doi: 10.1111/j.1420-9101.2005.00983.x

Cophylogenetic relationships between penguins and their chewing lice

J. C. BANKS,* R. L. PALMA† & A. M. PATERSON*

*Bioprotection and Ecology Division, Lincoln University, Canterbury, New Zealand
†Museum of New Zealand Te Papa Tongarewa, Wellington, New Zealand

Keywords:

chewing lice;
coevolution;
cophylogeny;
cospeciation;
failure to speciate;
inertia;
penguins;
Phthiraptera;
Sphenisciformes.

Abstract

It is generally thought that the evolution of obligate parasites should be linked intimately to the evolution of their hosts and that speciation by the hosts should cause speciation of their parasites. The penguins and their chewing lice present a rare opportunity to examine codivergence between a complete host order and its parasitic lice. We estimated a phylogeny for all 15 species of lice parasitising all 17 species of penguins from the third domain of the mitochondrial 12S ribosomal rRNA gene, a portion of the mitochondrial cytochrome oxidase subunit 1 gene and 55 morphological characters. We found no evidence of extensive cospeciation between penguins and their chewing lice using TreeMap 2.02β. Despite the paucity of cospeciation, there is support for significant congruence between the louse and penguin phylogenies due to possible failure to speciate events (parasites not speciating in response to their hosts speciating).

Introduction

It is generally thought that the evolution of obligate parasites, with few means of transferring between different host species, should be tightly linked to the evolution of their own host species (Klassen, 1992). Comparing host and parasite evolution is known as the study of codivergence, coevolution or cophylogenetic descent (Paterson & Banks, 2001). A major aim of coevolutionary studies is to determine the cophylogenetic events that have generated the present distribution of parasite species and to explain how parasites have become associated with their hosts (Brooks & McLennan, 1991). The two major paradigms to explain current host-parasite associations are either that a parasite species has switched to a new host lineage, or that each host species has inherited the association from its ancestor. The two paradigms can be summarized as 'association by colonization' and 'association by descent' (Brooks & McLennan, 1991) or as 'souvenirs' and 'heirlooms' (Kliks, 1990). Association by colonization proposes that a parasite's presence on a host is due to parasites 'switching' between hosts (Brooks & McLennan, 1991). Association by colonization suggests that a parasite will speciate following isolation from its ancestral population after colonizing the new host, analogous to speciation following dispersal to new areas by free-living organisms. Association by descent suggests parasites have been inherited from the ancestral host. Association by descent suggests that parasites will speciate when hosts speciate, which is analogous to speciation by free-living organisms following a vicariant event (Brooks & McLennan, 1991), and is known as cospeciation or codivergence (Ronquist, 2003).

Chewing lice are a useful group on which to conduct cophylogenetic studies because they are considered to have limited mobility, rely mainly on host-to-host contact for transmission (Hafner & Nadler, 1988), usually appear to have little effect on their host (Rothschild & Clay, 1952; Marshall, 1981) and survive only briefly away from their hosts (Marshall, 1981). For these reasons pocket gophers and their lice, with their remarkably congruent phylogenies (Hafner & Nadler,

Correspondence: Jonathan Banks, Entomology, 320 Morrill Hall, University of Illinois, 505 S. Goodwin Ave, Urbana, IL 61801, USA.
Tel.: (+1) (217) 265 8123; fax: (+1) (217) 244-3499;
e-mail: jbanks@life.uiuc.edu

1988; Hafner *et al.*, 1994), have become the textbook models, e.g. Ridley (1996), of association by descent and of cospeciation between two lineages. Pocket gopher lice have also become the group in which to trial and demonstrate new methods of analysing the extent of cospeciation (for example, Page (1990), Ronquist (1995), Huelsenbeck *et al.* (1997,2000), Legendre *et al.* (2002)). However, many other host-parasite systems have considerably less codivergence than the pocket gopher-louse group. It is now recognized that events other than host switching may explain incongruence between host and parasite phylogenies without ruling out a history of association by descent. For example, duplication events (speciation by the parasite without the host speciating), and sorting events, such as missing the boat (the parasite is absent from the host population founding the new host species) or parasite extinctions (Paterson & Banks, 2001), may allow apparently incongruent parasite phylogenies to support a hypothesis of association by descent. Indeed, an analysis of pocket gopher and louse phylogenies allowing these events found even more codivergence between the hosts and their parasites (Page, 1990).

A rigorous cophylogenetic study incorporates four stages: first, a robust alpha taxonomy of both hosts and parasites; second, construction of accurate host and parasite phylogenies; third, quantitative comparison of the host and parasite phylogenies; fourth, statistical testing for congruence between the two phylogenies (Clayton *et al.*, 1996). Methods to evaluate the extent of cophylogenetic descent can be divided into two groups. The first group assesses the extent of codivergence by comparing the topology of the independently derived host and parasite phylogenies. Brooks Parsimony Analysis (Brooks *et al.*, 2001), the generalized parsimony method (Ronquist, 1995) implemented in Treefitter, reconciliation analysis (Page, 1994b) as implemented in TreeMap 1 (Page, 1995), and Jungles (Charleston, 1998) as implemented in TreeMap 2.02β (Charleston & Page, 2002), are methods that assume that accurate phylogenies for hosts and parasites are known (Huelsenbeck *et al.*, 2000). The second group of methods does not assume accurate phylogenies are known. Methods such as Data Based Parsimony (Johnson *et al.*, 2001), Parafit (Legendre *et al.*, 2002), and statistical methods based on maximum likelihood (Huelsenbeck *et al.*, 1997) or Bayesian methods (Huelsenbeck *et al.*, 2000); allow the evaluation of codivergence between less than optimal host and parasite phylogenies. While a comparison of the merits of each method is beyond the scope of this paper, TreeMap is the leading method to analyse phylogenetic aspects of host-parasite coevolution (Brooks & McLennan, 2003).

The ideal cophylogenetic study should also extensively sample parasites from the host group of interest as it increases the probability of detecting evolutionary changes (Page, 1996). In the past, cophylogenetic studies have tended to choose hosts and parasites that represent various taxonomic levels such as host families or orders and wing or body lice. For example, the gopher-louse study examined 17 louse species that were representative taxa from larger clades containing 122 recognized louse species and the 15 gopher taxa were examples from a group containing 40 species and 450 subspecies (Page, 1996). Similarly an analysis of cophylogeny between seabirds and their lice examined 14 louse species parasitising 11 host species from two relatively large host orders containing over 100 species (Paterson *et al.*, 2000). Choosing taxa to represent higher taxonomic groups has resulted in some aspects of cophylogenetic relationships being neglected. Parasite species with multiple host species (multi-host parasites) are one group that has been especially neglected as they often parasitise closely related hosts.

We compared a phylogeny estimated for all 17 species of penguins (Sphenisciformes) (Giannini & Bertelli, 2004) to a phylogeny estimated for all of the 15 species of chewing lice (Phthiraptera: Philopteridae) parasitising penguins. Our study, which is the first to examine all species of chewing lice parasitising an entire host order, did not find evidence for extensive cospeciation. Because we did not exclude any penguin louse species *a priori* we found several examples where it appears the lice have failed to speciate, i.e. the lice not speciating in response to their hosts speciating (Page, 1994a; Johnson *et al.*, 2003). Failure to speciate has also been called cophylogenetic inertia (Paterson & Banks, 2001) or cophylogeny without cospeciation (Hugot *et al.*, 2001). We suggest that failure to speciate is an additional event that needs to be considered in cophylogenetic studies that examine host parasite groups with multi-host parasites as it can markedly affect the extent of association by descent.

