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Phylogenetic analysis of nuclear and mitochondrial genes supports species groups for *Columbicola* (Insecta: Phthiraptera)

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Abstract

The dove louse genus *Columbicola* has become a model system for studying the interface between microevolutionary processes and macroevolutionary patterns. This genus of parasitic louse (Phthiraptera) contains 80 described species placed into 24 species groups. Samples of *Columbicola* representing 49 species from 78 species of hosts were obtained and sequenced for mitochondrial (COI and 12S) and nuclear (EF-1α) genes. We included multiple representatives from most host species for a total of 154 individual *Columbicola*, the largest molecular phylogenetic study of a genus of parasitic louse to date. These sequences revealed considerable divergence within several widespread species of lice, and in some cases these species were paraphyletic. These divergences correlated with host association, indicating the potential for cryptic species in several of these widespread louse species. Both parsimony and Bayesian maximum likelihood phylogenetic analyses of these sequences support monophyly for nearly all the non-monotypic species groups included in this study. These trees also revealed considerable structure with respect to biogeographic region and host clade association. These patterns indicated that switching of parasites between host clades is limited by biogeographic proximity.

Keywords: Coevolution; Parasitism; Lice; Phylogeny; Molecular systematics; Ischnocera

1. Introduction

Parasitic lice (Insecta: Phthiraptera) are a model system for research on coevolution (Hafner et al., 1994; Clayton et al., 2004). Recent studies involving avian feather lice (Ischnocera) have linked two aspects of coevolution: cophylogenetic patterns and coadaptational processes (Clayton et al., 1999, 2003; Clayton and Johnson, 2003). The interface of coevolutionary history and coadaptation has been particularly well studied in the wing lice (*Columbicola*) of pigeons and doves (Aves: Columbidae). Species of *Columbicola* vary in their level of host specificity, which is related to their ability to disperse across host species (Johnson et al., 2002), but limited by their ability to survive on hosts of different sizes (Clayton et al., 2003; Johnson et al., 2005). Recent experimental work demonstrates that host specificity is determined in part by the ability of species of *Columbicola* to establish viable populations on hosts of different sizes. Species of *Columbicola* cannot survive on hosts that are markedly different in size from their native host (Clayton et al., 2003; Bush and Clayton, 2006). These experiments show that host size mediates the ability of lice to escape from preening, the main form of host defense against feather lice (Bush and Clayton, 2006). Thus, host size interacts with dispersal limitation to determine host specificity and ultimately the coevolutionary history of *Columbicola* lice with their hosts (Clayton and Johnson, 2003; Clayton et al., 2003).

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While considerable understanding of the linkages between microevolutionary processes and macroevolutionary patterns have been gained by studies of *Columbicola*, most of the phylogenetic studies of *Columbicola* have either focused on only New World species (Clayton and Johnson, 2003) or a relatively small, scattered sample of worldwide species (Johnson et al., 2003). Like their hosts, these parasites are distributed on all continents except Antarctica, as well as most oceanic islands (Price et al., 2003). Currently, 80 species of *Columbicola* are recognized, and these are treated in three recent taxonomic revisions of the genus (Clayton and Price, 1999; Adams et al., 2005; Bush and Price, 2006). The revision of Adams et al. (2005) recognized 24 species groups distinguished on the basis of morphological features. Many of these species groups are also confined to particular biogeographic regions. For example, five of these species groups are distributed only in the New World. The goal of the current study is to evaluate whether these species groups form monophyletic groups in trees based on molecular data and to evaluate biogeographic and host association patterns of this genus in a phylogenetic framework. This study represents the largest molecular phylogeny for any genus of parasitic louse.

2. Methods

To reconstruct a phylogeny of *Columbicola* we used DNA sequences from one nuclear (elongation factor-1α [EF-1α]) and two mitochondrial (12S rRNA and cytochrome oxidase I [COI]) genes. In this study, we included species from all continents and major lineages of hosts, for a total of 49 species of *Columbicola* from 78 species of hosts. Considerable divergence in mitochondrial gene sequences has been identified across host species within some species of *Columbicola* in the New World (Johnson et al., 2002). Thus, when possible, we included multiple representatives of species of *Columbicola* from different hosts, to evaluate the potential for cryptic species as well as identify possible paraphyletic species. In

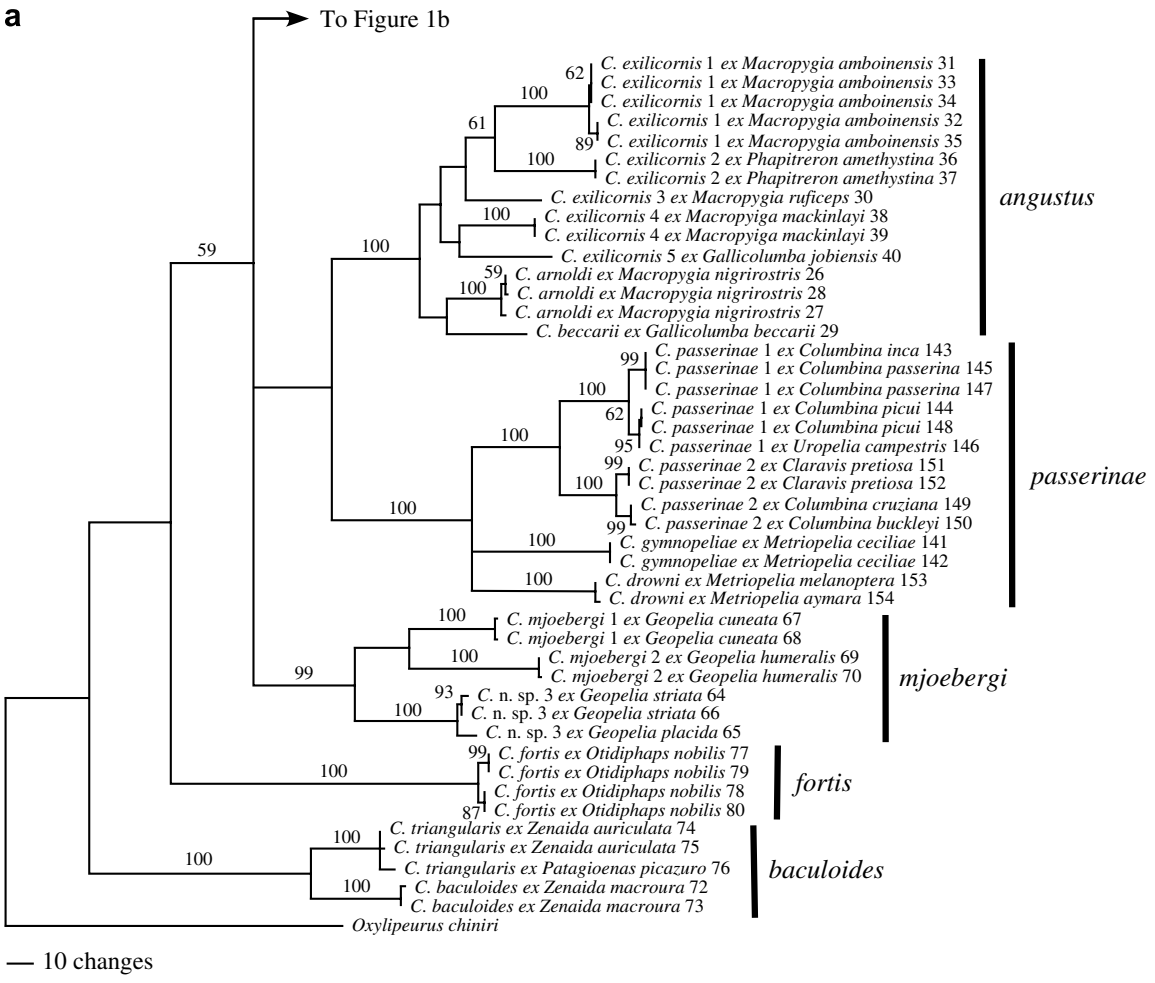

Fig. 1. Strict consensus of 800 most parsimonious trees (length = 4509, CI = 0.201) based on unweighted analysis of combined COI, 12S, and EF-1α sequences for *Columbicola*. Branches proportional to number of inferred changes (scale indicated). Numbers associated with nodes are percentage of 1000 bootstrap replicates containing the clade (only values >50% are shown). Numbers after *Columbicola* species names indicate presumed "cryptic" species based on sequence divergence and pattern of host specificity. Numbers after each host name refer to numbers for these individuals in Appendix A. Species groups indicated by vertical bars. *Indicates *cavifrons* species group recognized by Bush and Price (2006) but not included in Adams et al. (2005). *C.* = *Columbicola*. Tree partitioned into three portions (a–c).

