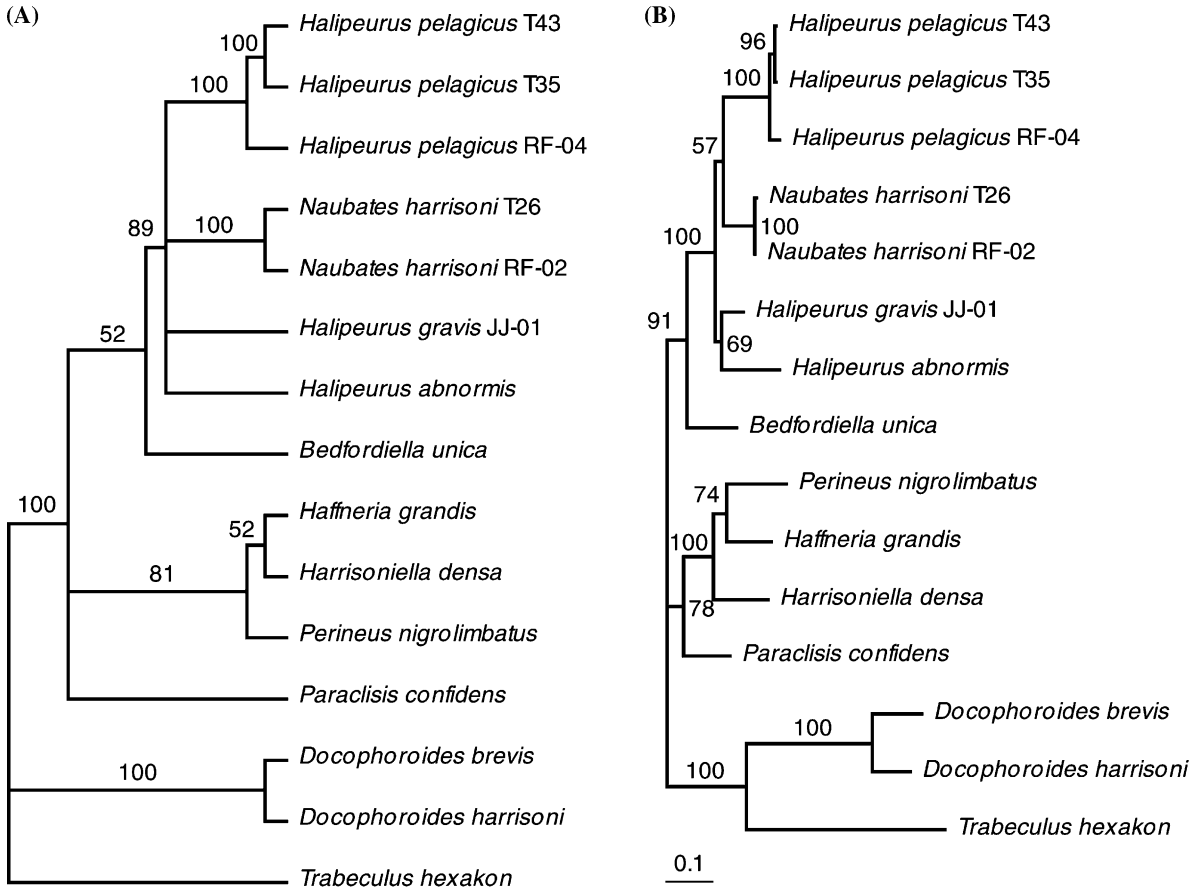

Fig. 6. Trees for the 15 taxa for which mitochondrial 12S rRNA, COI, and nuclear EF1α sequences are available. (A) Strict consensus of 6 equally parsimonious trees from a branch and bound analysis. Numbers on branches are bootstrap support values (where greater than 50%). (B) Consensus of Bayesian analysis with support values indicated (where greater than 50%). Sequences from the same louse species are distinguished by specimen code (see Appendix B). Scale bar represents 0.1 substitutions per site.

Harrisoniella lice are sister to the genus *Haffneria* which is found on skuas (Charadriiformes). The genus *Perineus* is also found on fulmars. TreeMap found a maximum of 14 codivergence events, which is not significant ($P = 0.25 \pm 0.043$). Some eight reconstructions were found with 14 codivergence events, and these had 0–2 host switches. These predominantly involved switches between *Thalassarche* and *Fulmarus* (*Perineus* lice) and between *Diomedea* and *Thalassarche* (*Harrisoniella ferox*). One reconstruction is shown in Fig. 8B.

Given the uncertain relationships of the petrel lice (particularly those of the smaller genera) we focus here on just the genus *Halipeurus* (Fig. 9). Prior to analysis of *Halipeurus* we excluded the sequence of *H. pelagicus* specimen T35 from *Bulweria bulweri* as we believe this is either a straggler or a contaminant. The normal parasite of *B. bulweria* is *H. bulweriae*, for which we do not have mitochondrial sequence data. There are some parallels between *Halipeurus* and host phylogeny: storm-petrels are the most basal petrels and host the basal louse lineage *Halipeurus pelagicus*, and the lice from *Pterodroma* form a clade. Interestingly, *Halipeurus* from shearwaters (*Calonectris* and *Puffinus*) do not form a clade. The largest number of codivergence events we found was 14 (=7 cospeciation events), which is not significant ($P = 0.46 \pm 0.050$). The bulk of the host switches postulated were between *Pterodroma* and *Calonectris* (involving *H. abnormis*), within *Puffinus* (involving *H. diversus*), and between the storm petrels (*H. pelagicus*) (Fig. 9B).

The outgroup genus *Docophoroides* is also a parasite of albatrosses, and its phylogeny shows some parallels with the host tree (Fig. 10). For the Bayesian trees in Fig. 10 the maximum number of codivergence events is 10 (=5 cospeciation events), which is not significant ($P = 0.36 \pm 0.015$). However, much of the apparent conflict between bird and louse tree concerns relationships among the sequences of *Docophoroides brevis*. Given that there is little support for the resolution of relationships within *Docophoroides brevis* shown in Fig. 10, there are alternative resolutions which show less conflict with the host tree.