Methods

Molecular methods

Lice were collected from penguins at various southern hemisphere locations (Table 1). Live penguins were restrained, sprayed with pyrethrin insecticide and the plumage searched manually for lice. Penguins found dead were taken back to field camps where they could be searched more thoroughly for lice. Louse specimens were stored in 100% ethanol at room temperature until they could be refrigerated. Frozen penguin carcasses from institutions, such as museums, were also searched and these provided numerous louse specimens from which we could extract DNA suitable for sequencing. Louse specimens were identified from morphological characters (Clay & Moreby, 1967; Banks & Palma, 2003) before the DNA was extracted.

Initially, DNA was extracted from lice using the high salt method (White *et al.*, 1990), but later Qiagen DNeasy

Table 1 Louse collection sites and hosts.

Louse species	Host species	Location	Coordinates latitude	Longitude	Collector	Host status	Gene region sequenced
A. antarcticus	*P. adeliae*	Ross Island, Antarctica	77.17 °S	166.83 °W	J. C. Banks	Live	COI, 12S
A. bifasciatus	*S. humboldti*	Coquimbo, Chile	30.75 °S	71.00 °E	J. C. Banks	Dead	COI
A. bifasciatus	*S. magellanicus*	Sea Lion Island, Falklands	51.75 °S	59.42 °W	J. C. Banks	Live	COI, 12S
A. brevipes	*A. patagonicus*	Kerguelen Is.	49.25 °S	69.17 °W	M. Gaulthier-Clerc	Live	COI, 12S
A. concii	*E. pachyrhynchus*	Jackson Bay, New Zealand	43.97 °S	168.70 °E	J. C. Banks	Live	12S
A. concii	*E. robustus*	Snares Islands, New Zealand	48.04 °S	166.55 °E	J. C. Banks	Live	COI, 12S
A. concii	*M. antipodes*	Otago Peninsula (Genbank accession number Y14910)	45.92 °S	170.48 °E	A. M. Paterson	Live	12S
A. cristati	*E. chrysocome chrysocome*	New Island, Falklands	51.70 °S	61.28 °E	A. van Buren	Live	COI
A. cristati	*E. chrysocome filholi*	Snares Islands, New Zealand	48.04 °S	166.55 °E	J. C. Banks	Live	COI, 12S
A. cristati	*E. robustus*	Snares Islands, New Zealand	48.04 °S	166.55 °E	J. C. Banks	Live	COI, 12S
A. demersus	*S. demersus*	Cape Town, South Africa	33.92 °S	18.42 °E	J. C. Banks	Dead	COI, 12S
A. gressitti	*P. papua*	Sea Lion Island, Falklands	52.00 °S	60.28 °E	J. C. Banks	Live	COI, 12S
A. hamiltoni	*E. schlegeli*	Macquarie Island, Australia	54.62 °S	158.93 °E	K. Edge	Live	COI, 12S
A. keleri	*E. chrysocome chrysocome*	New Island, Falklands	51.70 °S	61.28 °E	A. van Buren	Live	COI, 12S
A. macquariensis	*E. chrysocome filholi*	Macquarie Island, Australia	54.62 °S	158.93 °E	K. Edge	Live	COI, 12S
A. mawsoni	*A. forsteri*	Ross Island, Antarctica	77.17 °S	166.83 °W	P. Ponganis	Live	COI, 12S
A. vanalphenae	*M. antipodes*	Otago Museum carcass	Unrecorded	Unrecorded	J. C. Banks	Dead	COI, 12S
A. waterstoni	*E. minor*	Coromandel Peninsula	36.83°S	175.58 °E	J. C. Banks	Dead	COI, 12S
		Wellington Harbour	41.28 °S	174.90 °E	J. C. Banks	Live	COI, 12S
		Chatham Island	44.00 °S	176.5 °W	J. C. Banks	Live	COI, 12S
		Banks Peninsula	43.75 °S	173.00 °E	J. C. Banks	Live	COI, 12S
		Otago Harbour	45.92 °S	170.48 °E	J. C. Banks	Dead	COI, 12S
		Phillip Island, Australia	38.49 °S	145.23 °E	R. Jessop	Dead	COI, 12S
N. demersus	*A. patagonicus*	Macquarie Island, Australia	54.62 °S	158.93 °E	K. Edge	Live	COI, 12S

COI, cytochrome oxidase subunit 1.

kits were used following the protocol developed by Cruickshank *et al.* (2001). The head of the louse was separated from the body and then incubated for 48 h at 55 °C in a solution containing proteinase K. The DNA was extracted as outlined in the DNeasy protocol. The head and body were retained as voucher specimens. Among the louse species parasitising penguins, we were unable to obtain specimens of *A. bicornutus* for molecular analysis. Molecular characters for *A. bicornutus* were coded as missing for the phylogenetic analysis.

Portions of the 12S and COI regions were amplified from total genomic extracts using polymerase chain reaction (PCR). All PCR was carried out using a Perkin Elmer 2400 thermal cycler. Reaction profiles for each region were 94 °C for 4 min, 40 cycles of 94 °C for 20 s, annealing temperature as in Tables 3 and 4 for 30 s, 72 °C for 50 s, and finally 72 °C for 5 min. See Table 2 for primer sequences. PCR consisted of 2.5 μL of 10 × buffer (Roche), 2.5 μL of dNTPs (1 mM), $MgCl_2$ (25 mM) as outlined in Tables 3 and 4, 1 μL of each primer (10 μM), 0.25 μL *Taq* (5 units μL^{-1}, Roche), 0.5–1 μL of DNA and water to 25 μL for each reaction. A negative control was incorporated in each amplification round using water rather than DNA.

Initially, excess primers and salts were removed from the PCR product by precipitation with isopropanol in the presence of 2.5 M NH_4Ac followed by a 70% ethanol wash. Later, PCR product was purified using Qiagen Concert rapid PCR purification system kits. Purified PCR fragments were sequenced using BigDye Termination Mix (Perkin-Elmer) and run out on an ABI 373 automated sequencer. Both the sense and antisense strands were sequenced. Sequences were deposited in GenBank (http://www.ncbi.nlm.nih) (Benson *et al.*,

Table 2 Primer sequences used in PCR.

Primer 1	Sequence	Reference
12sai	AAACTAGGATTAGATACCCTATTAT	Simon *et al.* (1994)
12sbi	AAGAGCGACGGGCGATGTGT	Simon *et al.* (1994)
L1091	AAAAAGCTTCAAACTGGGATTA GATACCCCACTAT	Kocher *et al.* (1989)
12sfia	CGGGCGATGTGTRCATTMTT	This paper
C1-J-1718	GGAGGATTTGGAAATTGATTAGTTCC	Simon *et al.* (1994)
C1-N-2191	CCCGGTAAAATTAAAATATAAACTTC	Simon *et al.* (1994)
COI1	TAAATAAYATRAGDYTTTGDCTKCT	This paper
COI1R	CCYCCNGMNGGRTCAAAAAARGA	This paper

COI, cytochrome oxidase subunit 1.

Table 3 Reaction conditions and primers used for PCR of the third domain of the mitochondrial 12S ribosomal rRNA region (see text for more details).

