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Reinterpreting the origins of flamingo lice: cospeciation or host-switching?

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The similarity of the louse faunas of flamingos and ducks has been used as evidence that these two groups of birds are closely related. However, the realization that ducks actually are more closely related to Galliformes caused many workers to reinterpret this similarity in parasite faunas as host switching from ducks to flamingos. Recent unexpected phylogenetic results on the relationships of waterbirds and their lice call for a reinterpretation of the origins of the lice of the enigmatic flamingos. Here, we bring together new evidence on the phylogenetic relationships of flamingos and their lice and show that the lice of flamingos and grebes are closely related because their hosts share a common ancestor (cospeciation). We also demonstrate that the similarity of the louse faunas of flamingos and ducks is a result of host switching from flamingos to ducks, rather than from ducks to flamingos.

Keywords: coevolution; Phthiraptera; avian systematics; Phoenicopteridae; ducks; grebes

1. INTRODUCTION

Flamingos (Phoenicopteridae) are among the most phylogenetically enigmatic groups of birds (Sibley & Ahlquist 1990). Previous classifications of flamingos have alternatively placed them near herons and storks (Ciconiiformes), shorebirds (Charadriiformes) or ducks (Anseriformes). Hopkins (1942) pointed out that flamingos share three genera of lice in common with ducks (Anseriformes). As genera of lice are generally specific to single families or orders of birds (Hopkins & Clay 1952; Price *et al.* 2003), Hopkins suggested that this was strong evidence that flamingos and ducks are closely related. However, most current classifications place the flamingos in the Ciconiiformes and the ducks sister to the Galliformes (chickens, quail, partridge, pheasants, etc.; Sibley & Ahlquist 1990; Dyke & van Tuinen 2004). Subsequent workers have interpreted the extant flamingo louse fauna as the result of multiple host switches from ducks to flamingos, rather than as indicating a shared ancestry between the two groups (Clay 1974; Olson & Feduccia 1980; Sibley & Ahlquist 1990; Rozsa 1991).

This interpretation may not be appropriate, however, as recent work on the phylogenetic position of flamingos has produced an unexpected result. Molecular and morphological data both suggest that flamingos are actually closely related to grebes (Podocipediformes; Van Tuinen *et al.* 2001; Mayr 2004). Other recent molecular studies have also supported this relationship (Johnson 2001 (not shown); Chubb 2004). Molecular studies also consistently recover a sister relationship between the Anseriformes (ducks) and Galliformes, rather than between ducks and flamingos, and this view is now widely accepted. These findings further suggest that the lice shared by flamingos and ducks represent host-switching events, rather than parasites inherited from a common ancestor.

A recent analysis of three genes from avian feather lice (Ischnocera) indicated that the grebe louse genus *Aquanirmus* is the sister taxon of *Anaticola*, one of the genera that parasitizes both flamingos and ducks (Smith *et al.* 2004). This intriguing result suggests that a reinterpretation of the history of flamingo lice is required. The goal of the present study is to reconstruct the coevolutionary history of lice and their flamingo, grebe and duck hosts. In this paper, we examine in more detail the phylogenetic relationships of the lice on flamingos, grebes and ducks to assess whether their distribution is a result of shared ancestry (i.e. cospeciation) or is a result of host switching. For this analysis, we use the relevant taxa from higher level phylogenies of waterbirds (Van Tuinen *et al.* 2001) and their lice (Smith *et al.* 2004). We also provide new data and analyses to evaluate whether the *Anaticola* of flamingos are indeed closely related to those of ducks, because previous studies of avian louse phylogenies did not include this genus from flamingos. We test for the monophyly of *Anaticola* by including a diversity of species from both ducks and flamingos in this study.

