CODISTRIBUTED LINEAGES OF FEATHER LICE SHOW DIFFERENT PHYLOGENETIC PATTERNS

A Dissertation

by

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ABSTRACT

Recent molecular phylogenies have suggested that hawks (Accipitridae) and falcons (Falconidae) form 2 distantly related groups within birds. Avian feather lice have often been used as a model for comparing host and parasite phylogenies, and in some cases there is significant congruence between them. Using 1 mitochondrial and 3 nuclear genes, I inferred a phylogeny for the feather louse genus *Degeeriella* (which are all obligate raptor ectoparasites) and related genera. This phylogeny indicated that *Degeeriella* is polyphyletic, with lice from falcons and hawks forming 2 distinct clades. Falcon lice were sister to lice from African woodpeckers, while *Capraiella*, a genus of lice from rollers lice, was embedded within *Degeeriella* from hawks. This phylogeny showed significant geographic structure, with host geography playing a larger role than host taxonomy in explaining louse phylogeny, particularly within clades of closely related lice. However, the louse phylogeny broadly reflects host phylogeny, for example *Accipiter* lice form a distinct clade.

Unlike most bird species, individual kingfisher species (Aves: Alcidae) are typically parasitized by 1 of 3 genera of lice (Insecta: Phthiraptera). These lice partition hosts by subfamily: *Alcedoecus* and *Emersoniella* parasitize Daceloninae whereas *Alcedoffula* parasitizes both Alcedininae and Cerylinae. While *Emersoniella* is geographically restricted, *Alcedoecus* and *Alcedoffula* are widespread. I used 2 molecular markers, the nuclear gene EF-1α and mitochondrial gene COI to infer phylogenies for both widespread genera of kingfisher lice, *Alcedoffula* and *Alcedoecus*. Additinally, I combined published host records with new host records reported here and used ancestral state reconstruction to identify patterns of host parasitism. Lastly, I compared louse phylogenies to host phylogenies to reconstruct their cophylogenetic history. I determined there are 2 distinct clades within

Alcedoffula, 1 infesting Alcedininae, and the other infesting Cerylinae. Ancestral state reconstruction of kingfisher lice across the kingfisher phylogeny showed *Alcedoecus* and *Emersoniella* parasitize distinct clades within the kingfisher subfamily Daceloninae, and a single host switch by *Alcedoecus* onto the portion of the Daceloninae clade, which typically hosts *Emersoniella*. Cophylogenetic analysis indicated that although *Alcedoecus* and the lineage of *Alcedoffula* occurring on Alcedininae did not show evidence of cospeciation, the lineage of *Alcedoffula* occurring on Cerylinae showed strong evidence of cospeciation.

The chewing louse genus *Colpocephalum* parasitizes nearly a dozen distantly related orders of birds. Such a broad host range is uncommon among lice. However, the monophyly of the genus *Colpocephalum* with respect to a group of morphologically similar genera has never been tested. Using 1 nuclear and 1 mitochondrial gene, I inferred a phylogeny for 54 lice sampled from across the *Colpocephalum*-complex. The resulting phylogeny demonstrates several lineages were restricted to single host orders. These lineages corresponded to previously described genera. Maddison-Slatkin tests were performed on the resulting phylogeny and showed that host order, host family, and biogeographic region had significant phylogenetic signals when mapped onto the *Colpocephalum*-complex phylogeny. A PARAFIT analysis comparing the overall *Colpocephalum*-complex phylogeny to a host phylogeny revealed significant congruence between host and parasite trees. I also compared the cophylogenetic history of *Colpocephalum* and their hosts to that of a second distantly related feather louse genus, *Degeeriella*, which also infests diurnal birds of prey. Using PARAFIT to identify individual host-parasite links that contributed to overall congruence, I found no evidence of correlated cophylogenetic patterns between these 2 lice groups, which suggested that their distribution patterns were shaped by divergent evolutionary processes.

DEDICATION

I dedicate this to the letter B, the letter all of my favorite things start with. In no particular order: baseball, beer, blue (the color), bbq, birds, bird dogs, beef, and big trucks.

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ago we were starting a journey that includes close encounters with the police, killer deer, and hopefully bungee jumping.

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All work conducted for the dissertation was completed by the student independently with the exception of louse identification which was performed by Michel Valim.

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CHAPTER I

INTRODUCTION

Feather lice (Insecta: Phthiraptera) are obligate avian ectoparasites that typically spend their entire life on their host. Most birds are infested with multiple genera of feather lice with different natural history characteristics, including dispersal capabilities and host defense avoidance strategies. The phenomenon of multiple, independent lineages occurring on the same host creates replicates of lice differing in 1 or more characteristics. Comparing phylogenies between groups of codistributed lice (e.g., tests of cophylogeny) allows identification of life history characters that can influence phylogenetic patterns. These characteristics make lice important models for understanding cophylogenetic history. Lice also have been used to test hypotheses about host-parasite codiversification, in which parasite evolutionary history mirrors the host group's history. Cospeciation studies typically focus on systems where parasite transmission is either via contact between parent and offspring host, or via contact between unrelated host individuals, such as during copulation. In both instances, transmission modes lead to louse transfer between individuals of the same species. However, a number of feather louse species are capable of dispersal via phoresy, a process by which the louse disperses by riding on on a winged fly (Keirans 1975; Harbison and Clayton 2011). Thus, phoresy can result in colonization of distantly related host species which may explain cases in which there is little congruence between host and parasite phylogenies (Johnson et al. 2002, Weckstein 2004). Phoresy may be common; e.g., a survey of 3 species of hippoboscid flies by Bennett (1961) found over 40% of flies had attached lice. However, successful host switching is expected to be uncommon as lice have low survival rates on novel host species (Clayton et al. 2003).

While there is considerable correspondence between orders/families of birds and generic host associations of lice within the *Degeeriella* and *Colpocephalum* complexes of lice, it appears that this is an artifact of traditional louse classification. Historically, louse taxonomy was highly influenced by host taxonomy, so that many currently recognized genera are not monophyletic in molecular phylogenies (Johnson et al. 2002). However, higher level host classification can be reflected in lice, as many currently unnamed lineages correspond to host groups (Johnson et al. 2002). In both *Colpocephalum* and *Degeeriella*, while individual clades are well supported (posterior probability $\geq$0.95), backbone support (the support for relationships between clades) was generally low (less than 0.80; Johnson et al. 2002; Johnson et al. 2003; Catanach and Johnson 2015). In my research, the addition of 2 new nuclear genes improved support for individual clades, but did little to improve backbone support in the *Degeeriella* complex. Based on these results, and other louse phylogenies, it is not expected that a small number of nuclear genes will improve support in the *Colpocephalum* complex (Pereyra, personal communication). Instead, future studies should employ a phylogenomic approach, an approach that has worked for other groups that had been difficult to resolve (Jarvis et al. 2014; Misof et al. 2014).

Lineages within the *Degeeriella* and *Colpocephalum* complexes, are exclusive to diurnal birds of prey (including hawks, eagles, and falcons). These codistributed lineages are ideal for studying the impacts of natural history traits, such as phoresy, on louse phylogeny. The effects of phoresis can be seen at both the microevolutionary level (with sympatric host species sharing the same species of feather louse), and the microevolutionary level, with multiple presumed intrafamilial and intraordinal host switching events (Johnson et al. 2002; Pereyra, personal communication). While diurnal birds of prey have long been treated as a

single order, recent avian molecular phylogenies suggest that falcons (Falconidae) are not closely related to other diurnal birds of prey (Accipitridae) (Hackett et al. 2008, Jarvis et al. 2014). This distinction also is reflected in their lice; *Degeeriella* from falcons form a monophyletic clade to the exclusion of *Degeeriella* from hawks (Catanach and Johnson 2015). While taxon sampling of the *Colpocephalum* complex is limited, there also is evidence for a distinct falconid louse lineage in this group (Price and Beer 1963b,c). Current phylogenies have limited taxon sampling with lice, with approximately 10% of raptor species included (Catanach and Johnson 2015; Catanach et al. accepted). Lice from diurnal birds of prey are ideal for studying phoresy because their hosts are solitary (except for breeding season when pairs are formed), while many other bird species are found in aggregations for all or part of the year. These aggregations often include multiple bird species which could allow for lice to be transmitted to novel species through direct contact or shared dust baths and roosts, rather than through phoresy. Additionally, distantly related, but similar sized raptors occur in most areas. As lice are thought to be more likely to colonize novel hosts which are similar in size to their typical hosts, this sets up scenarios where phoresy and successful colonization can occur.

Although most birds are infested with lice from different families, kingfishers are only parasitized by 3 genera of lice, all from a single family, Philopteridae. One of these philopterid genera, *Emersoniella*, is limited to Australasia, the other 2, *Alcedoffula* and *Alcedoecus*, are geographically widespread. The distribution patterns of these widespread genera also are unusual. Typically, bird species (and often even an individual bird) is infested with multiple genera. However, each kingfisher species, with rare exceptions, is parasitized by lice from a single genus. Based on published host records, *Alcedoecus*

parasitizes 1 kingfisher subfamily, while *Alcedoffula* occurs on the other 2 subfamilies. Additionally, patterns of parsasite distribution are unclear in the third subfamily which is parasitized by both *Alcedoecus* and *Emersoniella*.

RESEARCH OBJECTIVES

I will explore relationships within 4 genera of lice, 2 found on diurnal birds of prey and 2 found on kingfishers. The first 2 objectives of my dissertation focus on reconstructing the evolutionary histories for 2 louse genera infesting diurnal birds of prey, *Degeeriella* which are frequently found attached to hippoboscid flies (Diptera: Hippoboscidae) and the *Colpocephalum* complex, a group of feather lice genera parasitizing many of the same bird species as *Degeeriella*, but that does not disperse via hippoboscid flies. Patterns linked to phoretic behavior can be identified by comparing these 2 phylogenies. The third objective investigates relationships among the kingfisher lice and tests the resulting phylogenies for evidence of cospeciation with their kingfisher hosts.

CHAPTER II

INDEPENDENT ORIGINS OF THE FEATHER LICE (INSECTA: *DEGEERIELLA*) OF RAPTORS[1]

Insight into factors leading to the diversification of parasites can be gained from either comparing a parasite phylogeny directly with that of its hosts or by studying patterns of host association with respect to parasite phylogeny (Page, 2003, de Vienne et al. 2013). Several studies focusing on comparisons of host and parasite phylogenies (Johnson et al. 2003, Page et al. 2004, Hughes et al. 2007, Johnson et al. 2002, Weckstein 2004, Banks et al. 2006) or on phylogenetic patterns of host specificity (Johnson et al.2009; Johnson et al. 2011) and host association (Johnson et al. 2001) have involved feather lice. Feather lice (Insecta: Ischnocera: Philopteridae) are obligate ectoparasites of birds that complete their entire life cycle on their host. Transfer between host individuals typically requires direct contact, such as while rearing young or copulation. Dispersal opportunities between species of hosts are generally rare. However, dispersal by attaching to winged hippoboscid flies (phoresy) has been documented for some groups of feather lice (Clay and Meinertzhagen 1943; Keirans 1975). Although phoresy potentially results in lice dispersing to a novel species of host (Harbison and Clayton 2011), survival might be low on these novel hosts, potentially due to differences in feather morphology, which result in lice being more susceptible to host defense mechanisms such as preening (Clayton et al. 2003; Malenke et al. 2009).

The generally low dispersal ability of feather lice, combined with reduced survival on foreign hosts, results in the phylogeny of these parasites often reflecting host relationships, due to the process of cospeciation. However, the degree to which the phylogeny of lice

1. Reprinted with permission from "Independent origins of the feather lice (Insecta: *Degeeriella*) of raptors" by TA Catanach and KP Johnson, 2015. Biological Journal of the Linnaean Society. Copyright 2015 The Linnean Society of London

matches that of their hosts varies from strong phylogenetic congruence (Clayton and Johnson 2003; Hughes et al. 2007), to matching higher level groups of birds and lice (Johnson et al. 2001), to no significant congruence between host and parasite phylogenies (Johnson et al. 2002; Weckstein 2004; Banks et al. 2006). This diversity of patterns makes feather lice an important model system in studying the processes that influence codiversification of hosts and parasites. In general, there is considerable correspondence between the higher level classification of birds (e.g., orders and families) and the generic host associations of feather lice (Price et al. 2003). However, because traditional louse classification was heavily influenced by host taxonomy, these looser relationships could be an artifact of taxonomic practice, rather than a reflection of actual relatedness (Johnson et al. 2002). In addition, several orders of birds have recently been shown to be paraphyletic (Hackett et al. 2008), which further compounds any evaluation of congruence assessed from classification alone.

Raptors (all diurnal birds of prey including hawks, falcons, and eagles) have historically been placed a single order. However, recent molecular phylogenies have suggested that falcons (Falconidae) are distantly related to the other diurnal raptors (hawks, eagles, vultures, etc.), which are now placed together in a single group Accipitriformes, to the exclusion of falcons (Hackett et al. 2008, Jetz et al. 2012). One genus of parasitic feather louse, *Degeeriella*, curiously occurs on both hawks and falcons, but not on other groups of birds (Price et al. 2003). However, morphological and molecular evidence has brought into question the monophyly of *Degeeriella*. Clay (1958) suggested *Degeeriella fulva* (from hawks) and *Capraiella*, a genus of louse only recorded from rollers (Coraciidae, a family of birds unrelated to birds of prey), are closely related based on similarities in the male genitalia and head shape. Additionally, Dalgleish (1969) found evidence that *Degeeriella* from

falcons are morphologically similar to some Old World *Picicola* of woodpeckers. A molecular phylogeny (Johnson et al. 2002) of the *Degeeriella* complex (as defined by Clay 1958), which included only a single exemplar each of lice from falcons, hawks, and rollers, indicated some support for these relationships and polyphyly of *Degeeriella*. However, detailed assessment of this genus could not be made because of limited sampling.

Species delineation in *Degeeriella* also is potentially problematic. Currently, all *Degeeriella* from Falconidae (with the exception of *Degeeriella carruthi* from American kestrel [*Falco sparverius*]) are currently placed in a single species, *Degeeriella rufa*. Similarly, *Degeeriella fulva* is recorded from a variety of hawk and eagle species (Price et al. 2003; Gonzalez-Acuña et al. 2008). Phoresy is well documented in *Degeeriella* (Keirans 1975) and could result in a single parasite species found across a variety of hosts. However, studies of feather lice from pigeons and doves (Columbidae) have indicated that widespread taxa could in fact represent cryptic species, particularly in groups with a wide range of host sizes (Johnson et al. 2002; Malenke et al. 2009). Therefore it is unknown if taxa currently recognized as widespread species of *Degeeriella* are truly a single species or represent distinct evolutionary lineages.

Using sequences from one mitochondrial and 3 nuclear genes, I reconstructed the phylogeny of the louse genus *Degeeriella* and relatives by sampling lice widely from many of the major groups of diurnal birds of prey along with *Capraiella* from rollers and *Picicola* from woodpeckers. I include raptor lice from most continents to evaluate the degree of biogeographic structure in parasite phylogeny. In addition, I include multiple representatives of some host genera to evaluate in more detail phylogenetic patterns of host association, with multiple samples from the same louse species in some cases.

MATERIALS AND METHODS

Specimen Acquisition

Lice were collected from host birds in various ways including ethyl acetate fumigation (Clayton et al. 1992), dust ruffling (Walther and Clayton 1997), and manual searches of birds for lice from a variety of sources. A total of 58 specimens of *Degeeriella* from 37 host species were included, along with 5 *Capraiella* specimens from 5 host species (Table 2.1). *Degeeriella* were obtained from a wide variety of raptor groups including falcons, soaring hawks, forest hawks, sea eagles, booted eagles, kites, and harriers, and *Capraiella* was sampled from both described genera of rollers (Table 2.1). A single representative of *Acutifrons*, a morphologically similar genus recorded from caracaras (Falconidae), also was included. Additionally, other members of the *Degeeriella* complex (all from non-raptor hosts, including woodpeckers) included in the study by Johnson et al. (2002) were used as outgroups.

Sequencing

Lice were collected and stored in 95% ethanol at -70°C. The head and body were separated and placed together in digestion buffer. DNA was extracted from each specimen using a DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA) following a modified version of protocol for Total DNA from Animal Tissues. Modifications include lengthening the incubation period in step 2 to 36 hours and decreasing the amount of Buffer AE in step 7 to 50µ (which was repeated twice in different 1.5mL collection tubes). The head and body were removed from buffer and mounted on a microslide in balsam as a voucher.

Table 2.1. List of louse taxa and host data from which DNA was included in my study. An "X" represents successful sequencing.