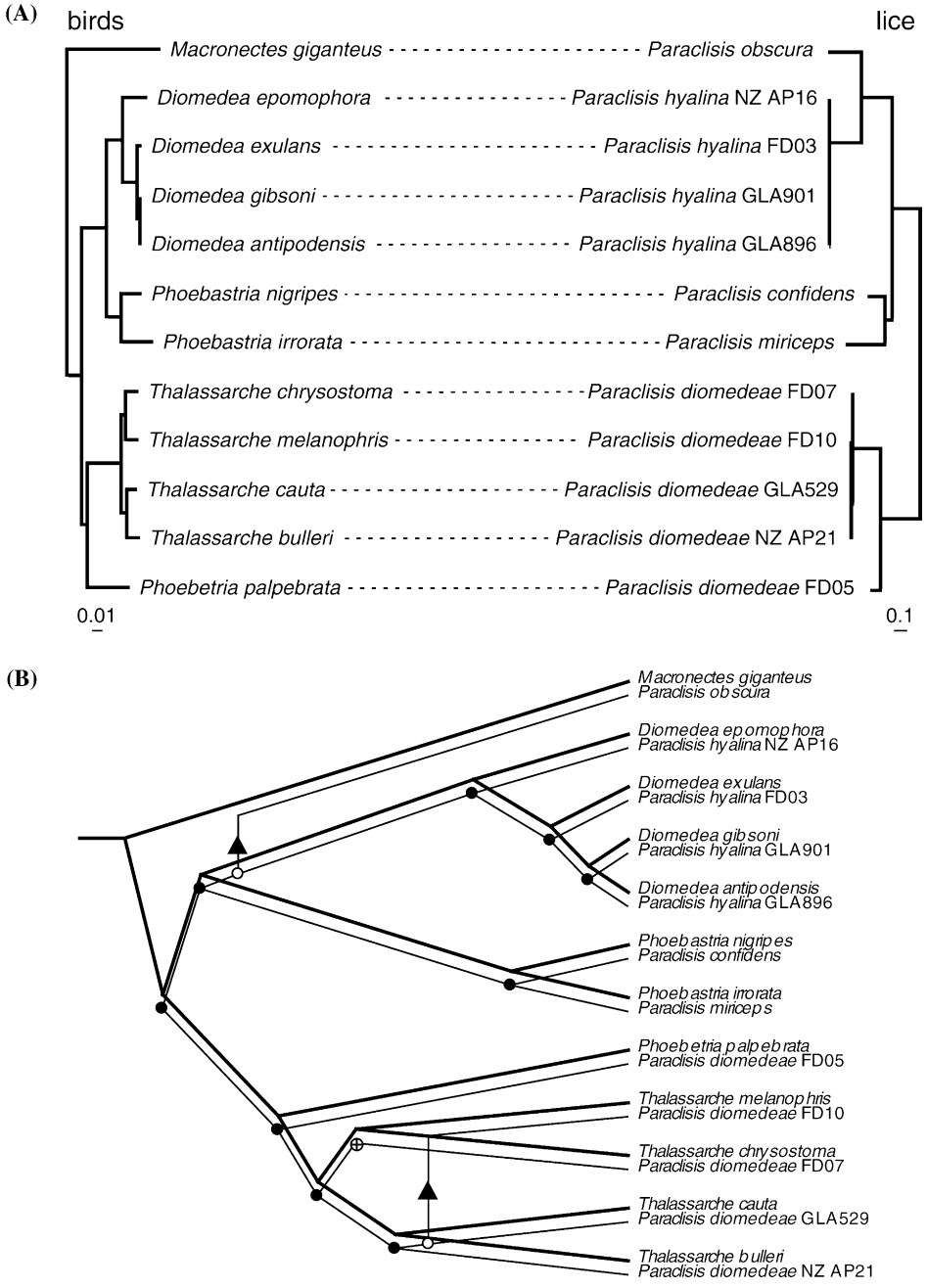

Fig. 7. (A) Tanglegram for *Paraclisis* lice and their hosts (albatrosses and the giant petrel). Each louse is connected to its host by a dashed line. Louse species that occur on more than one host are distinguished by specimen code (see Appendix A). Tree for lice is taken from the Bayesian tree in Fig. 5, tree for hosts from a Bayesian analysis of mitochondrial cytochrome *b* sequences. The scale bar for host and parasite trees represents 0.1 substitutions per site. (B) A possible reconstruction for the two trees shown in A found by the program TreeMap. Key to symbols: (●) cospeciation event; (○) duplication event; (⊕) sorting event; (→) host switch.

4. Discussion

4.1. Sequence divergence

Comparison of divergence in mitochondrial and nuclear genes suggests that both 12S rRNA and COI genes show the effects of multiple substitutions (Fig. 11). This is more pronounced in the COI sequences, for which within ingroup sequence divergence overlaps ingroup–outgroup sequence divergence to a greater degree than for 12S rRNA. This suggests that comparisons of COI within the *Philoceanus* complex will be affected by multiple substitutions. Both mitochondrial genes are more divergent than the nuclear EF1α sequences. However, the poor resolution of the trees based on EF1α sequences (Fig. 3) suggests that this gene is of limited use at this level in lice.