2. MATERIAL AND METHODS

To examine the higher level coevolutionary history of flamingo lice, specifically wing lice in the genus *Anaticola* (Ischnocera), we performed a cophylogenetic analysis using the jungles method (Charleston 1998), which allows the reconstruction of cospeciation, sorting, duplication and host-switching events. Costs can be assigned to each of these events and the optimal solution reconstructed. This method is still in developmental stages and is computationally intensive for large numbers of taxa and many host-switching events. Thus, we used simplified phylogenies for the relevant groups of birds and their lice. For the avian phylogeny, we used a composite of the phylogeny presented by Van Tuinen *et al.* (2001) based on DNA–DNA hybridization and nuclear DNA sequences. This phylogeny included the sister relationship between flamingos and grebes, which is strongly supported in the Van Tuinen *et al.* (2001) study. For the louse phylogeny, we pruned a molecular phylogeny presented by Smith *et al.* (2004) based on Bayesian ML analysis of three genes (nuclear EF-1α, mitochondrial 12S and cytochrome oxidase I (COI)) for 43 genera of avian Ischnocera. This pruned phylogeny includes louse genera from the relevant birds in Van Tuinen *et al.* 2001 study, including ischnoceran genera from flamingos, grebes and ducks. In this analysis we assume that *Anaticola* is monophyletic, such that the *Anaticola* of flamingos is closely related to those of waterfowl. We compared these host and parasite trees using TreeMap 2 (Charleston & Page 2002), with costs of 0 for cospeciation, 1 for sorting, 1 for duplication and 1 for host-switching events. Any

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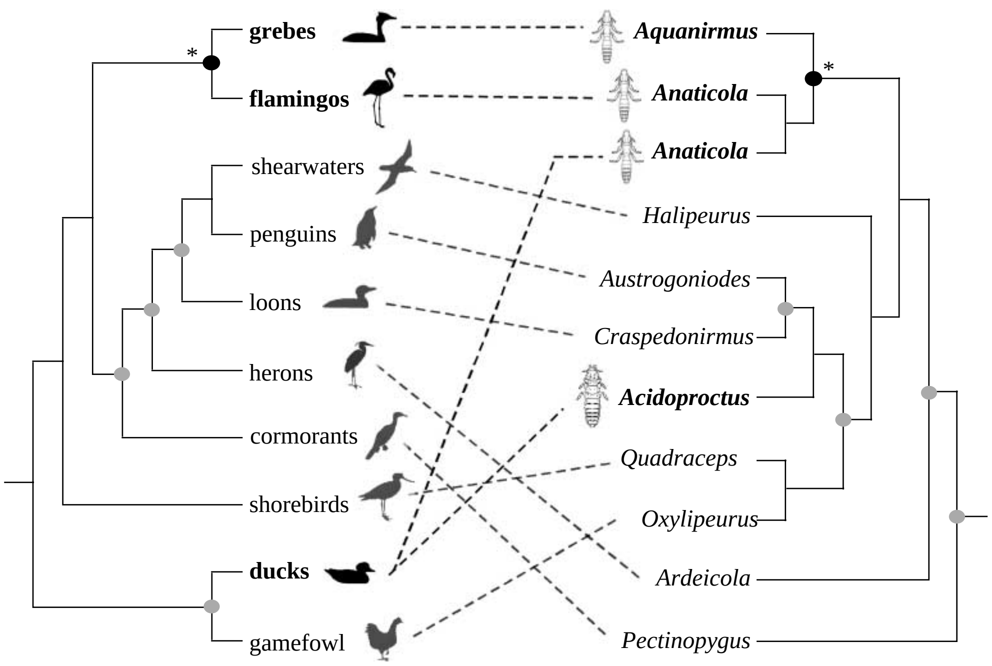

Figure 1. A tanglegram of waterbirds and louse phylogenies based on Van Tuinen *et al.* (2001) and Smith *et al.* (2004), respectively. Circles indicate the five cospeciation events inferred from the optimal jungles cophylogenetic analysis (TreeMap 2). The asterisk marks the cospeciation event between grebes and flamingos and their lice (*Anaticola* and *Aquanirmus*). Dashed lines connect bird families with their associated louse genera.

number of host-switching events were allowed and we searched for the optimal reconstruction(s).