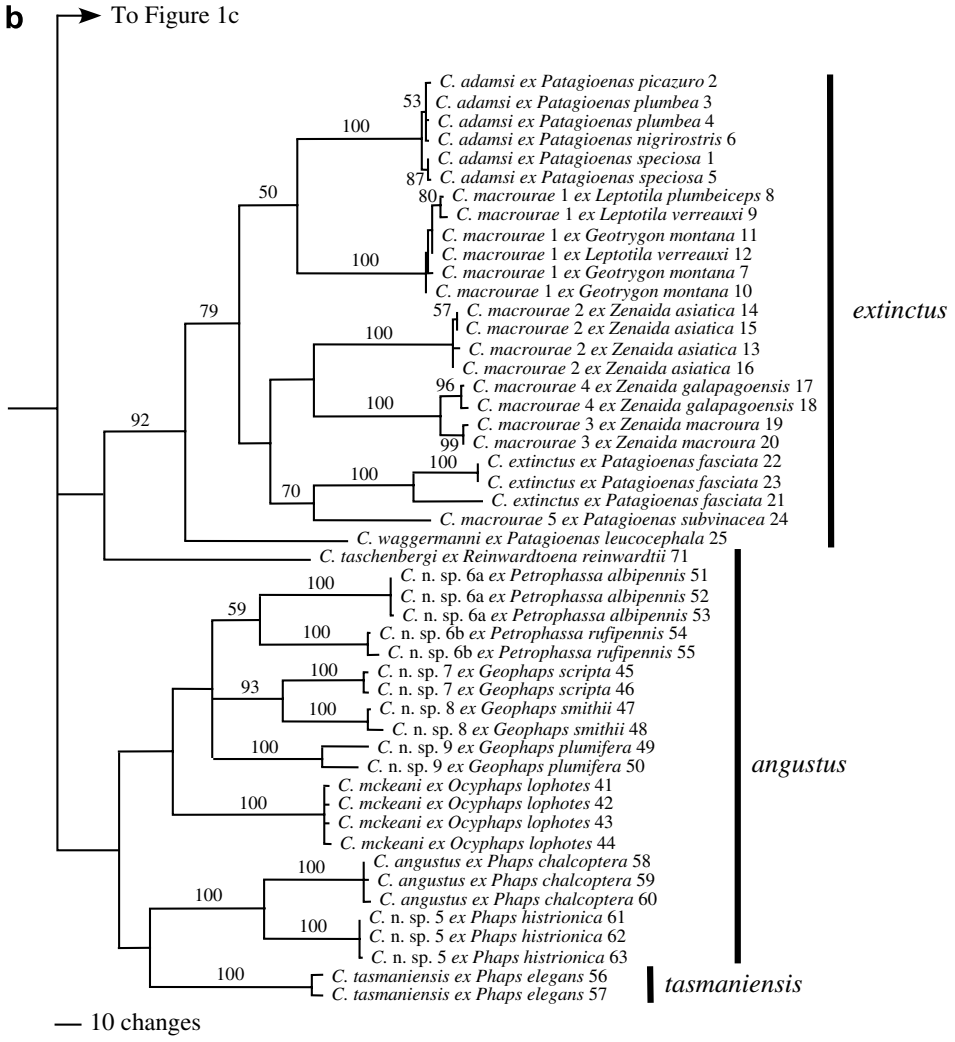

Fig. 1 (*continued*)

most cases, we also included multiple individuals from the same host species to assess the level of genetic variation within and among populations. This study includes a total of 154 individual lice sequenced for each gene. We used multiple methods to reconstruct the phylogeny for this genus and examined prior species group classification as well as patterns of biogeographic distribution and host association with respect to the phylogeny.

2.1. Specimen collection and DNA sequencing

We collected lice from hosts using the ethyl acetate fumigation method described by Clayton and Drown (2001). Individual hosts were kept separate at all times in paper or plastic bags and care was taken to clean all working surfaces between host fumigation. Lice were stored either frozen at −70 °C or in 95% ethanol at −20 °C. Samples of *Columbicola* were collected from 78 host species (Appendix A). These samples were chosen to span the diversity of hosts on which *Columbicola* occurs. We used *Oxylipeurus chiniri* as an outgroup (Johnson et al., 2003). We extracted DNA from individual lice by removing the head from the body with a pair of jeweler's forceps. These parts were placed in an extraction buffer and DNA was extracted from individual lice using a Qiagen Dneasy Tissue Extraction Kit. At the end of the digestion procedure, the head and the body of the louse were removed from the digestion buffer and reassembled in basalm on a microslide. This procedure, which does not damage fine structure, including setae, allows for morphological identification of louse specimens. Voucher slides are deposited in the Price Institute of Phthirapteran Research, University of Utah and at the Illinois Natural History Survey Insect Collection. Using other comparative slide material, we identified each species (using keys in Clayton and Price, 1999; Adams et al., 2005; Bush and Price, 2006) and noted general morphological differences between species for comparison with our molecular phylogeny. Many of the specimens included in this study represent new host records and new species, which await formal description (Bush et al., unpublished data).

DNA extracts of individual lice were used in PCR amplifications of the mitochondrial cytochrome oxidase I