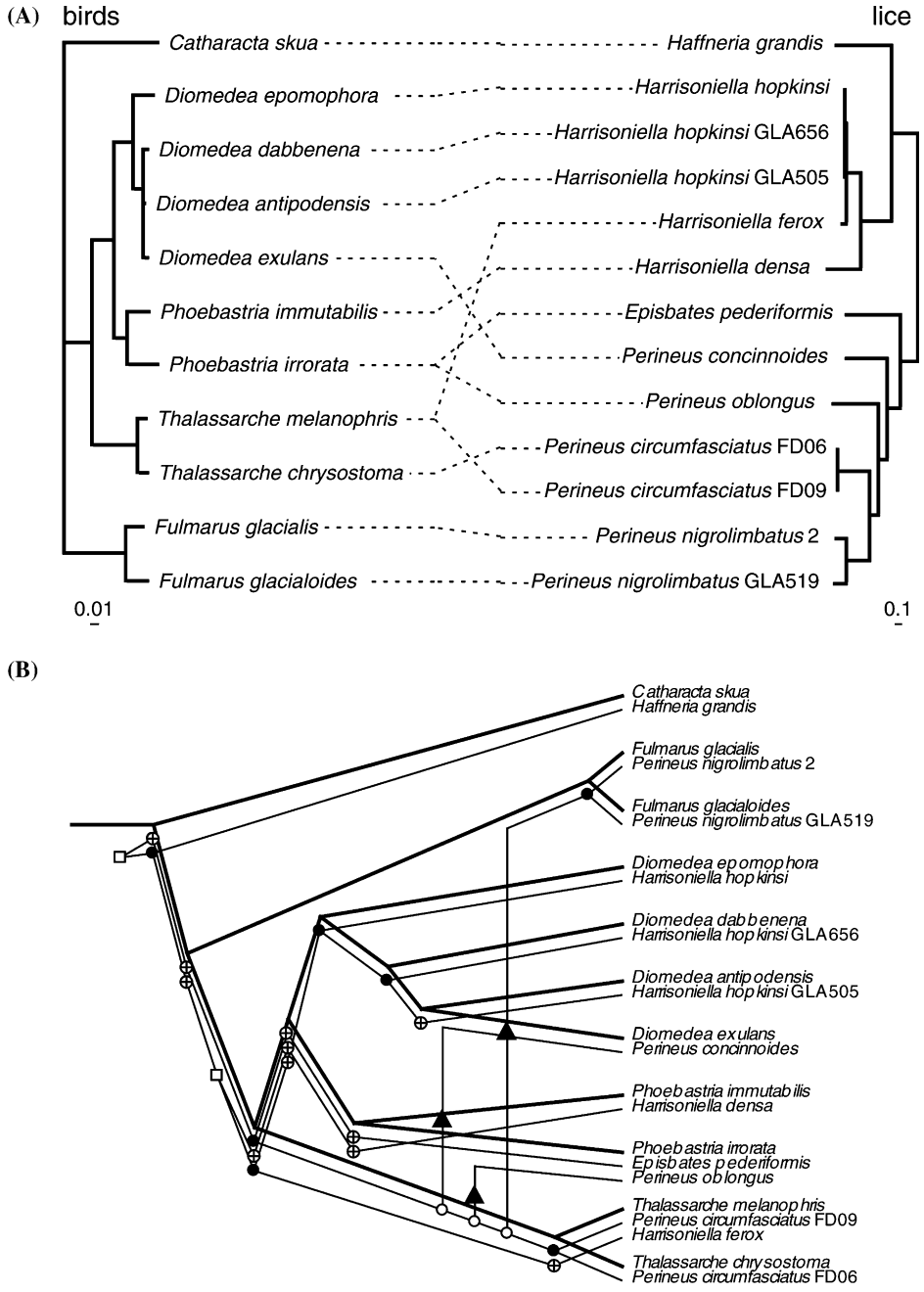

Fig. 8. Tanglegram (A) and reconstruction (B) for *Episbates*, *Harrisoniella*, and *Perineus* lice and their hosts. See Fig. 7 for key to symbols.

4.2. Taxonomic implications for genera

Based on our results the genus *Naubates* is not monophyletic. The two representatives of the subgenus *Naubates* (*Naubates*), *N. fuliginosus* and *N. harrisoni* are consistently grouped together, but are never grouped with the other members of *Naubates*: *N. heteroproctus*, *N. prioni*, *N. pterodromi*, and *N. ultimae*. These remaining *Naubates* species belong to the recently created subgenus *N.* (*Guenterion*) (Palma and Pilgrim, 2002). This subgenus is recovered in the combined mtDNA tree, but without convincing support. The relationships of the smaller genera *Bedfordiella*, *Pelmatocerandra*, *Philoceanus*, and *Pseudonirmus* are not satisfactorily resolved. Different datasets and analyses yield different possible placements, none with any confidence. Among the genera of lice on albatrosses, *Paraclisis* and *Harrisoniella* are both monophyletic. The monotypic genus *Episbates* is consistently grouped with *Perineus*, from which it differs in head morphology and other features (Thompson, 1947).

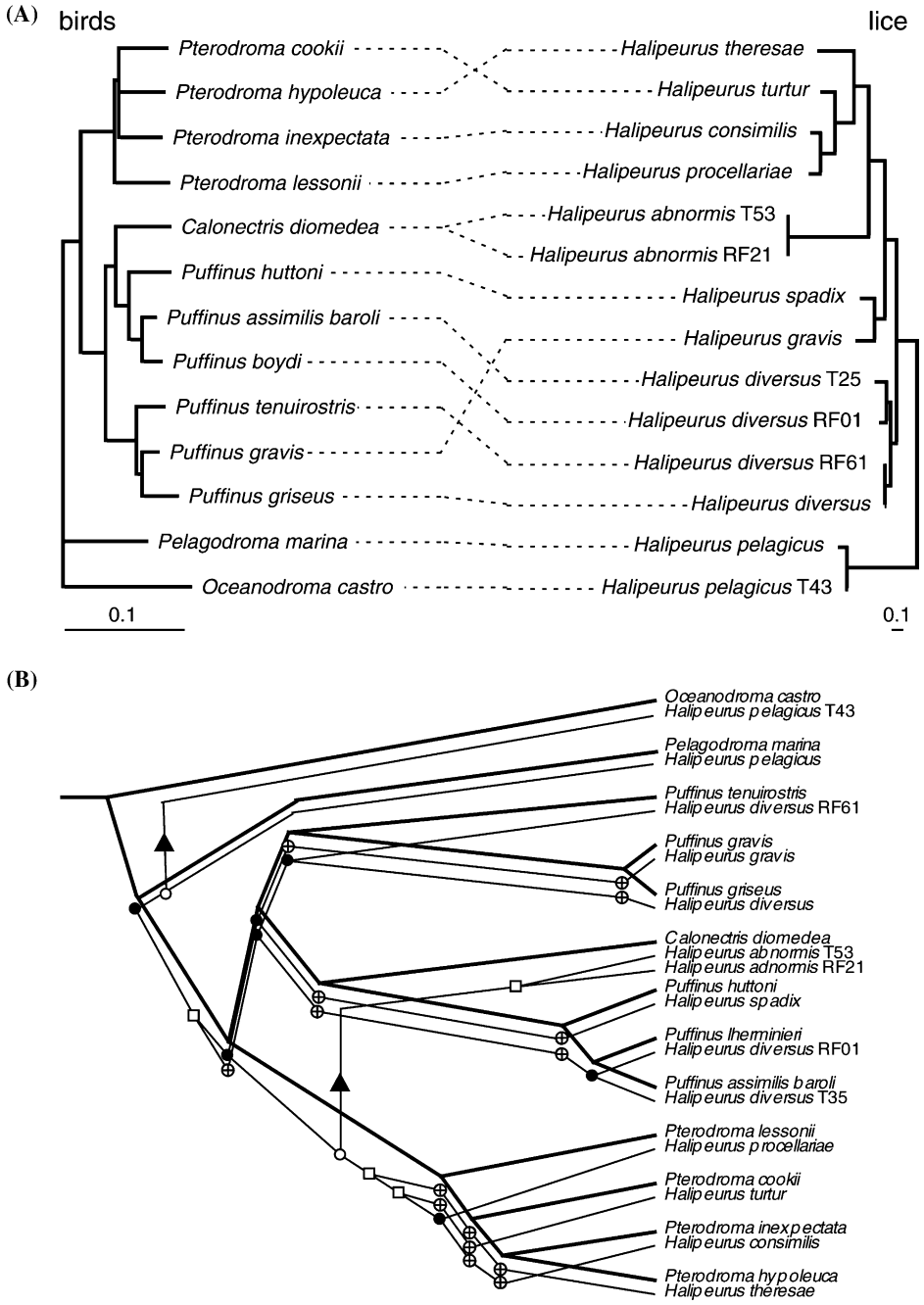

Fig. 9. Tanglegram (A) and reconstruction (B) for *Halipeurus* and its hosts (gadfly petrels, storm petrels, and shearwaters). See Fig. 7 for key to symbols.

