The LINNEAN SOCIETY of London BIOLOGICAL Journal of the Linnean Society

Biological Journal of the Linnean Society, 2011, **102**, 614–625. With 3 figures

There and back again: switching between host orders by avian body lice (Ischnocera: Goniodidae)

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Received 17 August 2010; accepted for publication 4 October 2010

Studies of major switches by parasites between highly divergent host lineages are important for understanding new opportunities for parasite diversification. One such major host switch is inferred for avian feather lice (Ischnocera) in the family Goniodidae, which parasitize two distantly-related groups of birds: Galliformes (pheasants, quail, partridges, etc.) and Columbiformes (pigeons and doves). Although there have been several cophylogenetic studies of lice at the species level, few studies have focused on such broad evolutionary patterns and major host-switching events. Using a phylogeny based on DNA sequences for goniodid feather lice, we investigated the direction of this major host switch. Unexpectedly, we found that goniodid feather lice have switched host orders, not just once, but twice. A primary host switch occurred from Galliformes to Columbiformes, leading to a large radiation of columbiform body lice. Subsequently, there was also a host switch from Columbiformes back to Galliformes, specifically to megapodes in the Papua–Australasian region. The results of the present study further reveal that, although morphologically diagnosable lineages are supported by molecular data, many of the existing genera are not monophyletic and a revision of generic limits is needed. *Biological Journal of the Linnean Society*, 2011, **102**, 614–625.

ADDITIONAL KEYWORDS: coevolution – Columbiformes – ectoparasites – Galliformes – host-switching – phylogeny.

INTRODUCTION

Cophylogenetic studies of parasitic lice (Insecta: Phthiraptera) have focused mainly on species level studies within orders or families of birds and mammals. These studies have revealed a variety of patterns, from tight cospeciation (Hafner *et al*., 1994; Page *et al*., 1998; Clayton & Johnson, 2003; Hughes *et al*., 2007) to a lack of significant congruence between host and parasite phylogenies (Johnson, Adams & Clayton, 2002). Studies of coevolutionary history at higher taxonomic scales (across families or orders) are rare (Johnson, Kennedy & McCracken, 2006). Understanding processes at these higher levels is important to determine whether species level processes, such as cospeciation, simply scale up to broader macroevolutionary patterns or whether host shifts between major host lineages have broad consequences for parasite diversification.

Among feather lice, such host-switching between families or orders of birds is considered to be rare because most genera of lice are confined to a single host family or order (Price *et al*., 2003). However, one such opportunity for exploring major host shifts lies within the body louse family Goniodidae. These lice parasitize two distantly-related orders (Hackett *et al*., 2008): Galliformes (pheasants, quail, partridges, megapodes, etc.) and Columbiformes (pigeons and doves). The presence of related genera of lice on these hosts is likely the result of one or more major host-

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switching events. Lice in the family Goniodidae have a rounded body form and are generally confined to the belly and rump regions of the host, which is why they are often called 'body' lice (Clay, 1949). These lice are closely related to body lice in the family Heptapsogasteridae (Smith, 2000; Cruickshank *et al*., 2001; Johnson, Adams & Clayton, 2001), which are confined to the avian order Tinamiformes (tinamous), an ancient lineage of South American birds that is closely related to the flightless ratites (ostriches, emus, rheas, cassowaries, and kiwis; Hackett *et al*., 2008).

Previous phylogenetic studies of the family Goniodidae have used both morphological (Smith, 2000) and molecular (Johnson *et al*., 2001) data for phylogeny reconstruction. The morphological study of Smith (2000), which used 62 morphological characters for 15 species of Goniodidae, failed to recover monophyly of either the lice parasitizing Galliformes or Columbiformes, suggesting multiple switching events between these host orders. By contrast, a molecular study by Johnson *et al*. (2001), which involved maximum likelihood analysis of two gene regions for 24 species of Goniodidae, recovered reciprocal monophyly for the lice parasitizing Galliformes with respect to those parasitizing Columbiformes. These results supported previous work separating the family into Goniodinae (from Galliformes) and Physconelloidinae (from Columbiformes). The molecular phylogenetic tree suggested a single inter-ordinal host switch, although the direction of the switch was ambiguous. However, only three species of lice from Galliformes were included in the study by Johnson *et al*. (2001).

The present study aimed to expand both the taxon sampling and number of gene regions in a more detailed molecular phylogenetic study of Goniodidae. The ultimate purpose of the study was to test further whether lice from Galliformes and Columbiformes are reciprocally monophyletic, and also to provide additional inferences regarding possible switching of lice between these host orders. We present analyses of DNA sequences from three gene regions (one nuclear and two mitochondrial) for expanded sampling of 89 taxa of Goniodidae.

MATERIAL AND METHODS

Lice were collected, stored, and prepared according to procedures described by Johnson *et al*. (2001). Species were identified from voucher specimen slides according to the generic level taxonomy of Price *et al*. (2003). However, we also applied generic names recognized by Tendeiro (1969a, b, 1973) for columbiform body lice as a potential subgeneric classification. DNA was extracted from individual lice using a Qiagen Tissue Extraction Kit and the exoskeleton was retained and slide mounted as a voucher specimen. Voucher slides are deposited in the Illinois Natural History Survey Insect Collection and in the Price Institute for Phthirapteran Research, University of Utah. Portions of the mitochondrial cytochrome oxidase I (COI; 379 bp) and nuclear elongation factor 1α (EF1α; 347 bp) were amplified using primers and polymerase chain reaction (PCR) protocols described by Johnson *et al*. (2001). Furthermore, a portion of the mitochondrial 16S ribosomal DNA gene (16S; 573 aligned bp) was amplified using the primers 16Sar and 16Sbr (Simon *et al*., 1994). PCR conditions were similar to those for COI and EF1α, although a 46 °C annealing temperature was used. PCR products were purified using a Qiagen PCR Purification Kit and sequenced using an ABI BigDye fluorescent cycle sequencing kit (Applied Biosystems). Sequences were run on an ABI 3730xl capillary sequencer (GenBank Accession Numbers: AF278644, AF278646-AF278647, AF278652, AF278655, AF278659, AF278662–AF278665, AF278670, AF278673, AF278678–AF278679, AF320403-AF320404, AF348644–AF348647, AF348650, AF348654–AF348655, AF348657, AF348668, AF348837–AF348842, AF348844–AF348845, AF348847–AF348849, AF348851–AF348853, AF414769, AF414772, AF414777, AF414780, AF414785, AF414787, AF414789, AF414805, and HQ332786–HQ333008).