To test the assumption made in the above cophylogenetic analysis—that the *Anaticola* of flamingos are closely related to those of ducks (i.e. that *Anaticola* is monophyletic)—we conducted a broader analysis of the genus *Anaticola* and close relatives including the grebe louse *Aquanirmus*. We investigate the phylogenetic relationships among species of *Anaticola* from flamingos and ducks by sequencing a number of taxa from a range of host species, including a flamingo (*Phoenicopterus ruber*). We obtained partial sequences of the mitochondrial COI and nuclear elongation factor-1α (EF-1α gene), using methods described by Johnson *et al.* (2003). We analysed the DNA sequence data (GenBank Accession numbers AF320353, AF320362, AF320404, AF320408, AF320410, AF320461, AF320468, AF348851, AF348864-6, AF348872, AF385004, AF385025, AF396559, AF444861, AF447197, AF447209, AF497799, AF545671, AF545712, AF545760, AY149435, AY314808, AY314810, AY314817, AY314823, AY314826, AY314828, AY314834, AY314836, AY314843, DQ314498–DQ314517) using maximum likelihood and Bayesian maximum likelihood analyses. Bayesian posterior probabilities were calculated by discarding the first 300 000 generations as burn-in from a 10 million generation Markov Chain Monte Carlo (MCMC) chain, and calculating a 50% majority consensus tree of the remaining trees sampled every 1000 generations. Trees were rooted on a composite outgroup of various genera of avian and mammalian Ischnocera, focusing on lice from waterbirds present in the phylogeny of Van Tuinen *et al.* (2001).

Table 1. Reconstructions from jungles analysis of bird and louse phylogenies from figure 1.

# reconstructions	cospeciations	duplications	losses	switches	cost
1	4	6	32	0	38
1	4	6	27	1	34
1	4	6	24	2	32
1	4	6	19	3	28
3	4	6	10	4	20
1[a]	5	5	3	5	13
6	4	6	1	6	13

[a] reconstruction that minimizes cost and maximizes number of inferred cospeciation events.

3. RESULTS

Cophylogenetic analyses, using TreeMap 2, of previously published trees (figure 1) recovered 14 possible reconstructions (table 1). Reconstructions involving five or six host switches were optimal, with costs greatly reduced over reconstructions with fewer host switches. All reconstructions, except one allowing no host switching or that with two host switches (table 1), indicated a cospeciation event between grebe lice (*Aquanirmus*) and flamingo lice (*Anaticola*), followed by host switching from flamingos to ducks by members of *Anaticola*. The optimal solution with the highest number of cospeciation events involves five cospeciation events, five duplication events, three sorting events and five host switches (figure 1; table 1). Randomization of the parasite tree with respect to the host tree did not indicate that the amount of cospeciation observed was greater than that expected by chance ($p=0.402$), indicating that the phylogeny of these lice does not closely mirror that of their waterbirds hosts.

Maximum likelihood and Bayesian analyses of our sequence data produced a well-resolved tree (figure 2) that was generally relatively well supported by bootstrap resampling and Bayesian posterior probabilities. In this tree, the Bayesian posterior probability and bootstrap support for the sister relationship between *Aquanirmus* and *Anaticola* was 100 and 81%, respectively. Bayesian posterior probability and bootstrap support for monophyly of *Anaticola* was 100 and 65%, respectively.

4. DISCUSSION

Higher level phylogenies of birds and their parasites can provide important insights into the