4.3. Species concepts in lice

The history of louse taxonomy at the species level has been driven by two opposing approaches (Mey, 1998). One emphasises host specificity, and treats lice on different hosts as belonging to different species, even if morphologically indistinguishable. The other approach resists recognising species on the basis of criteria other than clear morphological differentiation. These two approaches can have very different implications for estimates of host specificity in lice. A complicating factor is that lice are often morphologically conservative, so that consistent differences between related lice from different hosts may only emerge if multivariate morphometric techniques are used (Ramli et al., 2000). However, morphologically similar lice may be genetically very distinct. For example, individuals of *Dennyus carljonesi* from different hosts are morphologically very similar (Clayton et al., 1996) but have highly divergent mitochondrial cytochrome *b* sequences (Page et al., 1998). If

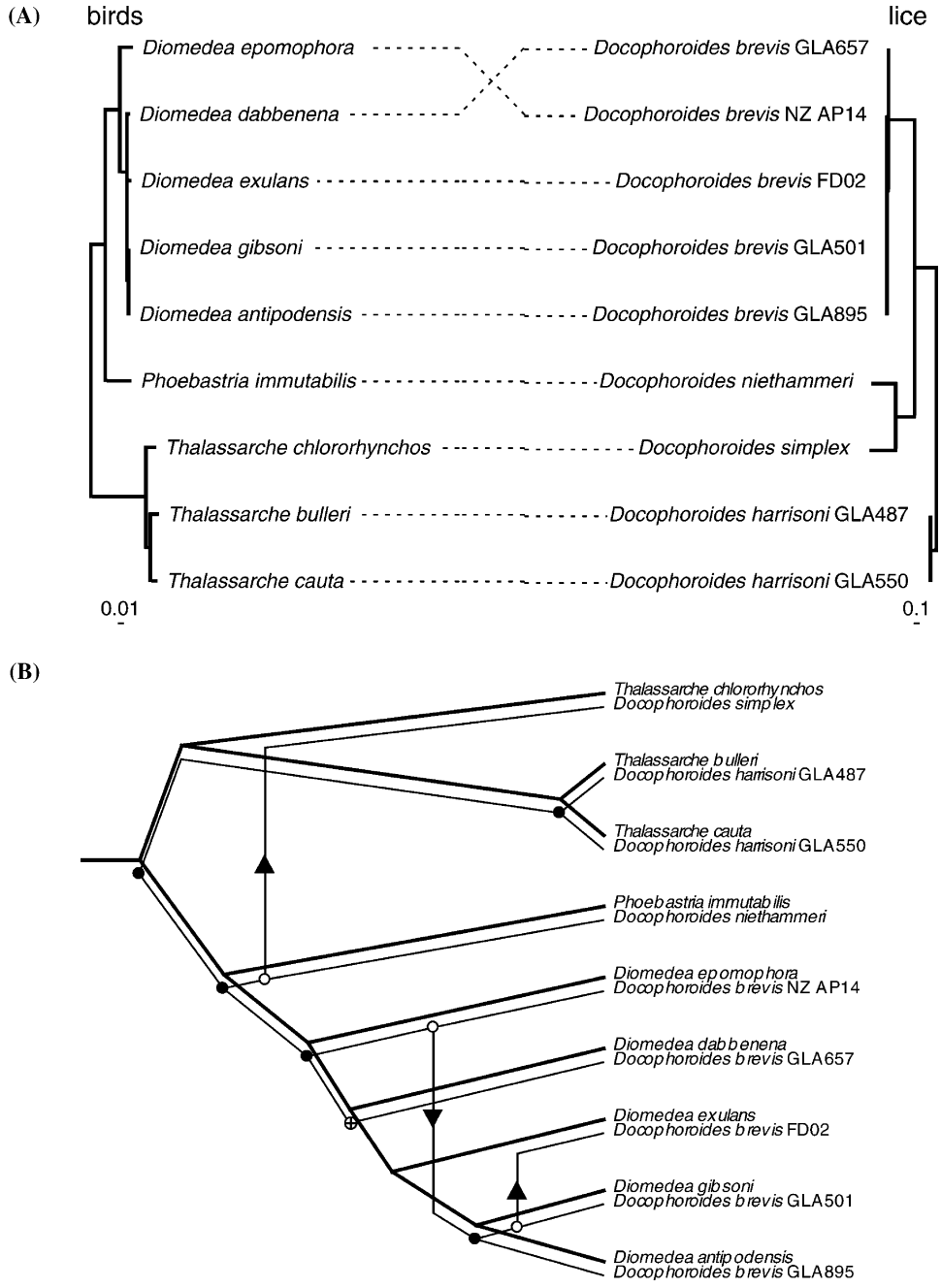

Fig. 10. Tanglegram (A) and reconstruction (B) for *Docophoroides* lice and their hosts. See Fig. 7 for key to symbols.

such examples of cryptic species are common in lice, then many cases of the "same" louse species occurring on different hosts may in fact be artefacts of poor taxonomy. This is not to deny that there are well-supported cases of low host specificity in lice (Johnson et al., 2002).