Louse Species	Host Species	Host Order	Country	Extraction Code	COI	EF-1α	*hyp*	TMEDE6
Capraiella sp.	*Coracias abysinicus*	Coraciiformes	Ghana	Cbsp.Coaby.9.6.2012.11	x	x	x	x
Capraiella sp.	*Coracias caudata*	Coraciiformes	Malawi	Cbsp.Cocau.9.6.2012.3	x	x	-	x
Capraiella sp.	*Coracias spatula*	Coraciiformes	Malawi	Cbsp.Cospa.9.6.2012.4	-	x	x	x
Capraiella sp.	*Eurystomus orientalis*	Coraciiformes	Australia	Cbsp.Euori.9.6.2012.12	x	x	x	x
Capraiella sp.	*Eurystomus gularis*	Coraciiformes	Ghana	Cbsp.Eugul.4.3.2000.5	AF444852	AF447190	-	-
Degeeriella carruthi	*Falco sparverius*	Falconiformes	USA	Dgcar.Faspa.6.13.2012.6	x	x	x	x
Degeeriella carruthi	*Falco sparverius*	Falconiformes	USA	Dgcar.9.8.1999.7	AF444860	AF447196	x	-
Degeeriella frater	*Accipiter tachiro*	Accipitriformes	Malawi	Dgsp.Actac.9.6.2012.2	x	x	x	x
Degeeirella frafer	*Acciptier virgatus*	Accipitriformes	China	Dgsp.Acvir.11.2.2012.3	x	x	x	-
Degeeriella fulva	*Buteo augur*	Accipitriformes	Kenya	Dgsp.Buaug.5.24.2013.11	x	x	-	x
Degeeriella fulva	*Buteo jamaicensis*	Accipitriformes	USA	Dgsp.Bujam.6.4.2012.4	x	-	-	-
Degeeriella fulva	*Buteo jamaicensis*	Accipitriformes	Canada	Dgsp.Bujam.8.2.2013.1	x	-	-	-
Degeeriella fulva	*Buteo jamaicensis*	Accipitriformes	Canada	Dgsp.Bujam.8.2.2013.3	x	-	x	-
Degeeriella fulva	*Buteo jamaicensis*	Accipitriformes	USA	Dgsp.Bujam.9.6.2012.6	x	x	x	x
Degeeriella fulva	*Buteo lagopus*	Accipitriformes	Japan	Dgful.Bulag.12.3.2012.2	x	x	x	x
Degeeriella fulva	*Buteo lagopus*	Accipitriformes	Canada	Dgsp.Bulag.8.19.2013.10	x	-	-	-
Degeeriella fulva	*Buteo regalis*	Accipitriformes	USA	Dgsp.Bureg.5.24.2013.10	x	x	-	x
Degeeriella fulva	*Buteo regalis*	Accipitriformes	USA	Dgful.1.15.2000.5	AF444861	AF447197	x	x
Degeeriella fusca	*Circus assimilis*	Accipitriformes	Australia	Dgfus.Ciass.6.13.2012.2	x	x	x	x
Degeeriella fusca	*Circus cyaneus*	Accipitriformes	Canada	Dgsp.Cicya.8.2.2013.8	x	-	-	-
Degeeriella haydocki	*Accipiter minullus*	Accipitriformes	Mozambique	Dgsp.Acmin.9.6.2012.5	x	x	x	-
Degeeriella nisus	*Accipiter nisus*	Accipitriformes	Sweden	Dgnis.Acnis.12.3.2012.6	x	x	-	-
Degeeriella nisus	*Accipiter nisus*	Accipitriformes	Sweden	Dgnis.Acnis.12.3.2012.1	x	x	x	-
Degeeriella nisus	*Accipiter striatus*	Accipitriformes	USA	Dgnis.Acstr.6.4.2012.3	x	x	x	-
Degeeriella quatei	*Henicopernis longicauda*	Accipitriformes	Papua New Guinea	Dgqua.Helon.6.13.2012.1	-	x	-	x
Degeeriella regalis	*Buteo galapagoensis*	Accipitriformes	Galapagos	Dgreg.Bugal.6.13.2012.5	x	x	x	-
Degeeriella regalis	*Buteo galapagoensis*	Accipitriformes	Galapagos	Dgsp.Bugal.5.24.2013.5	-	x	x	x
Degeeriella regalis	*Haliastur sphenurus*	Accipitriformes	Australia	Dgsp.Hasph.11.2.2012.4	x	x	x	x
Degeeriella rima	*Kaupifalco monogrammicus*	Accipitriformes	Malawi	Dgsp.Kamon.9.6.2012.10	x	x	x	x
Degeeriella rufa	*Falco berigora*	Falconiformes	Australia	Dgruf.Faber.6.4.2012.1	x	x	x	x
Degeeriella rufa	*Falco cenchroides*	Falconiformes	Australia	Dgruf.Facen.6.4.2012.5	-	x	x	x
Degeeriella rufa	*Falco longipennis*	Falconiformes	Australia	Dgruf.Falon.6.4.2012.6	x	x	x	x
Degeeriella sp.	*Accipiter cirrocephalus*	Accipitriformes	Australia	Dgsp.Accir.6.13.2012.7	x	x	x	x
Degeeriella sp.	*Accipiter fasciatus*	Accipitriformes	Australia	Dgsp.Acfas.6.13.2012.3	x	-	x	x
Degeeriella sp.	*Accipiter francesii*	Accipitriformes	Madagascar	Dgsp.Acfra.6.4.2012.2	x	x	x	-
Degeeriella sp.	*Accipiter striatus*	Accipitriformes	Canada	Dgsp.Acstr.8.2.2013.11	x	-	x	-
Degeeriella sp.	*Aquila morphnoides*	Accipitriformes	Australia	Dgsp.Himor.11.2.2012.2	x	-	-	-
Degeeriella sp.	*Aquila wahlbergi*	Accipitriformes	Malawi	Dgsp.Aqwah.9.6.2012.9	x	-	x	-
Degeeriella sp.	*Buteo jamaicensis*	Accipitriformes	Canada	Dgful.Bujam.8.2.2013.6	x	-	-	-
Degeeriella sp.	*Buteo jamaicensis*	Accipitriformes	USA	Dgsp.Bujam.11.2.2012.5	x	-	-	x
Degeeriella sp.	*Buteo magnirostris*	Accipitriformes	Peru	Dgsp.Bumag.1.31.2014.11	x	x	-	x
Degeeriella sp.	*Buteo platypterus*	Accipitriformes	Panama	Dgsp.Bupla.6.4.2012.8	x	x	x	x
Degeeriella sp.	*Buteo swainsoni*	Accipitriformes	Canada	Dgsp.Buswa.1.31.2014.2	x	x	-	x

Table 2.1 Continued.

Louse Species	Host Species	Host Order	Country	Extraction Code	COI	EF-1α	*hyp*	TMEDE6
Degeeriella sp.	*Falco sparverius*	Falconiformes	Canada	Dgcar.Faspa.1.31.2014.1	x	x	-	x
Degeeriella sp.	*Falco sparverius*	Falconiformes	Canada	Dgsp.Faspa.2.21.2014.11	x	x	x	x
Degeeriella sp.	*Falco sparverius*	Falconiformes	Canada	Dgsp.Faspa.2.21.2014.12	x	x	x	-
Degeeriella sp.	*Falco sparverius*	Falconiformes	Canada	Dgsp.Faspa.2.21.2014.13	x	x	x	-
Degeeriella sp.	*Falco sparverius*	Falconiformes	Canada	Dgsp.Faspa.2.21.2014.14	x	x	x	-
Degeeriella sp.	*Falco sparverius*	Falconiformes	Canada	Dgsp.Faspa.2.21.2014.15	x	x	x	-
Degeeriella sp.	*Falco sparverius*	Falconiformes	Canada	Dgsp.Faspa.2.21.2014.16	x	x	x	-
Degeeriella sp.	*Falco sparverius*	Falconiformes	Canada	Dgsp.Faspa.8.2.2013.10	x	-	x	-
Degeeriella sp.	*Falco sparverius*	Falconiformes	Canada	Dgsp.Faspa.8.2.2013.16	x	-	-	-
Degeeriella sp.	*Haliaeetus leucocephalus*	Accipitriformes	Canada	Dgdis.Haleu.8.2.2013.5	x	-	x	-
Degeeriella sp.	*Haliaeetus pelagicus*	Accipitriformes	Japan	Dgsp.Hapel.12.3.2012.5	-	-	x	-
Degeeriella sp.	*Haliastur indus*	Accipitriformes	Australia	Dgsp.Haind.6.13.2012.4	x	x	x	-
Degeeriella sp.	*Henicopernis longicauda*	Accipitriformes	Papua New Guinea	Dgsp.Helon.11.2.2012.1	-	x	x	x
Degeeriella sp.	*Ictinia mississippiensis*	Accipitriformes	USA	Dgsp.Icmis.11.2.2012.6	x	x	-	x
Degeeriella sp.	*Ictinia mississippiensis*	Accipitriformes	USA	Dgsp.Icmis.6.4.2012.7	x	x	-	x
Degeeriella sp.	*Ictinia plumbea*	Accipitriformes	Brazil	Dgsp.Icplu.9.6.2012.8	x	x	x	-
Degeeriella sp.	*Leucopternis semiplumbeus*	Accipitriformes	Panama	Dgsp.Lesem.6.13.2012.8	x	x	x	x
Degeeriella sp.	*Pseudastur albicollis*	Accipitriformes	Brazil	Dgsp.Lealb.9.6.2012.7	x	x	x	-
Degeeriella vagans	*Accipiter cooperi*	Accipitriformes	USA	Dgsp.Accoo.9.6.2012.1	-	x	x	-
Degeeriella vagans	*Accipiter gentilis*	Accipitriformes	Sweden	Dgsp.Acgen.2.1.2013.1	x	x	-	x
Outgroup								
Acutifrons sp.	*Caracara cheriway*	Falconiformes	USA	Assp.Cache.5.24.2013.6	x	x	x	-
Austrophilopterus pacificus	*Andigena nigrirostris*	Piciformes	Peru	Appac.1.17.2000.8	AF444846	AF447184	-	x
Austrophilopterus sp.	*Selenidera gouldi*	Piciformes	Brazil	Apsp.Segou.1.17.2000.7	AF444848	AF447186	-	x
Austrophilopterus sp.	*Ramphastos brevis*	Piciformes	Ecuador	Apsp.Rabre.1.17.2000.6	AF444847	AF447185	x	x
Austrophilopterus subsimilis	*Ramphastos sulfuratus*	Piciformes	Mexico	Ausub.1.27.1999.12	AF444850	AF447188	x	-
Austrophilopterus torquatus	*Pteroglossus torquatus*	Piciformes	Mexico	Ausp.Pttor.1.27.1999.1	AF444849	AF447187	x	x
Buceromersonia sp.	*Tockus erythrorhynchus*	Coraciiformes	Tanzania	Bmsp.Toery.5.24.2013.9	x	x	-	x
Colinicola docophoroides	*Callipepla californica*	Galliformes	USA	Cxdoc.1.15.2000.1	AF444859	AF38666	x	x
Cotingacola sp.	*Querula purpurata*	Passeriformes	Brazil	Issp.Qupur.10.12.1999.12	AF444863	AF447198	-	x
Cotingacola stotzi	*Querula purpurata*	Passeriformes	Brazil	Cnsto.10.12.1999.11	AF444854	AF447192	-	x
Cuclotogaster hopkinsi	*Francolinus africanus*	Galliformes	South Africa	Cusp.Frafr.2.3.1999.11	AF444858	AF447195	-	x
Cuculicola atopus	*Piaya cayana*	Cuculiformes	Mexico	Cuato.1.27.1999.4	AF444856	AF447193	-	-
Cuculicola sp.	*Chrysococcyx klaas*	Cuculiformes	Ghana	Cusp.Chkla.4.3.2000.10	AF444857	AF447194	-	-
Picicola capitatus	*Dendropicos fuscescens*	Piciformes	South Africa	Picap.2.3.1999.10	AF444866	AF447201	x	x
Picicola porisma	*Colaptes auratus*	Piciformes	USA	Pipor.10.17.2000.5	AF444867	AF447202	x	x
Picicola snodgrassi	*Melanerpes carolinensis*	Piciformes	USA	Pisno.10.5.1999.8	AF444868	AF447203	-	-
Picicola sp.	*Chelidoptera tenebrosa*	Piciformes	Brazil	Pisp.Chten.1.17.2000.12	AF444869	AF447204	x	x
Picicola sp.	*Galbula albirostris*	Piciformes	Brazil	Pisp.Gaalb.1.17.2000.10	AF444870	AF447205	-	x
Picicola sp.	*Monasa nigrifrons*	Piciformes	Bolivia	Pisp.Monig.1.17.2000.3	AF444872	AF447207	x	x
Picicola sp.	*Nystalus chacuru*	Piciformes	Bolivia	Pisp.Nycha.1.17.2000.1	AF444873	AF447208	x	-
Picicola sp.	*Mesopicus pyrrhogaster*	Piciformes	Ghana	Pisp.Mepyr.4.11.2000.9	AF444871	AF447206	-	-
Rhynonirmus sp.	*Scolopax bukidnonensis*	Charadriiformes	Philippines	Rhsp.Scsp.7.14.1999.9	AF444875	AF447210	x	x
Trogoninirmus sp.	*Trogon melanocephalus*	Trogoniformes	Mexico	Trsp.Trmel.1.27.1999.3	AF444876	AF447211	x	-

After extraction, PCR was performed in 50 µL reactions to amplify 4 genes: 1 mitochondrial protein coding gene: cytochrome oxidase I (COI), and 3 nuclear protein coding genes: elongation factor-1α (EF-1α), hypothetical protein EOG9XHC5 (*hyp*), and transmembrane emp24 domain-containing protein 6 precursor (TMEDE6). Primers L6625 and H7005 (Hafner et al. 1994) were used for COI; Ef1-For3 and Ef1-Cho10 (Danforth and Ji 1998) were used for EF-1α, BR50-181L and BR50-621R (Sweet et al. 2014) were used for *hyp*, and BR69-190F and BR69-432R (Sweet et al. 2014) were used for TMEDE6. PCR conditions follow those for Sweet et al. (2014) with an annealing temperature of 46°C except for EF-1 α for which the annealing temperature was set at 50°C. Sequencing reactions were performed using 1µL of BigDye and then submitted for sequencing on an ABI 3730xl capillary machine at the University of Illinois Keck Center for Comparative and Functional Genomics. Raw forward and reverse strands of each sequence were aligned and assembled in Sequencher 4.8 (minimum match = 60, minimum overlap = 20) and manually adjusted. Each gene was then assembled into a single contig and exported to seaview 4.3.0 as a FASTA file. The built-in MUSCLE aligner in seaview was used to produce multiple alignments with all alignment settings at default values, followed, when necessary, by manual adjustments by eye (Edgar 2004; Gouy et al. 2010).

Sequence data for 1 sample, *Degeeriella rufa*, from brown falcon (*Falco berigora*), was assembled from a paired end Illumina run using the automated Target Restriction Assembly Method (aTRAM DOI: 10.5281/zenodo.10431) using sequences of each target gene from other falconid *Degeeriella* (Allen et al. 2015).

Analysis

Each gene was first analyzed separately to ensure that gene trees were not in conflict (posterior probability greater than 0.95). This included selecting an evolutionary model for each gene using modelgenerator with the model having the best AIC score selected (Keane et al. 2006). GTR + I + G was selected for COI, HKY + G was selected for EF-1α, GTR + G was selected for *hyp*, and TrN + G was selected for TMEDE6 (with HKY + G, which was the second best model, used in programs where TrN + G is not available). Gene trees were inferred using 40 million generation BEAST runs under the model selected by modelgenerator (Drummond and Rambaut 2007). Excluding the placement of specimens collected from American kestrel (*Falco sparverius*), for which the COI gene tree conflicted with gene trees from nuclear genes, trees inferred from individual genes did not include any well supported (posterior probability above 0.95) topological conflicts. Thus, gene sequences were concatenated for analysis. In the case of lice from American kestrels, these formed a monophyletic clade when individual nuclear gene trees were inferred. This clade was well supported (above 0.95 posterior probability) in EF-1α and TMEDE6 gene trees while the *hyp* gene tree had a posterior probability of 0.85 for this arrangement. However, the mitochondrial COI gene tree conflicted strongly with the nuclear gene trees. The COI gene supported 2 distinct clades (each with posterior probability of 1.0) containing American kestrel lice, one composed solely of lice from this host species while the other also contained lice from falcons other than American kestrel.

In the combined analysis, each gene was treated as a separate partition to allow for different models of evolution for each gene. Phylogenies based on all genes together were inferred using Bayesian methods (MrBayes: 20 million generations, nrun = 4, nchain = 4,

sampling every 1,000 generations, burnin = 5,000 samples and BEAST: 40 million generations, sampling every 1,000 generations, burnin = 10,000 samples; Huelsenbeck and Ronquist 2001, Ronquist and Huelsenbeck 2003, Drummond and Rambaut 2007), ML (garli: 10 independent runs, default settings, automated stop criterion = 50,000; Zwickl 2006), and MP (using PAUP*, 1000 random addition sequences with TBR branch swapping; Swofford 2003). Posterior probabilities (using BEAST), ML bootstrap values (using garli, 500 bootstap replicates on default settings with automated stop criterion = 5,000), and parsimony bootstrap values (using PAUP*, 1000 replicates of 100 random addition sequences with maxtrees set at 100 due to computational constraints) were used to evaluate branch support (Swofford 2003).

RESULTS

The tree for *Degeeriella* and relatives resulting from combined analyses of 3 nuclear and one mitochondrial gene was well resolved and generally highly supported (Fig. 2.1). *Degeeriella* was not monophyletic, instead being separated into 2 well-supported clades that included other genera (Fig. 2.1). *Degeeriella* from members of the Falconidae formed a monophyletic group (94 MP bootstrap, 99 ML bootstrap, 1.0 posterior probability) that was sister to some (but not all) representatives of the genus *Picicola*, a group of lice that parasitizes woodpeckers. This arrangement also results in *Picicola* being paraphyletic. All the *Degeeriella* from Accipitriformes (hawks, eagles, and their allies) together with the genus *Capraiella* from rollers (Coraciidae) formed a well-supported monophyletic group (83 MP bootstrap, 98 ML bootstrap, 1.0 posterior).

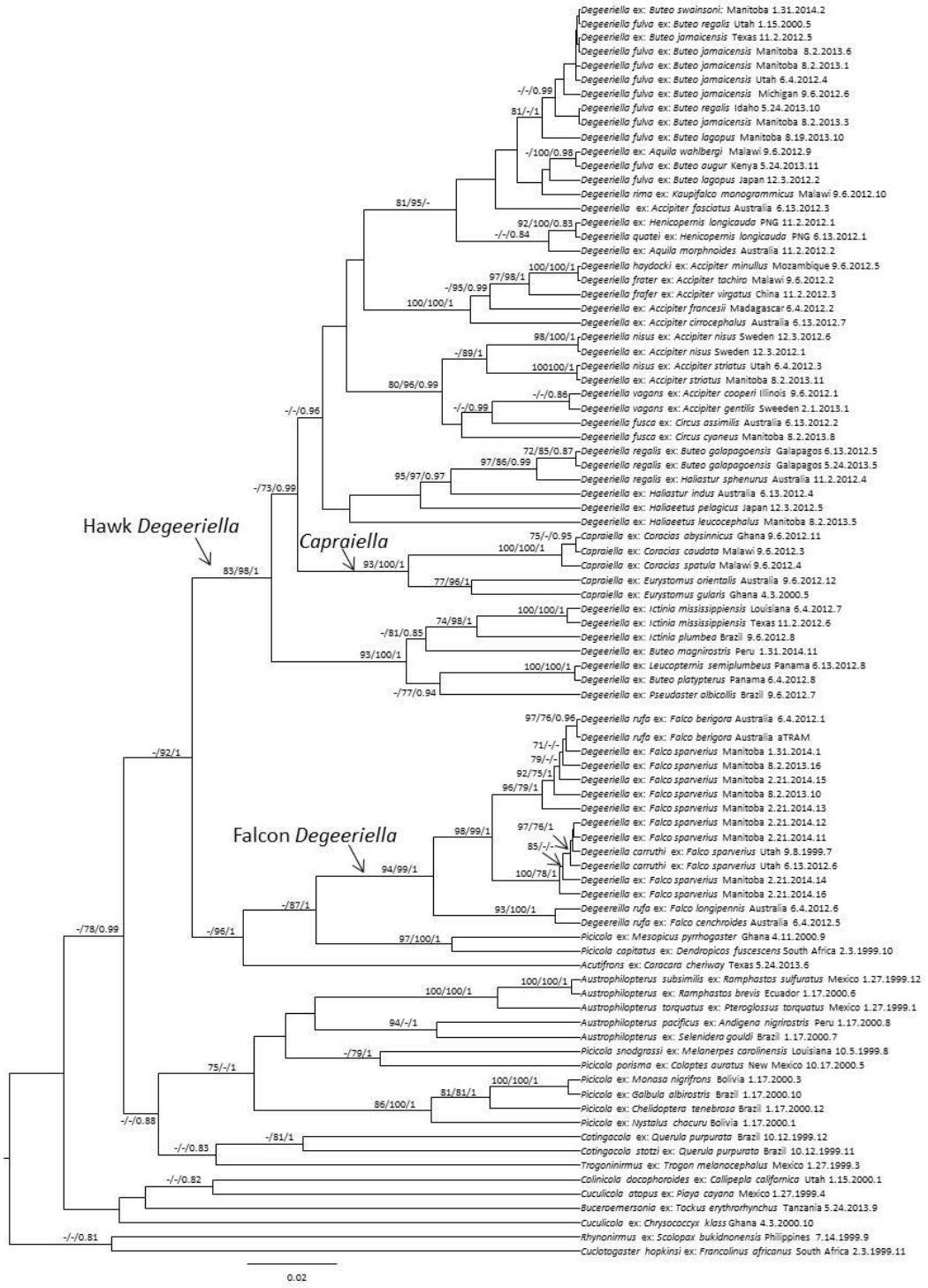

Figure 2.1. Phylogeny of *Degeeriella* and selected outgroups based on the results of the Bayesian Analysis after 20 million generations. Numbers on branches denote MP bootstrap/ML bootstrap/posterior probability. Cutoff for MP and ML bootstrap is 70 while cut off for posterior probabilities was set at 0.80. Note the hawk *Degeeriella* clade contains lice from a variety of accipitrid birds including hawks, eagles, and kites along with lice from rollers.