For protein coding genes, sequences were aligned by eye according to codons. There were no observed codon indels. For 16S rDNA, sequences were aligned using CLUSTALX (Thompson *et al*., 1997). This alignment resulted in several regions that appeared to have ambiguous alignments with many indels. These regions were removed from analyses to avoid any confounding influence of problematic homology among sites in the alignment (98 bp in total). For all analyses, trees were rooted using *Strongylocotes orbiculatus*, a representative of the Heptapsogasteridae, which parasitizes tinamous (Aves: Tinamidae). Both morphological (Smith, 2000) and molecular (Cruickshank *et al*., 2001; Johnson *et al*., 2001) data indicate that Heptapsogasteridae is the sister taxon of Goniodidae.

To evaluate the stability of trees to method of analysis, we used parsimony (using PAUP*; Swofford, 2000), Bayesian inference (using MrBayes; Ronquist & Huelsenbeck, 2003), and maximum likelihood (Zwickl, 2006) reconstruction methods. For parsimony, we conducted 100 random addition replicates of all three gene regions combined (1202 bp) with tree bisection–reconnection branch swapping. We also conducted analyses of each gene separately to evaluate any major conflicts between gene regions. We used bootstrapping (Felsenstein, 1985) to assess the stability of this tree to character re-sampling. We calcu-

lated consistency indices to evaluate and compare the relative substitution patterns of the three genes.

We conducted Bayesian analyses on three different partitioning schemes: (1) all data combined; (2) two-partitions [mitochondrial (mt)DNA and EF1α], and (3) three-partitions (COI, 16S, and EF1α). We used MRMODELTEST, version 2.3 (Nylander, 2004) to determine which model of molecular evolution was most appropriate for each partition and then chose among the three partitioning schemes using Bayes factors (Brandley, Schmitz & Reeder, 2005), calculated using the harmonic mean from the sump command within MrBayes (Ronquist & Huelsenbeck, 2003). We considered a difference of 2 ln Bayes factor > 10 as the minimum value to discriminate between partitioning schemes. The Bayes factor analysis determined that the three-partition scheme is most appropriate and is thus the one presented here. The three-partition scheme had likelihood models set for the two mtDNA genes (COI and 16S) as GTR + I + G with a flat Dirichlet prior for state frequencies and for EF1α as HKY + I + G with the state frequencies fixed as equal. All model parameters except the topology and branch lengths were unlinked between partitions and were estimated from the data as part of the analysis. We ran two parallel runs for ten million generations, each with four Markov chains, to ensure that our analyses were not stuck at local optima (Huelsenbeck & Bollback, 2001). Markov chains were sampled every 500 generations, yielding 20 000 parameter point estimates. We used these 20 000 point estimates minus the burn-in generations (500) to create a 50% majority-rule consensus tree and to calculate Bayesian posterior probabilities, which we used to assess nodal support.

As an alternative assessment of phylogenetic support, we conducted a maximum likelihood bootstrap analysis using Garli, version 1.0 (Zwickl, 2006). We used a six parameter model with invariant sites and a gamma shape parameter for rate heterogeneity. Values of the parameters that best fit the data are estimated during the analysis. We performed 100 maximum likelihood bootstrap replicates.

RESULTS

Substantial variation between species was evident in each gene region, with COI (CI = 0.14) being the most variable, followed by 16S (CI = 0.23) and nuclear EF1α (CI = 0.45). Earlier studies of substitution rates in mitochondrial versus nuclear genes in lice, including Goniodidae, have shown a dramatically elevated substitution rate in mitochondrial compared to nuclear genes (Johnson *et al*., 2003). Even very closely-related species exhibit large divergences in mitochondrial genes with almost no divergence in EF1α. Thus, mitochondrial genes should be useful for resolving relationships among closely-related species, whereas multiple substitution interferes with the ability of such genes to resolve deeper relationships. Even given these differences, parsimony trees from individual gene regions were broadly congruent (not shown). Results from a partition homogeneity test (Farris *et al*., 1994, 1995; Swofford, 2000) comparing all three gene regions were not significant ($P = 0.22$), again indicating that data from these three gene regions were broadly concordant. Given that each gene fragment is less than 1000 bp, a combined analysis of all three genes should improve resolution and support.

Combined unweighted parsimony searches recovered only two most parsimonious trees (Fig. 1). A consensus of these trees was highly resolved and revealed several notable groups of species. Among taxa parasitic on pigeons and doves (Columbiformes), support for several large clades was recovered. These included two large clades of *Physconelloides* species that primarily parasitize: (1) small-bodied New World doves (*Columbina*, *Uropelia*, *Claravis*, and *Metriopelia*) and (2) New World mid-sized doves (*Leptotila* and *Geotrygon*) and large bodied pigeons (*Patagioenas*). A monophyletic group of *Campanulotes* (*Saussurites*) parasitic on Australian phabine doves (*Phaps*, *Geophaps*, *Ocyphaps*, *Petrophassa*, *Geopelia*, *Leucosarcia*) was recovered, as was a large clade of *Coloceras* (*Coloceras*) species parasitic on a variety of Old World pigeons and doves. Among columbiform lice, the most basal split was between *Coloceras museihalense*, a parasite of the Great Cuckoo-Dove (*Reinwardtoena reinwardtsi*) of New Guinea, and all other species of lice on Columbiformes. Above this node, a group of four species (Subgenera: *Nitzschiella* and *Nitzschielloides*) was the sister taxon of the remaining columbiform lice. Although bootstrap support for some of these major clades, as well as more terminal species level relationships, is high (> 75%), support for relationships among major groups within columbiform lice is relatively weak (< 50%). This may be a result of the relatively short branches in this region of the tree, as well as the relatively high homoplasy in mitochondrial genes at these divergences.