We have sequenced conspecific lice from different hosts, and in several cases these lice are genetically distinct. The most striking example of this is *P. diomedeae*, which has been recorded from *Thalassarche* and *Phoebetria* albatrosses (Palma and Barker, 1996). *P. diomedeae* from *Thalassarche* species have nearly identical sequences (0–1% difference for 12S rRNA, 0–1% for COI), but *P. diomedeae* from the Light-mantled Sooty albatross (*Phoebetria palpebrata*) is genetically very different from its conspecifics on mollymawks (5% for 12S rRNA, 13% for COI). *Perineus nigrolimbatus* populations on the two species of fulmar, *Fulmarus glacialis* (Northern Fulmar) and *F. glacialoides* (Southern Fulmar) show slight morphological differences which have not been thought sufficient to regard the populations as belonging to different species (Palma and Pilgrim, 1988). Our molecular data suggests that the populations of *P.*

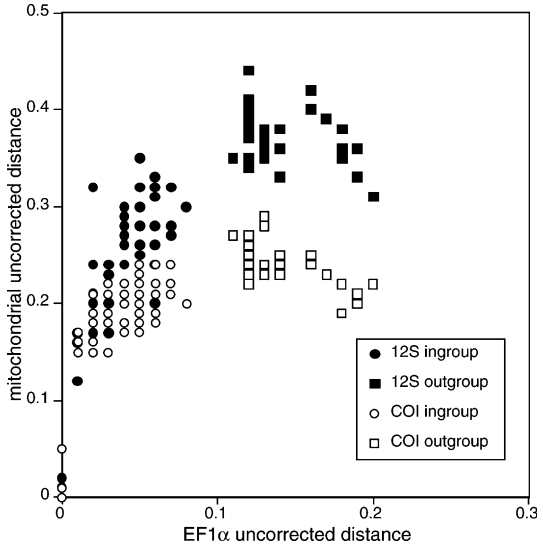

Fig. 11. Comparison of uncorrected sequence divergence in mitochondrial and nuclear sequences from seabird lice. Comparisons amongst ingroup (*Philoceanus* complex) and outgroup (*Docophoroides* and *Trabeculus*) sequences are distinguished.

nigrolimbatus on the Northern and Southern Fulmars are probably distinct species. Two species of *Trabeculus* show considerable genetic differentiation. Our results provide further evidence to support Paterson et al.'s (2000) finding that *T. hexakon* from *Procellaria* petrels and *Puffinus* shearwaters are genetically distinct. *Trabeculus schillingi* obtained from different species of *Pterodroma* are also as genetically different as currently recognised species in this genus. However, because most of our louse sequences have been obtained from single individuals from each host species, it would be highly desirable to obtain more sequences to assess within and between host-population variation in louse genetic diversity.

Based on these findings, the species we discuss above should probably be split further. Note however that there are clear examples of louse species recorded from more than one host that show little or no evidence of differentiation. Examples include *Paraclisis hyalina* on albatrosses (*Diomedea*), *Perineus circumfasciatus* on mollymawks (*Thalasarche*), *Naubates prioni* on prions (*Pachyptila*), and *Harrisoniella hopkinsi* and *Docophoroides brevis* on albatrosses (*Diomedea*).

4.4. Rates of evolution in birds and lice

Cospeciating host–parasite assemblages provide a unique framework for comparing rates of evolution in divergent organisms (Hafner and Nadler, 1990; Hafner and Page, 1995; Hafner et al., 1994; Huelsenbeck et al., 1997; Page, 1996, 2002; Page et al., 1998). If a pairs of hosts and their parasites have cospeciated then those two pairs of taxa are of the same age. We can use this fact to compare relative rates of evolution in hosts and parasites without requiring a fossil record (or some other means of calibrating the rate of evolution). Comparisons between mammals and their lice (Hafner et al., 1994; Huelsenbeck et al., 1997; Page, 1996) and between birds and their lice (Page et al., 1998; Paterson et al., 2000) suggest that louse mtDNA evolves 2–5 times more rapidly than that of their vertebrate hosts. Amongst the explanations that have been put forward are the shorter generation time of the lice (Hafner et al., 1994) and the possibility that louse populations undergo founder events as they colonise new host individuals (Page et al., 1998).

Direct comparison of rates of evolution in host and parasite requires homologous genes (Page et al., 1996). For procellariiform seabirds the largest number of sequences available are for cyt *b* (Nunn and Stanley, 1998), whereas we have louse sequences for 12S rRNA and COI. There is limited 12S rRNA data for seabirds (Cooper and Penny, 1997; Hedges and Sibley, 1994; Mindell et al., 1997; Paterson et al., 1995; van Tuinen et al., 2000), and no COI. Although a detailed comparison of rates is therefore not feasible, it is worth noting that our phylogeny has implications for the results reported by Paterson et al. (2000) and by Paterson and Banks (2001). Paterson et al. found that seabird louse 12S rRNA sequences were evolving 5.5 times more rapidly than those of their avian hosts, whereas Paterson and Banks (2001) found *Halipeurus* lice to be evolving only 1.53 times as fast as seabirds. This later rate is in line with estimates of the rate of evolution in other bird lice (Page et al., 1998).

Although Paterson and Banks speculated that this difference could be due to the large size of *Halipeurus* lice relative to most other procellariiform lice, it is more likely due to the inclusion of non-cospeciation events in their analysis. The result of Paterson et al. seems to be strongly influenced by the two deepest divergence events on their louse tree, events B and J (see Fig. 1). If we remove these two points and redo the regression (Fig. 12) we get a relative rate of 2.1, which is nearer the relative rate of 1.53 found for *Halipeurus* lice by Paterson and Banks (2001). Point B is the divergence between penguin lice *Austrogonoides* and procellariiform lice (*Trabeculus* and the *Philoceanus* complex). Although the relationships of *Austrogonoides* are still unclear (Cruickshank et al., 2001; Smith, 2000, 2001), there is no evidence that this genus is closely related to the *Philoceanus* complex. Hence, it is unlikely that event B represents cospeciation. For event J to be a cospeciation event the most recent common ancestor of *Harrisoniella* and *Halipeurus* would have to correspond to the split between albatrosses and petrels. While we cannot entirely rule this out, it seems unlikely given that in all of our trees the

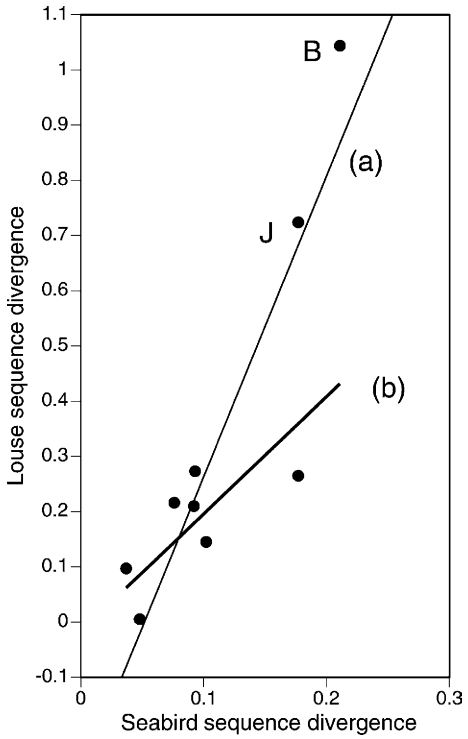

Fig. 12. Plot of 12S rRNA sequence divergence in seabird lice and their hosts. The original regression line of Paterson et al. (2000) is marked (a), the second line (b) is the reduced major-axis regression for the same data but with points B and J omitted (data from Paterson et al., 2000, Table 3).

path between *Harrisoniella* and *Halipeurus* crosses other louse lineages found on albatrosses and petrels. Hence, the estimate of relative rates of evolution found by Paterson and Banks (2001) is more likely to be more accurate than that of Paterson et al. (2000).