Within the *Degeeriella* complex recognized by Clay (1958) more broadly, the *Picicola* from African woodpeckers, *Capraiella*, *Acutifrons* (a genus of lice primarily from caracaras, Falconidae), and all *Degeeriella* comprised a well-supported monophyletic group (92 ML bootstrap, 1.0 posterior probability).

Considering first the lice of the Falconidae, the sole representative of *Acutifrons*, a *Degeeriella*-like genus from caracaras (a group of species within Falconidae that are placed in a different subfamily from the majority of falcons) was recovered as sister to a clade comprising the *Degeeriella* from falcons + *Picicola* from African woodpeckers (52 MP bootstrap, 96 ML bootstrap, 1.0 posterior probability; Fig. 2.1). *Degeeriella rufa* and *D. carruthi* are the only 2 species of lice recorded from the diverse falcon genus *Falco*, although *D. rufa* is not monophyletic with respect to *D. carruthi*. Surprisingly, for the mitochondrial COI gene tree, 2 genetically distinct and distantly related *Degeeriella* were found on American kestrels, which previously had been known to host only *D. carruthi*, and this result also appears in the combined analysis. Some of the specimens of lice from American kestrels grouped with *D. rufa*, while others formed a distinct clade containing only lice from American kestrels. This could explain Clay's (1958) observation that some specimens from American kestrel have head morphology more similar to *D. rufa*. This species has been treated by some authorities as a subspecies of *D. rufa* (which has a high degree of morphological variation), although many (but not all) specimens of *D. carruthi* have different head morphology from *D. rufa* (Clay 1958). However, because the nuclear gene trees strongly conflicted with this result, mitochondrial introgression could also explain these results. COI divergence ranged from 13 to 17% between the 2 clades of lice from American kestrels, but was less than 3% among members of the same clade. The results from

mitochondrial COI conflicted with all nuclear gene trees, which placed all lice from American kestrel in a single clade, which was typically well-supported. Although *Degeeriella* species have traditionally been defined based on host associations (and often specimen identification is based on the host species), there are instances where multiple *Degeeriella* species have been found on a single host species (Mey 1997; Price et al. 2003). Since raptors are sparsely sampled and lice identifications have often been based on host records rather than morphological examination, it is possible that it is not uncommon for a bird species to host multiple *Degeeriella* species.

Among the *Degeeriella* of hawks (Accipitriformes), clades tended to be structured by both geography and host taxonomy. The earliest diverging clade in the group includes lice from a variety of kite and hawk species that are all Neotropical residents or migrants to the Neotropics. The genus *Capraiella*, from rollers, is then sister to the remaining *Degeeriella* from Accipitriformes (93 MP bootstrap, 96 ML bootstrap, 0.99 posterior probability). Resolution among the other major lineages in this group is relatively poor. However, the *Degeeriella* of northern hemisphere *Accipiter* and *Circus* form a group (80 MP bootstrap, 0.99 posterior probability) as do the *Degeeriella* of southern hemisphere *Accipiter* (100 bootstrap, 100 ML bootstrap, 1.0 posterior probability).

In some cases lice collected from the same host species do not form monophyletic groups, although this could be an example of geographic sub-structure in the case of the 2 *Degeeriella fulva* specimens from rough-legged hawk (*Buteo lagopus*) since 1 host was sampled from North America and the other from Asia. While both *Degeeriella fulva* and *D. regalis* have been recorded from red-tailed hawks (*Buteo jamaicensis*) (and a few other raptor species), all samples from red-tailed hawks had a COI pairwise distance no greater

than 1.3%. This low divergence suggests I had only sampled 1 species (*D. fulva*) from red-tailed hawks, and this result was consistent with specimens for which morphological species determinations could be made.

In some of the cases in which lice from the same host species do not form a monophyletic group, lice from the same geographic region tend to be more closely related to each other regardless of host taxonomy. For example, a clade of closely related lice from red-tailed hawk, ferruginous hawk (*Buteo regalis*), and Swainson's hawk (*Buteo swainsoni*) from western North America are virtually identical in their COI sequences, while the COI sequence from a red-tailed hawk from eastern North America had a pairwise distance of 1.3% from the western North America specimens. Geographic structuring of the *Degeeriella* phylogeny also occurs for host species that occur throughout the Holarctic, such as the rough-legged hawk. Lice from rough-legged hawks in North America are in the North American clade previously mentioned, while those from Eurasia are in a distinct Old World clade. Phoresis on hippoboscid flies is known for *Degeeriella*, which could explain how birds in a given geographic region could share lice.

DISCUSSION

A phylogeny based on 1 mitochondrial and 3 nuclear genes for the feather louse genus *Degeeriella* agrees with the assessment of relationships based on morphology by Clay (1958) and Dalgleish (1969). Clay (1958) suggested the *Degeeriella* from falcons are closely related to *Picicola* from African woodpeckers, while the *Degeeriella* from hawks are more closely related to *Capraiella* from rollers. These results extend the conclusions of Johnson et al. (2002) by more densely sampling within *Degeeriella*, confirming the existence of only 2 distinct clades, but also that *Degeeriella* as currently defined is paraphyletic. With this

denser taxon sample, I find that roller lice (*Capraiella*) are in embedded within *Degeeriella* from hawks, although *Capraiella* does form a monophyletic group. Lice from the 2 genera of rollers, *Coracias* and *Eurystomus*, form 2 distinct subclades within *Capraiella*.

No prior molecular phylogenetic study has included *Acutifrons*, a louse genus found on caracaras. Here, I find it to be sister to the clade comprising lice from African woodpeckers and falcons. Given that caracaras are the sister taxon of true falcons (Fuchs et al. 2012), I interpret a host switch occurred from Falconidae to woodpeckers. However, additional taxon sampling is required to determine if *Acutifrons* is monophyletic with respect to *Degeeriella*. Similarly, the genus *Capraiella* is placed within the hawk *Degeeriella* clade and the most parsimonious explanation would be that host switch occurred from an accipitriform to a roller. However, further taxon sampling is again required to further refine understanding of the direction of the host switch. In both instances, a clade of lice from non-raptorial birds is embedded within a clade of raptorial birds. If a host switch by lice from predators to prey occurred, this would conflict with the hypothesis that lice would transfer from prey to predator as lice attempted to flee a dead host (Clay 1949; Whiteman et al. 2004). Instead the phylogenetic arrangement suggests some other method of host switching could be responsible, such as phoresy. This interpretation, however, relies on the assumption of equally weighting host-switches from predators to prey as from prey to predators. Another possibility is that lice switched from prey to raptors twice in each clade, although it is a less parsimonious interpretation.

When possible, lice were identified to species. Some specimens could not be conclusively identified because they were nymphs or not the right sex for species identification. With respect to previous taxonomic arrangements in *Degeeriella*, Clay (1958)

divided members of *Degeeriella* into 7 species groups, the most diverse being the *fulva* group. My topology supports this group with specimens of *D. fulva, D. rima, D. nisus, D. vagans, D. frater, D. haydocki*, and *D. fusca* forming a clade. Additionally, Elbel and Price (1973) described *D. quateri* and placed it within the *fulva* group. My analysis also supports this placement. Clay treated *D. vagans, D. frater*, and *D. haydocki* as subspecies of *D. nisus*, all of which are included in my phylogeny. While my topology places *D. haydocki* and *D. frater* as sister species, *D. nisus* and *D. vagans* are placed in a different clade (which also contains *D. fusca*). I also sampled multiple representatives of the *rufa* group and also found it to be well supported by my phylogeny. Testing the remaining species groups will require additional taxon sampling.

Interesting phylogenetic patterns of host association also emerge at lower taxonomic levels. The earliest diverging clade of *Degeeriella* from hawks includes lice from a wide range of hosts including 2 species of *Ictinia* kites and 3 hawks. While these hosts are not closely related, they are all either residents of the Neotropics or Neotropical migrants and similar in size. Other clades of *Degeeriella* occurring on hawks are also structured by both geography and body size. *Degeeriella* from large North American soaring hawks (including red-tailed hawk, ferruginous hawk, Swainson's hawk, and the North American exemplar of rough-legged hawk) all form a single, well-supported clade, which is sister to a group of large African or Euro-African migrants including the Old World exemplar of rough-legged hawk, Augur buzzard (*Buteo augur*), and Wahlberg's eagle (*Aquila wahlbergi*; although this group lacks support in analyses). Additionally, lice from 5 small (between 75 and 380 g) *Accipiter* species from Africa, southern Asian, and Australia, form a well-supported clade. A correlated relationship between host and parasite body size (known as Harrison's rule;

Harrison 1915) is well documented for a wide variety of feather lice (Clayton et al. 2003; Johnson, Bush and Clayton 2005; Tryjanowski et al. 2007; Malenke et al. 2009; Yamagishi et al. 2014) and may explain some of these patterns of host association. A second clade of *Accipiter* lice includes hosts from the Holarctic region plus 2 species of *Circus*. Wink and Sauer-Gurth (2004) recovered a sister relationship between *Circus* and *Accipiter* which might explain the closely phylogenetic relationship of their lice. This division within the *Degeeriella* of *Accipiter* also reflects host relationships recovered by Breman et al. (2013), who placed all of the host species included in the African/Asian/Australian clade in as sister to a group of all hosts from the Holarctic clade of *Accipiter* lice. Within the Holarctic clade, lice from sharp-shinned hawk (*Accipiter striatus*) and Eurasian sparrowhawk (*Accipiter nisus*) (2 specimens of each) were sister taxa congruent with the proposed close relationship between these host taxa (Wink and Sauer-Gurth 2004; Breman et al. 2013). Lice from the Brown Goshawk (*Accipiter fasciatus*), was placed outside of these clades, and instead placed as the sister to the large hawk clade although this placement was weakly supported. This Australian accipiter (weighing over 500 g), is much larger than the other accipiters sampled in this region. Further sampling of *Degeeriella* from *Accipiter* species in southeast Asia and Australia is required to further resolve these patterns.

When possible, I included multiple individuals of lice from a single host species. While lice from the same host species usually formed monophyletic clades, there were several examples where this was not the case. Most notable were lice from the rough-legged hawk. This species has a Holarctic distribution and both an Old World and New World sample was included in my study. The Old World specimen fell within the clade of lice from large hawks from the Old World and the New World specimen fell within the clade of lice

from large hawks in the New World (pairwise distance for COI is 8.7%). These relationships suggest that host geography can be as important in structuring louse phylogeny as host phylogeny, at least at the fine scale. Johnson et al. (2001) found similar levels of COI species-level divergence within other ischnoceran lice. This pattern also is supported by the relationships between lice collected from the red-tailed hawk, Swainson's hawk, and ferruginous hawk when looking only at COI. Lice from these species in flyways west of the Mississippi River are genetically nearly identical (pairwise distances for COI are all 0.0%), while a red-tailed hawk louse from east of the Mississippi is more divergent (pairwise distance for COI is 1.3% from the other members of this clade). Further sampling of other large raptor species in this flyway are needed to determine if this is an example of flyway homogenization, where birds in a given flyway share closely related lice. Some evidence of flyway homogenization was found for the lice of small sandpipers and stints (Scolopacidae), but not in lice of large sandpipers (Gustafsson and Olsson 2011). Interestingly, they also found no evidence of flyway differentiation of lice, whereas I found that lice from Old and New World rough-legged hawks were genetically differentiated into geographically structured clades.

In another case, 11 lice from American kestrel (from which only *Degeeriella carruthi* is recorded) were included in my study, 2 from the western US (from the same host individual) and 9 from central Canada (from 3 different individuals). The western US lice, along with half the Canadian lice formed a clade, while the remaining Canadian samples did not. These remaining Canadian samples were placed as more closely related to *Degeeriella* from brown falcon, but did not themselves form a clade. Additional taxon sampling from the host genus *Falco* is needed. This, along with the placement of lice from Australian Hobby

and Australian Kestrel as distinct from lice from brown falcon suggest *Degeeriella rufa* might contain multiple cryptic species and American kestrels may be host to more than one species of *Degeeriella*.

CHAPTER III

RELATIONSHIPS WITHIN THE ISCHNOCERAN LICE (INSECTA: PHTHIRAPTERA) OF KINGFISHERS (CORACIIFORMES: ALCEDINIDAE)

Permanent parasites are not only reliant on a host (or hosts) to complete all life stages, but live their entire life on a given host. At the extreme end of obligate parasitism are parasitic lice, which have adapted to survive only within the microclimatic conditions provided by their host's body and typically die within hours or days after becoming separated from the host (Price et al. 2003). This typically limits dispersal opportunities to direct physical contact between individuals during copulation or between parents and offspring during brooding. Over macroevolutionary time scales this lack of dispersal opportunities also limits the abilities of most lice to switch to novel host species. For some chewing lice parasitizing birds, switches to novel host species could occur via phoresy (lice attaching to hippoboscid flies which are winged generalist parasites), via takeover of nest cavities, or via physical contact during intraspecific territorial disputes (Clayton 1990; Harbison and Clayton 2011). However, survival on novel hosts is thought to be low, potentially due to difficulties overcoming novel host defenses (Clayton et al. 2003; Malenke et al. 2009).

If parasites are mainly transmitted vertically via close contact between conspecifics, populations of parasites on different host taxa can differentiate over time to form host specific lineages. If this happens in conjunction with the hosts themselves speciating then the phylogenies of both host and parasite would be largely congruent. However, if lice colonized a group of hosts after the hosts diverged, or if horizontal transfer of lice between different host taxa is common, then host and parasite phylogenies would differ. These 2 different patterns of cophylogenetic history are ends of a continuum exhibited by lice, which

vary both in terms of host specificity and the degree of cospeciation with their hosts. For example, *Pectinopygus* lice and their Pelecaniform hosts show strong evidence of cospeciation, whereas louse genera within the *Degeeriella* complex match higher-level classifications of their hosts, but toucan lice within the *Degeeriella* complex show no evidence of cospeciation with their hosts (Hughes et al. 2007; Weckstein 2004; Johnson et al. 2001). Different louse genera codistributed on the same host group often show differing patterns of host specificity and cophylogenetic history. For example, dove (Columbidae) body lice show evidence of cospeciation whereas dove wing lice do not (Clayton and Johnson 2003).

Kingfishers (Alcedinidae) include 117 species divided into 3 subfamilies: Daceloninae, Alcedininae, and Cerylinae. Daceloninae and Alcedininae are limited to the Old World, and Cerylinae occurs worldwide. The monophyly of each subfamily is strongly supported by morphological and molecular characters with Alcedininae as sister to Cerylinae + Daceloninae (Maurer and Raikow 1981; Johansson and Ericson 2003; Moyle 2006). The cosmopolitan distribution (New and Old World) of Cerylinae is likely the result of 2 New World invasions (Moyle 2006). Daceloninae is mainly restricted to Australia and southern Asia, with a single genus, *Halcyon*, also occurring in Africa. Alcedininae is widespread across the Old World. Moyle (2006) and Moyle et al. (2007) found the majority of kingfisher genera were not monophyletic resulting in a substantial taxonomic reorganization. Furthermore, species level relationships and species limits within the kingfishers are also in a state of flux, for example 26 new species have been recognized since 2013, mostly due to molecular studies supporting the elevation of island subspecies to full species status (Andersen et al. 2013; 2015).

Kingfishers are known to have 3 louse genera, *Alcedoffula*, *Alcedoecus*, and *Emersoniella*, all chewing lice within the family Philopteridae (Price et al. 2003 Johnson et al. 2012). Although many bird species are host to multiple genera of lice, kingfishers are typically only infected with a single louse genus and each kingfisher louse genus is specific to one or more kingfisher subfamilies. In the majority of instances where a kingfisher species is parasitized by 2 louse species, one is a species of *Alcedoecus* and the other a species of *Emersoniella*. Both *Alcedoecus* and *Emersoniella* are limited to Daceloninae kingfishers although *Emersoniella* is uncommonly encountered and with 7 described species one of the smallest genera of chewing lice. While *Emersoniella* is only known from Indo-Pacific kingfishers, both *Alcedoecus* (limited to Daceloninae) and *Alcedoffula* (found on the other 2 subfamilies) are geographically widespread. Although lice are known from 54 (46%) of currently recognized kingfisher species (Price et al 2003; personal records) there are only 2 instances where both *Alcedoffula* and *Alcedoecus* have been collected from the same kingfisher species, and 2 instances where multiple louse species from the same genus are known from the same host species. Here I used 2 markers (1 mitochondrial and 1 nuclear) to infer phylogenies for both widespread genera of kingfisher lice, *Alcedoffula* and *Alcedoecus*. Lastly, I compared the louse phylogenies with a molecular phylogeny of the kingfishers to reconstruct their cophylogenetic history.

MATERIALS AND METHODS

Specimen Acquisition

Lice were collected from host birds in various ways, including ethyl acetate fumigation and dust ruffling (Clayton et. al. 1992; Walther and Clayton 1997). Lice were stored in 95% ethanol at −70 °C until sequencing. In total, 47 kingfisher lice were sequenced from 11 of the 19 currently recognized genera of kingfishers (Table 3.1). When possible, lice

were sequenced from multiple host individuals (up to 4 specimens per host taxon), particularly in cases of geographically widespread host species or island populations. Additionally, 35 lice from various species were used as outgroup taxa (Table 3.1).