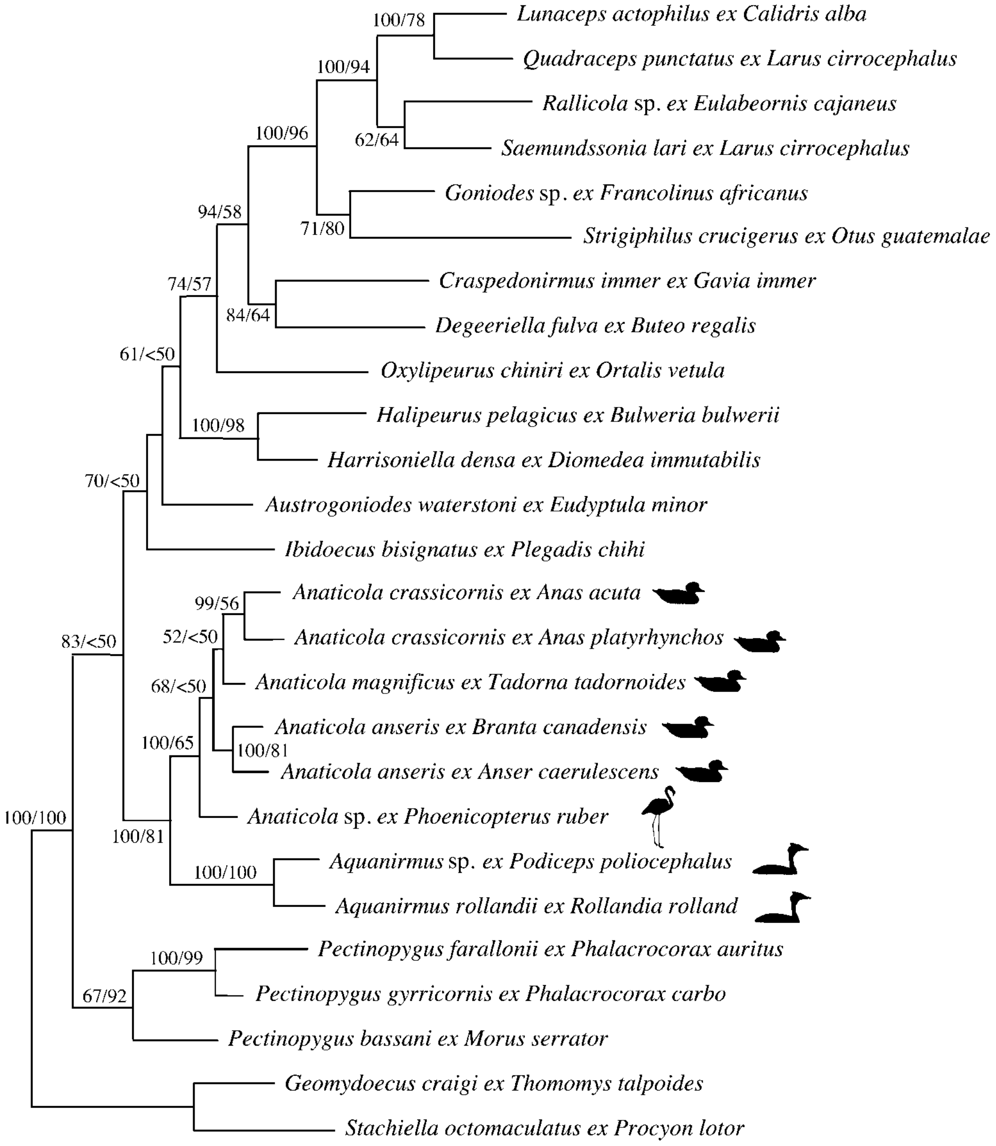

Figure 2. Phylogeny of *Aquanirmus* and *Anaticola* from grebes, flamingos and ducks inferred by consensus of 9700 trees from Bayesian maximum likelihood analyses. Numbers above branches are Bayesian posterior probability/maximum likelihood bootstrap values. Branches are proportional to inferred substitutions per site. Grebes, flamingos and duck lice are marked with icons. Tree rooted on mammal lice (Trichodectidae).

origins of birds and their parasite faunas. Unexpected results for the host lineage can shed new light on the interpretation of coevolutionary patterns for the parasite lineage. Lice (Insecta: Phthiraptera) have provided a model system for cophylogenetic studies because they typically are highly host-specific and are permanent parasites of their hosts (Clayton *et al.* 2004). Avian feather lice (Ischnocera) are particularly diverse among lice, and while phylogenetic information for many groups of birds is becoming more readily available, the phylogeny of avian Ischnocera is still poorly understood, although progress is being made on some groups (Cruickshank *et al.* 2001; Johnson *et al.* 2003). In addition, the higher level phylogenetic relationships of birds is still relatively poorly known, although this lack of knowledge is also changing (Johnson 2001; Van Tuinen *et al.* 2001; Chubb 2004). Thus, until recently, it has been difficult to interpret the history of higher level bird-louse associations.