Taxa parasitic on Galliformes (i.e. *Goniodes* and *Goniocotes*) for the most part formed a paraphyletic grade at the base of the tree, with lice from Columbiformes embedded within those from Galliformes. Interestingly, one louse species parasitic on Galliformes:Megapodidae (*Goniodes biordinatus* ex *Megapodius reinwardt*) is embedded within those parasitizing Columbiformes, making the lice from Columbiformes paraphyletic. Some of the relationships among the lice of Galliformes were relatively well supported by bootstrapping, including a sister

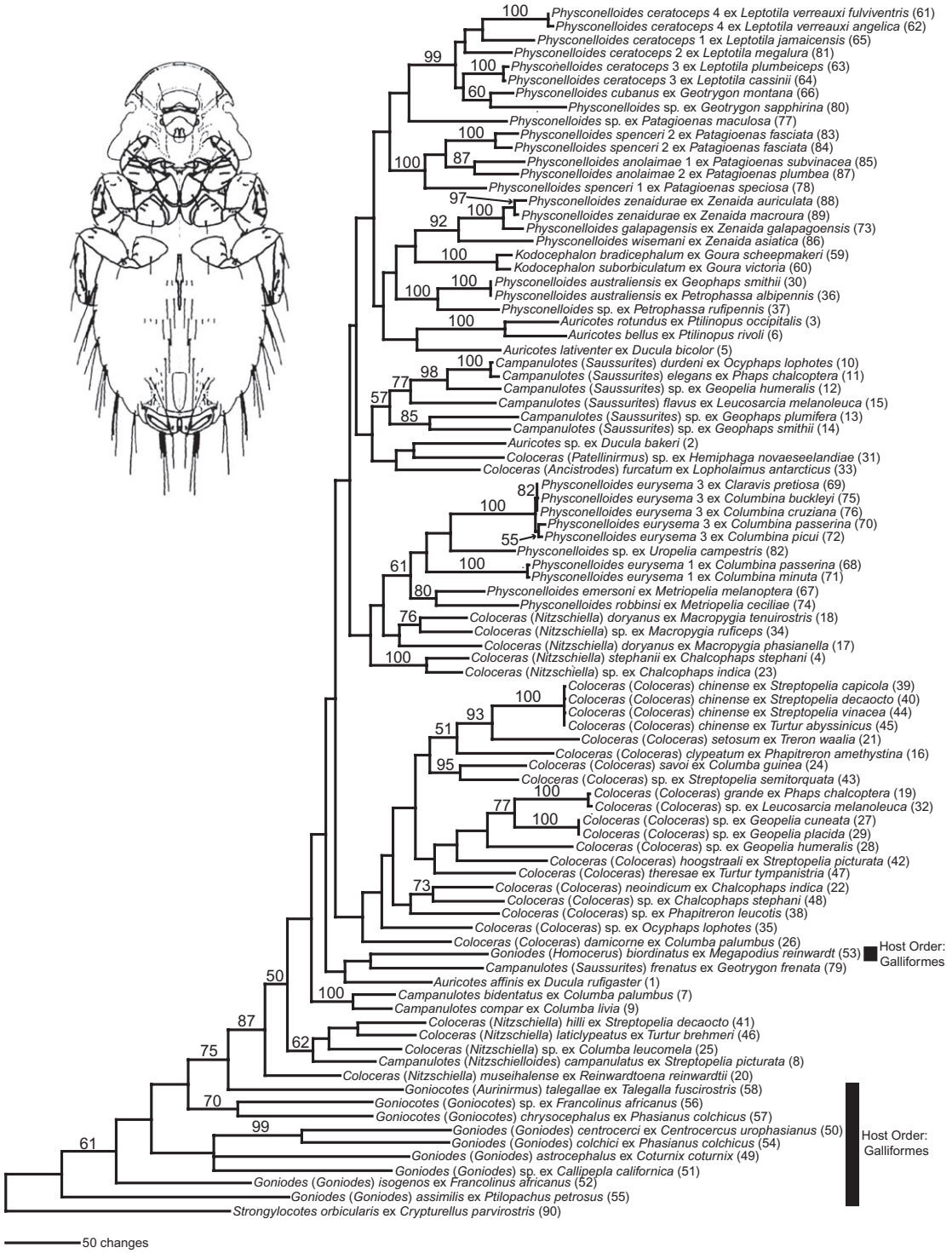

Figure 1. Consensus of two trees (length = 5401, consistency index = 0.195, rescaled consistency index = 0.096) from unweighted parsimony analysis of cytochrome oxidase I, 16S, and elongation factor 1-α combined. Branch lengths are proportional to the number of reconstructed substitutions. Numbers associated with branches are from 1000 parsimony bootstrap replicates. Lice associated with the Order Galliformes are indicated by vertical bars, with all other ingroup taxa occuring on pigeons and doves (Columbiformes). Numbers after louse species names indicate potentially cryptic species (*sensu* Johnson *et al*., 2001). Names in parentheses are generic names for lice *sensu* Tendeiro (1969a, b, 1973) and are used here as tentative subgenera. Numbers in parentheses correspond to the specimen numbers given in Table 1.