Parasite DNA Sequencing

DNA was extracted from specimens by creating a small incision between the head and thorax and a second incision between 2 abdominal sclerites then placing the specimen in digestion buffer. A QIAamp DNA Micro Kit (Qiagen, Valencia, CA) was used for DNA extractions using a modified version of protocol for total genomic DNA from tissues. Modifications include lengthening the incubation period in step to 36 hours for step 4, incubating the sample for 10 minutes at 70 °C for step 6, and decreasing the amount of Buffer AE to 50µ (which was repeated twice in different 1.5mL collection tubes) for step 12. During step 12 the Buffer AE is incubated for 5 minutes at 70 °C prior to centrifuging rather than performing step 13. After digestion, louse exoskeletons were retained, cleared, and mounted on a microslide in balsam as a voucher following the protocols of Palma (1978). After extraction, PCR was performed in 25µL reactions to amplify 2 genes, the mitochondrial protein coding gene cytochrome oxidase I (COI) and the nuclear protein coding gene elongation factor-1α (EF-1α). Primers L6625 and H7005 (Hafner et al. 1994) were used for COI and Ef1-For3 and Ef1-Cho10 (Danforth and Ji 1998) were used for EF-1α. PCR conditions follow those for Smith et al. (2004) except an annealing temperature of 50°C was used for EF-1α. Sequencing reactions were performed using 1µL of BigDye and then submitted for sequencing on an ABI 3730xl capillary machine at the University of Illinois Keck Center for Comparative and Functional Genomics.

Table 3.1. List of louse taxa and host data from which DNA was included in my study. An "X" denotes successful DNA sequencing of a given gene.

Genus	Code	Host Species	Country	COI	EF1a
Alcedoecus orientalis	Alori.1.16.2001.7	*Ceyx erithaca*	Borneo		X
Alcedoecus sp.	Alsp.Chama.1.16.2001.10	*Chloroceryle amazona*	Peru	x	X
Alcedoecus sp.	Alsp.Alcri.1.16.2001.12	*Corythornis cristatus*	Ghana	x	X
Alcedoecus sp.	Alsp.Alleu.1.16.2001.9	*Corythornis leucogaster*	Uganda	x	X
Alcedoecus sp.	Issp.Dalea.10.16.2002.11	*Dacelo leachii*	Australia	x	X
Alcedoecus sp.	Alsp.Danov.8.27.2014.3	*Dacelo novaeguineae*	Australia	x	X
Alcedoecus sp.	Alsp.Haalb.7.1.2014.6	*Halcyon albiventris*	Malawi		X
Alcedoecus sp.	Alsp.Haalb.7.1.2014.12	*Halcyon albiventris orientalis*	Malawi	x	X
Alcedoecus sp.	Alsp.Habad.8.27.2014.5	*Halcyon badia*	Ghana	x	X
Alcedoecus sp.	Alsp.Hache.7.1.2014.16	*Halcyon chelicuti*	Malawi	x	X
Alcedoecus sp.	Alsp.Hacor.7.1.2014.11	*Halcyon coromanda*	Malaysia	x	X
Alcedoecus sp.	Alsp.Hacor.7.1.2014.10	*Halcyon coromanda*	Philippines	x	X
Alcedoecus sp.	Alsp.Hamel.4.3.2000.3	*Halcyon malimbica*	Ghana	x	X
Alcedoecus sp.	Alsp.Hamal.1.16.2001.11	*Halcyon malimbica*	Ghana	x	x
Alcedoecus sp.	Alsp.Hasen.8.27.2014.6	*Halcyon senegalensis*	Ghana	x	x
Alcedoecus sp.	Alsp.Hasen.8.27.2014.11	*Halcyon senegalensis*	Ghana	x	x
Alcedoecus sp.	Alsp.Hasen.7.1.2014.14	*Halcyon senegalensis cyanoleuca*	Malawi	x	
Alcedoecus sp.	Alann.Hasmy.CT091	*Halcyon smyrnensis*	Vietnam	x	
Alcedoecus sp.	Mealc.1.16.2001.8	*Megaceryle alcyon*	Louisana	x	x
Alcedoecus sp.	Alsp.Cetor.8.12.2014.1	*Megaceryle torquata*	Peru	x	x
Alcedoecus sp.	Alsp.Hachl.7.1.2014.4	*Todiramphus sacer*	Solomon Islands	X	x
Alcedoecus sp.	Alsp.Tochl.8.12.2014.6	*Todiramphus sordidus*	Australia (northern)	X	x
Alcedoecus sp.	Alsp.Tosan.8.27.2014.4	*Todiramphus sanctus*	Australia (western)	x	x
Alcedoffula alcyonae	Afalc.Mealc.8.12.2014.7	*Megaceryle alcyon*	Canada	x	x
Alcedoffula duplicata	Afdup.Cerud.4.3.2000.4	*Ceryle rudis*	Ghana	x	
Alcedoffula duplicata	Afdup.3.16.2001.10	*Ceryle rudis*	Ghana	x	x
Alcedoffula sp.	Afsp.Alsem.7.1.2014.5	*Alcedo semitorquata*	Malawi	x	
Alcedoffula sp.	Afsp.Alazu.8.27.2014.7	*Ceyx azureus*	Australia	x	x
Alcedoffula sp.	Afsp.Ceeri.8.27.2014.8	*Ceyx erithaca*	Malaysia	x	x
Alcedoffula sp.	Afsp.Ceeri.7.1.2014.1	*Ceyx erithaca*	Malaysia		x
Alcedoffula sp.	Afsp.Ceruf.7.1.2014.9	*Ceyx rufidorsa*	Malaysia		x
Alcedoffula sp.	Afsp.Chame.8.27.2014.9	*Chloroceryle americana*	Panama	x	x
Alcedoffula sp.	Afsp.Chame.7.18.2014.3	*Chloroceryle americana*	Peru	x	
Alcedoffula sp.	Alsp.Chind.8.12.2014.2	*Chloroceryle inda*	Peru	x	x
Alcedoffula sp.	Afsp.Alleu.7.18.2014.4	*Corythornis leucogaster*	DRC	x	x
Alcedoffula sp.	Afsp.Alleu.3.16.2001.11	*Corythornis leucogaster*	Uganda	x	x
Alcedoffula sp.	Afsp.Coleu.8.27.2014.2	*Corythornis leucogaster*	Uganda	x	x
Alcedoffula sp.	Afsp.Ismad.8.12.2014.3	*Corythornis madagascariensis*	Madagascar	x	x
Alcedoffula sp.	Afsp.Alcri.8.12.2014.4	*Corythornis vintsioides*	Madagascar	x	x

Table 3.1. Continued

Genus	Code	Host Species	Country	COI	EF1a
Alcedoffula sp.	Afsp.Ispic.8.27.2014.10	*Ispidina picta*	DRC	x	x
Craspedorhynchus hirsuts	Cfhir.1.15.2000.6	*Buteo regalis*	USA	x	x
Emersoniella braeteata	Embra.2.4.2002.11	*Dacelo novaeguinnea*	NSW Australia	x	x
Emersoniella sp.	Alsp.Tasyl.8.12.2014.5	*Tanysiptera sylvia*	Australia	x	x
Icidifrons transpositus	Intra.1.15.2000.9	*Fulica americana*	USA	x	x
Lunaceps actophilus	Issp.Caalb.1.15.2000.7	*Calidris alba*	USA	x	x
Quadraceps aethereus	Quaet.11.22.2001.4	*Aethia pusilla*	Buldir Is, AK	x	x
Quadraceps impar	Quimp.3.16.2001.7	*Heteroscelus brevipes*	Australia	x	x
Quadraceps punctatus	Qupun.3.24.2001.8	*Larus californica*	Utah	x	
Quadraceps puntatus	Qupun.2.3.1999.2	*Larus cirrocephalus*		x	x
Quadraceps quadrisetaceus	Ququa.4.11.2000.5	*Rostratula benghalensis*	Ghana	x	x
Quadraceps sp.	Qusp.Aecri.11.22.2001.2	*Aethia cristatella*	Buldir Is, AK		x
Quadraceps sp.	Qusp.Esmag.1.9.2001.6	*Esacus magnirostris*	Australia	x	x
Quadraceps sp.	Qusp.Haful.3.16.2001.8	*Haematopus fuliginosus*	Australia	x	x
Quadraceps sp.	Qusp.Hihim.3.24.2001.6	*Himantopus himantopus*	Australia	x	x
Quadraceps sp.	Qusp.Himex.3.16.2001.9	*Himantopus mexicanus*	Louisana	x	x
Quadraceps sp.	Qusp.Renov.3.24.2001.5	*Recurvirostra novaehollandiae*	Australia	x	
Quadraceps sp.	Qusp.Stisa.10.16.2002.12	*Stiltia isabella*	Australia	x	
Quadraceps strepsilaris	Qustr.3.16.2001.13	*Arenaria interpes*	Australia	x	x
Quadraceps zephyra	Quzep.4.11.2000.11	*Recurvirostra americana*	Utah	x	x
Rallicola sp.	Rasp.Arcaj.3.29.1999.2	*Aramides cajanea*		x	x
Rallicola sp.	Raad.1.3.2011.11	*Fulica americana*	Illinois	x	x
Rallicola sp.	Rasp.Apsp.3.3.2011.4	*Apteryx sp.*	New Zealand	x	x
Saemundssonia haematopi	Sahae.1.9.2001.7	*Haematopus ostralegus*	Australia	x	x
Saemundssonia lari	Salar.4.7.1999.12	*Larus cirrocephalus*		x	x
Saemundssonia sp.	Sasp.Aepus.11.22.2001.5	*Aethia pusilla*	Buldir Is, AK	x	x
Saemundssonia sp.	Sasp.Aepyg.2.4.2002.8	*Aethia pygmaea*	Alaska	x	
Saemundssonia sp.	Sasp.Scsp.7.14.1999.8	*Scolopax*		x	x
Saemundssonia wumisuzume	Sawum.11.22.2001.3	*Aethia cristatella*	Buldir Is, AK	x	x
Saemundssonia wumisuzume	Sawum.11.22.2001.7	*Aethia cristatella*	St. Lawrence, AK	x	
Strigiphilus sp.	Stcru.Otgua	*Otus guatemalae*	Mexico	x	x
Unknown Ischnocera	Issp.Reame.4.11.2000.10	*Recurvirostra americana*	Utah	x	x
Unknown Ischnocera	Issp.Trsub.9.27.2000.7	*Tryngites subruficollis*	Louisana	x	x

Raw forward and reverse strands of each sequence were assembled into contigs in Geneious 8.0.4 (Biomatters Ltd.) and manually adjusted to produce consensus sequences. The resulting consensus sequences were aligned in Geneious using the MUSCLE plugin and

exported to Seaview 4.3.0 where they were checked and adjusted by eye (Edgar 2004; Gouy et al. 2010).

Host DNA Sequencing

Not all host species or subspecies for which I had sampled lice were included in existing kingfisher phylogenies (Moyle 2006; Moyle et al. 2007). Thus to conduct a cophylogenetic analysis with all parasite terminal taxa I needed to acquire sequences for some additional host species or subspecies. For a few host species, *Halycon coromanda* and *H. smyrensis*, DNA sequences from portions of the mitochondrial nicotinamide adenine dinucleotide dehydrogenase subunit 2 (ND2) and nuclear recombination activating gene (RAG-1) genes were available on GenBank. For 4 other host taxa, *H. chelicuti*, *H. albiventris orientalis*, *H. senegalensis cyansleuca*, and *Ispidina picta natalensis*, I extracted DNA from tissues, amplified ND2 and RAG-1 genes, and then sanger sequenced the resulting PCR products (Table 3.2). Host DNA was extracted using a DNeasy Blood and Tissue Kit following the manufactures protocols for tissue samples (Qiagen, Valencia, CA). After extraction, PCR was performed in 25µL reactions to amplify the ND2 and RAG-1 genes. For ND2 amplifications, I used primers L5215 (Hackett 1996) and H6313 (Johnson and Sorenson 1998) following the protocol described in Weckstein (2005). For sequencing, I also used internal primers ND2Hal and ND2Alc (Moyle 2006). For RAG-1, all amplification and sequencing primers except for RagB (5'- TGGCTCCTGGTTATGGAGTGG-3', a kingfisher specific primer designed by R. Moyle, pers. comm.) are from (Groth and Barrowclough 1999).

Two initial PCRs for RAG-1 were performed using the PCR protocol described in Groth and Barrowclough (1999). One set used primers R7 and R4B and the other used

primers R13 and R8 (Groth and Barrowclough 1999). For sequencing reactions, additional internal primers R9, R10, R11B, and R16 were used to completely sequence the fragment between R13B and R8 and R3E and RagB were used to sequence between R7 and R4B. PCR products were submitted to Functional Biosciences for sanger sequencing. Host sequence processing followed the same procedure outlined above for louse DNA sequences. The resulting consensus sequences were combined with the sequences acquired from GenBank and aligned to the data published by Moyle (2006).

Table 3.2 List of host taxa sequenced for my study. ND2 and RAG-1 were sampled for all hosts.

Species	Tissue Number	Location	Extraction
Halecyon albiventris orientalis	MLW-3737	Malawi	Haalb.2.23.2016.1
Halycyon senegalensis cyansleuca	MLW-4185	Malawi	Hasen.2.23.2016.2
Halcyon chelicuti	MLW4604	Malawi	Hache.2.23.2016.3
Ispidina picta natalensis	MLW-3781	Malawi	Ispic.2.23.2016.4

Phylogenetic Analysis of Parasites

The 2 genes were first analyzed separately to ensure that gene trees for each ingroup (*Alcedoecus* (Fig 3.1) and *Alcedoffula* (Fig 3.2)) were not in conflict (posterior probability

great than 0.95. Gene trees were inferred using 40 million generation BEAST runs under the model selected by PartitionFinder 1.1.1 (branchlength= linked; model_selection= AIC; search= greedy; Drummond & Rambaut, 2007, Lanfear et al. 2012). Although some nodes were in conflict between the 2 gene trees, these were typically limited to relationships among outgroups (potentially due to long branches and sparse outgroup taxon sampling) and the placement of 2 ingroup taxa (*Alcedoffula duplicate* and *Chloroceryle inda*), both of which were placed on long branches sister to a given clade in one gene tree, but within the clade in the other. Since conflict was limited, genes were concatenated for further analysis.

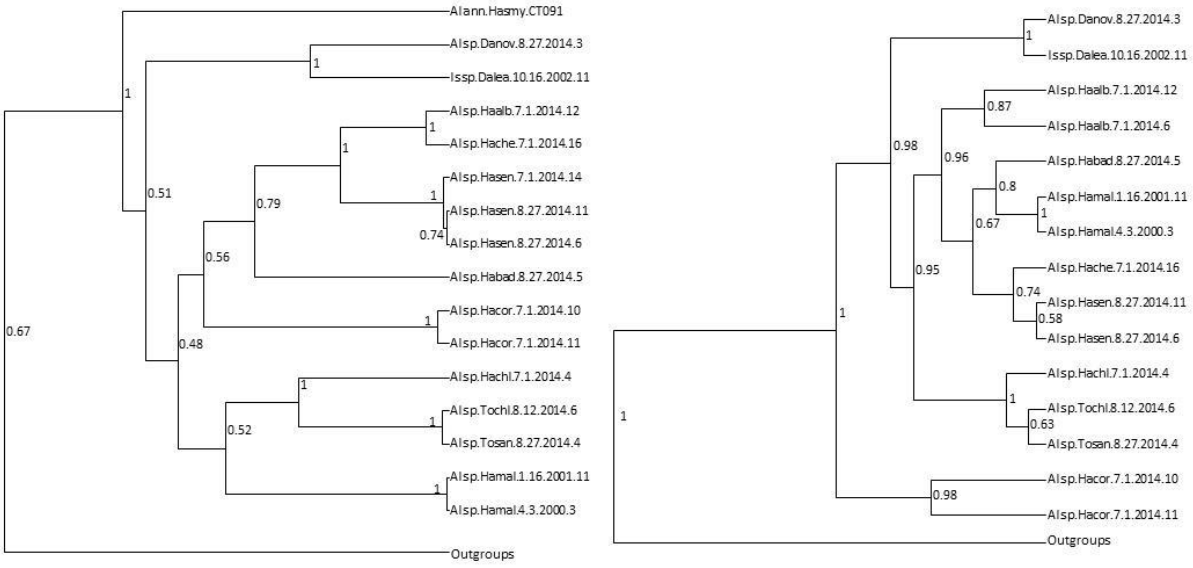

Figure 3.1. *Alcedoecus* phylogeny resulting from Bayesian Analysis of COI (left) and EF-1α (right). Numbers on branches represent posterior probabilities.

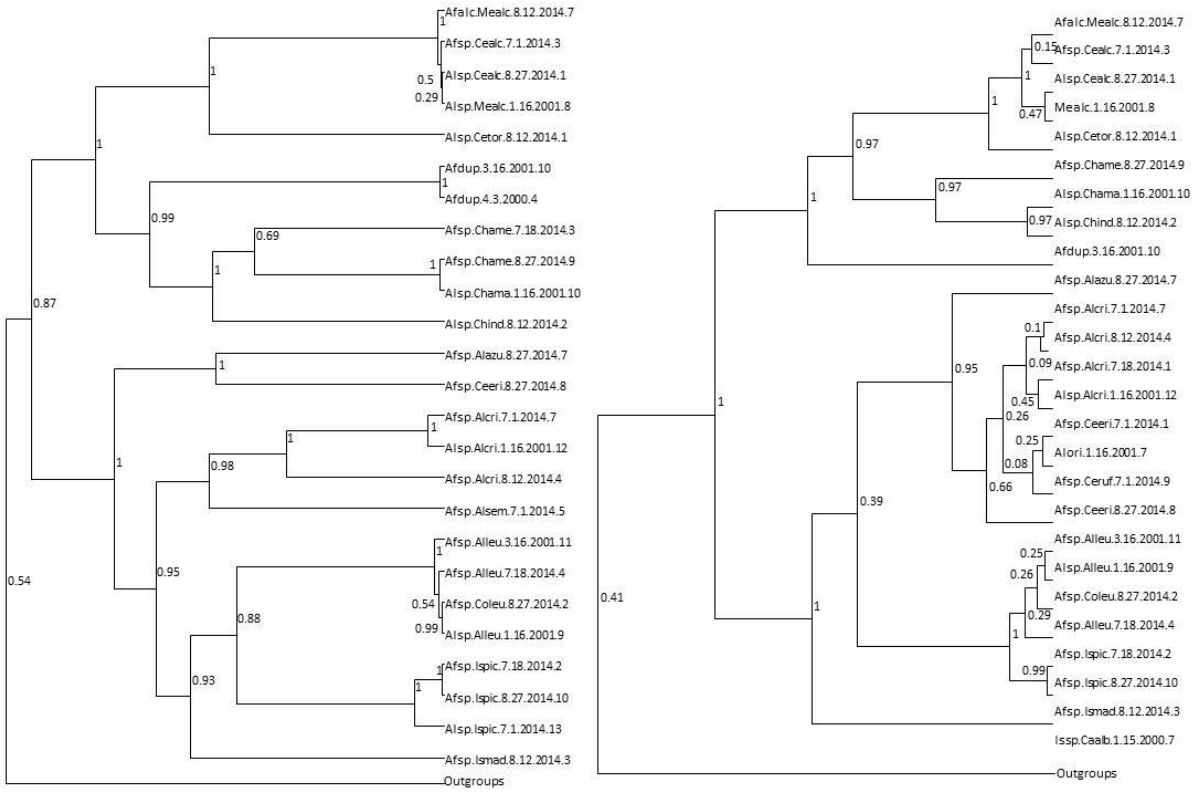

Figure 3.2. *Alcedoffula* phylogeny resulting from Bayesian Analysis of COI (left) and EF-1α (right). Numbers on branches represent posterior probabilities.