4.5. Taxonomic sampling and cospeciation

Taxonomic sampling is important for unravelling the history of an association (Page et al., 1996). Interestingly, one of the clearest associations we have is that between *Paraclisis* and its hosts (Fig. 7). This is also an association that we have sampled extensively, having lice from all four albatross genera. For other taxa the situation is not so good. The relationship between albatrosses and *Episbates*, *Harrisoniella*, and *Perineus* appears more complex, but part of this may be due to limited sampling. We have a single specimen of *E. pederiformis* from the Waved Albatross (*Phoebastria irrorata*), whereas it is also known from the genus *Diomedea* (Palma and Barker, 1996). Our sampling of *Harrisoniella* and *Perineus* from the genus *Thalassarche* is also poor (Palma and Pilgrim, 1984, 1988). Our sample of *Halipeurus* is larger than Paterson et al.'s, but still we have only a fraction of the known species available for sequencing.

4.6. Host switching

The genus *Haffneria* is unusual amongst the *Philoceanus* complex as it is not hosted by a procellariiform seabird. Instead, *Haffneria* parasitises skuas (Charadriiformes). Although there is morphometric variation amongst *Haffneria* populations on different host species (Ramli et al., 2000), most authors recognise only a single species, *H. grandis*. Its position in our trees suggests that skuas acquired this louse from an albatross. Note that the reconstruction depicted in Fig. 8B does not show a host switch from procellariiform seabirds to the skua. This is because both the host and louse trees are subtrees of much larger trees (e.g., Fig. 5). Considered in isolation, it is plausible that *Haffneria grandis* is an ancient parasite of skuas. However, once we consider that *Haffneria* is embedded in a much larger clade of procellariform lice it seems much more likely that *Haffneria* is an albatross louse that has secondarily colonized skuas.

The other clear instance of host switching involves the presence of *P. obscura* on the Southern Giant-petrel *Macronectes giganteus* (Fig. 7). Giant petrels are also host to *Perineus* and *Docophoroides*, although we were unable to obtain specimens of these lice from this host. *Fulmarus* is host to the otherwise typical albatross louse *Perineus*, suggesting a further host switch between albatrosses and fulmars, reflecting the heterogeneous louse community found on fulmars (Timmermann, 1965).

It is clear that the association between procellariiform birds and their lice has involved a mixture of cospeciation and host switching, with some clades of lice (e.g., *Paraclisis*) showing close fidelity to their hosts, and other clades showing higher levels of host switching (e.g., *Perineus* and *Halipeurus*).

4.7. Future work

Our data suggest that *Philoceanus* complex lice may be broadly divided into an albatross louse clade comprising *Episbates*, *Haffneria*, *Harrisoniella*, and *Perineus* (and possibly *Paraclisis*) and a petrel louse clade comprising *Bedfordiella*, *Halipeurus*, and the two *Naubates* subgenera (Fig. 13). The affinities of the small genera *Pelmatocerandra*, *Philoceanus*, and *Pseudonirmus* are not clear. Most genera for which we have representatives of more than one species are monophyletic, with the notable exception of *Naubates*. Resolution of generic relationships within the complex will require identifying a better marker than those so far employed in louse systematics.

Although we have not resolved the phylogeny of the *Philoceanus* complex, it is clear that some groups within this complex are candidates for detailed cospeciation analysis. Given the desirability of extensive sampling, the

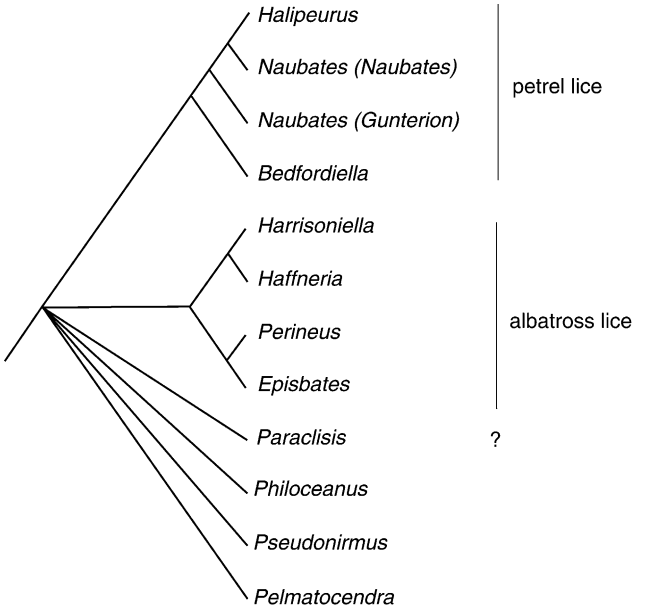

Fig. 13. Summary of relationships among genera of the *Philoceanus* complex. We recognise a clade of albatross lice (which may include *Paraclisis*), a clade of petrel lice, and three petrel louse genera of uncertain affinities. The genus *Naubates* is not monophyletic.

albatross louse genera are the most promising for investigation. These genera are particularly appealing because they share the same hosts, permitting replicated comparisons of the degree of cospeciation, host switching, and rates of molecular evolution. Detailed analysis of these associations is currently in progress.