Table 1. Specimens used in the present study

Number	Extract code	Louse species	Host species	Host order	Country
1	Auaff.5.18.2004.13	*Auricotes affinis*	*Ducula rufigaster*	Columbiform.	New Guinea
2	Aumar.5.18.2004.14	*Auricotes* sp.	*Ducula bakeri*	Columbiform.	Vanuatu
3	Aurot.5.26.1999.1	*Auricotes rotundus*	*Ptilinopus occipitalis*	Columbiform.	Philippines
4	Ausp.Chste.5.18.2004.11	*Coloceras* (*Nitzschiella*) *stephanii*	*Chalcophaps stephani*	Columbiform.	New Guinea
5	Ausp.Dubic.2.9.2004.11	*Auricotes lativenter*	*Ducula bicolor*	Columbiform.	Australia
6	Ausp.Ptriv.5.18.2004.12	*Auricotes bellus*	*Ptilinopus rivoli*	Columbiform.	New Guinea
7	Cabid.2.9.2004.6	*Campanulotes bidentatus*	*Columba palumbus*	Columbiform.	UK
8	Cacam.2.9.2004.8	*Campanulotes* (*Nitzschielloides*) *campanulatus*	*Streptopelia picturata*	Columbiform.	Madagascar
9	Cacom.1.16.2001.4	*Campanulotes compar*	*Columba livia*	Columbiform.	USA
10	Cadur.2.9.2004.4	*Campanulotes* (*Saussurites*) *durdeni*	*Ocyphaps lophotes*	Columbiform.	Australia
11	Cafla.2.9.2004.2	*Campanulotes* (*Saussurites*) *elegans*	*Phaps chalcoptera*	Columbiform.	Australia
12	Casp.Gehum.2.24.2004.4	*Campanulotes* (*Saussurites*) sp.	*Geopelia humeralis*	Columbiform.	Australia
13	Casp.Geplu.2.24.2004.8	*Campanulotes* (*Saussurites*) sp.	*Geophaps plumifera*	Columbiform.	Australia
14	Casp.Gesmi.4.26.2004.16	*Campanulotes* (*Saussurites*) sp.	*Geophaps smithii*	Columbiform.	Australia
15	Casp.Lemel.2.24.2004.10	*Campanulotes* (*Saussurites*) *flavus*	*Leucosarcia melanoleuca*	Columbiform.	Australia
16	Cccly.5.26.1999.2	*Coloceras* (*Coloceras*) *clypeatum*	*Phapitreron amethystina*	Columbiform.	Philippines
17	Ccdor.2.9.2004.1	*Coloceras* (*Nitzschiella*) *doryanus*	*Macropygia phasianella*	Columbiform.	Australia
18	Ccdor.7.1.1999.8	*Coloceras* (*Nitzschiella*) *doryanus*	*Macropygia tenuirostris*	Columbiform.	Philippines
19	Ccgra.2.9.2004.3	*Coloceras* (*Coloceras*) *grande*	*Phaps chalcoptera*	Columbiform.	Australia
20	Ccmus.4.26.2004.7	*Coloceras* (*Nitzschiella*) *museihalense*	*Reinwardtoena reinwardtii*	Columbiform.	New Guinea
21	Ccset.3.21.2000.10	*Coloceras* (*Coloceras*) *setosum*	*Treron waalia*	Columbiform.	Ghana
22	Ccsp.Chind.3.21.2000.4	*Coloceras* (*Coloceras*) *neoindicum*	*Chalcophaps indica*	Columbiform.	Philippines
23	Ccsp.Chind.5.18.2004.1	*Coloceras* (*Nitzschiella*) sp.	*Chalcophaps indica*	Columbiform.	Vanuatu
24	Ccsp.Cogui.2.10.1999.10	*Coloceras* (*Coloceras*) *savoi*	*Columba guinea*	Columbiform.	South Africa
25	Ccsp.Colcm.5.18.2004.4	*Coloceras* (*Nitzschiella*) sp.	*Columba leucomela*	Columbiform.	Australia
26	Ccsp.Copal.2.9.2004.7	*Coloceras* (*Coloceras*) *damicorne*	*Columba palumbus*	Columbiform.	UK
27	Ccsp.Gecun.5.18.2004.8	*Coloceras* (*Coloceras*) sp.	*Geopelia cuneata*	Columbiform.	Australia
28	Ccsp.Gehum.12.6.2004.8	*Coloceras* (*Coloceras*) sp.	*Geopelia humeralis*	Columbiform.	Australia
29	Ccsp.Gepla.5.18.2004.7	*Coloceras* (*Coloceras*) sp.	*Geopelia placida*	Columbiform.	Australia
30	Ccsp.Gesmi.5.18.2004.3	*Physconelloides australiensis*	*Geophaps smithii*	Columbiform.	Australia
31	Ccsp.Henov.4.26.2004.4	*Coloceras* (*Patellinirmus*) sp.	*Hemiphaga novaeseelandiae*	Columbiform.	N. Zealand
32	Ccsp.Lemel.2.24.2004.9	*Coloceras* (*Coloceras*) sp.	*Leucosarcia melanoleuca*	Columbiform.	Australia
33	Ccsp.Loant.5.18.2004.2	*Coloceras* (*Ancistrodes*) *furcatum*	*Lopholaimus antarcticus*	Columbiform.	Australia
34	Ccsp.Maruf.11.15.1999.4	*Coloceras* (*Nitzschiella*) sp.	*Macropygia ruficeps*	Columbiform.	Borneo
35	Ccsp.Oclop.2.9.2004.5	*Coloceras* (*Coloceras*) sp.	*Ocyphaps lophotes*	Columbiform.	Australia
36	Ccsp.Pealb.5.18.2004.6	*Physconelloides australiensis*	*Petrophassa albipennis*	Columbiform.	Australia
37	Ccsp.Peruf.5.18.2004.9	*Physconelloides* sp.	*Petrophassa rufipennis*	Columbiform.	Australia
38	Ccsp.Phleu.5.26.1999.4	*Coloceras* (*Coloceras*) sp.	*Phapitreron leucotis*	Columbiform.	Philippines
39	Ccsp.Stcap.1.12.1999.5	*Coloceras* (*Coloceras*) *chinense*	*Streptopelia capicola*	Columbiform.	South Africa
40	Ccsp.Stdct.12.6.2004.7	*Coloceras* (*Coloceras*) *chinense*	*Streptopelia decaocto*	Columbiform.	USA
41	Ccsp.Stdec.11.15.1999.2	*Coloceras* (*Nitzschiella*) *hilli*	*Streptopelia decaocto*	Columbiform.	Netherlands
42	Ccsp.Stpic.2.9.2004.9	*Coloceras* (*Coloceras*) *hoogstraali*	*Streptopelia picturata*	Columbiform.	Madagascar
43	Ccsp.Stsem.4.26.2004.5	*Coloceras* (*Coloceras*) sp.	*Streptopelia semitorquata*	Columbiform.	Ghana
44	Ccsp.Stvin.4.26.2004.2	*Coloceras* (*Coloceras*) *chinense*	*Streptopelia vinacea*	Columbiform.	Ghana
45	Ccsp.Tuaby.4.26.2004.15	*Coloceras* (*Coloceras*) *chinense*	*Turtur abyssinicus*	Columbiform.	Ghana
46	Ccsp.Tubre.3.21.2000.7	*Coloceras* (*Nitzschiella*) *laticlypeatus*	*Turtur brehmeri*	Columbiform.	Ghana
47	Ccsp.Tutym.2.3.2001.6	*Coloceras* (*Coloceras*) *theresae*	*Turtur tympanistria*	Columbiform.	Uganda
48	Ccste.5.18.2004.10	*Coloceras* (*Coloceras*) sp.	*Chalcophaps stephani*	Columbiform.	New Guinea
49	Gdast.4.26.2004.10	*Goniocotes* (*Goniodes*) *astrocephalus*	*Coturnix coturnix*	Galliformes	Russia
50	Gdcen.2.24.2004.7	*Goniodes* (*Goniodes*) *centrocerci*	*Centrocercus urophasianus*	Galliformes	USA
51	Gdsp.Cacal.1.15.2000.2	*Goniodes* (*Goniodes*) sp.	*Callipepla californica*	Galliformes	USA
52	Gdsp.Frafr.2.3.1999.12	*Goniodes* (*Goniodes*) *isogenos*	*Francolinus africanus*	Galliformes	South Africa
53	Gdsp.Merei.2.24.2004.3	*Goniod.* (*Homocerus*) *biordinatus*	*Megapodius reinwardt*	Galliformes	Australia
54	Gdsp.Phcol.2.24.2004.1	*Goniodes* (*Goniodes*) *colchici*	*Phasianus colchicus*	Galliformes	USA
55	Gdsp.Ptpet.4.26.2004.3	*Goniodes* (*Goniodes*) *assimilis*	*Ptilopachus petrosus*	Galliformes	Ghana
56	Gosp.Frafr.1.12.1999.12	*Goniocotes* (*Goniocotes*) sp.	*Francolinus africanus*	Galliformes	South Africa
57	Gosp.Phcol.11.10.2001.2	*Goniocotes* (*Goniocotes*) *chrysocephalus*	*Phasianus colchicus*	Galliformes	USA
58	Gosp.Tafla.4.26.2004.9	*Goniocot.* (*Aurinirmus*) *talegallae*	*Talegalla fuscirostris*	Galliformes	New Guinea
59	Kobra.3.24.2001.1	*Kodocephalon bradicephalum*	*Goura scheepmakeri*	Columbiform.	New Guinea
60	Kosub.4.26.2004.8	*Kodocephalon suborbiculatum*	*Goura victoria*	Columbiform.	New Guinea