In the combined concatenated analysis, each gene codon was treated as a separate partition, each with model parameters as determined by PartitionFinder (GTR+I+G for codons 1 and 2 and GTR+G for codon 3 of COI and TrN+G for codons 1 and 2 and K80+G for codon 3 for EF-1α). Phylogenies based on the combined analysis were inferred using Bayesian inference (BEAST: 40 million generations, sampling every 1,000 generations, burnin = 10,000 samples), Maximum Likelihood (ML; garli: 10 independent runs, default settings, automated stop criterion = 50,000; Zwickl 2006, Drummond and Rambaut 2007), and Maximum

Parsimony (MP; using PAUP*, 1000 random addition sequences with TBR branch swapping; Swofford 2003). I used posterior probabilities (using BEAST), ML bootstrap values (using garli, 500 bootstrap replicates on default settings with automated stop criterion = 5,000), and parsimony bootstrap values (using PAUP*, 1,000 replicates of 100 random addition sequences with maxtrees set at 500 due to computational constraints) to evaluate branch support.

Phylogenetic Analysis of Hosts

The host phylogeny was inferred using a 40 million generation BEAST run under the model selected by PartitionFinder 1.1.1 (branchlength= linked; model_selection= AIC; search= greedy; Drummond and Rambaut 2007, Lanfear et al. 2012). PartitionFinder selected GTR+I+G for each partition, with the exception of RAG-1 third positions, for which SYM+G was the best model. I evaluated branch support using posterior probabilities (generated by BEAST) and parsimony bootstrap values (generated by PAUP*, 100 replicates of 100 random addition sequences with maxtrees allowed to automatically increase by 100; Swofford 2003).

Tests of Codivergence

I used the louse tree generated from the Bayesian analysis and either the Alcedininae phylogeny inferred by Moyle et al. (2007) or the kingfisher phylogeny inferred above to conduct statistical tests of cospeciation using Jane4 (Conow et al. 2010). Parasite tips were collapsed to ensure that each tree topology only included a single representative of each putative louse species, and terminals that did not form a parasite/host pair were removed. As *Alcedoffula* contains 2 lineages, which do not parasitize sister kingfisher subfamilies, the 2 lineages were analyzed separately. These analyses were run on the actual tree topology

(using default costs). To assess statistical significance, I generated 1,000 random tip mappings and 1,000 randomly generated parasite trees in Stats Mode to assess if the results of Jane4's cophylogenetic analysis were lower than expected from random chance.

Ancestral state reconstruction- Mesquite (Maddison and Maddison 2011) was used to perform ancestral state reconstruction where each kingfisher species for which lice have been recorded was coded base on louse genus (or genera) known to occur on that host. These data were acquired from Price et al. (2003), Najer et al. (2012), Gustafsson and Bush (2014), and specimens used this this study. This was then mapped across a species-level kingfisher tree generated from Jetz et al. (2012). A random sampling of 1,000 Ericson All Species trees was downloaded from birdtree.org then summarize into a single tree using TreeAnnotator (Drummond and Rambaut 2007). This tree was compared to existing kingfisher phylogenies and the host phylogeny inferred here to identify potential areas of conflict. The impact of discrepancies between the trees will be described below.

Biogeographic Reconstruction

Using BioGeoBEARS (Matzke 2013), I reconstructed the biogeographic history of the kingfishers themselves and both *Alcedoecus* and *Alcedoffula*. Within BioGeoBEARS, I estimated ancestral-areas using DEC, likelihood interpretations of a dispersal-vicariance model (DIVALIKE), and a Bayesian binary model (BAYAREALIKE). Reconstructions were calculated twice for each method, once including the j (long distance dispersal) parameter and once without. For kingfishers, tips from the same summarized Jetz et al. (2012) tree used for ancestral state reconstruction were coded to reflect the 6 major biogeographic regions. For the lice, tips were collapsed if COI divergence was less than 2.5% and outgroup taxa removed. I coded geographic range at 2 scales, one of the 6 major

biogeographic regions (5 states as no lice are available from Palearctic kingfishers) and one breaking continents into major ecosystems (8 states; i.e., the regions of Sub-Saharan Africa as defined by Linder et al. [2012]). For the broad scale coding, lice collected from hosts on Indo-Pacific Island were placed in either southeast Asia or Australia based on Wallace's Line while in fine scale coding lice from the Indo-Pacific region were split between Australian, Solomon Islands, and southeast Asia (including Borneo and the Philippines). In all instances, maxareas was set to 2. Results from each method were compared using AIC scores.

RESULTS

The phylogeny resulting from a combined analysis of COI and EF-1α was well resolved and reasonably well supported at most nodes. Both *Alcedoffula* and *Alcedoecus* were recovered as monophyletic (posterior probability [PP] = 1.0 for both clades Fig. 3.3 and 3.4). Within *Alcedoffula* (Fig. 3.3), 2 well-supported clades (PP = 1.0 for both clades) were recovered, each infesting a single kingfisher subfamily. The clade infesting the Cerylinae also contains 2 well-supported clades (both with PP = 1.0). There are only 9 host species within this kingfisher subfamily and I have sampled lice from 6 of them (lice from a 7th species, American pygmy kingfisher (*Chloroceryle aenea*) failed to sequence and molecular grade specimens are not available from the 2 Old World *Megaceryle* species). One *Alcedoffula* clade contains lice limited to New World *Megaceryle* kingfishers, whereas the other clade of *Alcedoffula* is more geographically widespread and is found in both the New World (on the various *Chloroceryle* species) and in the Old World (on pied kingfisher, *Ceryle rudis*, a monotypic genus). In this geographically widespread clade *Alcedoffula duplicata*, from pied kingfisher, is sister to lice from the New World genus *Chloroceryle*. Within *Alcedoffula* parasitizing *Chloroceryle*, 2 samples of lice from green kingfisher

(*Chloroceryle americana*) are not each other's closest relatives with respect to the lice from the 2 other *Chloroceryle* species. One green kingfisher louse is sister to the sample of lice from Amazon kingfisher (*Chloroceryle amazona*). The COI sequences of these 2 samples are almost identical, with uncorrected p distances of 0.2%.

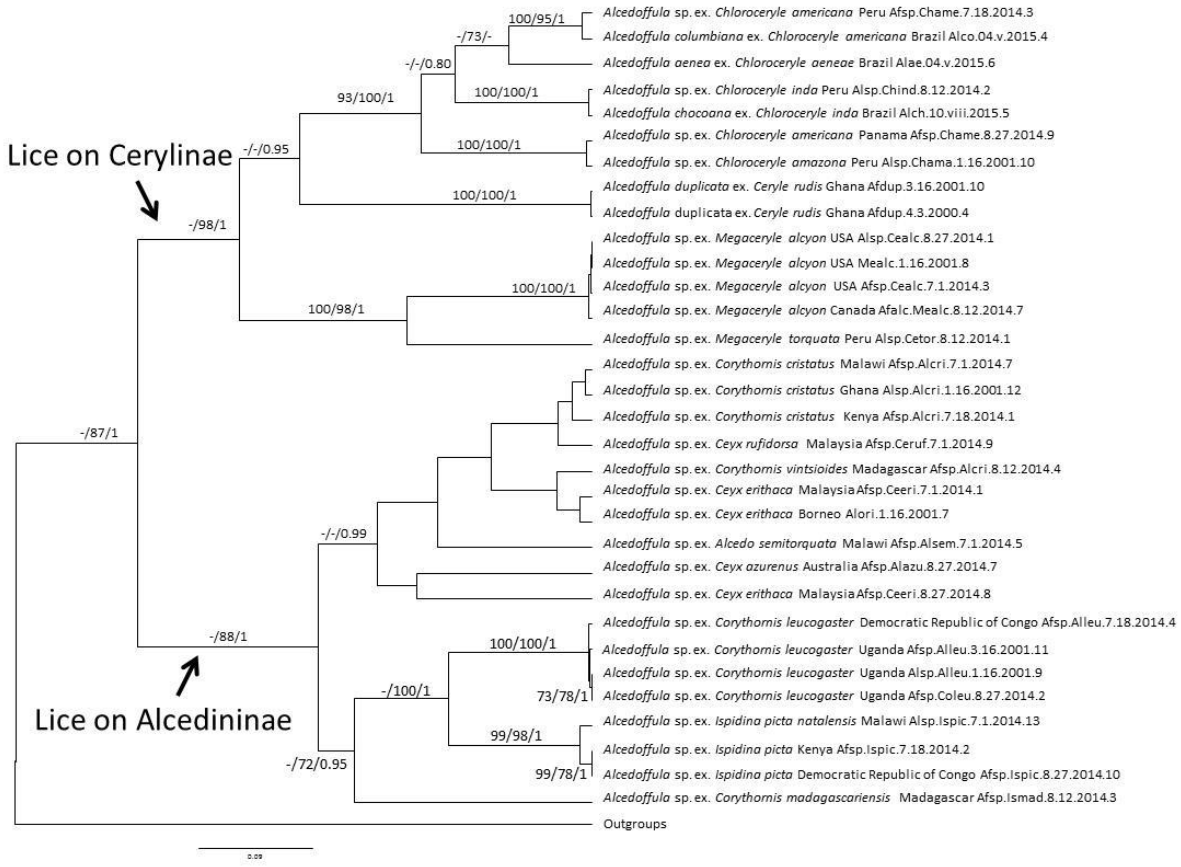

Figure 3.3. Parasite phylogeny resulting from Bayesian Analysis of COI and EF-1α. Numbers on branches represent MP bootstrap values followed by ML bootstrap values then posterior probabilities. Bootstrap values below 70 and posterior probabilities below 0.80 not shown.

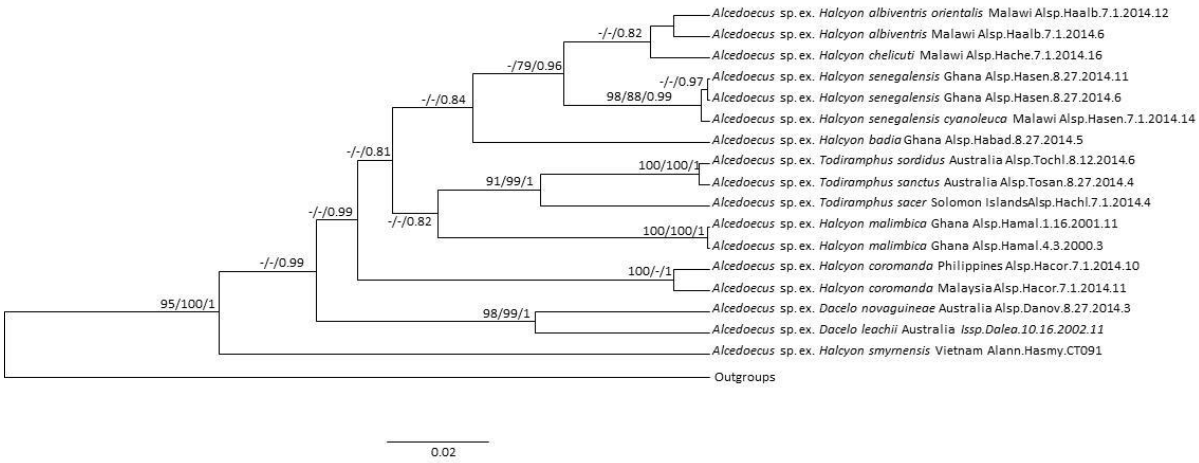

Figure 3.4. *Alcedoecus* phylogeny resulting from Bayesian Analysis of EF-1α and COI. Numbers on branches represent MP bootstrap values followed by ML bootstrap values then posterior probabilities. Bootstrap values below 70 and posterior probabilities below 0.80 not shown.

The other *Alcedoffula* clade is found exclusively on the kingfisher subfamily Alcedininae. This *Alcedoffula* clade is comprised of 2 clades, one of which is well supported (PP = 0.95) with well supported relationships between members within this clade. The second, is supported in BI but not in either ML or MP (PP=0.99). Additionally, relationships between members in this clade lack statistical support. The well supported clade is comprised of lice from the African kingfisher's 2 species of *Corythornis* (*C. madagascariensis* and *C. leucogaster*) and *Ispidina picta*. Two of these kingfisher species were represented by multiple host individuals collected from across the host's range. In both of these instances lice collected from the same host species were each other's closest relatives (PP = 1.0). *Ispidina picta* was represented by lice from all 3 recognized host subspecies. Although all 3 were placed in a clade, the louse from *Ispidina picta natalensis* was 3% divergent for the COI gene (uncorrected p distance) from lice parasitizing the other 2

subspecies. While this level of COI divergence is normal within louse species in other genera, kingfisher lice collected from a single host species show very little divergence (1.5% uncorrected p distance or lower). This level of divergence between lice collected from *Ispidina picta natalensis* and the other *Ispidina picta* subspecies provides potential evidence of population structure in the host. Further sampling from across Africa could shed further light on this host species and the taxonomic status of *I. natalensis*. Additionally, the lice from the host genus *Corythornis* as a whole are not monophyletic, although support within this clade is lacking (Fig. 3.3). First, *Alcedoffula* from *Corythornis leucogaster* are more closely related to *Alcedoffula* from *Ispidina picta* to the exclusion of lice from *Corythornis madagascariensis* (PP = 1.00). The other lice sampled from *Corythornis* are placed within a well-supported clade (PP = 0.90) which also contains lice from *Ceyx rufidorsa* and *Ceyx erithaca*. Although relationships within this clade are unresolved in the combined analysis, the COI gene tree includes a well-supported clade of lice from *Corythornis cristatus*, which is sister to a louse from *Corythornis vintsioides*. This clade is placed within an unsupported clade which also contains lice collected from *Alcedo* and *Ceyx* kingfishers. Lice within this clade were collected from throughout the tropical and subtropical Old World.

The most basal node within the louse genus *Alcedoecus* tree (Fig. 3.4) unites the louse collected from *Halcyon smyrnesis* with all other *Alcedoecus*. The remaining members of the genus *Alcedoecus* form a well-supported clade (PP = 0.99). Within this clade, lice from 2 species of kookaburra (*Dacelo* spp.) are sister to a well-supported clade (PP = 0.99) containing lice collected from 6 species of *Halcyon* and 3 species of *Todiramphus*. Within this clade, lice from *Todiramphus* form a well-supported monophyletic clade (posterior probability = 1.0), which is embedded within a larger clade containing lice from *Halcyon*. In

all instances where *Alcedoecus* from multiple host individuals were sampled from a given host species they fall out as sisters in the phylogeny, although not all of these sister relationships were well-supported.

The kingfisher phylogeny (Fig. 3.5) recovered the 3 subfamilies and all genera with multiple representatives as monophyletic with high support. In instances where multiple subspecies were included subspecies were recovered as sisters.

The results of the Jane4 cophylogenetic analyses were variable (Table 3.3; Figs. 3.6 and 3.7). The cophylogenetic analysis of *Alcedoffula* from cerylinine kingfishers returned only 1 distinct result with 4 instances of cospeciation, and the total cost was significantly different than expected by random chance ($P = 0.01$). Cophylogenetic reconstructions of both *Alcedoffula* and their alcedinine kingfishers and *Alcedoecus* with the kingfisher subfamily Daceloninae showed no evidence for cospeciation between louse and host phylogenies (all $P > 0.21$).

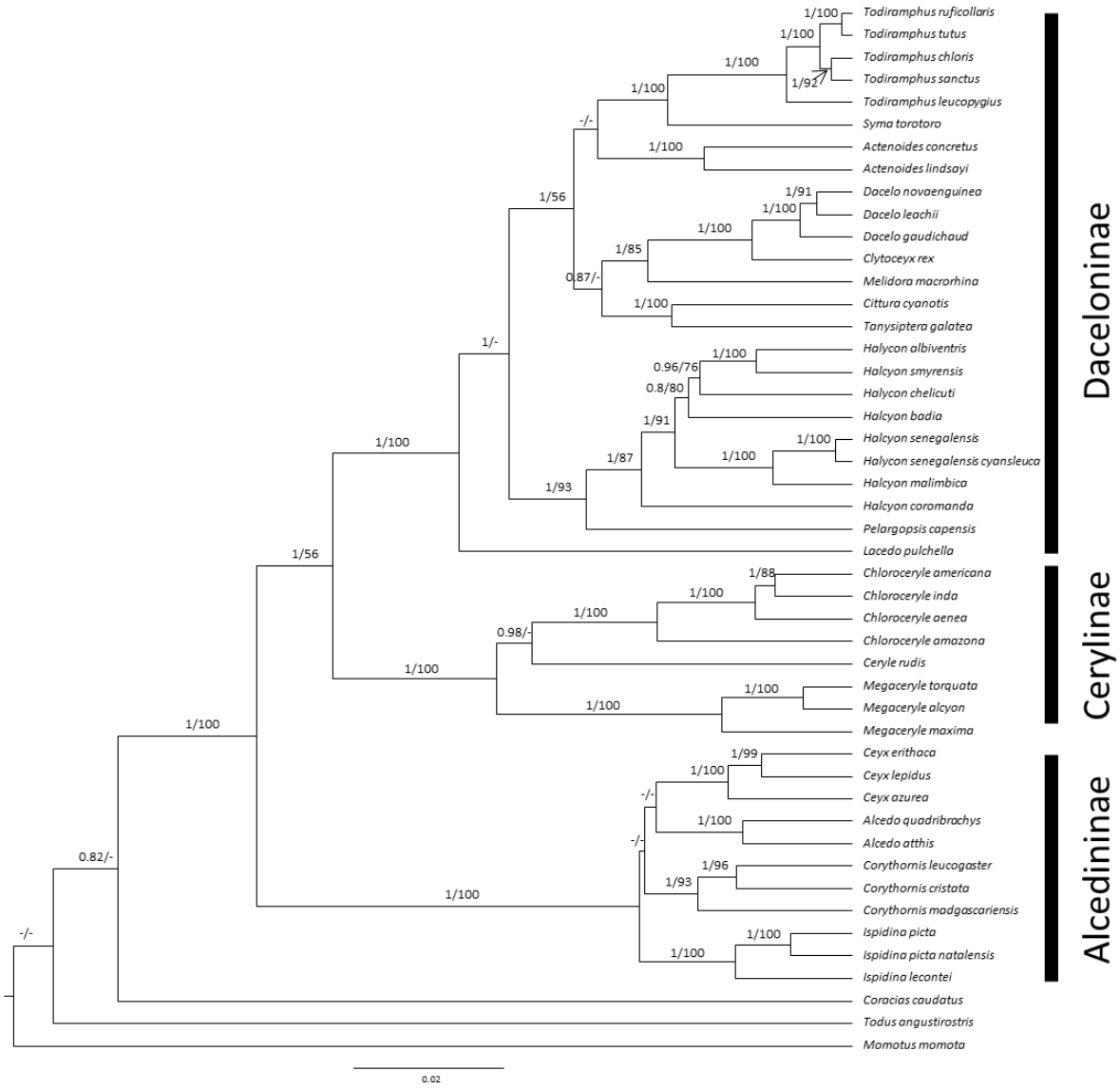

Figure 3.5. Kingfisher phylogeny resulting from Bayesian Analysis of ND2 and RAG-1. Numbers on branches represent posterior probability followed by maximum parsimony bootstrap values. Bootstrap values below 50 and posterior probabilities below 0.80 not shown. Thick black bars to the right of the phylogeny denote the 3 kingfisher subfamilies

Table 3.3. Results of Jane Analysis on actual data by host subfamily (upper) and using the statistical solutions option based on 1,000 random samples (lower).