Species	Primer 1	Primer 2	Extra $MgCl_2$ (μL of 25 mM)	Annealing temp (°C)
A. antarcticus	12sai	12sfia	1.2	45
A. bifasciatus	L1091	12sbi	1.2	47
A. brevipes	12sai	12sfia	0	56
A. concii	L1091	12sbi	1.2	45
A. cristati	12sai	12sfia	0	47
A. demersus	L1091	12sbi	1.2	45
A. gressitti	12sai	12sfia	0	47
A. hamiltoni	L1091	12sbi	1.2	45
A. keleri	12sai	12sfia	0	47
A. macquariensis	L1091	12sbi	1.2	45
A. mawsoni	12sai	12sfia	0	47
A. vanalphenae	12sai	12sfia	0	47
A. waterstoni	L1091	12sbi	1.2	45
N. demersus	L1091	12sbi	1.2	47

Table 4 Reaction conditions and primers used for PCR of a portion of the COI region.

Species	Primer 1	Primer 2	Extra $MgCl_2$ (μL of 25 mM)	Annealing temp (°C)
A. antarcticus	C1-J-1718	C1-N-2191	1.2	47
A. bifasciatus	C1-J-1718	C1-N-2191	1.2	47
A. brevipes	C1-J-1718	C1-N-2191	0	47
A. concii	C1-J-1718	C1-N-2191	1.2	47
A. cristati	COI1	COI1R	1.2	47
A. demersus	C1-J-1718	C1-N-2191	1.2	47
A. gressitti	C1-J-1718	C1-N-2191	1.2	47
A. hamiltoni	C1-J-1718	C1-N-2191	1.2	47
A. keleri	COI1	COI1R	1.2	47
A. macquariensis	COI1	COI1R	1.2	47
A. mawsoni	COI1	COI1R	1.2	47
A. vanalphenae	C1-J-1718	C1-N-2191	1.2	47
A. waterstoni	C1-J-1718	C1-N-2191	1.2	47
N. demersus	C1-J-1718	C1-N-2191	1.2	47

COI, cytochrome oxidase subunit 1.

2002) (GenBank accession numbers AF491754–AF491758, AY229898–AY229936).

Our PCR results did not contain ghost bands and had few sequence ambiguities that are both usual signs of the amplification of pseudogenes (Bensasson *et al.*, 2001). For species, such as *A. antarcticus*, with large insertions, we sequenced 12S twice, using different primer sets and obtained identical sequences. The COI sequences were not punctuated by stop codons when translated into amino acids.

Louse taxonomy followed Hopkins & Clay (1953), Clay (1967), and Banks & Palma (2003). We considered the louse species *A. strutheus* as a *nomen dubium* following the discussion in Clay (1967). Because there were few genetic differences between individual lice of the same species parasitising different host species (see Table 1 for duplicates sequenced), we used one representative sequence for each gene region from each louse morpho-species in the phylogenetic analysis.

COI sequences were aligned using Clustal X (Thompson *et al.*, 1997), then adjusted manually using sequential pairwise comparisons. Alignment of the 368 base pair fragment of COI was straightforward because there were few insertions or deletions (indels). Postulated gaps within COI were adjusted with respect to the amino acid sequence so that codons were not split. Alignment of 12S was somewhat problematic and we aligned 12S manually with respect to the secondary structure of *A. waterstoni* (Page *et al.*, 2002). Unalignable regions were deleted leaving 293 base pairs of 12S for analysis.

Louse phylogeny

A mixed model Bayesian analysis using Mr Bayes 3 (Ronquist & Huelsenbeck, 2003) was conducted on 55 louse morphological characters (Banks & Paterson, 2004) and the COI and 12S molecular data. The general time reversible model plus gamma (Rodríguez *et al.*, 1990; Yang *et al.*, 1994) was chosen to analyse the genetic data based on the Akaike information criteria in ModelTest (Posada, 2000). Three independent Bayesian analyses were run for each gene to ensure proper sampling of tree space. Two runs were made of 2 000 000 generations, and one of 1 000 000 generations, with four chains using flat priors and mixed models, saving trees every 100 generations. All trees prior to stationarity were discarded (1000 or 2000 trees depending on the number of generations). Each run produced 50% majority rule trees of the same topology and converged on similar likelihood values after trees prior to stationarity were discarded. A single run of 5 000 000 generations was also conducted with 5000 trees prior to stationarity discarded.

Nesiotinus demersus was chosen as the outgroup as, although deeper relationships of ischnoceran lice are not well resolved (Cruickshank *et al.*, 2001), we are confident that *N. demersus* is not part of *Austrogoniodes* based on substantial morphological differences.

Cophylogenetic analysis

Penguin louse associations were collated from several sources (Clay, 1967; Watson, 1967; Pilgrim & Palma, 1982; Palma, 1996,1999; Price *et al.*, 2003) and are shown in Fig. 1. Associations of doubtful validity, for example, lice collected from penguins kept in zoos were not considered. A full list of doubtful records is given in Banks & Paterson (2004).

We analysed cophylogenetic relationships between penguins and their chewing lice using a penguin phylogeny estimated from 70 integumentary and breeding characters (Giannini & Bertelli, 2004). However, the

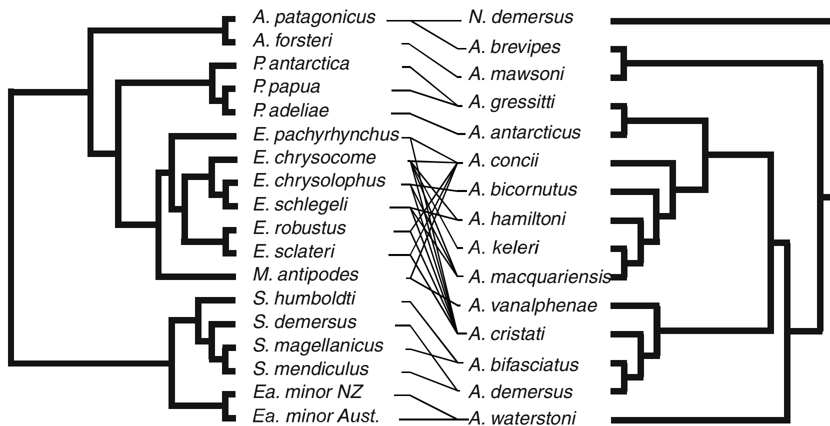

Fig. 1 Tanglegram of penguins and their lice. Lines connecting penguins and lice indicate associations.

penguin phylogeny we analysed differed slightly from the published phylogeny as we split *Eudyptula minor* into two taxa following the discussion in Banks *et al.* (2002) and combined the two *Eudyptes chrysocome* subspecies as a single terminal taxon.

The cophylogenetic history of the penguins and their lice was reconstructed using TreeMap 1 and TreeMap 2.02β. TreeMap 2.02β requires that multi-host lice (lice parasitising several host species) are subdivided into dummy lineages (Page & Charleston, 2002) to generate hypotheses of cophylogeny correctly. The topology of that portion of the phylogeny containing dummy lineages mirrored that of the host phylogeny. Because TreeMap 2.02β requires the addition of dummy taxa, the significance of the cophylogenetic relationship between the penguins and their lice, without dummy taxa, was assessed using TreeMap 1.

Parafit (Legendre *et al.*, 2002), which uses the patristic distances of a host and parasite phylogeny transformed into principle coordinates (Gower, 1966), was used to test the extent of a global hypothesis of coevolution between the lice and their hosts. Treefitter 1.0 (http://www.ebc.uu.se/systzoo/research/treefitter/treefitter.html) was also used to implement the generalized parsimony method of testing for a cophylogenetic relationship between penguins and their chewing lice. Costs used were cospeciation = 0, duplication and sorting = 1, and switching = 1–10. The cost of fitting the penguin phylogeny to the louse phylogeny was compared to the cost of fitting the host tree to 10 000 random parasite trees. Both of these methods are able to deal with multi-host lice.