Acknowledgments

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Appendix A

Specimens from which mitochondrial DNA sequences were obtained, and GenBank accession numbers for mtDNA sequences. The specimen codes refer to specimens in LouseBASE (http://r6-page.zoology.gla.ac.uk/lousebase/2). If more than one specimen code is listed then the 12S rRNA and COI sequences were obtained from different specimens. If no code is given (represented by —), then the sequence was obtained by Paterson et al. (2000) (GenBank accession numbers starting with "Y"). Specimens identified by * are not vouchered, all other specimens were determined by RLP

Louse species	Host species (Common name)	Specimen code(s)	12S rRNA	COI
Bedfordiella unica	*Aphrodroma brevirostris* (Kerguelen Petrel)	V.18*	AF396487	AF396546
Docophoroides brevis	*Diomedea antipodensis* (Antipodean Wandering Albatross)	GLA895	AY160058	AY160033
Docophoroides brevis	*Diomedea dabbenena* (Tristan Albatross)	GLA657	AY160057	AY160031
Docophoroides brevis	*Diomedea epomophora* (Royal Albatross)	NZ AP14	AF396488	AF396547
Docophoroides brevis	*Diomedea exulans* (Wandering Albatross)	FD02	AF396489	AF396548
Docophoroides brevis	*Diomedea gibsoni* (Gibson's Wandering Albatross)	GLA501	AY160054	AY160029
Docophoroides harrisoni	*Thalassarche bulleri* (Short-tailed Albatross)	GLA487	AY160053	AY160028
Docophoroides harrisoni	*Thalassarche cauta* (Shy Albatross)	GLA550	AY160056	AY160032
Docophoroides levequei	*Phoebastria irrorata* (Waved Albatross)	NZ71	—	AF396550
Docophoroides niethammeri	*Phoebastria immutabilis* (Laysan Albatross)	NH-03	AF396490	AF396551
Docophoroides simplex	*Thalassarche chlororhynchos* (Atlantic Yellow-nosed Albatross)	GLA655	AY160055	AY160030

Appendix A (*continued*)

Louse species	Host species (Common name)	Specimen code(s)	12S rRNA	COI
Episbates pederiformis	*Phoebastria irrorata* (Waved Albatross)	NZ70	AF396491	AF396552
Haffneria grandis	*Catharacta skua* (Great Skua)	T5*, RF-29*	AF189135	AF396553
Halipeurus abnormis	*Calonectris diomedea* (Cory's Shearwater)	T53	AF396492	AF396554
Halipeurus abnormis	*Calonectris edwardsii* (Cape Verde Shearwater)	RF-21, RF-22	AF396493	AF396555
Halipeurus attenuatus	*Puffinus lherminieri subalaris* (Galapagos Shearwater)	GLA906	AY160079	—
Halipeurus consimilis	*Pterodroma inexpectata* (Mottled Petrel)	—, NZ AP31	Y14914	AF396556
Halipeurus diversus	*Puffinus boydi* (Cape Verde Little Shearwater)	RF-01	AF396498	AF396564
Halipeurus diversus	*Puffinus assimilis baroli* (Canary Island Little Shearwater)	T25	AF396497	AF396563
Halipeurus diversus	*Puffinus griseus* (Sooty Shearwater)	GLA515	AY160060	AY160052
Halipeurus diversus	*Puffinus mauretanicus* (Balearic Shearwater)	N.Z. 41	AY160059	—
Halipeurus diversus	*Puffinus tenuirostris* (Short-tailed Shearwater)	RF-61	AF396494	AF396557
Halipeurus falsus	*Pelecanoides urinatrix* (Common Diving-petrel)	—	Y14913	—
Halipeurus priapulus	*Puffinus carneipes* (Flesh-footed Shearwater)	N.Z. 43	AF396496	—
Halipeurus gravis	*Puffinus gravis* (Great Shearwater)	JJ-01	AF396495	AF396558
Halipeurus pelagicus	*Oceanodroma castro* (Band-rumped Storm-petrel)	T43, RF-13	AF189137	AF396560
Halipeurus pelagicus	*Pelagodroma marina* (White-faced Storm-petrel)	—, RF-04	Y14915	AF396560
Halipeurus procellariae	*Pterodroma lessonii* (White-headed Petrel)	GLA517	AY160061	AY160051
Halipeurus pelagicus	*Bulweria bulwerii* (Bulwer's Petrel)	T35*	AF189136	AF396559
Halipeurus spadix	*Puffinus huttoni* (Hutton's Shearwater)	—, NZ AP29	Y14916	AF396562
Halipeurus theresae	*Pterodroma hypoleuca* (Bonin Petrel)	NH-06	AF396499	AF396565
Halipeurus turtur	*Pterodroma cookii* (Cook's Petrel)	NZ AP30	AF396500	AF396566
Harrisoniella densa	*Phoebastria immutabilis* (Laysan Albatross)	NH-02	AF396501	AF396567
Harrisoniella ferox	*Thalassarche melanophris* (Black-browed Albatross)	FD08	AF396502	AF396568
Harrisoniella hopkinsi	*Diomedea antipodensis* (Antipodean Wandering Albatross)	GLA505	AY160062	AY160045
Harrisoniella hopkinsi	*Diomedea dabbenena* (Tristan Albatross)	GLA656	AY160063	AY160046
Harrisoniella hopkinsi	*Diomedea epomophora* (Royal Albatross)	—, NZ AP15	Y14918	AF396569
Naubates fuliginosus	*Procellaria aequinoctialis* (White-chinned Petrel)	GLA900	AY160065	AY160034
Naubates fuliginosus	*Procellaria westlandica* (Westland Petrel)	NZ AP25	AF396503	AF396570
Naubates harrisoni	*Puffinus assimilis baroli* (Canary Island Little Shearwater)	T26*	AF396504	AF396571

Appendix A (*continued*)