	Actual Solutions						
Host Family	#of isometric solutions	# of inferred cospeciations	# of inferred duplications	# of inferred duplications + host switches	# of inferred losses	# of inferred failures to diverge	Total Cost
Alcedininae solution 1	2-	5	0	4	2	0	10
Alcedininae solution 2	15	5	0	4	2	1	10
Cerylinae	4	4	0	2	1	1	6
Daceloninae	5	4	0	1	1	0	3

	Statistical Solutions					
	Random Parasite Tree			Random Tip Mapping		
Host Family	Mean cost	Standard deviation	%Solution with lower cost than actual solutions	Mean cost	Standard deviation	%Sample with lower cost than actual solutions
Alcedininae	12.41	1.39	8.80%	12.59	1.42	8.00%
Cerylinae	10.00	1.46	3.10%	9.81	1.85	4.40%
Daceloninae	5.86	1.46	8.40%	5.71	1.31	11.50%

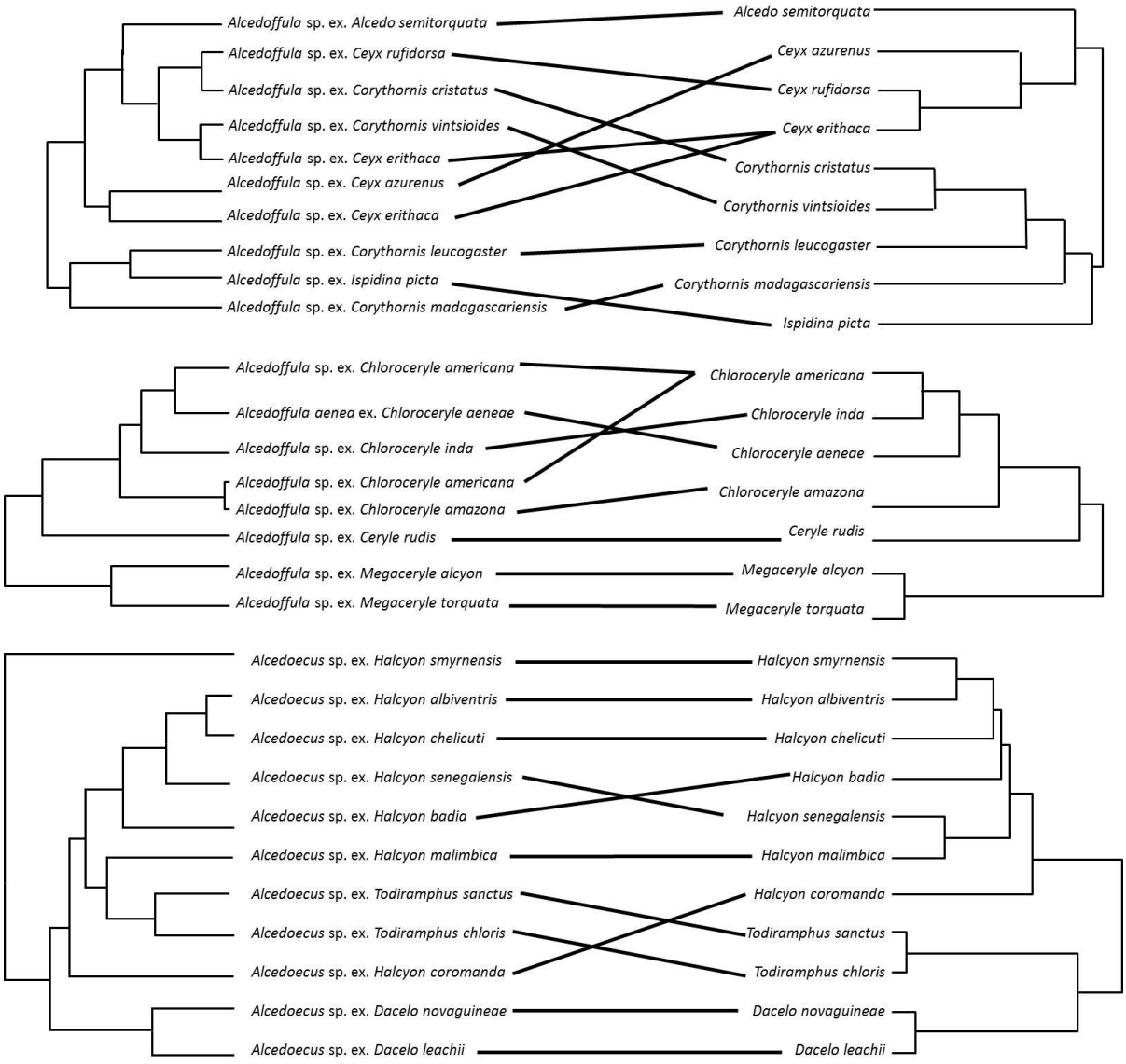

Figure 3.6 Tanglegrams showing links between lice (left) and host (right) broken up by host subfamily. Top pair is Alcedininae, middle Cerylinae, bottom Daceloninae.

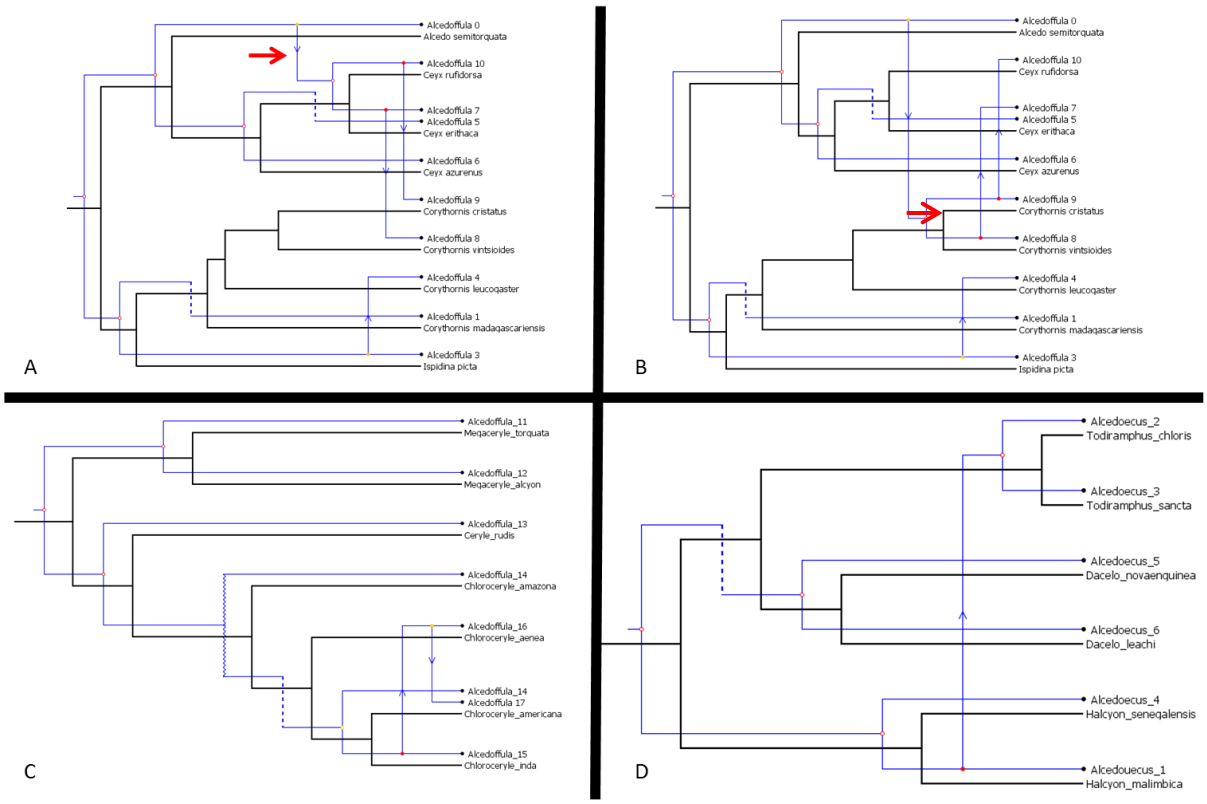

Figure 3.7. Inferred patterns of cospeciation for Alcedininae (A and B), Cerylinae (C), and Daceloninae (D). Open circles mark nodes of cospeciation while the filled circle represents a duplication coupled with a host switch, or only a duplication in the case of C. Arrows in A and B denote where the 2 equally costly solutions differ in their reconstructions.

The results of the ancestral state character (Fig 3.8) reconstruction in Mesquite showed that which genus of louse parasitizes a species of kingfisher, based on the 51 kingfishers for which lice are known, is strongly influenced by host phylogeny. As expected from existing literature, 2 kingfisher subfamilies are parasitized by *Alcedoffula*. Surprisingly, I found the other 2 genera of kingfisher lice, *Alcedoecus* and *Emersoniella*, each parasitize distinct clades within Daceloninae,

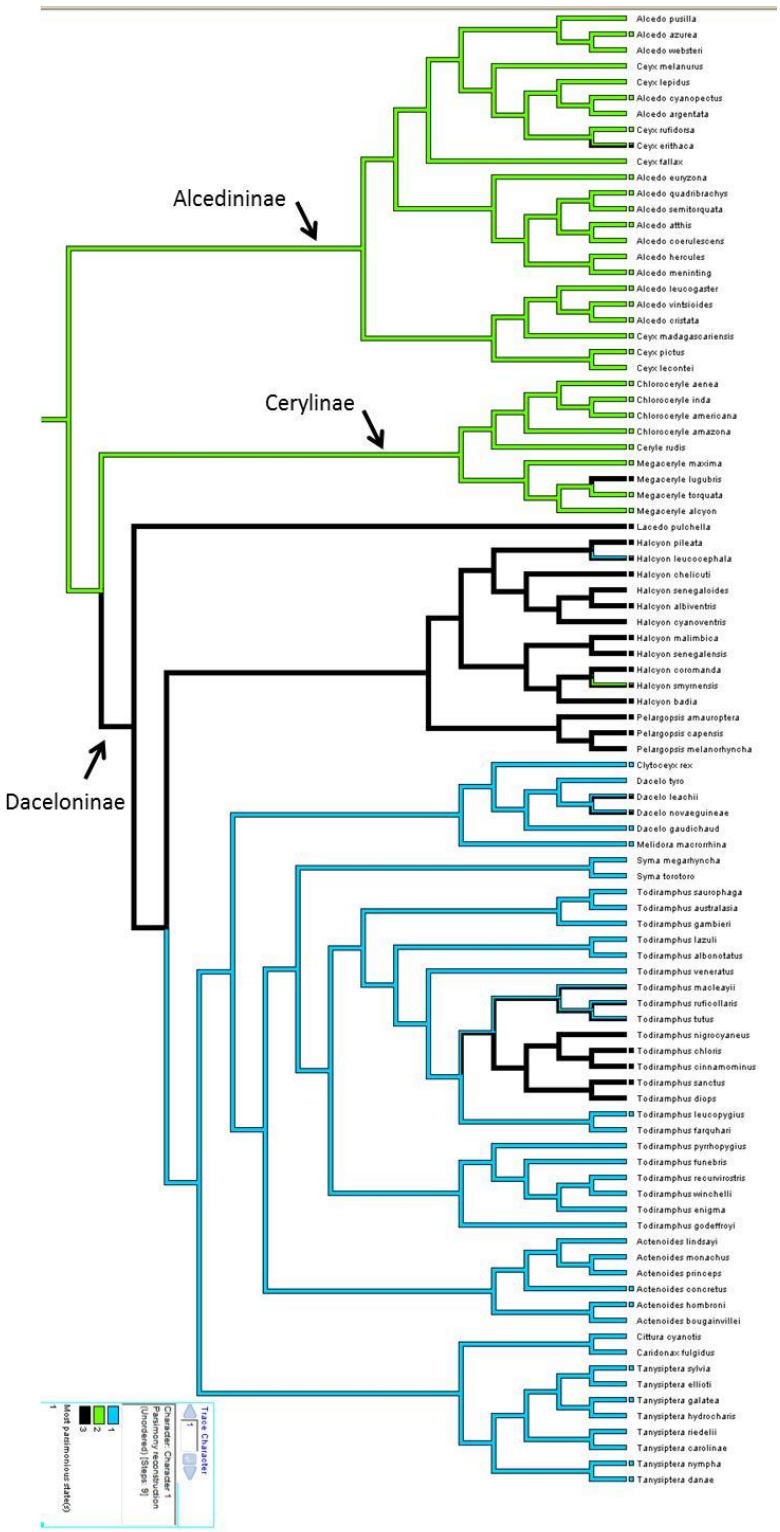

Figure 3.8. Ancestral state reconstruction of louse parasitism by genus. Tips with colored squares represent known host records (i.e., blue = *Emersoniella*, black = *Alcedoecus*, green = *Alcedoffula*).

a widespread clade and one restricted to the Indo-Pacific region, respectively. This reconstruction infers *Alcedoecus* as being the ancestral louse on Daceloninae, which stems from the placement of *Lacedo pulchella* a known host of *Alcedoecus* (Fig. 3.5, 3.8 and Moyle 2006). There also is evidence for a single host switch of *Alcedoecus* onto the Indo-Pacific kingfisher clade.

DEC+J yielded the best AIC score for the kingfisher tree, and fine scale biogeographic reconstructions in both *Alcedoecus* and *Alcedoffula*. Conversely, in the broad scale coding DIVALIKE+J was the best for *Alcedoffula* while *Alcedoecus* had equal likelihood scores for both DIVALIKE+J and BAYAREALIKE+J. While the results for the fine scale coding for *Alcedoffula* were equivocal, the broadscale coding recovered an African + South American origin. I recovered a single South American origin for lice parasitizing cerylinine kingfishers, with subsequent colonization of Africa. An African origin was inferred for lice parasitizing alcedinine kingfishers with 2 invasions into southeast Asia. One of these lineages also spread into Australia. Both coding schemes recovered an Australian + southeast Asian origin for *Alcedoecus*. A single radiation of lice from African hosts was slightly favored. Two invasions of Australia also were slightly favored. The first, originating from southeast Asia contains lice from kookaburras while the second, from Africa, contained lice from *Todiramphus* kingfishers.

DISCUSSION

Two louse genera, *Alcedoecus* and *Alcedoffula*, broadly parasitize kingfishers while a third, *Emersoniella*, is only known from a few species of Australasian kingfishers and kookaburras (Fig. 3.8). A 2 gene phylogeny recovered both *Alcedoecus* and *Alcedoffula* as strongly supported monophyletic clades. While many groups of birds are infested by multiple species of lice, typically from different genera, kingfisher lice are unusual in the vast

majority of host records indicate that each host species is infested with just a single species of louse. Louse genera are specific to particular host subfamilies with *Alcedoffula* parasitizing both ceryline and alcedinine kingfishers and *Alcedoecus* parasitizing the Daceloninae. This arrangement is interesting; the kingfisher phylogeny inferred by Moyle (2006) placed Alcedininae as sister to a clade made up of Daceloninae and Cerylinae. One way to explain this host-parasite association is that *Alcedoffula* was lost (i.e., went extinct) from the ancestor of Daceloninae and subsequently "replaced" by *Alcedoecus*. *Emersoniella* colonized one clade of Daceloninae but was subsequently replaced again by *Alcedoecus* on two clades (two species of *Dacelo* and a clade of *Todiramphus*). This is more parsimonious than these two clades retaining *Alcedoecus* while all other lineages within this kingfisher clade replacing *Alcedoecus* with *Emersoniella*. This is based on a limited number of host associations, particularly within the clade parasitized by *Emersoniella*.

There are a few examples in the literature where a kingfisher is parasitized by the louse from the "wrong" genus and it is possible these are examples of species being placed into an incorrect genus. For example, we sequenced 3 lice from *Ceyx erithaca*, 2 identified as *Alcedoffula* and 1 as *Alcedoecus*. The resulting topology included 2 distinct lineages; however both fell within *Alcedoffula*. Notably, the relationship between the 2 lineages of lice from *Ceyx erithaca* lacked statistical support. The only record of *Alcedoecus* on a ceryline kingfisher is *Alcedoecus nepalensis* on *Megaceryle lugubris*, an Old World species. This kingfisher genus occurs in both the New and Old World. *Megaceryle maxima*, the other Old World *Megaceryle* species, is parasitized by *Alcedoffula* as are both New World *Megaceryle* species. This suggests the *Alcedoecus* found on *Megaceryle lugubris* is the result of a host switch, which is possible as this bird species overlaps with many taxa known to harbor

Alcedoecus. Conversely, this could be a morphologically extreme *Alcedoffula* species incorrectly placed in *Alcedoecus*. Unfortunately, I do not have material from *Megaceryle lugubris* to test these hypotheses.