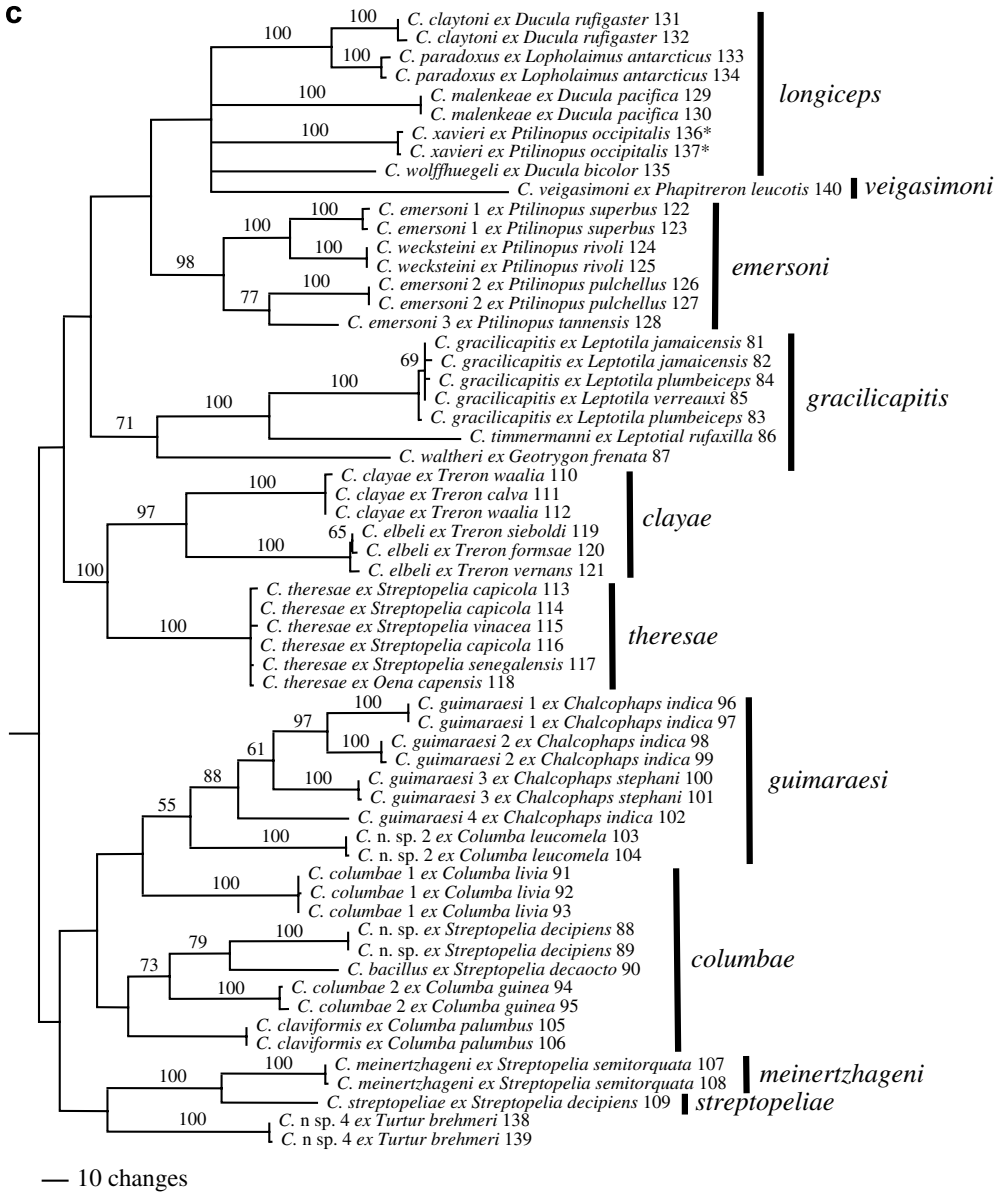

Fig. 1 (*continued*)

(COI), 12S rRNA (12S), and nuclear elongation factor 1-α (EF1) genes. We used the primers L6625 and H7005 (Hafner et al., 1994) to amplify COI, 12Sai, and 12Sbi (Simon et al., 1994) to amplify 12S, and EF1-For3 and EF1-Cho10 (Danforth and Ji, 1998) to amplify EF-1α (reaction conditions described by Johnson and Clayton, 2000). We purified PCR products using a Qiagen PCR purification kit and used the amplification primers in sequencing reactions. DNA cycle sequencing was performed using ABI Prism BigDye Terminators (Perkin-Elmer). We resolved complementary chromatograms using Sequencher 4.1 (GeneCodes). The mitochondrial 12S gene was aligned using Clustal X (Thompson et al., 1997). This alignment revealed several regions of ambiguous alignment and these were excluded from phylogenetic analyses. Alignment of protein coding genes was straightforward based on amino acid sequence (365 bp for COI and 360 bp for EF-1α). The aligned 12S sequence was 450 bp in length and 180 of these were excluded. The total length of analyzed sequences was 1017 bp. All single gene sequences are deposited in GenBank (Accession Nos. EF678749–EF679153).

2.2. Phylogenetic analysis

Both parsimony and Bayesian maximum likelihood methods were used to reconstruct phylogenetic trees for *Columbicola*. A partition homogeneity test (Farris et al., 1994, 1995; Swofford, 2001) did not reveal any significant conflict among the three gene regions ($P > 0.05$). In addition, independent parsimony analyses of these gene regions did not reveal conflict between trees that was supported by bootstrapping, therefore we combined these three gene regions for all analyses. Parsimony analyses were per-

formed using PAUP* (Swofford, 2001), whereas Bayesian analyses were performed with MrBayes 3.0 (Huelsenbeck and Ronquist, 2001). We conducted parsimony searches using 100 random addition replicates with TBR branch swapping.

MrBayes was used to run Metropolis-coupled MCMC chains (one cold and three heated chains) for Bayesian inference of phylogeny. The chains were run for 10 million generations and conservatively the first 1 million generations were discarded as burn-in, because plots of likelihood scores showed considerable stability by this point. The model of nucleotide evolution was the general time reversible model with 6 rate classes and unequal base frequencies with parameters estimated separately for nuclear and mitochondrial partitions. Trees were sampled every 1000 generations and majority rule consensus trees were constructed to estimate the posterior probabilities of each branch.

3. Results

Within *Columbicola*, uncorrected pairwise sequence divergences ranged up to 27% for COI, 26% for 12S