Table 1. *Continued*

Number	Extract code	Louse species	Host species	Host order	Country
61	Phcer.1.25.1999.10	*Physconelloides ceratoceps* 4	*L. verreauxi fulviventris*	Columbiform.	Mexico
62	Phcer.1.25.1999.11	*Physconelloides ceratoceps* 4	*L. verreauxi angelica*	Columbiform.	USA
63	Phcer.11.15.1999.9	*Physconelloides ceratoceps* 3	*Leptotila plumbeiceps*	Columbiform.	Mexico
64	Phcer.2.24.2004.5	*Physconelloides ceratoceps* 3	*Leptotila cassinii*	Columbiform.	Costa Rica
65	Phcer.9.29.1998.10	*Physconelloides ceratoceps* 1	*Leptotila jamaicensis*	Columbiform.	Mexico
66	Phcub.1.25.1999.2	*Physconelloides cubanus*	*Geotrygon montana*	Columbiform.	Mexico
67	Pheme.2.9.2004.10	*Physconelloides emersoni*	*Metriopelia melanoptera*	Columbiform.	Argentina
68	Pheur.1.16.2001.5	*Physconelloides eurysema* 1	*Columbina passerina*	Columbiform.	USA
69	Pheur.1.25.2000.1	*Physconelloides eurysema* 3	*Claravis pretiosa*	Columbiform.	Mexico
70	Pheur.1.25.2000.4	*Physconelloides eurysema* 3	*Columbina passerina*	Columbiform.	Mexico
71	Pheur.2.24.2004.6	*Physconelloides eurysema* 1	*Columbina minuta*	Columbiform.	Costa Rica
72	Pheur.5.18.2004.5	*Physconelloides eurysema* 3	*Columbina picui*	Columbiform.	Bolivia
73	Phgal.7.1.1999.1	*Physconelloides galapagensis*	*Zenaida galapagoensis*	Columbiform.	Galapagos
74	Phrob.10.5.1999.11	*Physconelloides robbinsi*	*Metriopelia ceciliae*	Columbiform.	Bolivia
75	Phsp.Cobuc.4.26.2004.13	*Physconelloides eurysema* 3	*Columbina buckleyi*	Columbiform.	Peru
76	Phsp.Cocru.4.26.2004.14	*Physconelloides eurysema* 3	*Columbina cruziana*	Columbiform.	Peru
77	Phsp.Comcs.4.26.2004.12	*Physconelloides* sp.	*Patagioenas maculosa*	Columbiform.	Peru
78	Phsp.Cospe.4.19.1999.9	*Physconelloides spenceri* 1	*Patagioenas speciosa*	Columbiform.	Mexico
79	Phsp.Gefre.1.9.2001.16	*Cam.* (*Saussurites*) *frenatus*	*Geotrygon frenata*	Columbiform.	Peru
80	Phsp.Gesap.3.24.2001.7	*Physconelloides* sp.	*Geotrygon sapphirina*	Columbiform.	Peru
81	Phsp.Lemeg.1.25.2000.6	*Physconelloides ceratoceps* 2	*Leptotila megalura*	Columbiform.	Bolivia
82	Phsp.Urcam.10.12.1999.6	*Physconelloides* sp.	*Uropelia campestris*	Columbiform.	Bolivia
83	Phspe.1.16.2001.6	*Physconelloides spenceri* 2	*Patagioenas fasciata*	Columbiform.	USA
84	Phspe.10.12.1999.3	*Physconelloides spenceri* 2	*Patagioenas fasciata*	Columbiform.	Peru
85	Phtal.4.19.1999.8	*Physconelloides anolaimae* 1	*Patagioenas subvinacea*	Columbiform.	Guyana
86	Phwis.9.29.1998.11	*Physconelloides wisemani*	*Zenaida asiatica*	Columbiform.	USA
87	Phwol.4.24.1999.4	*Physconelloides anolaimae* 2	*Columba plumbea*	Columbiform.	Guyana
88	Phzen.2.24.2004.2	*Physconelloides zenaidurae*	*Zenaida auriculata*	Columbiform.	Bolivia
89	Phzen.5.4.1999.2	*Physconelloides zenaidurae*	*Zenaida macroura*	Columbiform.	USA
90	Sgorb.11.10.2001.10	*Strongylocotes orbicularis*	*Crypturellus parvirostris*	Tinamiformes	Bolivia

relationship (75%) between *Goniocotes tallegallae* and all of the body lice of Columbiformes (including the *G. biordinatus* ex *Megapodius*). However, in this tree, neither *Goniodes*, nor *Goniocotes*, were monophyletic.

Despite the problem of high levels of multiple substitution in mitochondrial genes, the results from the Bayesian analyses (Fig. 2) were quite similar to those of parsimony. In particular, two large but separate, clades of New World *Physconelloides* were recovered. However, unlike the parsimony trees, the Bayesian tree included the *Physconelloides* parasitic on New World mid-sized doves in the genus *Zenaida* in the same group as those from other New World mid-sized doves (*Leptotila* and *Geotrygon*). There was high (100% posterior probability) support for a group of Australian *Campanulotes* (*Saussurites*), as well as for monophyly of a large clade, comprising the Old World *Coloceras* (*Coloceras*) species (Fig. 3). The most basal splits among the columbiform lice were identical to those recovered by parsimony, with *C. museihalense* again being the sister taxon of all other lice parasitizing Columbiformes. Furthermore, as in the parsimony tree, the next node up the tree was the split between the group of four *Coloceras* (*Nitzschiella*/*Nitzschielloides*) species and all other columbiform lice, indicating that the most basal relationships within columbiform lice are stable to method of analysis. Relationships among the major clades of columbiform lice were relatively weakly supported by Bayesian posterior probabilities.