Similarities between the ectoparasitic louse faunas of flamingos and ducks were previously used as evidence that either flamingos and ducks are closely related or that lice switched from ducks to flamingos. However, by bringing together phylogenies for birds and their lice, we show that flamingos inherited at least one louse lineage (*Anaticola*) via their shared ancestry with grebes. In addition, by using cophylogenetic reconstruction methods, we show that the genus *Anaticola* is shared between flamingos and ducks because of host-switching from flamingos to ducks rather than the reverse (as has traditionally been inferred). The phylogeny of avian feather lice also provides illumination on the phylogenetic position of flamingos, lending additional support to the finding that flamingos are closely related to grebes. Although taking such a parasitological approach to host phylogeny must be used with caution (and indeed caused problems in identifying the closest relatives of flamingos in the past; Hopkins 1942), other parasites are also shared by flamingos and grebes (Storer

2000; Mayr 2004), indicating that a parasitological approach, combined with additional phylogenetic information on hosts and parasites might prove useful.

The host distribution of *Anaticola* within waterfowl also shows interesting patterns that bear on the present results. While *Anaticola* has a worldwide distribution, this genus is not found on screamers (Anhimidae) or the magpie goose (Anseranatidae), which are basal to all other waterfowl (Anatidae). Rather, these two lineages of waterfowl, together with two basal lineages within Anatidae (whistling ducks and geese and swans), harbour members of the *Acidoproctus* species group of Ischnocera, which are restricted to these large-bodied lineages of waterfowl. *Anaticola* and *Acidoproctus* are both wing lice, escaping host preening by inserting themselves between the barbs of the wing feathers. Previous work has shown that the size of these wing lice is closely matched to the size of the host and that this is selected for by host-preening defences (Johnson *et al.* 2005). It seems likely that *Acidoproctus* is adapted for large-bodied waterfowl, but as host size decreased, *Anaticola* was able to fill an open niche on smaller bodied hosts. Further support for the idea that ancestral small-bodied ducks were an open niche for lice comes from the fact that two basal Australian relict waterfowl species, *Biziura lobata* and *Malacorhynchus membranaceus*, possess lice more typically found on penguins and shorebirds, respectively (Price *et al.* 2003). The two other genera of lice, *Anatoecus* (Ischnocera) and *Trinoton* (Amblycera), that are shared between flamingos and ducks, are also not found on the screamers or the magpie goose, suggesting that their distribution on ducks is also the result of a host-switch from flamingos to ducks. However, their phylogenetic relationships could not be addressed in detail in the current study because fresh specimens of *Anatoecus* and *Trinoton* from flamingos were not available for study.

Our results highlight how new insights in avian phylogenetics can lead to new insights about the origins and diversification of their parasite faunas. In turn, an understanding of parasite phylogenetic relationships can shed light on the predicted phylogenetic relationships among their hosts, as well as provide evidence that ancestral hosts were in geographic proximity when host switching is inferred.

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Charleston, M. A. 1998 Jungles: a new solution to the host/parasite phylogeny reconciliation problem. *Math. Biosci.* **149**, 191–223. (doi:10.1016/S0025-5564(97)10012-8)

Charleston, M. A. & Page, R. D. M 2002. *TreeMap 2*. http://taxonomy.zoology.gla.ac.uk/%7emac/treemap/index.html.