Results

The 50% majority rule consensus tree of 45 000 trees estimated from the Bayesian analysis is shown in Fig. 2. The analysis found strong support for two groups within *Austrogoniodes*. The first was a '*concii*' clade of *A. concii, A. bicornutus, A. hamiltoni, A. keleri* and *A. macquariensis* and the second was a '*cristati*' clade of *A. vanalphenae, A. cristati,*

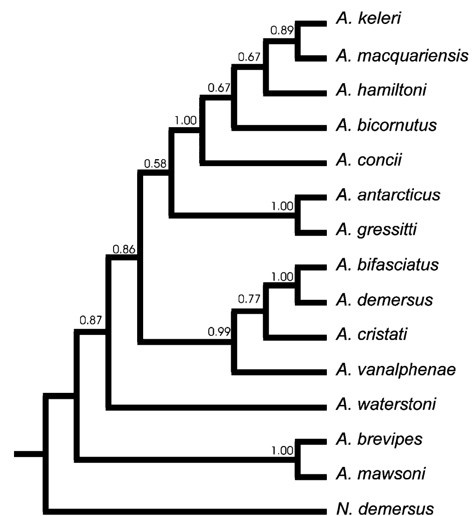

Fig. 2 Fifty per cent majority rule consensus tree of 45 000 trees for the penguin chewing lice estimated from a Bayesian analysis of sequences from portions of the third domain of the mitochondrial 12S ribosomal rRNA gene (12S), cytochrome oxidase subunit 1 (COI) gene and 55 morphological characters. Clade support values are shown above the lines.

A. demersus and *A. bifasciatus*. *Austrogoniodes antarcticus* and *A. gressitti*, as well as *A. brevipes* and *A. mawsoni* were also strongly supported as two pairs of sister taxa.

Cophylogenetic analysis

TreeMap

TreeMap 2.02β (Charleston & Page, 2002), with cospeciation events weighted as 0, duplications, lineage losses and host switching events weighted as one and up to two host switches allowed (the maximum number that was feasible with the computer power available), found six scenarios that maximized cospeciation. With zero host switches only three cospeciation events were found

(Figs 3 and 4). With one host switch there were four cospeciation events (Figs 4 and 5) or three cospeciation events with two host switches. TreeMap 1, with zero host switches, found the same reconstruction as TreeMap 2.02β using similar constraints.

When 'cospeciation' events due to dummy taxa were excluded, the number of cospeciation events did not differ significantly from the number obtained when 1000 random louse phylogenies were compared to the host phylogeny ($P = 0.89$) using TreeMap 1. When dummy

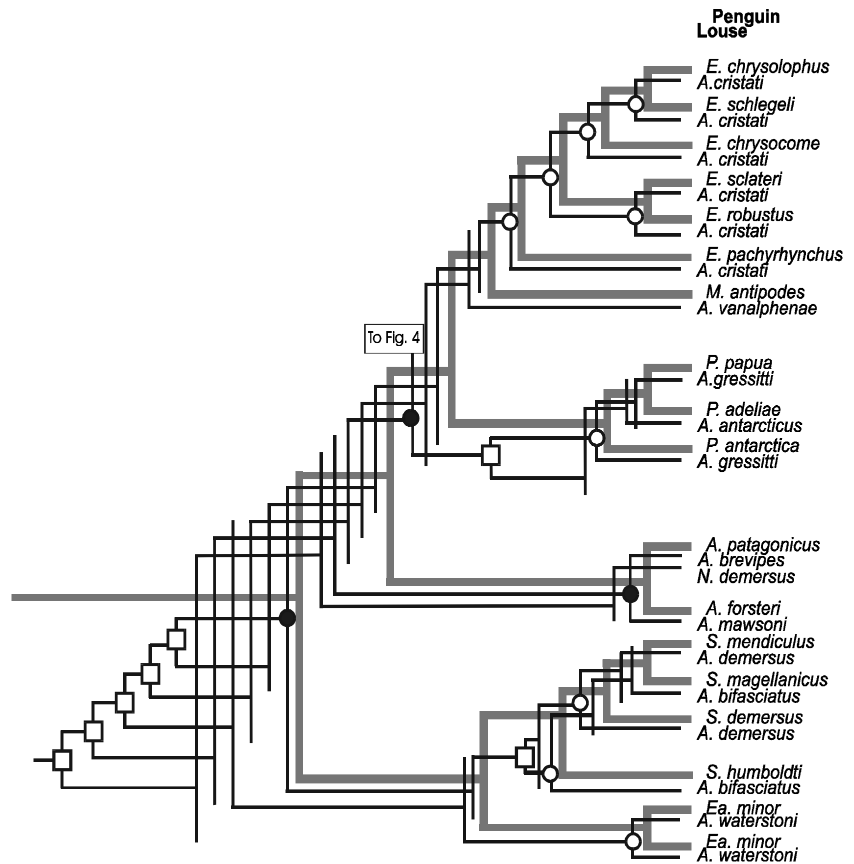

Fig. 3 Penguin and louse cophylogenetic history estimated using TreeMap 2.02β with no host switching (the *concii* lineage is shown in Fig. 4 for clarity). The thick lines represent the penguin phylogeny, thin lines the louse phylogeny. The louse phylogeny is stacked over the penguin phylogeny but offset to the left and down relative to the penguin phylogeny. Filled circles = cospeciation events, open circles = possible failure to speciate events, squares = duplication events. Louse lineages that do not form terminal branches have undergone sorting events.

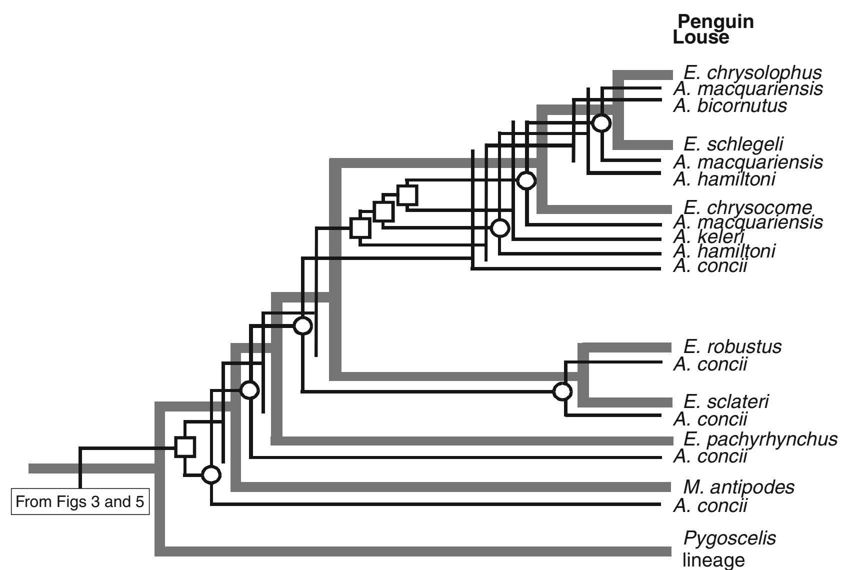

Fig. 4 *Megadyptes* and *Eudyptes* penguin and the louse *concii* clade (*A. bicornutus, A. concii, A. hamiltoni, A. keleri* and *A. macquariensis*) cophylogenetic history estimated using TreeMap 2.0β with zero or one host switch. The thick lines represent the penguin phylogeny, thin lines the louse phylogeny. The louse phylogeny is stacked over the penguin phylogeny but offset to the left and down relative to the penguin phylogeny. Open circles = possible failure to speciate events, squares = duplication events. Louse lineages that do not form terminal branches have undergone sorting events.