The tree recovered by Bayesian analysis also included a paraphyletic grade of galliform lice in which the lice of Columbiformes were embedded. In addition, *G.* (*Homocerus*) *biordinatus* from *M. reinwardt* (Galliformes: Megapodidae) was well embedded within the lice of Columbiformes. As in the parsimony tree, *G.* (*Aurinirmus*) *talegallae* from *Talegalla fuscirostris* (Galliformes: Megapodidae) was sister to the columbiform louse group (100% posterior probability). Some of the other relationships among the galliform lice were different from those recovered by parsimony, although many were strongly supported by Bayesian posterior probability (> 95%). For example, monophyly of a group containing all the species sampled from the genus *Goniodes* (minus *G. biordinatus*) was supported in the Bayesian tree. The results of the maximum likelihood bootstrap analysis were concordant with the Bayesian analysis. Most of the nodes

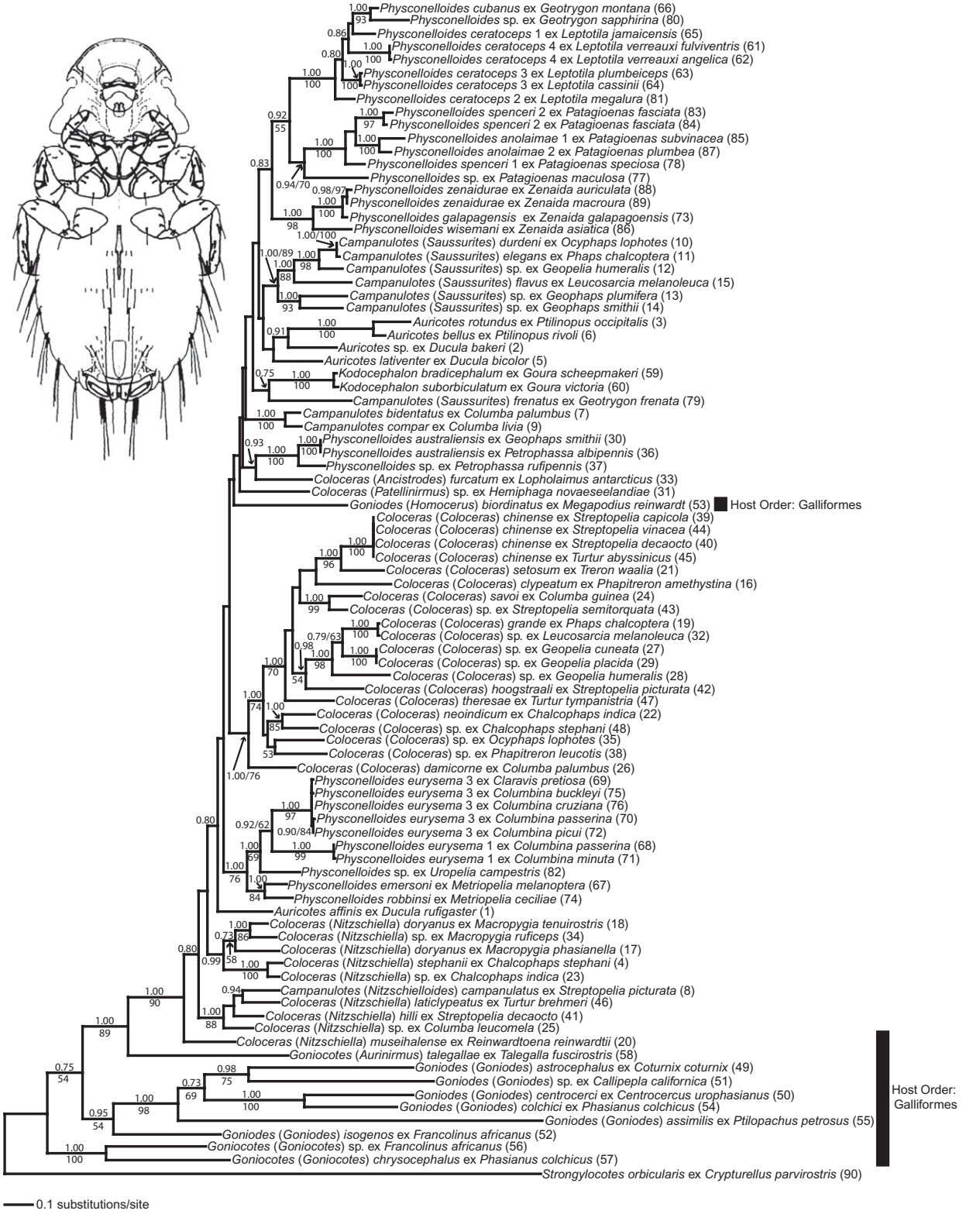

Figure 2. Bayesian consensus tree from the three partition analysis scheme (cytochrome oxidase I, 16S, and elongation factor 1-α). Branch lengths are proportional to substitutions per site. Numbers associated with nodes are Bayesian posterior probabilities (above branches or slashes) and maximum likelihood bootstrap values (below branches or slashes). Other conventions follow those of Fig. 1.