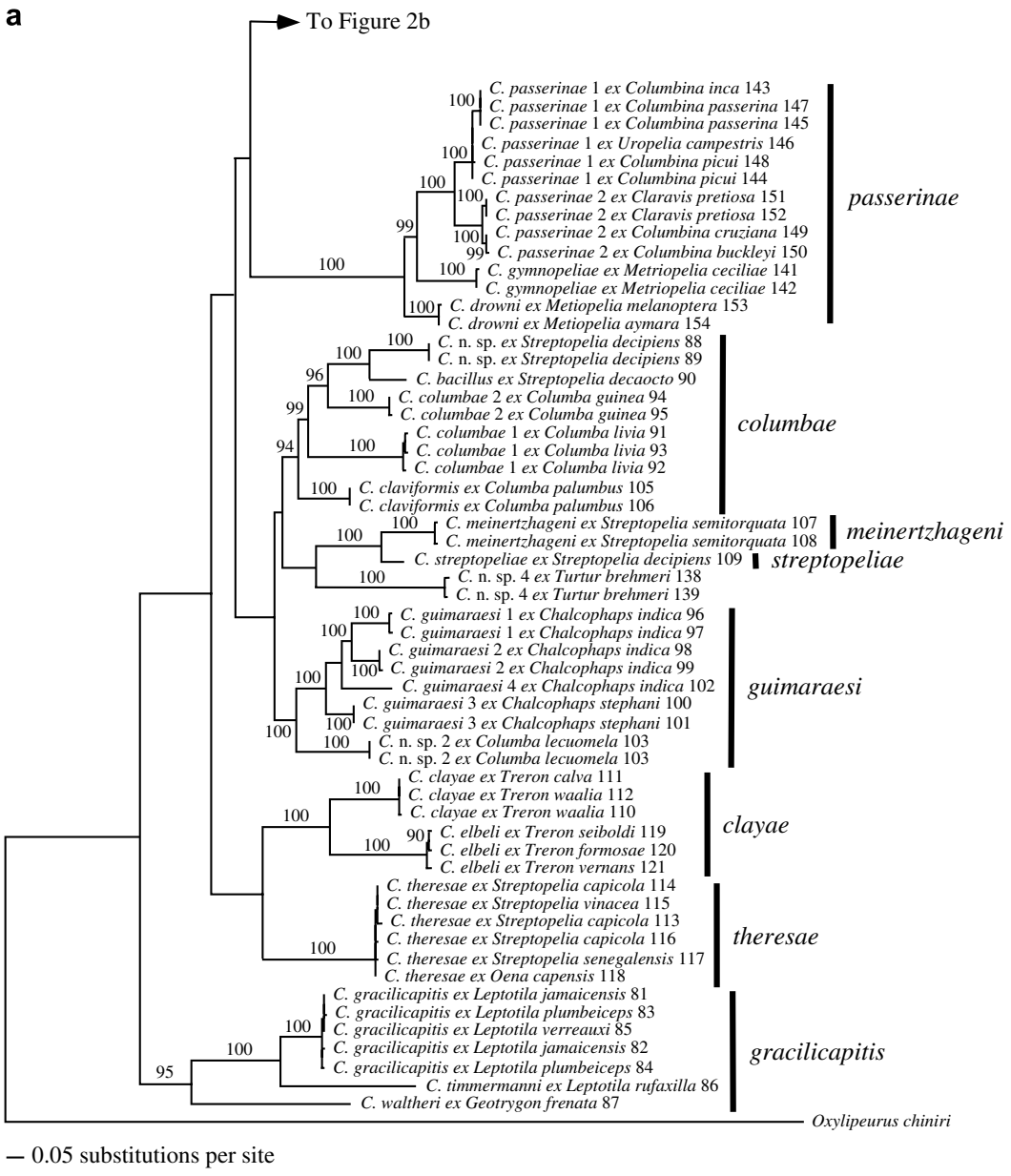

Fig. 2. Most likely tree from Bayesian maximum likelihood analysis of combined COI, 12S, and EF-1α sequences for *Columbicola*. Branches proportional to substitutions per site for the most likely tree (scale indicated). Numbers associated with nodes are posterior probabilities for the clade from a 10 million generation MCMC analysis, sampled every 1000 generations and excluding the first 1 million generations as burn-in (only values >90% are shown). Numbers after *Columbicola* species names indicate presumed "cryptic" species based on sequence divergence and pattern of host specificity. Numbers after each host name refer to numbers for these individuals in Appendix A. Species groups indicated by vertical bars. *Indicates *cavifrons* species group recognized by Bush and Price (2006) but not included in Adams et al. (2005). *C.* = *Columbicola*. Tree partitioned into three portions (Fig. 1a–c).

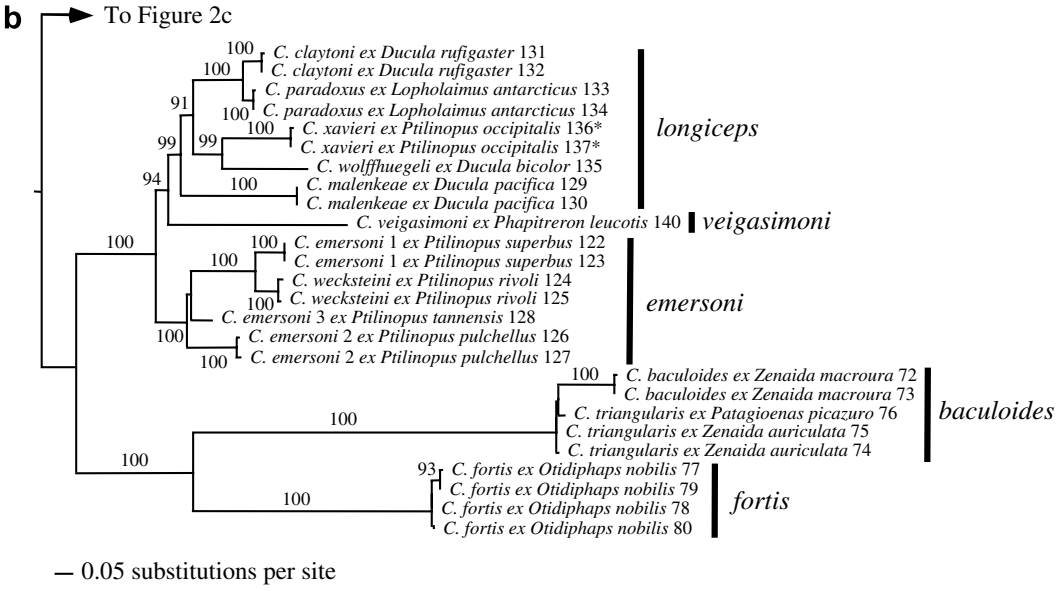

Fig. 2 (*continued*)

(aligned regions only), and 13% for EF-1α. Parsimony analysis revealed 800 most parsimonious trees (length = 4509, CI = 0.201). However, the differences between these trees largely involved minor rearrangements of individuals within species, while most of the relationships between species were stable across these trees. The combined strict consensus of these trees is well resolved (130/154 possible nodes) and a high fraction of these nodes are supported in over 50% of bootstrap replicates (Fig. 1). Several species of *Columbicola* that occur on multiple host species exhibit pronounced mitochondrial sequence divergences that correspond to different host species. Within a host species, uncorrected COI divergences between *Columbicola* generally are less than 1%. However, in several cases, divergences between *Columbicola* on different host species range from 5% to 20%. This pattern of pronounced between host mitochondrial differentiation occurs in several widespread species of *Columbicola*, including *C. emersoni*, *C. guimaraesi*, *C. columbae*, *C. macrourae*, *C. exilicornis*, *C. passerinae*, and *C. mjoebergi*. In many of these cases, other species of *Columbicola* are embedded within these widespread species making the widespread species paraphyletic: *C. wecksteini* within *C. emersoni*, *C. bacillus* within *C. columbae*, and *C. adamsi* and *C. extinctus* within *C. macrourae*. Given the morphological similarity of several described species of *Columbicola*, it is likely that these host specific populations represent cryptic host specialized species (Malenke et al., unpublished data). These divergent haplogroups have been given numbers for ease of reference, but await formal taxonomic description.

The parsimony tree also recovered monophyly for many non-monotypic species groups (from Adams et al., 2005) that were sampled by more than one species. These include the *extinctus*, *clayae*, *emersoni*, *gracilicapitis*, *passerinae*, and *baculoides* species groups. The *columbae*, *angustus*, and *longiceps* groups were paraphyletic in this tree, although in each case such paraphyly was not supported by over 50% of bootstrap replicates. In general, there was a good correspondence between morphological species and species groups and the molecular tree (Fig. 1).

Bayesian maximum likelihood analysis also produced a well resolved and well supported tree (Fig. 2). Many nodes were supported with over 90% posterior probability, including some more basal nodes not well supported by parsimony analysis. In particular, a group containing the *extinctus*, *mjoebergi*, *angustus*, and *tasmaniensis* species groups was supported with 99% posterior probability. Differences between the Bayesian and parsimony trees generally involved rearrangements among species groups. In particular in the parsimony tree the *baculoides* and *fortis* groups were at the base of the tree while in the Bayesian tree these groups were more derived and on long branches. However, these differences, which may be due to long branch attraction, were not well supported. In most cases, nodes that were well supported by parsimony bootstrapping (>75%) were also well supported by Bayesian posterior probability (>95%). The Bayesian tree recovered more monophyletic species groups than the parsimony tree, including the *extinctus*, *clayae*, *emersoni*, *gracilicapitis*, *passerinae*, *baculoides*, *columbae*, and *longiceps* species groups. Bush and Price (2006) split the *longiceps* species group into the *longiceps* and *cavifrons* species groups. The *cavifrons* species group was represented by *C. xavieri* in our study. *Columbicola xavieri* fell well within the *longiceps* species group with high posterior probability (99%) in the Bayesian tree, suggesting that recognition of the *cavifrons* species group may render the *longiceps* species group paraphyletic. Of the groups recognized by Adams et al. (2005), only the *angustus* species group was not recovered as monophyletic, because the monotypic *tasmaniensis* species