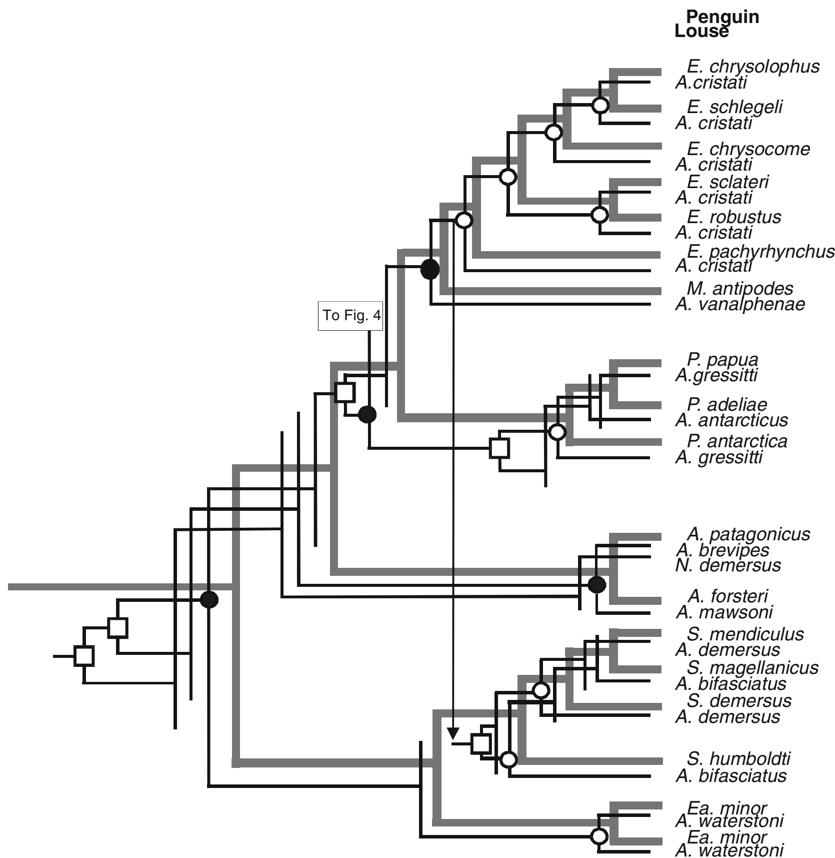

Fig. 5 Penguin and louse cophylogenetic history with one host switch (the *concii* lineage is shown in Fig. 4 for clarity) estimated using TreeMap 2.02β. The thick lines represent the penguin phylogeny, thin lines the louse phylogeny. The louse phylogeny is stacked over the penguin phylogeny but offset to the left and down relative to the penguin phylogeny. Filled circles = cospeciation events, open circles = possible failure to speciate events, squares = duplication events. The arrow indicates the host switch from the ancestor of *Eudyptes* to the ancestor of *Spheniscus*. Louse lineages that do not form terminal branches have undergone sorting events.

lineages were included in both TreeMap analyses, there was significantly more 'cospeciation' than if the penguin phylogeny had been compared to 1000 random louse phylogenies ($P < 0.01$).

Host switches

TreeMap 2.02β proposed a host switch from the ancestor of the crested penguins to the ancestor of the spheniscid penguins that gave rise to *A. bifasciatus* and *A. demersus* (Fig. 5). The scenario with two host switches included the switch to the spheniscid penguins and a switch from the ancestor of *Pygoscelis, Megadyptes* and *Eudyptes* to *Eudyptula*. TreeMap 1 also found the same scenarios. The three other scenarios from TreeMap 2.02β with two host switches suggested the host switches were by multi-host lice expanding their range to new host taxa. For example, one scenario proposed a switch by *A. hamiltoni* from *E. chrysocome* to *E. schlegeli*.

Parafit and Treefitter

Parafit rejected the null hypothesis that the penguins and their lice have evolved independently ($P = 0.001$), indicating that there is significant cophylogenetic history within the penguin-louse group. Treefitter also found that the cost of fitting the penguin phylogeny to the louse phylogeny was significantly less than the costs of fitting the penguin phylogeny to 10 000 random trees ($P < 0.003$) when the cost of switching was set from 2 to 10. The cost was not significantly different if the cost of switching was set to one.

Discussion

TreeMap 2.02β found significant shared cophylogenetic history if we included 'cospeciation' events due to 'dummy' taxa, i.e. branches added to the host and parasite trees so that all hosts have only one parasite. If dummy branches are included in a TreeMap 2.02β analysis it is more appropriate to consider cospeciation between dummy branches as 'host tracking' events, i.e. cospeciation and failure to speciate events. If this is done, TreeMap 2.02β then assesses the significance of the maximum extent of association by descent rather than the extent of cospeciation.

Parafit and Treefitter do not require the addition of dummy taxa. The Parafit analysis found that the penguin and louse phylogenies were significantly more similar to each other than 999 random phylogenies, suggesting a coevolutionary relationship between the penguins and the lice. Treefitter found the cost of fitting the penguin

phylogeny to the louse phylogeny was significantly less than cost of fitting it to 10 000 random phylogenies but only if host switching is made more difficult (i.e. cost >1) than duplication and sorting events. It has been argued that setting a higher cost for host switching is justified if parasites do not have a dispersal phase (Desdevies *et al.*, 2002). As lice rely on host-to-host contact for transmission (Hafner & Nadler, 1988), assigning a higher cost to host switching may be justified.

Interpreting the reasons for the presence of multi-host parasites markedly affects the extent of association by descent. It has been suggested that multi-host parasites could be genetically isolated, although morphologically conservative, and could be treated as separate taxa (Page *et al.*, 2004). It has also been suggested that a parasite species could parasitise several closely related hosts if it could maintain genetic contact between populations on divergent hosts and thus have failed to speciate (Paterson & Banks, 2001; Johnson *et al.*, 2003). Alternatively, it could be that only one host has inherited the parasite species and the rest of the associations are due to host switching (Ronquist, 2003). Several penguin species that share the same species of lice also share breeding islands or are occasional visitors, often during moulting, to the breeding sites of other penguin species (del Hoyo *et al.*, 1992; Miskelly *et al.*, 2001). However, there are examples of sympatric penguin species that do not share lice, for example emperor, *Aptenodytes forsteri*, and Adelie, *P. adeliae*, penguins mix at several sites in Antarctica (Marchant & Higgins, 1990) but are parasitised by different species of lice (Price *et al.*, 2003). Therefore, host switching cannot be inferred simply by host species living in sympatry. Our analysis of the cophylogenetic relationship between penguin lice, without excluding any parasite taxa *a priori*, shows that multi-host parasites can contribute either to the extent of association by descent or by association depending on the reason(s) for their multi-host parasitism (Banks & Paterson, 2005).