Louse species	Host species (Common name)	Specimen code(s)	12S rRNA	COI
Naubates harrisoni	*Puffinus boydi* (Cape Verde Little Shearwater)	RF-02	AF396505	AF396573
Naubates harrisoni	*Puffinus gravis* (Great Shearwater)	JJ-02	AF396506	AF396572
Naubates heteroproctus	*Pterodroma macroptera* (Great-winged Petrel)	N.Z. 46	AF396507	AF396574
Naubates prioni	*Pachyptila belcheri* (Slender-billed Prion)	BAS-6*	AY160066	AY160048
Naubates prioni	*Pachyptila crassirostris* (Fulmar Prion)	GLA518	AY160064	AY160047
Naubates prioni	*Pachyptila turtur* (Fairy Prion)	NZ AP34	AF396508	AF396576
Naubates prioni	*Pachyptila vittata* (Broad-billed Prion)	AP01	AF396509	AF396577
Naubates pterodromi	*Pterodroma inexpectata* (Mottled Petrel)	NZ AP32	AF396510	AF396578
Naubates ultimae	*Pterodroma ultima* (Murphy's Petrel)	GLA908	AY160076	AY160049
Paraclisis confidens	*Phoebastria nigripes* (Black-browed Albatross)	NH-01	AF396511	AF396579
Paraclisis diomedeae	*Phoebetria palpebrata* (Light-mantled Sooty albatross)	FD05	AF396514	AF396582
Paraclisis diomedeae	*Thalassarche bulleri* (Short-tailed Albatross)	NZ AP21	AF396512	AF396580
Paraclisis diomedeae	*Thalassarche cauta* (Shy Albatross)	GLA529	AY160068	AY160040
Paraclisis diomedeae	*Thalassarche chrysostoma* (Grey-headed Albatross)	FD07	AF396513	AF396581
Paraclisis diomedeae	*Thalassarche melanophris* (Black-browed Albatross)	FD10*	AY160067	AY160039
Paraclisis giganticola	*Phoebastria immutabilis* (Laysan Albatross)	NH-04	AF396515	—
Paraclisis hyalina	*Diomedea antipodensis* (Antipodean Wandering Albatross)	GLA896	AY160069	AY160041
Paraclisis hyalina	*Diomedea epomophora* (Royal Albatross)	NZ AP16	AF396516	AF396583
Paraclisis hyalina	*Diomedea exulans* (Wandering Albatross)	FD03	AF396517	AF396584
Paraclisis hyalina	*Diomedea gibsoni* (Gibson's Wandering Albatross)	GLA901	AY160070	AY160042
Paraclisis miriceps	*Phoebastria irrorata* (Waved Albatross)	NZ72	AF396518	AF396585
Paraclisis obscura	*Macronectes giganteus* (Southern Giant-petrel)	GLA914	AY160077	AY160037
Pelmatocerandra enderleini	*Pelecanoides georgicus* (South Georgia Diving-petrel)	GLA912	AY160078	AY160038
Pelmatocerandra setosa	*Pelecanoides urinatrix* (Common Diving-petrel)	GLA913	AY179332	—
Perineus circumfasciatus	*Thalassarche bulleri* (Short-tailed Albatross)	AP02	AF396519	—
Perineus circumfasciatus	*Thalassarche chrysostoma* (Grey-headed Albatross)	FD06	AF396520	AF396586
Perineus circumfasciatus	*Thalassarche melanophris* (Black-browed Albatross)	FD09	AF396521	AF396587
Perineus concinnoides	*Diomedea exulans* (Wandering Albatross)	FD04	AF396522	AF396588
Perineus nigrolimbatus	*Fulmarus glacialis* (Northern Fulmar)	2	AF189143	AF396589
Perineus nigrolimbatus	*Fulmarus glacialoides* (Southern Fulmar)	GLA519	AY160074	AY160043

Appendix A (*continued*)

Louse species	Host species (Common name)	Specimen code(s)	12S rRNA	COI
Perineus oblongus	*Phoebastria irrorata* (Waved Albatross)	GLA902	AY160075	AY160044
Philoceanus garrodiae	*Garrodia nereis* (Grey-backed Storm-petrel)	N.Z. 51	AF396523	—
Philoceanus robertsi	*Oceanites oceanicus* (White vented Storm-petrel)	RF60	AF396524	AF396590
Pseudonirmus gurlti	*Daption capense* (Cape Petrel)	AP03	AF396525	AF396591
Trabeculus flemingi	*Puffinus huttoni* (Hutton's Shearwater)	—, NZ AP28	Y14921	AF396613
Trabeculus hexakon	*Procellaria aequinoctialis* (White-chinned Petrel)	GLA899	AY160072	AY160027
Trabeculus hexakon	*Procellaria westlandica* (Westland Petrel)	—	Y14923	—
Trabeculus hexakon	*Pterodroma hypoleuca* (Bonin Petrel)	NH-07	AF396535	AF396614
Trabeculus hexakon	*Puffinus gravis* (Great Shearwater)	JJ-03	AF396536	AF396615
Trabeculus hexakon	*Puffinus griseus* (Sooty Shearwater)	GLA516	AY160073	AY160035
Trabeculus mirabilis	*Puffinus boydi* (Cape Verde Little Shearwater)	RF-03	AF396537	AF396616
Trabeculus schillingi	*Pterodroma inexpectata* (Mottled Petrel)	—, NZ AP33	Y14924	AF396617
Trabeculus schillingi	*Pterodroma lessonii* (White-headed Petrel)	GLA898	AY160071	AY160026
Trabeculus schillingi	*Pterodroma macroptera* (Great-winged Petrel)	N.Z. 48	AF396538	AF396618

Appendix B

Specimens used in this study, and GenBank accession numbers for EF1α sequences. The specimen codes refer to specimens in LouseBASE (http://r6-page.zoology.gla.ac.uk/lousebase/2). Specimens identified by * are not vouchered, all other specimens were determined by RLP

Louse species	Host species	Specimen code	GenBank Accession No.
Bedfordiella unica	*Aphrodroma brevirostris* (Kerguelen Petrel)	N.Z. (RP) 3	AF320369
Docophoroides brevis	*Diomedea epomophora* (Royal Albatross)	NZ AP14	AF320394
Docophoroides harrisoni	*Thalassarche bulleri* (Short-tailed Albatross)	NZ AP19	AF320395
Haffneria grandis	*Catharacta skua* (Great Skua)	T5*	AF320406
Halipeurus abnormis	*Calonectris diomedea* (Cory's Shearwater)	T53	AY179333
Halipeurus gravis	*Puffinus carneipes* (Flesh-footed Shearwater)	N.Z. 43	AY179334
Halipeurus gravis	*Puffinus gravis* (Great Shearwater)	JJ-01	AY179335
Halipeurus pelagicus	*Oceanodroma castro* (Band-rumped Storm-petrel)	T43*	AF320409
Halipeurus pelagicus	*Pelagodroma marina* (White-faced Storm-petrel)	RF-04	AY179336
Halipeurus pelagicus	*Bulweria bulwerii* (Bulwer's Petrel)	T35*	AF320408
Harrisoniella densa	*Phoebastria immutabilis* (Laysan Albatross)	NH-02	AF320410
Naubates harrisoni	*Puffinus assimilis baroli* (Canary Island Little Shearwater)	T26*	AF320432
Naubates harrisoni	*Puffinus boydi* (Cape Verde Little Shearwater)	RF-02	AY179337
Paraclisis confidens	*Phoebastria nigripes* (Black-browed Albatross)	NH-01	AF502566
Perineus nigrolimbatus	*Fulmarus glacialis* (Northern Fulmar)	0010*	AF320448
Trabeculus hexakon	*Puffinus griseus* (Sooty Shearwater)	NZ AP26	AY179338

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