The phylogeny recovered for *Alcedoffula* collected from ceryline kingfishers broadly resembles the ceryline kingfisher portion of the kingfisher phylogeny published by Moyle (2006). *Alcedoffula* from *Megaceryle* kingfishers formed a clade which was sister to a clade containing lice from the Neotropical *Chloroceryle*, and *Ceryle rudis*, a monotypic kingfisher genus that occurs throughout Africa and southern Asia (Fig 3.3). Moyle (2006) placed *Ceryle rudis* as sister to the *Chloroceryle* radiation which matches my louse phylogeny. However, the branching pattern of *Alcedoffula* on *Chloroceryle* kingfishers does not closely match the published host tree in Moyle (2006) or the phylogeny inferred here (Fig 3.5). Lice collected from *Chloroceryle americana* are paraphyletic, with 1 representative being placed with lice from *Chloroceryle amazona*, with an uncorrected p distance of 0.2%. This pair of samples as sister to the rest of *Chloroceryle* lice. Lice have never been recorded from *Chloroceryle amazona*, and samples were only available from a single individual host. Without additional sampling it is unclear whether this record is a novel host association caused by host-switching or if this is an example of straggling.

Moyle et al. (2007) found weak evidence for a clade of kingfishers containing *Corythornis* and *Ispidina*. My data set included *Alcedoffula* from 5 of 6 of the host species currently placed within these genera and I found close affinities between lice from *Corythornis leucogaster, Corythornis madagascariensis* and *Ispidina picta* (Fig. 3.3). Not all lice collected from these genera were placed together as *Alcedoffula* from *Corythornis cristatus* and *Corythornis vintsioides*, which Moyle et al (2007) found to be sister species,

fall outside of this clade (Fig. 3.3). Other relationships recovered within this clade of *Alcedoffula* from the Alcedininae lacked support similar to the poor support observed in the hosts (Moyle et al. 2007). Additionally, lice from these sister species are not recovered as sister, although statistical support for relationships within this clade are lacking. This suggests that something other than host relationships is driving patterns of louse distribution. Since louse species appear to be host species specific but lack evidence of cophylogeny with the host taxa, it is possible that *Alcedoffula* colonized Alcedininae kingfishers after the kingfishers themselves radiated. Furthermore, lice from both *Corythornis vintsioides* and *Corythornis madagascariensis* were collected in Madagascar while lice from *Corythornis cristatus* were sampled from regions overlapping with the sampling of *Corythornis leucogaster* and *Ispidina picta*. The lack of geographically structured clades suggest that lice are not circulating between members of different host species in the same region, be it phoretically or via shared nesting cavities or other means of indirect contact

In the *Alcedoecus* tree (Fig. 3.4), lice from kookaburras form a clade which is sister to virtually all other sampled *Alcedoecus*. I only have COI sequence data for *Alcedoecus* sp. ex. *Halcyon smyrnesis* so its placement as sister to the rest of *Alcedoecus* bears further study. While *Halcyon* lice are not monophyletic, it is surprising lice from this specimen are on such a long branch. Additionally, this placement could be due to the lack of EF-1α so additional samples with both genes sequenced are needed to determine its correct placement. . Also, and lice from *Todiramphus* were embedded within the lice from *Halcyon* kingfishers. *Todiramphus* itself was recently split from *Halcyon*, and the relationships between host species in these genera are uncertain (Moyle 2006). This host group will require further investigation with more thorough taxon sampling required for both the lice and their hosts.

Where specimens were available, I included samples from multiple individuals of the same host species to determine whether louse lineages are host specific. Within this dataset, I included 2–4 representatives from 11 of the 27 sampled host species. Lice from all but 2 hosts (*Ceyx erithaca* and *Chloroceryle americana*) were each other's closest relatives (posterior probability = 0.95 or greater). In the case of *Ceyx erithaca* , there are 2 taxa described from the host species (Price et al 2003). *Chloroceryle americana* is from a geographically widespread host species that breeds from the southern United States to Argentina (eBird-Clements-v2015-integrated-checklist-August-20).

In several cases, my louse phylogeny also mirrors recently proposed host splits. For example, *Todiramphus chloris* was recently split into 6 species and my dataset includes parasite data from 2 of these host taxa (*Todiramphus sacer* and *Todiramphus sordidus*). These 2 lice from *Todiramphus* are 16% divergent from one another in COI sequences and are not each other's closest relatives in the phylogeny (Fig 3.4). A second example involves lice from *Corythornis vintsioides* and *Corythornis cristatus,* which are sometimes treated as conspecific. My study includes lice from both host taxa, including multiple representatives of *Corythornis cristatus* from across Africa. My phylogeny recovered a clade containing all lice from *Corythornis cristatus*, but that excluded the louse from *Corythornis vintisioides*. In the COI gene tree, the louse from *Corythornis vintisioides* is sister to the *Corythornis cristatus* louse clade with a COI divergence of about 15% (Fig 3.1). This level of divergence between lice is high enough the lice from *Corythornis vintisioides* and *Corythornis cristatus* should be considered different species, corresponding to Price et al. (2003) which treats lice from these two hosts as distinct species. Given the louse data, these *Corythornis* species should continue to be treated as full species.

Varying degrees of cospeciation have been found in lice ranging from phylogenies which show strong congruence to virtually no similarity between host and parasite trees (Hughes et al. 2007, Weckstein 2004, Johnson et al. 2001). Here, I found the degree of congruence varied not only between the 2 kingfisher louse genera, but also within the 2 clades of *Alcedoffula*. This appears to be the first time in which different clades within a single louse genus have differing levels of congruence with the host tree. Within *Alcedoffula*, lice occurring on cerylinae kingfishers showed strong evidence of cospeciating with their hosts. In contrast, *Alcedoffula* from alcedinine kingfishers and *Alcedoecus* from Daceloninae do not show evidence of cospeciation with their hosts. Taxon diversity between the 3 subfamilies is uneven- Cerylinae contains 10 kingfisher species distributed in both the New and Old World (although I am missing lice from 2 Old World species). Conversely, the other 2 subfamilies have many more species, including many with extremely limited distributions meaning lice were only available from a limited number of host species. Further taxon sampling could result in increased evidence of cophylogeny between kingfishers and their lice. This is particularly the case in island archipelagos where one kingfisher species invaded an island and then spread down the island chain speciating as it went, a common pattern in Old World kingfishers.

The kingfisher phylogeny can strongly predict which louse genus infests a given host species (Fig. 3.8). Both Cerylinae and Alcedininae are parasitized by *Alcedoffula* while Daceloninae is parasitized by both *Alcedoecus* and *Emersoniella*. Daceloninae is composed of 2 major clades, one of which is widespread throughout the Old World, while the other is most specious in the Indo-Pacific region, and each is parasitized by a different louse genus. *Alcedoecus* is found on the widespread clade while *Emersoniella* occurs on the Indo-Pacific

kingfisher clade. Although reconstruction infers *Alcedoecus* as being the ancestral louse on Daceloninae, this stems from the placement of *Lacedo pulchella* a known host of *Alcedoecus*. This species is one in which the Jetz et al. (2012) tree differs from other existing kingfisher trees (Moyle 2006; Moyle et al. 2007; Fig 3.5). Jetz et al (2012) places this kingfisher as sister to all other Daceloninae while Moyle (2006) places this taxon as sister to the widespread clade within Daceloninae. *Emersoniella* is restricted to the Indo-Pacific clade with the exception of one record from *Halcyon leucocephalus* (Najer et al. 2012).

Although no formal assessment of kingfisher biogeographic history exists, Moyle (2006) discussed potential biogeographic patterns of the taxa included in his phylogeny. Due to high species diversity and levels of endemism in Australia and the Indo-Pacific Islands, this region has been suggested to be the kingfisher center of origin. However, Moyle (2006) found kingfishers from the Malesia region were not placed basally in the tree. This finding is echoed in the louse phylogenies inferred here as Australian lice from both *Alcedoecus* and *Alcedoffula* were embedded within large African louse clades. One clade of Australian lice, those from Kookaburras, is placed in a relatively basal position in the louse tree, leading to biogeographic reconstruction of *Alcedoecus* favoring an Australian + southeast Asian origin. This contrasts with the host phylogeny in which kookaburras are deeply embedded within Daceloninae. Wallace's line divides these 2 regions, and while kingfishers appear to be good dispersers across water barriers (having distributions including many remote oceanic islands) many of the land masses currently occupying this region did not form until after kingfishers appeared in the fossil records of Europe and North America.

Within lice parasitizing cerylinine kingfishers, I inferred a single South American origin. This clade subsequently spread into North America (Belted Kingfisher lice) and

Africa (Pied Kingfisher lice). The contrasts with the host biogeography detailed in Moyle (2006) where an Old World origin with 2 New World invasions was the most parsimonious explanation of host distribution patterns. Moyle (2006) recovered African and Asian kingfishers as the more basal members of Alcedinine with Australian representatives embedded deeply within the Alcedininae. This was similar my biogeographic reconstruction of alcedinine *Alcedoffula* which inferred an African origin of the clade followed by 2 southeast Asian invasions. One of these lineages later colonized Australia.

An Australian + southeast Asian origin was slightly favored for *Alcedoecus*, the lice parasitizing Daceloninae; however, a number of other potential histories also were inferred. While this region agrees with the historically suggested center of origin for kingfishers, it is unusual for a clade to be unaffected by Wallace's Line as is this case in inferring an Australian + southeast Asian origin (Moyle 2006). A single colonization of African hosts was slightly favored; 1 lineage of this clade later reinvaded Australia and the Solomon Islands. Within Africa, a clade containing most lice from *Halcyon* was inferred to have originated in the Congolian region (western African forests) and then colonized birds in the Zambezian region (central and eastern African forests).

In summary, kingfishers are parasitized by 3 genera. While *Emersoniella* is limited in distribution and only includes 7 described species, *Alcedoecus* and *Alcedoffula* are diverse and widespread. Here, I inferred phylogenies for *Alcedoecus* and *Alcedoffula* and determined both are monophyletic. Within *Alcedoffula*, 1 clade parasitizes alcedinine kingfishers while the other is limited to ceryline kingfishers. *Alcedoecus* is limited to dacelonine kingfishers. While *Emersoniella* is also known from dacelonine kingfishers ancestral state reconstruction revealed that these two genera actually parasitize separate clades within Daceloninae. Where

possible, lice from multiple representatives of the same host were included. In virtually all instances lice from a given host species formed monophyletic units, even when samples were taken from different portions of the range. This points to a high degree of host specialization by lice. In the case of ceryline kingfishers this was also accompanied by significant evidence of cospeciation between lice and their hosts. This high level of host specialization also presents the opportunity to test the taxonomic status of some hosts which are alternatively treated as full species or subspecies. For example, *Corythornis vintsioides* and *Corythornis cristatus* are alternatively treated as conspecifics or as separate taxa. COI divergence between lice form these taxa was 15%, suggesting that these bird taxa are not sharing lice and supporting the current placement of *vintsioides* as a full species rather than a subspecies of *cristatus*. Future studies should include additional sampling, particularly concentrating on hosts/lice collected from the Indo-Pacific where kingfisher diversity is highest.

CHAPTER IV

COPHYLOGENETIC ANALYSIS OF LICE IN THE *COLPOCEPHALUM*-COMPLEX (PHTHIRAPTERA: AMBLYCERA)

Chewing louse genera are typically restricted to a single avian host family or order. However, the louse genus *Colpocephalum* Nitzsch, 1818 (Phthiraptera: Amblycera: Menoponidae), as currently defined (Price et al. 2003), is found on 11 distantly related avian host groups. The type species of this genus is a parasite of white stork (*Ciconia ciconia*), and other *Colpocephalum* species have been described (Price et al. 2003) from a variety of different avian host orders including falcons (Falconiformes), pelicans and relatives (Pelecaniformes), gamebirds (Galliformes), flamingos and relatives (Ciconiiformes), and pigeons (Columbiformes). Although parasitizing a wide diversity of hosts, species placed within *Colpocephalum* are united by a comb of ctenidia on the sternites and femora and the presence of black occipital and preocular nodi (connected to each other). A diversity of other menoponid genera also fall within the *Colpocephalum*-complex on account of sharing these morphological features, and these other *Colpocephalum*-complex genera are each restricted to single avian host orders (e.g., *Psittacobrosus* on Psittaciformes and *Ciconiphilus* on Ciconiiformes). Some of these genera are morphologically well described, whereas others are not. Thus, taxonomic revisions and checklists (Hopkins and Clay1952; Price and Emerson 1966; Price et al. 2003) have synonymized many poorly described genera in the complex, considering them *Colpocephalum*, and have only retained those genera with detailed descriptions identifying significant morphological differences. As a result, in both past and present taxonomic classifications, the genus *Colpocephalum* is somewhat of a dumping ground and the generic limits in the complex as a whole are not well defined.

Clay (1968) placed a number of additional genera into the *Colpocephalum*-complex based on morphological characters of the head and legs. Interestingly, these genera have not been synonymized with *Colpocephalum* and many are codistributed on the same bird groups with *Colpocephalum sensu stricto* (*sensu* Hopkins and Clay 1952; Price and Beer 1963*a*, *b*). Although Clay (1968) placed some genera into the Colpocephalum-complex it was not a definitive list. In addition to *Colpocephalum* and *Kurodaia* we have identified 20-22 potential genera based on morphological characters: *Dicteisia*; *Epiara*; *Ardeiphilus*; *Colpocephalum*; *Ciconiphilus*; *Heterokodeia*; *Osborniella*; *Comatomenopon*; *Heteromenopon*; *Kurodaia*; *Psittacobrosus*; *Afrimenopon*, *Franciscoloa*; *Coramenopon*; *Turacoeca*; *Psittacomenopon*; *Falcomenopon*; *Odoriphila*; *Bucperocolpocehalum*; *Eomenopon*; and possibly *Mimemamenopon* and *Cuculiphilus*. The majority of these other genera in the *Colpocephalum*-complex have not been included in a molecular phylogeny, and therefore the relationships and monophyly of these genera with respect to *Colpocephalum* are unclear.

One of these morphologically similar genera is *Kurodaia* Uchida, 1926, which is differentiated from *Colpocephalum sensu stricto* by the lack of strongly defined occipital nodi in the head and differences in the female genitalia (Price and Beer 1963*c*, *d*). Furthermore, within *Kurodaia*, Price and Beer (1963b) recognized 2 subgenera, one parasitizing diurnal birds of prey (*Kurodaia*) and the other parasitizing owls (*Conciella*). No species of *Kurodaia* was included in Marshall's (2003) morphological phylogenetic analysis of Amblycera, but a molecular phylogeny with limited taxonomic sampling and sequences from 2 genes recovered *Colpocephalum* and *Kurodaia* as sister taxa (Johnson et al. 2003). However, Johnson et al. (2003) only included single representatives of these genera in their

phylogeny and therefore, monophyly of the genera and subgenera within the *Colpocephalum*-complex could not be assessed.

The monophyly of *Colpocephalum* has never been tested in a modern phylogenetic framework. If *Colpocephalum* is monophyletic, then interordinal and interfamilial host switching is likely rampant because the host orders and families of this louse genus are not all closely related and instead are scattered across the avian tree of life (Hackett et al. 2008; Jarvis et al. 2015; Prum et al. 2015). Additonally, the various host orders parasitized by *Colpocephalum* do not share life history characteristics, such as competition for nest cavities (Clayton 1990; Johnson et al. 2002) that could explain the widespread distribution pattern. Recently, molecular phylogenies have called into question the validity of taxonomically widespread louse genera. For example, the ischnoceran louse genus *Degeeriella* Neumann, 1906, which parasitizes hawks (Accipitriformes) and falcons (Falconiformes), consists of 2 distinct, unrelated lineages, one specific to each host order (Catanach and Johnson 2015) while the genus *Picicola* Clay & Meinertzhagen, 1938 is actually 5 different lineages corresponding to 3 different host orders (Pereyra et al. in prep.).

Here, I reconstruct a phylogeny for *Colpocephalum* and *Kurodaia* to: (1) test the monophyly of *Colpocephalum*, (2) test the validity of *Kurodaia*, (3) reconstruct the history of interordinal and interfamilial host switching events in the complex, and (4) directly compare the phylogeny of these lice to that for *Degeeriella* (Catanach and Johnson 2015), which is distributed on some of the same groups of birds. The goal of this comparison is to evaluate whether codistributed parasites exhibit correlated divergence events as a result of concordant evolutionary events.

MATERIALS AND METHODS

Specimen Acquisition

Lice were collected from avian hosts in various ways, including ethyl acetate fumigation of vouchered host specimens, dust ruffling, and manual searches of hosts that were banded and released (Clayton et al. 1992; Walther and Clayton 1997). In total, 39 *Colpocephalum* and 11 *Kurodaia* were included (Table 4.1). To test the monophyly of *Colpocephalum* and *Kurodaia* I also included representatives of 8 additional genera considered members of the *Colpocephalum*-complex by Clay (1968; Table 4.1). When possible, I included DNA sequences from multiple host individuals (up to 4 specimens per host species), particularly from geographically widespread host species.

DNA Sequencing

For each louse specimen prior to extraction, I made a small incision between the head and thorax as described by Valim and Weckstein (2011) and a second incision between 2 abdominal sclerites and then placed the specimen in digestion buffer. I used the QIAamp DNA Micro Kit (Qiagen, Valencia, CA) for DNA extraction following a modified version of the protocol for total genomic DNA from tissues. Modifications include lengthening the incubation period in step 4 to 36 hours, incubating the sample for 10 minutes at 70°C in step 6, and decreasing the amount of Buffer AE in elution step (step 12) to 50 µL (which was repeated twice in different 1.5 mL collection tubes). During step 12, once pipetted to the filter, the Buffer AE was incubated for 5 minutes at 70°C prior to centrifugation rather than performing step 13. Specimen exoskeletons were retained, cleared, and mounted on a microslide in balsam as vouchers, following the general protocols of Palma (1978). Slides were identified using the relevant taxonomic literature for these taxa.

Table 4.1. List of louse taxa and host data from which DNA was included in study.