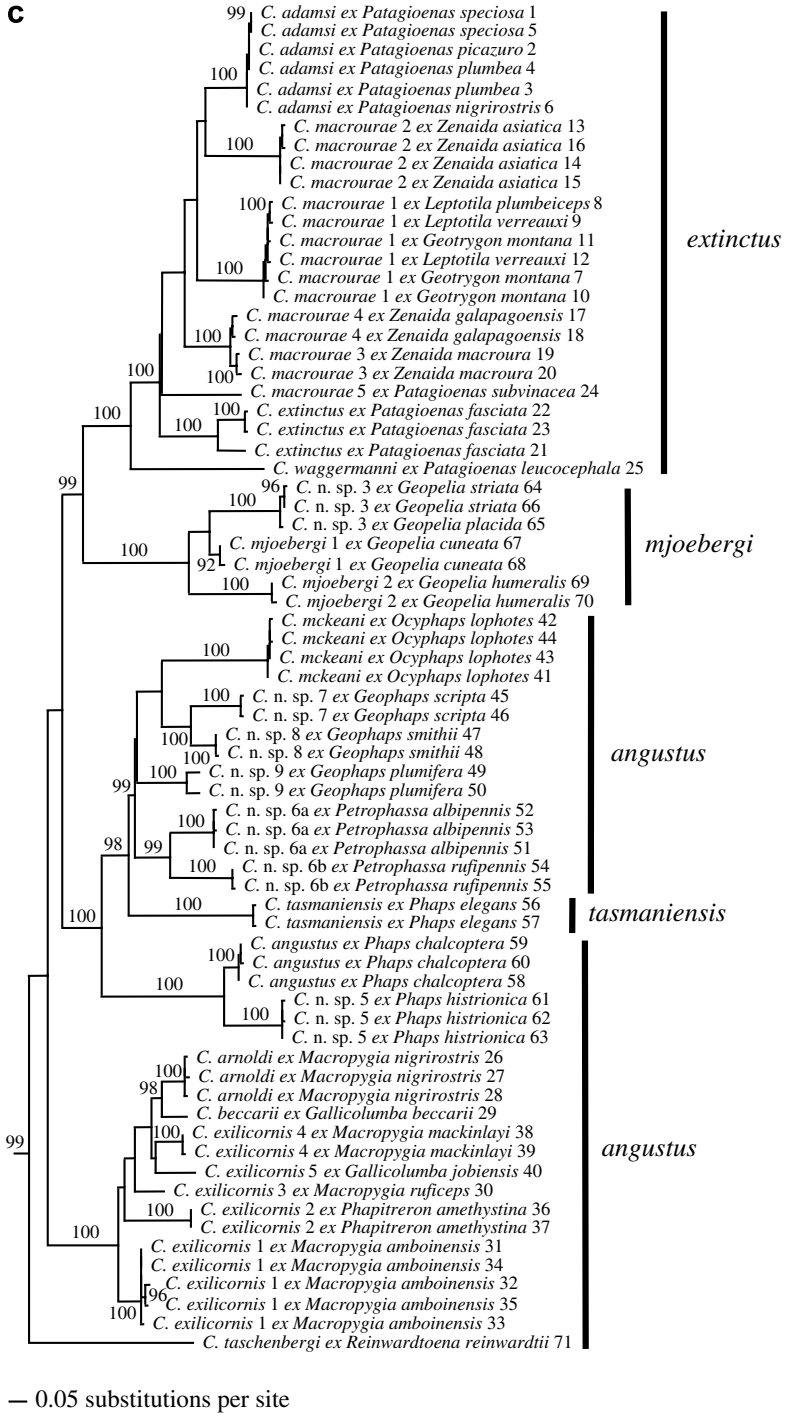

Fig. 2 (*continued*)

group was imbedded within the *angustus* group with 99% posterior probability.

4. Discussion

The largest molecular based tree for a single genus of parasitic louse (*Columbicola*) is well resolved and well supported. This tree based on two mitochondrial genes (COI and 12S) and one nuclear gene (EF-1α) for these wing lice of doves supports monophyly of most of the non-monotypic species groups of *Columbicola* identified by Adams et al. (2005). In this sense, the molecular phylogeny is highly concordant with morphology, and thus this molecular tree forms a reasonable hypothesis for the phylogeny of this genus. No morphological phylogenetic analysis with which to compare our molecular tree has been published for *Columbicola*.

Several morphologically described species of *Columbicola* occur on multiple host species (e.g. *C. macrourae* from 12 species of doves, Malenke et al., unpublished data).

Some of these species appear to actually be assemblages of "cryptic" species, as suggested by large mitochondrial genetic divergences between host specific haplotypes. Interestingly, however, not all non-specific species of *Columbicola* show such patterns of genetic differentiation (Johnson et al., 2002, Fig. 1). For example, *C. gracilicapitis* and *C. adamsi* show no evidence of genetic differentiation among host species, even though both parasitize more than two host species. Clearly there is variation in the degree of host specificity of species of *Columbicola*, even at the genetic level. More detailed morphological study may reveal subtle morphological differences consistent with recognizing these genetically differentiated forms as different species. Indeed, this was shown to be the case in the recent re-evaluation of *C. longiceps*, which split this widespread species into several, more host specific, species on the basis of morphology alone (Bush and Price, 2006). We feel that formal naming of other "cryptic" species should await more detailed morphological study of the species that exhibit such molecular differentiation, such as *C. macrourae* and *C. exilicornis*.

Avian feather lice are highly host specific, and *Columbicola* is no exception. This host specificity is generally reflected in a correspondence between the phylogeny for *Columbicola* and five major clades of hosts identified by Johnson and Clayton (2000): (A) small New World ground doves, (B) pigeons and cuckoo doves, (C) New World quail doves, (D) fruit doves and allies, (E) Australian phabines. For example, members of the *passerinae* species group occur only on host clade A, small New World ground doves (Figs. 3 and 4). The louse clade comprising the *longiceps*, *veigasimoni*, and *emersoni* species groups is restricted to host clade D, and another large clade of lice is restricted to clade E.

While there is a general correspondence between the molecular phylogeny for *Columbicola* and its host groups,

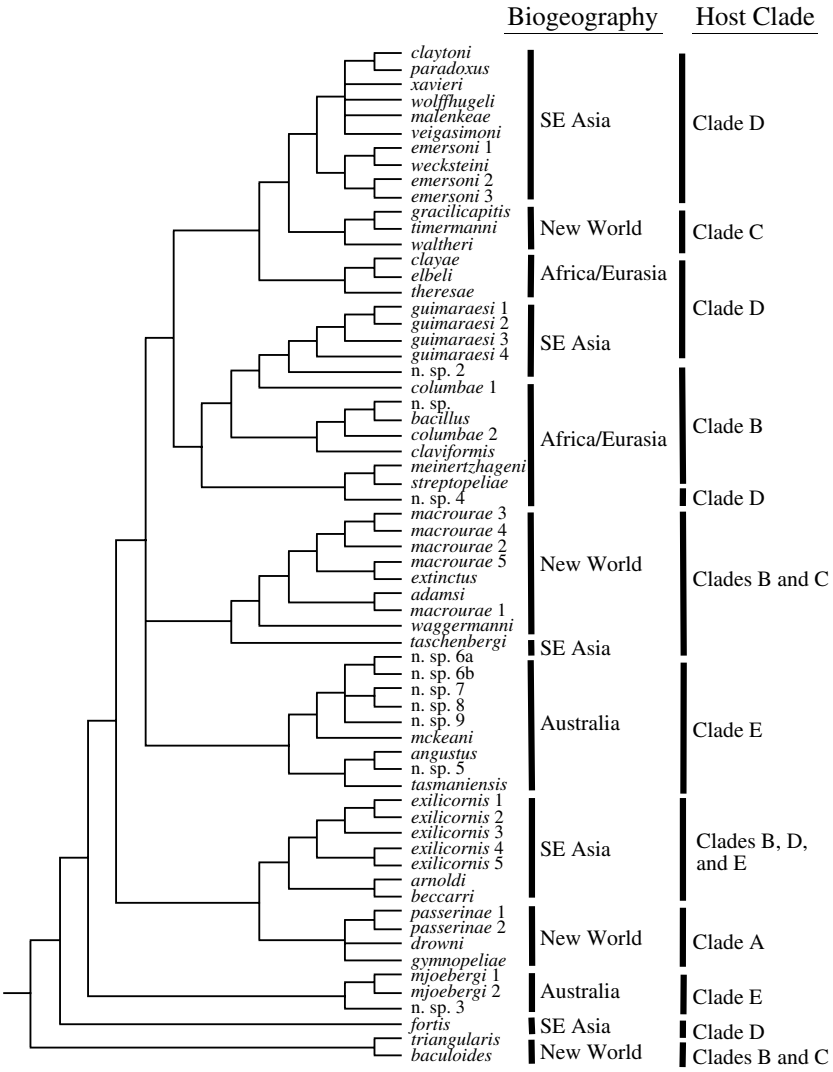

Fig. 3. Parsimony tree from Fig. 1 collapsed to show only a single representative of each species. Biogeographic region and host clade parasitized are indicated by vertical bars (A = small New World ground doves, B = pigeons and cuckoo doves, C = New World quail doves, D = fruit doves and allies, E = Australian phabines).