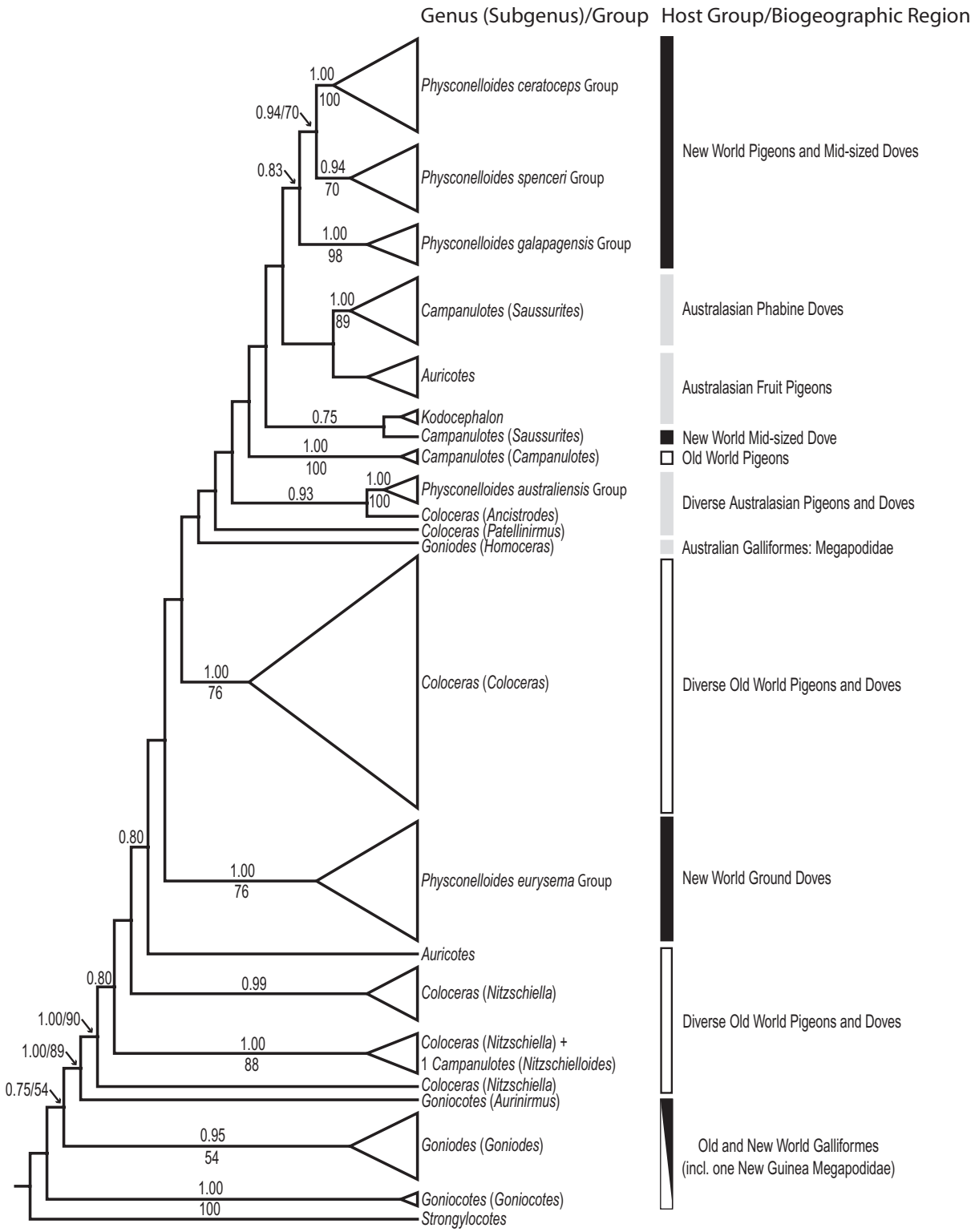

Figure 3. Schematic phylogenetic tree of Goniodidae based on the Bayesian tree showing generic classification (subgenus or species group), biogeographic distribution (vertical), and host group. Shading of vertical bars corresponds to biogeographic region: grey, Australasia; white, Old World; black, New World; white/black, lineages found in both the Old and New World. Numbers above and below the branches or slashes are Bayesian posterior probability (> 0.75) and maximum likelihood bootstrap (> 50) values, respectively.

strongly supported by Bayesian posterior probabilities also had strong maximum likelihood bootstrap support (Figs 2, 3).

In general, the phylogeny reflects the traditional generic classifications of Goniodidae. However, several genera are not monophyletic in either the parsimony or Bayesian trees. In the past, there have been two main classification schemes of the Goniodidae of Columbiformes. One is more conservative, recognizing fewer genera (Hopkins & Clay, 1952; Price *et al*., 2003). The other, developed by Tendeiro (1969a, b, 1971, 1973), over several revisions of this group, split taxa into many more genera (which we have indicated with subgeneric designations in parentheses). In several cases, the splitting of taxa into additional genera by Tendeiro appears to be justified. For example *Coloceras* (*Coloceras*) forms a large well-supported clade that is separated from other groups that have been lumped under the genus *Coloceras* (Price *et al*., 2003): *Nitzschiella*, *Patellinirmus*, *Ancistrodes*. A subgroup of *Campanulotes*, distributed on Australian phabine doves, is separated from other *Campanulotes*, and placed by Tendeiro in the genus *Saussurites*. However, Tendeiro (1971) also places *Campanulotes flavus* in this genus, but it appears to be distantly related to the Australian *Campanulotes* (*Saussurites*). Consistent with Tendeiro's interpretation, *Campanulotes* (*Nitzschielloides*) is separated from other species in the genus *Campanulotes*.

Tendeiro (1980a, 1983) also recognized separate genera (*Homocerus* and *Aurinirmus*) for some of the species of goniodid lice occurring on megapodes. In the present study, these are represented by *G.* (*Homocerus*) *biordinatus* and *G.* (*Aurinirmus*) *tallegallae*. In both trees, we find these separated from other members of *Goniodes* and *Goniocotes*, both having closer phylogenetic relationships with the lice of Columbiformes. Tendeiro (1980a) suggested that the species of *Homocerus* are closely related to *Coloceras* and *Patellinirmus*, and this is what we found for *G.* (*Homocerus*) *biordinatus*, which fell between *Coloceras* (*Coloceras*) and *Coloceras* (*Patellinirmus*). Similarly, Tendeiro (1983) suggested that *Aurinirmus* is more closely related to columbiform lice in the genera *Saussurites* and *Auricotes* than to the lice of Galliformes and, in all our analyses, *G.* (*Aurinirmus*) *tallegallae* was sister to the lice of Columbiformes, and not to other *Goniocotes*. Thus, the paraphyly of galliform goniodid lice based on our molecular data is in agreement with the taxonomic assessment of Tendeiro (1980a, 1983) based on morphology.

Although the morphological differences used by Tendeiro and colleagues to recognize additional genera within Goniodidae appear to largely reflect phylogenetic history, some of Tendeiro's genera still remain problematic. For example, subgenus *Nitzschiella* does not form a monophyletic group in either the parsimony or Bayesian tree. Furthermore, the subgenus *Saussurites* is not monophyletic, with the New World species being separated from the Australian taxa. Although taxon sampling of the large genus *Auricotes* is not high, this genus is also not monophyletic in either tree. Finally, Tendeiro recognized the genus *Physconelloides*; however, this genus also appears to involve at least three independent groups: one on Australian phabines, one on small New World ground doves, and one on larger New World doves and pigeons. Five species groups were recognized by Tendeiro (1980b) and Price, Clayton & Hellenthal (1999) on the basis of morphology, and the monophyly of each of these groups is generally well supported in the molecular phylogeny.

In the Bayesian tree, recognition of *Homocerus* and *Aurinirmus* as distinct genera would make both *Goniodes* and *Goniocotes* monophyletic. However, the problem of distinguishing *Goniodes* and *Goniocotes* morphologically has long been recognized (Clay, 1951; Ledger, 1980), and our limited taxon sampling of these genera does not enable a more detailed assessment of their status. Further morphological and molecular work on the Goniodidae of Galliformes is needed.