Chubb, A. L. 2004 New nuclear evidence for the oldest divergence among neognath birds: the phylogenetic utility of ZENK (i). *Mol. Phylogenet. Evol.* **30**, 140–151. (doi:10.1016/S1055-7903(03)00159-3)

Clay, T. 1974 The Phthiraptera (insecta) parasitic on flamingoes (Phoenicopteridae: Aves). *J. Zool.* **172**, 483–490.

Clayton, D. H., Bush, S. E. & Johnson, K. P. 2004 Ecology of congruence: past meets present. *Syst. Biol.* **53**, 165–173. (doi:10.1080/10635150490265102)

Cruickshank, R. H., Johnson, K. P., Smith, V. S., Adams, R. J., Clayton, D. H. & Page, R. D. M. 2001 Phylogenetic analysis of partial sequences of elongation factor 1 alpha identifies major groups of lice (Insecta: Phthiraptera). *Mol. Phylogenet. Evol.* **19**, 202–215. (doi:10.1006/mpev.2001.0928)

Dyke, G. J. & van Tuinen, M. 2004 The evolutionary radiation of modern birds (Neornithes): reconciling molecules, morphology and the fossil record. *Zool. J. Linn. Soc.* **141**, 153–177. (doi:10.1111/j.1096-3642.2004.00118.x)

Hopkins, G. H. E. 1942 The Mallophaga as an aid to the classification of birds. *Ibis* **84**, 94–106.

Hopkins, G. H. E. & Clay, T. 1952 *A check list of the genera and species of Mallophaga*. London: British Museum of Natural History.

Johnson, K. P. 2001 Taxon sampling and the phylogenetic position of Passeriformes: evidence form 916 avian cytochrome *b* sequences. *Syst. Biol.* **50**, 128–136.

Johnson, K. P., Cruickshank, R. H., Adams, R. J., Smith, V. S., Page, R. D. M. & Clayton, D. H. 2003 Dramatically elevated rate of mitochondrial substitution in lice (Insecta: Phthiraptera). *Mol. Phylogenet. Evol.* **26**, 231–242. (doi:10.1016/S1055-7903(02)00342-1)

Johnson, K. P., Bush, S. E. & Clayton, D. H. 2005 Correlated evolution of host and parasite body size: tests of Harrison's Rule using birds and lice. *Evolution* **59**, 1744–1753.

Mayr, G. 2004 Morphological evidence for sister group relationship between flamingos (Aves: Phoenicopteridae) and grebes (Podicipedidae). *Zool. J. Linn. Soc.* **140**, 157–169. (doi:10.1111/j.1096-3642.2003.00094.x)

Olson, S. L. & Feduccia, A. 1980 Relationships and evolution of flamingos (Aves: Phoenicopteridae). *Smithson. Contrib. Zool.* **316**, 1–73.

Price, R. D., Hellenthal, R. A., Palma, R. L., Johnson, K. P. & Clayton, D. H. 2003 The chewing lice: world checklist and biological overview. *Ill. Nat. Hist. Surv. Spec. Publ.*, 24.

Rozsa, L. 1991 Points in question: flamingo lice contravene Fahrenholz. *Int. J. Parasitol.* **21**, 151–152. (doi:10.1016/0020-7519(91)90003-P)

Sibley, C. G. & Ahlquist, J. E. 1990 *Phylogeny and classification of birds: a study in molecular evolution*. New Haven, CT: Yale University Press.

Smith, V. S., Page, R. D. M. & Johnson, K. P. 2004 Data incongruence and the problem of avian louse phylogeny. *Zool. Scr.* **33**, 239–259. (doi:10.1111/j.0300-3256.2004.00149.x)

Storer, R. W. 2000 The metazoan parasite fauna of grebes (Aves: Podicipediformes) and its relationship to the birds' biology. *Univ. Mich. Mus. Zool. Misc. Publ.* **188**, 1–90.

Van Tuinen, M., Butvill, D. B., Kirsch, J. A. W. & Hedges, S. B. 2001 Convergence and divergence in the evolution of aquatic birds. *Proc. R. Soc. B* **268**, 1345–1350. (doi:10.1098/rspb.2001.1679)