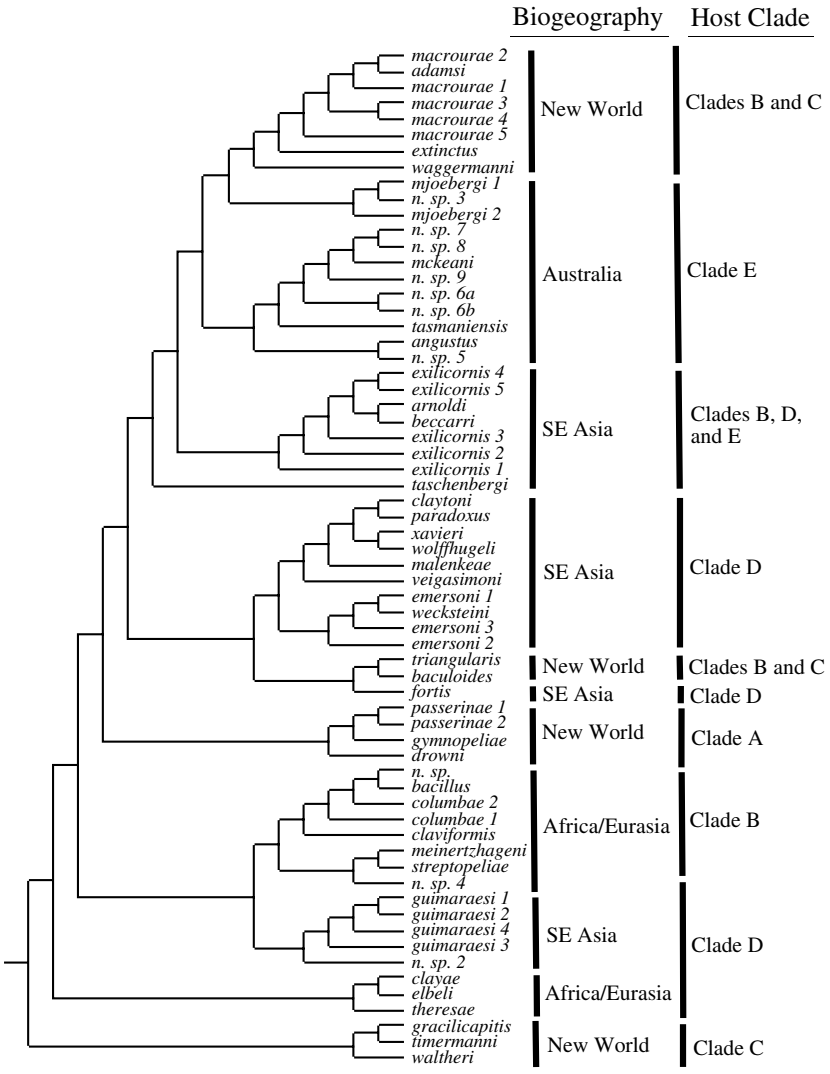

Fig. 4. Best Bayesian tree from Fig. 2 collapsed to show only a single representative of each species. Biogeographic region and host clade parasitized are indicated by vertical bars (A = small New World ground doves, B = pigeons and cuckoo doves, C = New World quail doves, D = fruit doves and allies, E = Australian phabines).

biogeography also plays an important role in structuring *Columbicola* phylogenetic relationships. The geographic distribution of the host genera (Columbiformes) and *Columbicola* wing lice can be best described by dividing their distributions into four major regions: New World, Papuan-Australian, South East Asian, and Eurasian-African. New World *Columbicola* form four distinct groups that are not closely related to each other: *baculoides*, *gracilicapitis*, *passerinae*, and *extinctus* species groups (Figs. 1–4). In both the parsimony (Fig. 1) and Bayesian (Fig. 2) trees one of these New World groups is sister to all other *Columbicola* (*baculoides* or *gracilicapitis* respectively). Such an early split in dove wing lice is concordant with phylogenetic analyses of doves which also indicates a basal split between New World lineages and other taxa (Johnson and Clayton, 2000; Pereira et al., 2007), though reconstructing an area of origin would be ambiguous in this case. Interestingly, the closest relative of each New World species group occurs in South East Asia (Figs. 1 and 2).

Other major clades of *Columbicola* also exhibit a strong biogeographic signal. For example, another large clade is confined to Australian phabine doves (most of the *angustus* and *tasmaniensis* species groups), and yet another large clade in the Bayesian tree (*columbae*, *meinertzhageni*, and *streptopeliae* species groups) occurs in the Eurasian-African region. In several cases, clades of lice from the same biogeographic region occur across multiple host groups. For example, the *extinctus* species group, confined to the New World, occurs on both clades B and C of doves and only on the New World representatives of clade B. A clade containing the *Columbicola* species *C. exilicornis*, *C. arnoldi*, and *C. beccarri* occurs only in South East Asia but on three host clades that have representatives in this region: B, D, and E. These patterns suggest that biogeographic overlap has provided opportu-

nities for these parasites to switch between host clades at some point in the past.

Our molecular phylogenetic tree for *Columbicola* is based on sequences of 154 individual lice, representing 49 species from 78 species of hosts. This tree provides a robust framework with which to conduct future comparative studies. The tree for *Columbicola* shows good correspondence with morphologically defined species groups, host groups, and biogeographic regions. The genetic differentiation detected within species of *Columbicola* across different host species provides a starting point for more detailed population genetic and phylogeographic analyses. The phylogeny presented here, combined with extensive ecological information on determinants of dispersal and host specificity of species of *Columbicola* (Clayton et al., 2003; Bush and Clayton, 2006; Bush et al., 2006), make this genus a valuable model system for understanding the links between microevolutionary processes (e.g. gene flow) and macroevolutionary patterns (e.g. cospeciation).