DISCUSSION

Phylogenetic analyses (parsimony and Bayesian inference) of sequences from mitochondrial COI and 16S and nuclear EF1α genes for parasitic lice in Goniodidae result in relatively well resolved and supported trees (Figs 1, 2). At the highest level, these trees indicate host-switching between avian orders. Given that Galliformes (pheasants, quail, partridges, megapodes, etc.) and Columbiformes (pigeons and doves) are very distantly related (Hackett *et al*., 2008), the host distribution of lice in these phylogenies indicates a major switch from Galliformes to Columbiformes because columbiform lice are well embedded within those of Galliformes (Fig. 3). More importantly, a host switch in the opposite direction (from Columbiformes to Galliformes) also appears to have happened more recently. Both parsimony and Bayesian trees place *G. biordinatus* from *M. reinwardt* (Galliformes: Megapodidae) well within the clade of lice from pigeons and doves. Unfortunately, the exact relationship of this species within this clade is still unclear because of low support for basal relationships within the columbiform louse group, making it difficult to reconstruct the details of this switch.

Although morphologically similar species tended to form well-supported clades, most genera were not recovered as monophyletic in either the parsimony or Bayesian trees. *Physconelloides* was split into two

(Bayesian) or three (parsimony) groups. The genus *Campanulotes*, which is largely recognized for its small size and morphological simplicity, fell into three separate groups. Finally, representatives of the genus *Coloceras* were spread throughout the tree. Much of the paraphyly of *Coloceras* can be accounted for by recognition of the subgenera *Ancistrodes*, *Patellinirmus*, and *Nitzschiella* as distinct from *Coloceras*. However, the subgenus *Nitzschiella*, which was recognized as a distinct genus by Tendeiro (1969a) but not by Price *et al*. (2003), formed three (Bayesian) or four (parsimony) distinct groups; therefore, adopting the classification of Tendeiro still leaves unresolved taxonomic problems. It should be noted, however, that because support for relationships among major clades was low, monophyly of many of these genera cannot be completely ruled out. Note, however, that both methods of analysis identified the same major groups.

Several other important biogeographic and host association patterns are also evident in the phylogeny of Gonididae (Fig. 3). Species parasitic on New World hosts are largely split into two main groups. These are mainly comprised of lice in the genus *Physconelloides*, which Price *et al*. (1999) divided into five main groups. The monophyly of each of the four New World groups is supported, and the Bayesian tree recovers a clade containing three of these four groups (Figs 2, 3). The New World species *Campanulotes frenatus*, from *Geotrygon frenata*, is not closely related to New World *Physconelloides* and appears to be an independent colonization of the New World. The large clade of *Coloceras* (*Coloceras*) occurs exclusively in the Old World including Australia. It is also widespread across distantly-related lineages of pigeons and doves (Johnson, 2004), thus showing correlation with biogeography but not host phylogeny. Lice from Australian phabine doves also form three distinct clades, suggesting three independent radiations in Australia on this group of hosts. Interestingly, the Australian phabines are the only group of Columbiformes to host three different genera of body lice, which differ markedly in size. There are also species of non-phabine doves in Australia that independently colonized Australia from South-East Asia (e.g. *Macropygia*, *Ptilinopus*, *Ducula*). Lice from these non-phabine doves are separated from the three groups of phabine lice, suggesting that these birds may have carried their lice with them when they colonized Australia (Pereira *et al*., 2007).

The sister taxon to all columbiform lice is *G. talegallae*, which parasitizes a megapode (*T. fuscirostris*) from New Guinea. Furthermore, the most basal split among columbiform lice occurs between *C.* (*Nitzschiella*) *museihalense* from *R. reinwardtii*, also from New Guinea. Together, this suggests that columbiform lice may have begun to radiate first in New Guinea, which is consistent with a South East Asian and Papua-Australian origin for Columbiformes identified by Pereira *et al*. (2007), with subsequent rapid dispersal to other regions. This early radiation in the Papua–Australian region also appears to have facilitated the host-switch back to Galliformes because *Megapodius* is distributed in Australia and New Guinea.

In conclusion, the results obtained in the present study provide an example of how major host switches by parasites between distantly-related groups of hosts can be important evolutionary events. As such, they provide novel opportunities for parasite diversification on these new hosts. The avian feather lice in the family Goniodidae have undergone two such major host shifts: one from Galliformes to Columbiformes and one back to Galliformes (in particular to megapodes) from Columbiformes. The first host switch provided an opportunity for these lice to radiate on pigeons and doves, in some cases with up to three genera on a single host. Given the lack of strong concordance between louse phylogeny and major host groups at deeper scales, and the very short branches connecting major lineages of lice in this group, it appears likely that much of the early radiation of these lice was fostered by host-switching among existing columbiform lineages. By contrast, the more terminal relationships in the louse phylogeny are concordant with host phylogeny (Clayton & Johnson, 2003; Johnson & Clayton, 2003), indicating a more recent history of cospeciation. Molecular dating of the louse and host phylogenies could aid in determing when the first host-switch from Galliformes to Columbiformes occurred with respect to the radiation of Columbiformes.

ACKNOWLEDGEMENTS

We thank the following individuals for their assistance with collecting the specimens used in the present study B. Benz, S. Bush, T. Chesser, S. de Kort, D. Drown, R. Faucett, S. Goodman, N. Ingle, A. Kratter, J. Malenke, B. Marks, I. Mason, K. McCracken, R. Moyle, A. Navarro, R. Palma, R. Palmer, A. T. Peterson, M. Robbins, V. Smith, D. Steadman, T. Valqui, D. Willard, R. Wilson, C. Witt, and J. Wombey. For assistance with collecting permits and field work, we also thank the Nature Conservation Research Centre (J. Mason, L. Ashrifie, P. Adjewodah, and M. Boateng); Ghana Wildlife Division (M. Adu-Nsiah, J. Oppong, and J. Braimah); Transvaal Museum (Tamar Cassidy); the South African offices of Environmental Affairs and Tourism in Free State, Gauteng; and the Mpumulanga Parks Board. R. J. Adams and R. D. Price assisted with the preparation of slide mounts and the identification of voucher specimens. We thank two anonymous reviewers for

helpful comments on the manuscript. The present study was supported in part by NSF PEET DEB-0118794 to D.H.C. and K.P.J.; NSF DEB-0107891 and DEB-0612938 to K.P.J.; NSF DEB-0743491 and DEB-0816877 to D.H.C.; NSF DEB-0515672 to J.D.W.; and the Field Museum's Emerging Pathogens Project, funded by the Davee Foundation and the Dr Ralph and Marian Falk Medical Research Trust.

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