We think it unlikely the multi-host penguin lice are cryptic species. We examined 10 multi-host lice and nine of the 10 within-species comparisons showed no differences in the 12S and COI sequences (data not shown). For example, Australian and New Zealand blue penguins, differ by 4% for COI and 2% for 12S (Banks *et al.*, 2002) and yet the sequences for the louse *A. waterstoni* collected from these hosts in Australia and New Zealand did not differ at all for the same gene regions.

The penguin lice contrast with several studies that have found that there are genetic differences between populations of louse species parasitising different host species. For example, there were differences in the sequences for COI from populations of the louse *Physconelloides eurysema* parasitising the pigeon hosts *Claravis pretiosa*, *Columbina inca* and *C. passerina*. COI sequences for *P. eurysema* even varied with the location of the host (Johnson *et al.*, 2002).

Failure to speciate is an alternative reason for multi-host parasites that supports association by descent. Penguin species with multi-host lice share several characteristics, such as sympatric distributions and morphological similarity that are thought to make failure to speciate possible (Clayton *et al.*, 2004). For example, all six of the morphologically similar *Eudyptes* species are parasitised by *A. concii*, and all six eudyptids have been reported from the Snares Islands (Miskelly *et al.*, 2001). Straggling by birds, especially during moulting, may provide sufficient opportunities for the lice to maintain genetic contact.

The absence of genetic differences found in our comparison of multi-host louse populations meant we could not distinguish failure to speciate from an extremely recent host switch. It may be that the absence of genetic differences between louse populations on different host species is due to a very recent host switch and insufficient time has elapsed since the switch for differences to accumulate between the louse populations. Hugot *et al.* (2001) suggested that where the same parasite species parasitises closely related hosts it is more parsimonious to propose failure to speciate than host switching. Application of this principle would suggest that the distribution of most of the multi-host penguin lice is due to failure to speciate. Additionally, we can think of no recent changes in the distribution of penguins that would enable so many very recent host switches. However, faster diverging genes are necessary to distinguish recent host switches from failure to speciate events.

Mapping the louse phylogeny onto the penguin phylogeny using TreeMap 2.02β (Charleston & Page, 2002) did not find evidence of extensive cospeciation. There were three or four cospeciation events, depending on the number of host switches allowed, which was not significantly more than would be expected if we compared the penguin phylogeny to 1000 random louse phylogenies. Cospeciation events were only 21–29% of the total number of speciation events, which contrasts with the strong evidence of codivergence found from gophers and their chewing lice (Hafner & Nadler, 1988; Hafner *et al.*, 1994), or seabirds and their chewing lice (Paterson *et al.*, 2000). The proportion of cospeciation events in the penguin-chewing louse assemblage was similar to the lowest values found for chewing lice parasitising a range of mammalian taxa (20% for horses and 25% for cats) (Taylor & Purvis, 2003).

Why is the extent of cospeciation in the penguin lice so low in comparison to the pocket gopher lice? Hafner *et al.* (2003) suggested that cospeciation is likely to occur when the hosts have a patchy distribution, the lice have low dispersal abilities and there are constraints that prevent lice establishing on new host taxa. These factors are likely to affect both the penguin and the gopher lice. However, pocket gophers differ from penguins in that gophers have a fossorial lifestyle, are geographically isolated and have small population sizes (Hafner *et al.*, 2003). It seems

likely these host specific factors contribute to the extent of cospeciation between gophers and their lice. Often several species of penguins use the same islands for breeding and/or straggle to other species' breeding areas (Marchant & Higgins, 1990). Contact at these times may offer lice opportunities to transfer between hosts and thus reduce the extent of cospeciation. Other cophylogenetic studies of lice parasitising bird species sharing nest holes have also found few cospeciation events. For example, toucan lice do not show cospeciation with their hosts (Weckstein, 2004). Although the pocket gopher chewing lice may be a 'text-book' example of cladogenesis by cospeciation, speciation of other parasite groups, such as the penguin chewing lice, is not as tightly linked to the divergence of their hosts.

The TreeMap 2.02β analysis suggested duplications were the predominant method by which penguin lice speciated. Duplication events require genetic divergence between louse populations parasitising the same host species. Band-tailed pigeons, *Patagioenas fasciata* are parasitised by the louse *Physcollenoides spenceri* in North and South America (Price *et al.*, 2003). *Physcollenoides spenceri* in North America show mitochondrial divergence levels of 9% from those in South America, which is similar to divergence levels found between different louse species (Johnson & Clayton, 2004). It is possible that these two louse populations are too divergent to interbreed if they were to be re-united in the future. Overlap of the ranges of *P. spenceri* in the future would give rise to a duplication event.

Penguin colonies tend to be geographically distant from each other, especially in the Southern Ocean and geographical isolation followed by re-contact may be a route for duplications to occur. Warham (1975) speculated that glaciation of breeding grounds during colder conditions or shifts in the hydrological convergences may have resulted in penguin populations and species being more isolated than they are now. However, little is known about the effect of climate variation on the historical distribution of penguins and conditions on the subantarctic island breeding grounds of the crested penguins (Warham, 1975).

The TreeMap 2.02β scenario with zero host switches postulated that the most recent common ancestor of the penguins was parasitised by six louse species. Six species of lice parasitising a single host taxon seems unlikely. Currently the maximum number of louse species parasitising a single penguin species, the rockhopper penguin, *E. chrysocome*, is five (Price *et al.*, 2003). However, two of the three subspecies of rockhopper penguins recognized on morphological differences are parasitised by three louse species, while the third subspecies harbours only two louse species (Price *et al.*, 2003; Banks & Paterson, 2004). The scenario with one host switch (Figs 4 and 5) postulated three louse species on the most recent common ancestor of the penguins. This situation seems more likely. However, without information on the probability of the occurrence of host switches, duplications and extinctions, it is difficult to justify the choice of TreeMap scenarios. Genetic data for the penguins would be useful in choosing between scenarios as relative branch lengths could be used to eliminate switches between hosts that were not extant with the lice.

The topology of the 50% majority rule consensus tree produced by the Bayesian analysis with *A. bicornutus* pruned from the phylogeny was identical to the topology of a tree estimated from a maximum likelihood analysis of the genetic data alone (result not shown). Also, *A. bicornutus* and *A. concii* were sister taxa in both the combined Bayesian analysis in this study and a maximum parsimony (MP) analysis of only morphological characters (Banks & Paterson, 2004). The Bayesian consensus tree presented here and a MP analysis of the morphological data (Banks & Paterson, 2004) found broadly similar groups. For example, the *concii* clade had the same members in the MP and Bayesian analyses. However, MP and Bayesian analyses found different relationships within the groups. For example, the Bayesian analysis found *A. concii* to be the basal member of the group whereas the morphological data alone had *A. concii* as the most derived member of the group.

Generally cophylogenetic studies have concentrated on host specific parasites while multi-host parasites have been ignored. Indeed, several methods of analysing cophylogenetic data at this point in time cannot deal with multi-host parasites (Charleston & Page, 2002) or else multi-host parasites make the methods unwieldy (Johnson *et al.*, 2001). Multi-host parasites have often been (1) treated as an unresolved clade, which more data will resolve (Page, 1994a), (2) eliminated from the analysis (Huelsenbeck *et al.*, 1997,2000) or (3) assumed to be widespread because of recent host switching (Dowling *et al.*, 2003). None of these methods of analysing cophylogenetic history explicitly allow failure to speciate to occur and yet, if closely related host species share a parasite species, it seems possible that the association has been inherited from an ancestor. Additionally, the method chosen to deal with multi-host parasites markedly affects the extent of association by descent. If every association between multi-host lice and their penguin hosts is due to failure to speciate, all of the penguin louse associations can be explained as 'association by descent'. Alternatively if multi-host lice have only recently colonized their present hosts only 51% of the penguin louse associations are due to association by descent. More study is required to distinguish these possibilities.