Acknowledgments

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Appendix A

Specimens of *Columbicola* included in study

Columbicola species	Host	Country	Extract voucher code	Nos.
adamsi	*Patagioenas speciosa*	Mexico	Coada.10.19.1998.7	1
adamsi	*Patagioenas picazuro*	Bolivia	Cotri.11.15.1999.3	2
adamsi	*Patagioenas plumbea*	Guyana	Cosp.Coplu.10.19.1998.8	3
adamsi	*Patagioenas plumbea*	Guyana	Cosp.Coplu.4.24.1999.3	4
adamsi	*Patagioenas speciosa*	Mexico	Coada.3.1.1999.7	5
adamsi	*Patagioenas nigrirostris*	Panama	Cosp.Conig.1.8.2003.14	6
macrourae 1	*Geotrygon montana*	Mexico	Comac.9.29.1998.1	7
macrourae 1	*Leptotila plumbeiceps*	Mexico	Cosp.plu.10.19.1998.4	8
macrourae 1	*Leptotila verreauxi*	Mexico	Cosp.ver.10.19.1998.2	9
macrourae 1	*Geotrygon montana*	Mexico	Comac.3.1.1999.4	10
macrourae 1	*Geotrygon montana*	Mexico	Comac.3.1.1999.1	11
macrourae 1	*Leptotila verreauxi*	Peru	Cosp.Lever.7.22.2004.13	12
macrourae 2	*Zenaida asiatica*	USA	Comac.10.2.1999.4	13
macrourae 2	*Zenaida asiatica*	USA	Comac.9.29.1998.5	14
macrourae 2	*Zenaida asiatica*	USA	Comac.9.14.1999.8	15
macrourae 2	*Zenaida asiatica*	USA	Comac.10.14.1999.5	16
macrourae 4	*Zenaida galapagoensis*	Galapagos	Comac.12.13.1999.7	17
macrourae 4	*Zenaida galapagoensis*	Galapagos	Comac.7.1.1999.2	18
macrourae 3	*Zenaida macroura*	USA	Cosp.mac.10.19.1998.5	19
macrourae 3	*Zenaida macroura*	USA	Cosp.Zemac.2.1.1999.9	20
extinctus	*Patagioenas fasciata*	Peru	Coext.10.12.1999.2	21
extinctus	*Patagioenas fasciata*	USA	Cosp.Cofas.9.27.2000.4	22
extinctus	*Patagioenas fasciata*	USA	Coext.1.20.2003.1	23
macrourae 5	*Patagioenas subvinacea*	Bolivia	Comac.11.15.1999.5	24
waggermanni	*Patagioenas leucocephala*	USA	Cowag.11.15.1999.8	25
arnoldi	*Macropygia nigrirostris*	Papua New Guinea	Coexi.5.14.2003.5	26
arnoldi	*Macropygia nigrirostris*	Papua New Guinea	Coexi.5.14.2003.6	27
arnoldi	*Macropygia nigrirostris*	Papua New Guinea	Cosp.Manig.7.22.2004.18	28
beccarii	*Gallicolumba beccarii*	Papua New Guinea	Cobec.1.8.2003.12	29
exilicornis 3	*Macropygia ruficeps*	Borneo	Coexi.11.15.1999.6	30

(*continued on next page*)

Appendix A (*continued*)

Columbicola species	Host	Country	Extract voucher code	Nos.
exilicornis 1	*Macropygia amboinensis*	Papua New Guinea	Cosp.Maamb.8.19.2003.7	31
exilicornis 1	*Macropygia amboinensis*	Australia	Cosp.Maamb.1.20.2003.9	32
exilicornis 1	*Macropygia amboinensis*	Papua New Guinea	Cosp.Maamb.5.14.2003.1	33
exilicornis 1	*Macropygia amboinensis*	Papua New Guinea	Cosp.Maamb.8.19.2003.8	34
exilicornis 1	*Macropygia amboinensis*	Australia	Cosp.Maamb.1.20.2003.8	35
exilicornis 2	*Phapitreron amethystina*	Philippines	Covei.5.26.1999.6	36
exilicornis 2	*Phapitreron amethystina*	Philippines	Cosp.Phame.7.22.2004.12	37
exilicornis 4	*Macropygia mackinlayi*	Vanuatu	Cosp.Mamac.1.27.2004.3	38
exilicornis 4	*Macropygia mackinlayi*	Vanuatu	Cosp.Mamac.1.27.2004.4	39
exilicornis 5	*Gallicolumba jobiensis*	Papua New Guinea	Coexi.1.12.1999.2	40
mckeani	*Ocyphaps lophotes*	Australia	Comck.1.20.2003.10	41
mckeani	*Ocyphaps lophotes*	Australia	Comck.5.14.2003.16	42
mckeani	*Ocyphaps lophotes*	Australia	Comck.5.14.2003.15	43
mckeani	*Ocyphaps lophotes*	Australia	Cosp.Oclop.7.20.2004.10	44
n. sp. 7	*Geophaps scripta*	Australia	Cosp.Gescr.1.8.2003.10	45
n. sp. 7	*Geophaps scripta*	Australia	Cosp.Gescr.7.27.2004.6	46
n. sp. 8	*Geophaps smithii*	Australia	Cosp.Gesmi.1.27.2004.10	47
n. sp. 8	*Geophaps smithii*	Australia	Cosp.Gesmi.1.27.2004.9	48
n. sp. 9	*Geophaps plumifera*	Australia	Cosp.Geplu.1.8.2003.16	49
n. sp. 9	*Geophaps plumifera*	Australia	Cosp.Geplu.7.7.2003.13	50
n. sp. 6a	*Petrophassa albipennis*	Australia	Cosp.Pealb.5.14.2003.13	51
n. sp. 6a	*Petrophassa albipennis*	Australia	Cosp.Pealb.5.14.2003.14	52
n. sp. 6a	*Petrophassa albipennis*	Australia	Cosp.Pealb.7.7.2003.16	53
n. sp. 6b	*Petrophassa rufipennis*	Australia	Cosp.Peruf.1.27.2004.12	54
n. sp. 6b	*Petrophassa rufipennis*	Australia	Cosp.Peruf.1.27.2004.13	55
tasmaniensis	*Phaps elegans*	Australia	Cosp.Phele.6.6.2005.7	56
tasmaniensis	*Phaps elegans*	Australia	Cotas.1.27.2004.14	57
angustus	*Phaps chalcoptera*	Australia	Cosp.Phcha.1.20.2003.11	58
angustus	*Phaps chalcoptera*	Australia	Cosp.Phcha.1.20.2003.12	59
angustus	*Phaps chalcoptera*	Australia	Cosp.Phcha.7.20.2004.15	60
n. sp. 5	*Phaps histrionica*	Australia	Cosp.Phhis.1.27.2004.16	61
n. sp. 5	*Phaps histrionica*	Australia	Cosp.Phhis.5.14.2003.9	62
n. sp. 5	*Phaps histrionica*	Australia	Cosp.Phhis.7.7.2003.7	63
n. sp. 3	*Geopelia striata*	Hawaii	Comjo.3.21.2000.5	64
n. sp. 3	*Geopelia placida*	Australia	Cosp.Gepla.5.14.2003.17	65
n. sp. 3	*Geopelia striata*	Hawaii	Comjo.1.20.2003.13	66
mjoebergi 1	*Geopelia cuneata*	Australia	Cosp.Gecun.1.27.2004.11	67
mjoebergi 1	*Geopelia cuneata*	Australia	Cosp.Gecun.7.26.2004.3	68
mjoebergi 2	*Geopelia humeralis*	Australia	Cosp.Gehum.5.14.2003.11	69
mjoebergi 2	*Geopelia humeralis*	Australia	Cosp.Gehum.5.14.2003.12	70
taschenbergi	*Reinwardtoena reinwardtii*	Papua New Guinea	Cotas.8.19.2003.9	71
baculoides	*Zenaida macroura*	USA	Cobac.10.19.1998.1	72
baculoides	*Zenaida macroura*	USA	Cobac.1.12.1999.1	73
triangularis	*Zenaida auriculata*	Argentina	Cosp.Zeaur.6.9.2001.5	74
triangularis	*Zenaida auriculata*	Argentina	Cosp.Zeaur.1.8.2003.6	75
triangularis	*Patagioenas picazuro*	Argentina	Cosp.Copic.1.20.2003.5	76
fortis	*Otidiphaps nobilis*	Papua New Guinea	Cofor.5.14.2003.7	77
fortis	*Otidiphaps nobilis*	Papua New Guinea	Cofor.7.7.2003.17	78
fortis	*Otidiphaps nobilis*	Papua New Guinea	Cofor.5.14.2003.8	79
fortis	*Otidiphaps nobilis*	Papua New Guinea	Cosp.Otnob.7.7.2003.18	80
gracilicapitis	*Leptotila jamaicensis*	Mexico	Cogra.9.29.1998.4	81
gracilicapitis	*Leptotila jamaicensis*	Mexico	Cosp.Lejam.2.1.1999.4	82
gracilicapitis	*Leptotila plumbeiceps*	Mexico	Cosp.Leplu.3.1.1999.2	83