Because we did not exclude any louse species from our coevolutionary analysis, we found that multi-host parasites, usually neglected in other studies, can critically affect the interpretation of the extent of cophylogenetic history in a lineage. Additionally, some of the methods used to analyse cophylogenetic history require that the phylogenies be manipulated to produce the correct

reconstruction. Identifying the reasons some parasite species appear to be able to maintain genetic contact despite their hosts diverging will be an interesting extension of cophylogenetic studies.

Acknowledgments

This work was supported logistically by Antarctica New Zealand, collecting was partially funded by Royal Society of New Zealand, Kelly Tarlton's Antarctica, and Claude McCarthy, Lincoln University doctoral and Gordon Williams scholarships. Kevin Johnson provided assistance with the analysis. We are grateful to many people who assisted with the collection of specimens especially Sonja van Alphen, Amy van Buren, Kerri-Anne Edge, Michel Gaulthier-Clerc, Paul Ponganis, Paul Sagar, Cor Vink and Kath Walker.

References

Banks, J.C., Mitchell, A.D., Waas, J.R. & Paterson, A.M. 2002. An unexpected pattern of molecular divergence within the blue penguin (*Eudyptula minor*) complex. *Notornis* **49**: 29–38.

Banks, J.C. & Palma, R.L. 2003. *Austrogoniodes vanalphenae*, a new species of chewing louse (Phthiraptera: Insecta) from the yellow-eyed penguin, *Megadyptes antipodes* and new host records for *A. demersus* and *A. bifasciatus*. *N. Z. J. Zool.* **30**: 69–75.

Banks, J.C. & Paterson, A.M. 2004. A penguin-chewing louse (Insecta: Phthiraptera) phylogeny derived from morphology. *Invert. Syst.* **18**: 89–100.

Banks, J.C. & Paterson, A.M. 2005. Multi-host parasite species in cophylogenetic studies. *Int. J. Parasitol.* **35**: 741–746.

Bensasson, D., Zhang, D., Hartl, D.L. & Hewitt, G.M. 2001. Mitochondrial pseudogenes: evolution's misplaced witnesses. *Trends Ecol. Evol.* **16**: 151–156.

Benson, D.A., Karsch-Mizrachi, I., Lipman, D.J., Ostell, J., Rapp, B.A. & Wheeler, D.L. 2002. GenBank. *Nucleic Acids Res.* **30**: 17–20.

Brooks, D.R. & McLennan, D.A. 1991. *Phylogeny, Ecology and Behaviour: A Research Program in Comparative Biology*. The University of Chicago Press, Chicago.

Brooks, D.R. & McLennan, D.A. 2003. Extending phylogenetic studies of coevolution: secondary Brooks parsimony analysis, parasites, and the Great Apes. *Cladistics* **19**: 104–119.

Brooks, D.R., van Veller, M.G.P. & McLennan, D.A. 2001. How to do BPA, really. *J. Biogeogr.* **28**: 345–358.

Charleston, M.A. 1998. Jungles: a new solution to the host/parasite phylogeny reconciliation problem. *Math. Biosci.* **149**: 191–223.

Charleston, M.A. & Page, R.D.M. 2002. *Treemap (Jungle Edition)*. http://taxonomy.zoology.gla.ac.uk/%7emac/treemap/index.html (accessed 29 December 2004).

Clay, T. 1967. Mallophaga (biting lice) and Anoplura (sucking lice). Part 1: *Austrogoniodes* (Mallophaga) parasitic on penguins (Sphenisciformes). In: *Antarctic Research Series 10: Entomology of Antarctica* (J. L. Gressitt, ed.), pp. 149–155. American Geophysical Union, Washington, D.C.

Clay, T. & Moreby, C. 1967. Mallophaga (biting lice) and Anoplura (sucking lice). Part II: Keys and locality lists of Mallophaga and Anoplura. In: *Antarctic Research Series 10: Entomology of Antarctica*, pp. 157–196. American Geophysical Union, Washington, D.C.

Clayton, D.H., Bush, S.E. & Johnson, K.P. 2004. Ecology of congruence: past meets present. *Syst. Biol.* **53**: 165–173.

Clayton, D.H., Price, R.D. & Page, R.D.M. 1996. Revision of *Dennyus* (*Collodennyus*) lice (Phthiraptera: Menoponidae) from swiftlets, with descriptions of new taxa and a comparison of host-parasite relationships. *Syst. Entomol.* **21**: 179–204.

Cruickshank, R., Johnson, K., Smith, V., Adams, R., Clayton, D. & Page, R. 2001. Phylogenetic analysis of partial sequences of elongation factor 1 alpha identifies major groups of lice (Insecta: Phthiraptera). *Mol. Phylogenet. Evol.* **19**: 202–215.

del Hoyo, J., Elliot, A. & Sargatal, J. 1992. *Handbook of the Birds of the World*. Lynx Edicions, Barcelona.

Desdevies, Y., Morand, S., Jousson, O. & Legendre, P. 2002. Coevolution between *Lamellodiscus* (Monogenea: Diplectanidae) and Sparidae (Teleostei): the study of a complex host-parasite system. *Evolution* **56**: 2459–2471.

Dowling, A.P.G., van Veller, M.P.G., Hoberg, E.P. & Brooks, D.A. 2003. A priori and a posteriori methods in comparative evolutionary studies of host-parasite associations. *Cladistics* **19**: 240–253.

Giannini, N.P. & Bertelli, S. 2004. Phylogeny of extant penguins based on integumentary and breeding characters. *Auk* **121**: 422–434.

Gower, J.C. 1966. Some distance properties of latent root and vector methods used in multivariate analysis. *Biometrika* **53**: 325–328.

Hafner, M.S., Demastes, J.W., Spradling, T.A. & Reed, D.L. 2003. Cophylogeny between pocket gophers and their chewing lice. In: *Tangled Trees. Phylogeny, Cospeciation, and Coevolution* (R. D. M. Page, ed.), pp. 195–220. The University of Chicago Press, Chicago.

Hafner, M.S. & Nadler, S.A. 1988. Phylogenetic trees support the coevolution of parasites and their hosts. *Nature* **332**: 258–259.

Hafner, M.S., Sudman, P.D., Villablanca, F.X., Spradling, T.A., Demastes, J.W. & Nadler, S.A. 1994. Disparate rates of molecular evolution in cospeciating hosts and parasites. *Science (Wash. D.C.)*. **265**: 1087–1090.

Hopkins, G.H.E. & Clay, T. 1953. Additions and corrections to the checklist of Mallophaga. *Ann. Mag. Nat. Hist.* **12**: 434–448.

Huelsenbeck, J.P., Rannala, B. & Larget, B. 2000. A bayesian framework for the analysis of cospeciation. *Evolution* **54**: 352–364.

Huelsenbeck, J.P., Rannala, B. & Yang, Z. 1997. Statistical tests of host-parasite cospeciation. *Evolution* **51**: 410–419.

Hugot, J.P., Gonzalez, J.P. & Denys, C. 2001. Evolution of the old world Arenaviridae and their rodent hosts: generalized host-transfer or association by descent? *Infect. Genet. Evol.* **1**: 13–20.