Appendix A (*continued*)

Columbicola species	Host	Country	Extract voucher code	Nos.
gracilicapitis	*Leptotila plumbeiceps*	Mexico	Cosp.Leplu.3.1.1999.5	84
gracilicapitis	*Leptotila verreauxi*	Mexico	Cosp.Lever.3.1.1999.12	85
timmermanni	*Leptotila rufaxilla*	Guyana	Cotim.4.24.1999.2	86
waltheri	*Geotrygon frenata*	Peru	Cosp.Gefre.1.20.2003.4	87
n. sp.	*Streptopelia decipiens*	Uganda	Cosp.Stdec.1.20.2003.3	88
n. sp.	*Streptopelia decipiens*	Uganda	Cosp.Stdec.2.3.2001.7	89
bacillus	*Stretopelia decaocto*	Netherlands	Cobcs.11.15.1999.1	90
columbae 1	*Columba livia*	USA	Cocol.6.29.1998.3	91
columbae 1	*Columba livia*	USA	Cocol.9.18.1997.1	92
columbae 1	*Columba livia*	USA	Cocol.6.29.1998.1	93
columbae 2	*Columba guinea*	South Africa	Cosp.Cogui.2.10.1999.9	94
columbae 2	*Columba guinea*	South Africa	Cosp.Cogui.7.22.2004.3	95
guimaraesi 1	*Chalcophaps indica*	Vanuatu	Cogui.1.27.2004.1	96
guimaraesi 1	*Chalcophaps indica*	Vanuatu	Cosp.Chind.7.26.2004.4	97
guimaraesi 2	*Chalcophaps indica*	Australia	Cosp.Chind.7.20.2004.12	98
guimaraesi 2	*Chalcophaps indica*	Australia	Cosp.Chind.7.20.2004.13	99
guimaraesi 3	*Chalcophaps stephani*	Papua New Guinea	Cosp.Chste.5.14.2003.4	100
guimaraesi 3	*Chalcophaps stephani*	Papua New Guinea	Cosp.Chste.5.14.2003.3	101
guimaraesi 4	*Chalcophaps indica*	China	Cosp.Chind.6.6.2005.1	102
n. sp. 2	*Columba leucomela*	Australia	Cosp.Coleu.1.27.2004.7	103
n. sp. 2	*Columba leucomela*	Australia	Cosp.Coleu.1.27.2004.8	104
claviformis	*Columba palumbus*	United Kingdom	Coclv.1.20.2003.15	105
claviformis	*Columba palumbus*	United Kingdom	Coclv.1.20.2003.16	106
meinertzhageni	*Streptopelia semitorquata*	Ghana	Cosp.Stsem.7.27.2004.11	107
meinertzhageni	*Streptopelia semitorquata*	Ghana	Cosp.Stsem.7.27.2004.12	108
streptopeliae	*Streptopelia decipiens*	Uganda	Cosp.Stdec.2.3.2001.8	109
clayae	*Treron waalia*	Ghana	Cocla.3.21.2000.9	110
clayae	*Treron calva*	Ghana	Cosp.Trcal.7.27.2004.3	111
clayae	*Treron waalia*	Ghana	Cosp.Trwaa.7.27.2004.1	112
theresae	*Streptopelia capicola*	South Africa	Cosp.Stcap.1.12.1999.4	113
theresae	*Streptopelia capicola*	South Africa	Cosp.Stcap.4.19.1999.5	114
theresae	*Streptopelia vinacea*	Ghana	Cosp.Stvin.3.21.2000.11	115
theresae	*Streptopelia capicola*	South Africa	Cosp.Stcap.4.19.1999.4	116
theresae	*Streptopelia senegalensis*	South Africa	Cosp.Stsen.3.29.1999.11	117
theresae	*Oena capensis*	South Africa	Cosp.Oecap.2.10.1999.8	118
elbeli	*Treron sieboldi*	China	Cosp.Trsie.6.6.2005.4	119
elbeli	*Treron formosae*	Japan	Cosph.1.8.2003.18	120
elbeli	*Treron vernans*	Borneo	Cosp.Trver.7.27.2004.8	121
emersoni 1	*Ptilinopus superbus*	Papua New Guinea	Coeme.7.7.2003.2	122
emersoni 1	*Ptilinopus superbus*	Papua New Guinea	Coeme.7.7.2003.3	123
wecksteini	*Ptilinopus rivoli*	Papua New Guinea	Cosp.Ptriv.1.8.2003.11	124
wecksteini	*Ptilinopus rivoli*	Papua New Guinea	Cosp.Ptriv.7.7.2003.1	125
emersoni 2	*Ptilinopus pulchellus*	Papua New Guinea	Cosp.Ptpul.8.19.2003.11	126
emersoni 2	*Ptilinopus pulchellus*	Papua New Guinea	Cosp.Ptpul.8.19.2003.12	127
emersoni 3	*Ptilinopus tannensis*	Vanuatu	Cosp.Pttan.7.26.2004.6	128
malenkeae	*Ducula pacifica*	Vanuatu	Colon.1.27.2004.2	129
malenkeae	*Ducula pacifica*	Vanuatu	Cosp.Dupac.7.26.2004.7	130
claytoni	*Ducula rufigaster*	Papua New Guinea	Colon.8.19.2003.13	131
claytoni	*Ducula rufigaster*	Papua New Guinea	Cosp.Duruf.8.19.2003.14	132
paradoxus	*Lopholaimus antarcticus*	Australia	Cosp.Loant.1.27.2004.5	133
paradoxus	*Lopholaimus antarcticus*	Australia	Cosp.Loant.1.27.2004.6	134
wolffhuegeli	*Ducula bicolor*	Australia	Cosp.Dubic.1.8.2003.8	135

(*continued on next page*)

Appendix A (*continued*)

Columbicola species	Host	Country	Extract voucher code	Nos.
xavieri	*Ptilinopus occipitalis*	Philippines	Cosp.Ptocc.7.22.2004.11	136
xavieri	*Ptilinopus occipitalis*	Philippines	Coxav.7.1.1999.4	137
n. sp. 4	*Turtur brehmeri*	Ghana	Cosp.Tubre.3.21.2000.6	138
n. sp. 4	*Turtur brehmeri*	Ghana	Cosp.Tubre.7.27.2004.2	139
veigasimoni	*Phapitreron leucotis*	Philippines	Codeb.5.26.1999.3	140
gymnopeliae	*Metriopelia ceciliae*	Peru	Cogym.10.5.1999.12	141
gymnopeliae	*Metriopelia ceciliae*	Peru	Cosp.Mecec.7.27.2004.5	142
passerinae 1	*Columbina inca*	USA	Copsr.9.29.1998.6	143
passerinae 1	*Columbina picui*	Argentina	Cosp.Copic.1.8.2003.3	144
passerinae 1	*Columbina passerina*	Mexico	Copsr.9.29.1998.2	145
passerinae 1	*Uropelia campestris*	Bolivia	Cosp.Urcam.10.12.1999.5	146
passerinae 1	*Columbina passerina*	USA	Copsr.9.14.1999.7	147
passerinae 1	*Columbina picui*	Argentina	Cosp.Copic.1.20.2003.7	148
passerinae 2	*Columbina cruziana*	Peru	Cosp.Cocru.7.27.2004.4	149
passerinae 2	*Columbina buckleyi*	Peru	Cosp.Cobuc.7.27.2004.7	150
passerinae 2	*Claravis pretiosa*	Mexico	Copsr.9.29.1998.3	151
passerinae 2	*Claravis pretiosa*	Mexico	Cosp.Clpre.2.1.1999.6	152
drowni	*Metriopelia melanoptera*	Argentina	Cosp.Memel.1.8.2003.2	153
drowni	*Metriopelia aymara*	Argentina	Coalt.1.8.2003.4	154

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