Johnson, K.P., Adams, R.J., Page, R.D.M. & Clayton, D.H. 2003. When do parasites fail to speciate in response to host speciation? *Syst. Biol.* **52**: 37–47.

Johnson, K.P. & Clayton, D.H. 2004. Untangling coevolutionary history. *Syst. Biol.* **53**: 92–94.

Johnson, K.P., Drown, D.M. & Clayton, D.H. 2001. A data based method of cophylogenetic analysis. *Zool. Scr.* **30**: 79–87.

Johnson, K.P., Williams, B.L., Drown, D.M., Adams, R.J. & Clayton, D.H. 2002. The population genetics of host specificity: genetic differentiation in dove lice (Insecta: Phthiraptera). *Mol. Ecol.* **11**: 25–38.

Klassen, G.J. 1992. Coevolution: a history of the macroevolutionary approach to studying host-parasite association. *J. Parasitol.* **78**: 573–587.
Kliks, M.M. 1990. Helminths as heirlooms and souvenirs: a review of new world paleoparasitology. *Parasitol. Today* **6**: 93–100.
Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Paabo, S., Villablanca, F.X. & Wilson, A.C. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Nat. Acad. Sci. USA.* **86**: 6196–6200.
Legendre, P., Desdevies, Y. & Bazin, E. 2002. A statistical test for host-parasite coevolution. *Syst. Biol.* **51**: 217–234.
Marchant, S. & Higgins, P.J. 1990. *Handbook of Australian, New Zealand and Antarctic Birds*. Oxford University Press, Oxford.
Marshall, A.G. 1981. *The Ecology of Ectoparasitic Insects*. Academic Press, London.
Miskelly, C.M., Sagar, P.M., Tennyson, A.J.D. & Scofield, R.P. 2001. Birds of the Snares Islands, New Zealand. *Notornis* **48**: 1–40.
Page, R.D.M. 1990. Temporal congruence and cladistic analysis of biogeography and cospeciation. *Syst. Zool.* **39**: 205–226.
Page, R.D.M. 1994a. Parallel phylogenies: reconstructing the history of host-parasite assemblages. *Cladistics* **10**: 155–173.
Page, R.D.M. 1994b. Maps between trees and cladistic analysis of historical associations among genes, organisms and areas. *Syst. Biol.* **43**: 58–77.
Page, R.D.M. 1995. TreeMap http://taxonomy.zoology.gla.ac.uk/rod/treemap.html. (accessed 29 December 2004).
Page, R.D.M. 1996. Temporal congruence revisited: comparison of mitochondrial DNA sequence divergence in cospeciating pocket gophers and their chewing lice. *Syst. Biol.* **45**: 151–167.
Page, R.D.M. & Charleston, M.A. 2002. Treemap versus BPA (again): a response to Dowling http://taxonomy.zoology.gla.ac.uk/publications/tech-reports/02-02.pdf (accessed 29 December 2004).
Page, R.D.M., Cruickshank, R., Dickens, M., Furness, R.W., Kennedy, M.J., Palma, R.L. & Smith, V.S. 2004. Phylogeny of the ''*Philoceanus*-complex'' seabird lice (Phthiraptera: Ischnocera) inferred from mitochondrial DNA sequences. *Mol. Phylogenet. Evol.* **30**: 633–652.
Page, R.D.M., Cruickshank, R. & Johnson, K.P. 2002. Louse (Insecta: Phthiraptera) mitochondrial 12S rRNA secondary structure is highly variable. *Insect Mol. Biol.* **11**: 361–369.
Palma, R.L. 1996. Philopteridae. In: *Zoological Catalogue of Australia, Vol. 26. Psocoptera, Phthiraptera, Thysanoptera* (A. Wells, ed.), pp. 149–230. CSIRO Publishing, Melbourne.
Palma, R.L. 1999. Amendments and additions to the 1982 list of chewing lice (Insecta: Phthiraptera) from birds in New Zealand. *Notornis* **46**: 373–387.
Paterson, A.M. & Banks, J.C. 2001. Analytical approaches to measuring cospeciation of host and parasites: through a glass darkly. *Int. J. Parasitol.* **31**: 1012–1022.
Paterson, A.M., Wallis, G.P., Wallis, L.J. & Gray, R.D. 2000. Seabird and louse coevolution: complex histories revealed by 12S rRNA sequences and reconciliation analyses. *Syst. Biol.* **49**: 383–399.
Pilgrim, R.L.C. & Palma, R.L. 1982. A list of the chewing lice (Insecta: Mallophaga) from birds in New Zealand. *Notornis* **29** (Suppl.): 1–32.
Posada, D. 2000. Testing Models of Evolution – Modeltest Version 3.06. http://darwin.uvigo.es/software/modeltest.html (accessed 29 December 2004).
Price, R.D., Hellenthal, R.A. & Palma, R.L. 2003. World checklist of chewing lice with host associations and keys to families and genera. In: *The Chewing Lice. World Checklist and Biological Overview* (R. D. Price, R. A. Hellenthal, R. L. Palma, K. P. Johnson & D. H. Clayton, eds), pp. 1–448. Illinois Natural History Survey Special Publication 24. Champaign, Illinois.
Ridley, M. 1996. *Evolution*, 2nd edn. Blackwell Science, Cambridge, Massachusetts.
Rodríguez, F., Oliver, J.F., Marín, A. & Medina, J.R. 1990. The general stochastic model of nucleotide substitution. *J. Theor. Biol.* **142**: 485–501.
Ronquist, F. 1995. Reconstructing the history of host-parasite associations using generalised parsimony. *Cladistics* **11**: 73–89.
Ronquist, F. 2003. Parsimony analysis of coevolving species associations. In: *Tangled Trees. Phylogeny, Cospeciation, and Coevolution* (R. D. M. Page, ed.), pp. 22–64. The University of Chicago Press, Chicago.
Ronquist, F. & Huelsenbeck, J.P. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
Rothschild, M. & Clay, T. 1952. *Fleas, Flukes and Cuckoos*. Collins, London.
Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. 1994. Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. *Ann. Entomol. Soc. Am.* **87**: 651–701.
Taylor, J. & Purvis, A. 2003. Have mammals and their lice diversified in parallel? In: *Tangled Trees. Phylogeny, Cospeciation, and Coevolution* (R. D. M. Page, ed.), pp. 240–261. The University of Chicago Press, Chicago.
Thompson, J.D., Gibson, T.J., Plewniak, F., Jeanmougin, F. & Higgins, D.G. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Research* **24**: 4876–4882.
Warham, J. 1975. The crested penguins. In: *The Biology of Penguins* (B. Stonehouse, ed.), pp. 189–270. The MacMillan Press Ltd, London.
Watson, K.C. 1967. The terrestrial arthropoda of Macquarie Island. III. Parasites of mammals and birds. *ANARE Sci. Rep. Ser. B (I) Zool.* **99**: 70–76.
Weckstein, J.D. 2004. Biogeography explains cophylogenetic patterns in toucan chewing lice. *Syst. Biol.* **53**: 154–164.
White, T.J., Bruns, T., Lee, S. & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: *PCR Protocols: A Guide to Methods and Applications* (M. A. Innis, H. Gelfand, J. J. Sninsky & T. J. White, eds), pp. 315–322. Academic Press, San Diego.
Yang, Z., Goldman, N. & Friday, A. 1994. Comparison of models for nucleotide substitution used in maximum-likelihood phylogenetic estimation. *Mol. Biol. Evol.* **11**: 316–324.

Received 4 February 2005; revised 27 April 2005; accepted 24 May 2005