Louse species	Extraction Code	Locality	Host species	COI	COIL	EF1a
Ciconiphilus decimfasciatus	Cisp.Bustr.7.18.2014.13	Brazil	*Butorides striata*	x	x	
Anseriphilus sp.	Ciconiphilus sp RF 49		*Cygnus olor*	x		X
Colpocephalum alecturae	Cwsp.Allat.8.19.2013.7	Australia	*Alectura lathami*		x	X
Colpocephalum turbinatum	Kusp.Bulac.1.31.2014.6	Malawi	*Bubo lacteus*	x	x	
Colpocephalum cristatae	Cwsp.Cabur.2.21.2013.4	Bolivia	*Chunga burmeisteri*		x	
Colpocephalum cucullare	Cwsp.Saser.5.21.2014.2	Kenya	*Sagittarius serpentarius*		x	
Colpocephalum fregili	Cwsp.Coalb.1.31.2014.8	Malawi	*Corvus albus*	x	x	
Colpocephalum fregili	Cwsp.Coalc.1.31.2014.9	Malawi	*Corvus albicollis*	x	x	
Colpocephalum heterosoma	Cwsp.Phand.5.24.2013.4	Argentina	*Phoenicoparrus andinus*	x	x	X
Colpocephalum heterosoma	Cwsp.Phchi.5.24.2013.3	Argentina	*Phoenicopterus chilensis*		x	X
Colpocephalum ibicter	Cwsp.Ibame.7.18.2014.6	Peru	*Ibycter americanus*			X
Colpocephalum indi	Cwsp.Icmis.2.21.2013.9	Louisiana	*Ictinia mississippiensis*	x		X
Colpocephalum kelloggi	Cwsp.Caaur.2.21.2013.2	Illinois	*Cathartes aura*	x	x	X
Colpocephalum kelloggi	Cwsp.Caaur.8.2.2013.14	Canada	*Cathartes aura*	x		X
Colpocephalum nanum	Cwsp.Accoo.10.31.2014.6	Canada	*Accipiter cooperii*	x	x	X
Colpocephalum nanum	Cwsp.Bujam.8.2.2013.13	Canada	*Buteo jamaicensis*		x	X
Colpocephalum nanum	Cwsp.Bujam.8.2.2013.7	Canada	*Buteo jamaicensis*		x	X
Colpocephalum nanum	Cwsp.Bulag.1.31.2014.5	USA	*Buteo lagopus*		x	
Colpocephalum napiforme	Cwsp.Bulag.10.31.2014.9	Canada	*Buteo lagopus*	x	x	X
Colpocephalum polybori	Cwsp.Cache.5.24.2013.7	Texas	*Caracara cheriway*	x	x	X
Colpocephalum sp.	Cwsp.Lecay.7.18.2014.5	Peru	*Leptodon cayanensis*		x	
Colpocephalum sp.	Cwsp.Faamu.5.21.2014.4	Kenya	*Falco amurensis*		x	
Colpocephalum spinicollis	Cwsp.Thsp.2.21.2013.10	Australia	*Threskiornis spinicollis*	x	x	
Colpocephalum subzerafae	Cwsp.Faber.2.21.2013.7	Australia	*Falco berigora*	x	x	
Colpocephalum subzerafae	Cwsp.Facol.8.19.2013.6	Canada	*Falco columbarius*			X
Colpocephalum subzerafae	Kufas.Facol.8.19.2013.4	Canada	*Falco columbarius*		x	X
Colpocephalum turbinatum	Cwsp.Bugal.5.24.2013.1	Galapagos	*Buteo galapagoensis*	x	x	X
Colpocephalum turbinatum	Cwsp.Ciapp.2.1.2013.6	New Zealand	*Circus approximans*	x	x	X
Colpocephalum turbinatum	Cwsp.Haleu.2.1.2013.9	Texas	*Haliaeetus leucocephalus*			X
Colpocephalum turbinatum	Cwsp.Haleu.8.2.2013.4	Canada	*Haliaeetus leucocephalus*	x	x	X
Colpocephalum turbinatum	Cwsp.Hasph.5.24.2013.2	Australia	*Haliastur sphenurus*	x		
Colpocephalum turbinatum	Cwsp.Helon.2.1.2013.4	PNG	*Henicopernis longicauda*		x	X
Colpocephalum turbinatum	Cwsp.Bulac.1.31.2014.7	Malawi	*Bubo lacteus*		x	
Colpocephalum unciferum	Cwsp.Peery.2.21.2013.1	Louisiana	*Pelecanus erythrorhynchus*	x	x	X
Colpocephalum unciferum	Cwsp.Peery.8.2.2013.9	Canada	*Pelecanus erythrorhynchus*		x	X
Cuculiphilus (*Cuculiphilus*) *fasciativentris*	Cqsp.Pimel.7.18.2014.12	Brazil	*Piaya melanogaster*	x		
Cuculiphilus (*Falcophilus*) *alternatus*	Cwsp.Coatr.2.1.2013.10	Texas	*Coragyps atratus*	x	x	X
Cuculiphilus sp.	Cqsp.Chkla.4.3.2000.2	Africa	*Chrysococcyx klaas*	x		X
Kurodaia (*Conciella*) *longipes*	Kusp.Buafr.1.31.2014.15	Malawi	*Bubo africanus*	x	x	X
Kurodaia (*Conciella*) sp.	Kumag.Stneb.10.31.2014.7	Canada	*Strix nebulosa*	x		X
Kurodaia (*Kurodaia*) *fulvofasciata*	Kusp.Acpol.2.1.2013.3	PNG	*Accipiter poliocephalus*	x	x	
Kurodaia (*Kurodaia*) *fulvofasciata*	Kuful.Bujam.8.19.2013.2	Canada	*Buteo jamaicensis*		x	X
Kurodaia (*Kurodaia*) *fulvofasciata*	Kusp.Bujam.1.31.2014.4	USA	*Buteo jamaicensis*	x	x	X
Kurodaia (*Kurodaia*) *fulvofasciata*	Kusp.Bumag.1.31.2014.12	Peru	*Buteo magnirostris*	x		
Kurodaia (*Kurodaia*) *fulvofasciata*	Kusp.Icplu.1.31.2014.10	Peru	*Ictinia plumbea*	x	x	
Kurodaia (*Kurodaia*) *haliaeeti*	Cwsp.Pahal.1.31.2014.14	USA	*Pandion haliaetus*	x	x	X
Kurodaia (*Kurodaia*) *haliaeeti*	Cwsp.Pahal.2.21.2013.8	Australia	*Pandion haliaetus*	x	x	X
Kurodaia (*Kurodaia*) *haliaeeti*	Cwsp.Pahal.5.24.2013.8	Texas	*Pandion haliaetus*	x		X
Kurodaia (*Kurodaia*) *haliaeeti*	Kuhal.Pahal.8.2.2013.2	Canada	*Pandion haliaetus*	x	x	
Microctenia major	Mtsp.Timaj.7.18.2014.15	Brazil	*Tinamus major*	x	x	
Piagetiella bursaepelecani	Qibur.5.1.2000.3	USA	*Pelecanus occidentalis*	x		X
Psittacobrosus sp.	Pssp.Amalb.5.4.1999.10	Central America	*Amazona albifrons*	x		X
Psittacobrosus anduzei	Psand.3.29.1999.3	Mexico	*Eupsittula nana astec*	x		X
Psittacobrosus molinae	Hmsp.Pymel.7.18.2014.14	Brazil	*Pyrrhura melanura*	x	x	
Psittacomenopon impar	Qmsp.Pocry.7.18.2014.16	Malawi	*Poicephalus cryptoxanthus*	x	x	
Psittacomenopon impar	Qmsp.Pomey.7.18.2014.8	Malawi	*Poicephalus meyeri*	x	x	
Trinoton querquedulae	Amsp.Anpla.4.19.1999.3	USA	*Anas platyrhynchos*	x		X

After extraction, PCR (25 µL reactions) was performed to amplify 3 fragments of DNA from 2 genes, including 2 fragments of the mitochondrial protein coding gene: cytochrome oxidase I (COI) and the nuclear protein coding gene: elongation factor-1α (EF-1α). For COI amplification, I used primers L6625 and H7005 (fragment 1) (Hafner et al. 1994) and LCO1490 and HCO2198 (fragment 2) (Folmer et al. 1994) and for EF-1α I used EF1-For3 and EF1-Cho10 (Danforth and Ji 1998). PCR conditions follow those for Smith et al. (2004) except that an annealing temperature of 50°C was used for EF-1α. Cycle sequencing reactions were performed using 1µL of BigDye, 2µL of sequencing buffer, 5.2µL of 12.5% glycerol and 2 µL of 1 µM primer. The resulting product was submitted for automated sequencing on an ABI 3730xl automated capillary sequencing machine at the University of Illinois Keck Center for Comparative and Functional Genomics. Raw forward and reverse strands of each sequence were assembled in Geneious 8.0.4 (Biomatters Ltd.) and manually reconciled. Resulting consensus sequences were aligned in Geneious using the MUSCLE plugin and exported to Seaview 4.3.0 where they were checked and adjusted by eye (Edgar 2004; Gouy et al. 2010).

Louse Identification

After extraction, all louse specimens were mounted permanently on slides and identified using available parasite literature for each host group. The genus *Colpocephalum* is a good example of the benefits of compiling a parasite specimen collection along with the relevant taxonomic literature. Many of taxa within *Colpocephalum* and related genera are poorly described and have never been redescribed using modern standards. In this study, I worked with Michel Valim to morphologically compared my specimens to those described from the same host (*sensu* Price et al. 2003). We then compared our specimens with those described or redescribed in taxonomic revisions for each bird group as listed here:

Accipitriformes (Price and Beer 1963b,c), Anseriformes (Clay and Hopkins 1960; Price and Beer 1965b), Cariamiformes (Price 1968), Cathartiformes (Price and Beer 1963b; Scharf and Price 1965), Cuculiformes (Scharf and Price 1965), Falconiformes (Price and Beer 1963b,c), Galliformes (Price and Beer 1964), Passeriformes (Price and Beer 1965b), Pelecaniformes (Price and Beer 1965a; Price 1970), Psittaciformes (Price and Beer 1966, 1968), Strigiformes (Price and Beer 1963a,d), and Tinamiformes (Guimarães 1947). Specimens used in this dataset which could not be positively identified to species based on available literature and reference specimens were considered as "sp.", regardless their host association. No identification was made based exclusively on host-parasite relationship.

Phylogenetic Analysis

The 3 gene regions were first analyzed separately to ensure that gene trees were not in conflict (Fig 4.1, posterior probability [PP] greater than 0.95). Gene trees were inferred using Bayesian Inference (BI) as implemented in BEAST 2.3.1 (Drummond and Rambaut 2007) run 40 million generations under the model selected by PartitionFinder 1.1.1 (Lanfear et al. 2012) with branchlengths = linked; model_selection = AIC; search = greedy). PartitionFinder favored an 8 partition model (each gene/codon position separate with the exception of the 2nd codon position for both regions of COI) with GTR+I+G selected for all COI partitions except the 3rd positions for which HKY+I+G was favored for both fragment 1. PartitionFinder favored a different model for each EF-1α codon position, selecting TrN + I, HKY+I, and GTR+G for codon positions 1, 2, and 3, respectively. During phylogenetic analyses each partition was unlinked. No major conflicts occurred among ingroup taxa, so I concatenated the gene sequences.

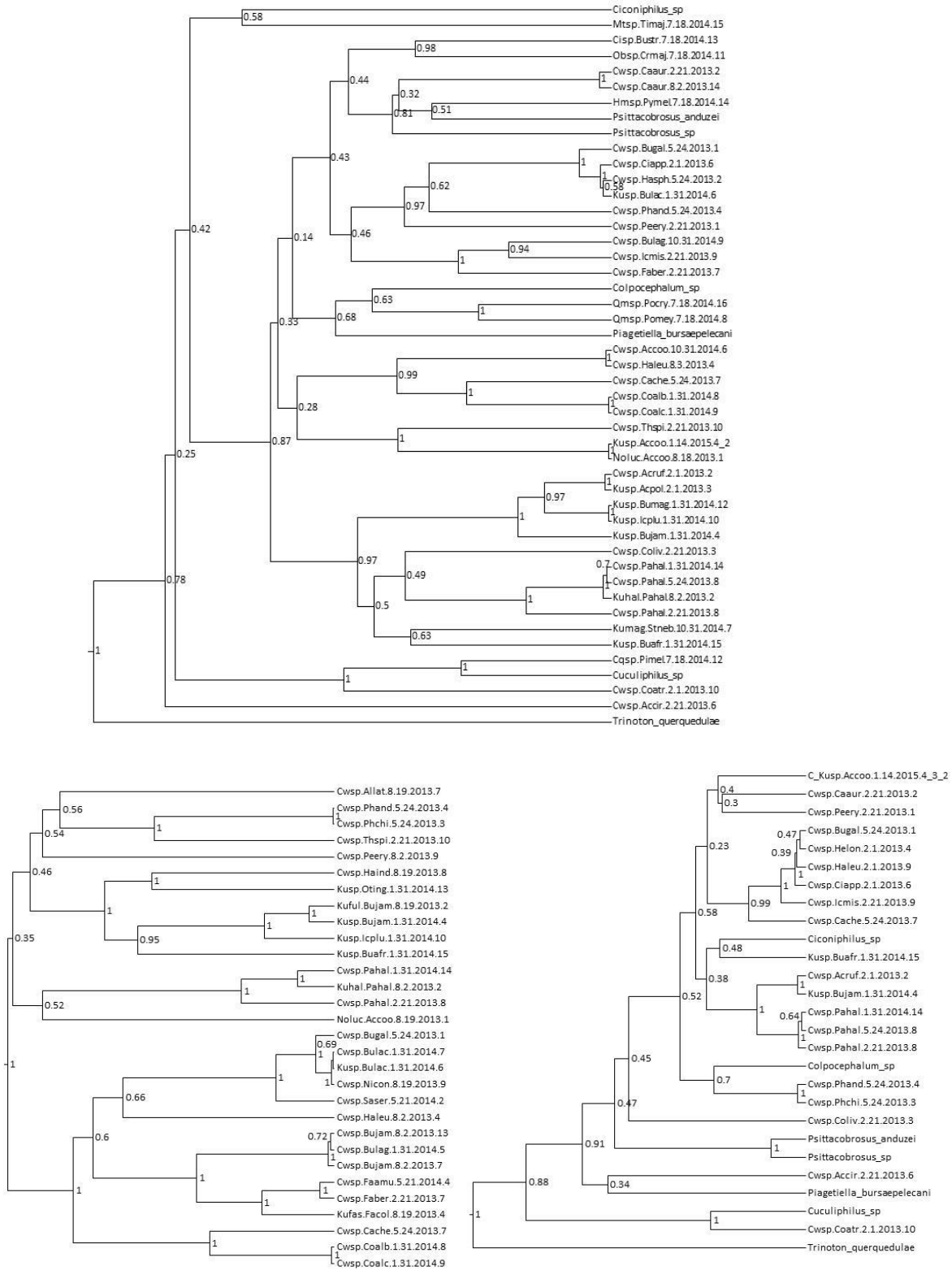

Figure 4.1 Individual gene trees of *Colpocephalum*-complex members. Top is COI fragment 1, bottom left is COI fragment 2, and bottom right is EF-1α. Values represent posterior probabilities

Phylogenies based on the combined analysis were inferred using BI as implemented in BEAST 2.3.1 (Drummond and Rambaut 2007; 40 million generations, sampled every 1,000 generations, burnin = 10,000), Maximum Likelihood (ML) as implemented in Garli version 2.01. (Zwickl 2006: 10 independent runs, default settings, automated stop criterion = 50,000), and Maximum Parsimony (MP) as implemented in PAUP* (Swofford 2003; 1,000 random addition sequences with TBR branch swapping). Bayesian posterior probabilities and both MP (1,000 replicates of 100 random addition sequences with maxtrees set at 1,000 due to computational constraints) and ML bootstrap values (500 bootstrap replicates on default settings with automated stop criterion = 50,000) were used to evaluate branch support. In BI analyses

Cophylogenetic Analysis

Phylogenetic signal for host taxonomy (order and family) and host geography was tested using a Maddison and Slatkin (1991) test. Host taxonomy was based on the eBird-Clements checklist (eBird-Clements-v2015-integrated-checklist-August-2015 available through Cornell University: http://www.birds.cornell.edu/clementschecklist/download/). Geography was coded based upon where the host was acquired – Nearctic, Neotropics, Ethiopian, Australasian, Palearctic, and Oriental regions. In cases where I sampled multiple host individuals from the same species and geographic region, I pruned tips to limit the tree to a single representative to prevent duplicate samples of the same louse from influencing the results (and bias results towards finding evidence of significant phylogenetic signal). The test was performed using R code (available at www.github.com/juliema/publications; Bush et al. 2016)

Twelve host species of *Colpocephalum* included in this phylogeny also harbor *Degeeriella*, a second genus of louse parasitizing diurnal birds of prey. These species of *Degeeriella* were previously included in a phylogeny of the genus (Catanach and Johnson 2015). Following the methods outlined in Sweet et al. (2016), I used the R implementation of PARAFIT (in package 'ape'; Legendre et al. 2002; Paradis et al. 2004) to evaluate whether cophylogenetic patterns were correlated between the 2 louse genera and their hosts. PARAFIT tests for evidence of congruence between host and parasite trees by randomizing the association matrix. In addition to calculating a global measure of congruence, individual links are also evaluated to determine how much each contributes to the global test statistic. This process results in an F1 (more conservative) and F2 (in some instances has greater power) statistic, both of which were retained in my analysis (Legendre et al. 2002). The host trees were created by selecting the relevant species using the Phylogeny Subsets tool from BirdTree.org (Jetz et al. 2012) and the *Degeeriella* tree is from Catanach and Johnson (2015). A random sampling of 1,000 Ericson All Species trees was downloaded then summarize into a single tree using TreeAnnotator (Drummond and Rambaut, 2007). Parasite trees were pruned in R (using package 'ape'; Paradis et al. 2004) to remove outgroups and duplicates (where a single louse species was sampled multiple times, based on sequence divergence and tree topology). PARAFIT was run for 999 permutations comparing the host tree to the *Colpocephalum* tree and the host tree to *Degeeriella* tree using an R script (available at https://github.com/adsweet/cophylogenetic_analyses).

To determine whether cophylogenetic patterns are correlated between *Colpocephalum* and *Degeeriella*, I analyzed a 2 x 2 contingency table of significant and non-significant links for each genus. In instances where 2 links existed for a single host species from one of the

suborders (i.e., a host species infested with 2 congeneric louse species from the Amblycera suborder), the louse from the other suborder (e.g., Ischnocera) was counted twice. For example, 2 different species of lice from the *Colpocephalum*-complex occur on red-tailed hawk (*Buteo jamaicensis*), whereas only a single *Degeeriella* taxon occurs. The *Degeeriella* link is therefore counted twice to fill the contingency table. I performed a Fisher's exact test (in R) to determine whether patterns between *Colpocephalum* and *Degeeriella* were correlated. A significant test would indicate these 2 genera had similar cophylogenetic patterns.

RESULTS

The tree resulting from Bayesian analysis of COI and EF-1α sequences for the *Colpocephalum*-complex (Fig 4.2) indicated that members of *Colpocephalum* were placed in several distinct lineages, most of which parasitize a single host order or clade (Fig 4.3). Although many of the lineages were strongly supported as monophyletic (posterior probabilities ≥ 0.95), some lacked statistical support, and support among lineages along the backbone of the tree was generally very low. The tree suggests that *Colpocephalum* is not monophyletic. However, there was not significant support for this result.

Kurodaia from diurnal and nocturnal birds of prey form a strongly supported monophyletic group (PP = 0.95; Fig. 4.2, clades O and P). Within *Kurodaia*, there are 3 well-supported (PP > 0.99) lineages. One includes lice from owls, from the subgenus *Conciella* (Fig 4.2, clade P), and this clade is sister to lice from the subgenus *Kurodaia* (Fig 4.2, clade O) parasitizing hawks (Accipitriformes).

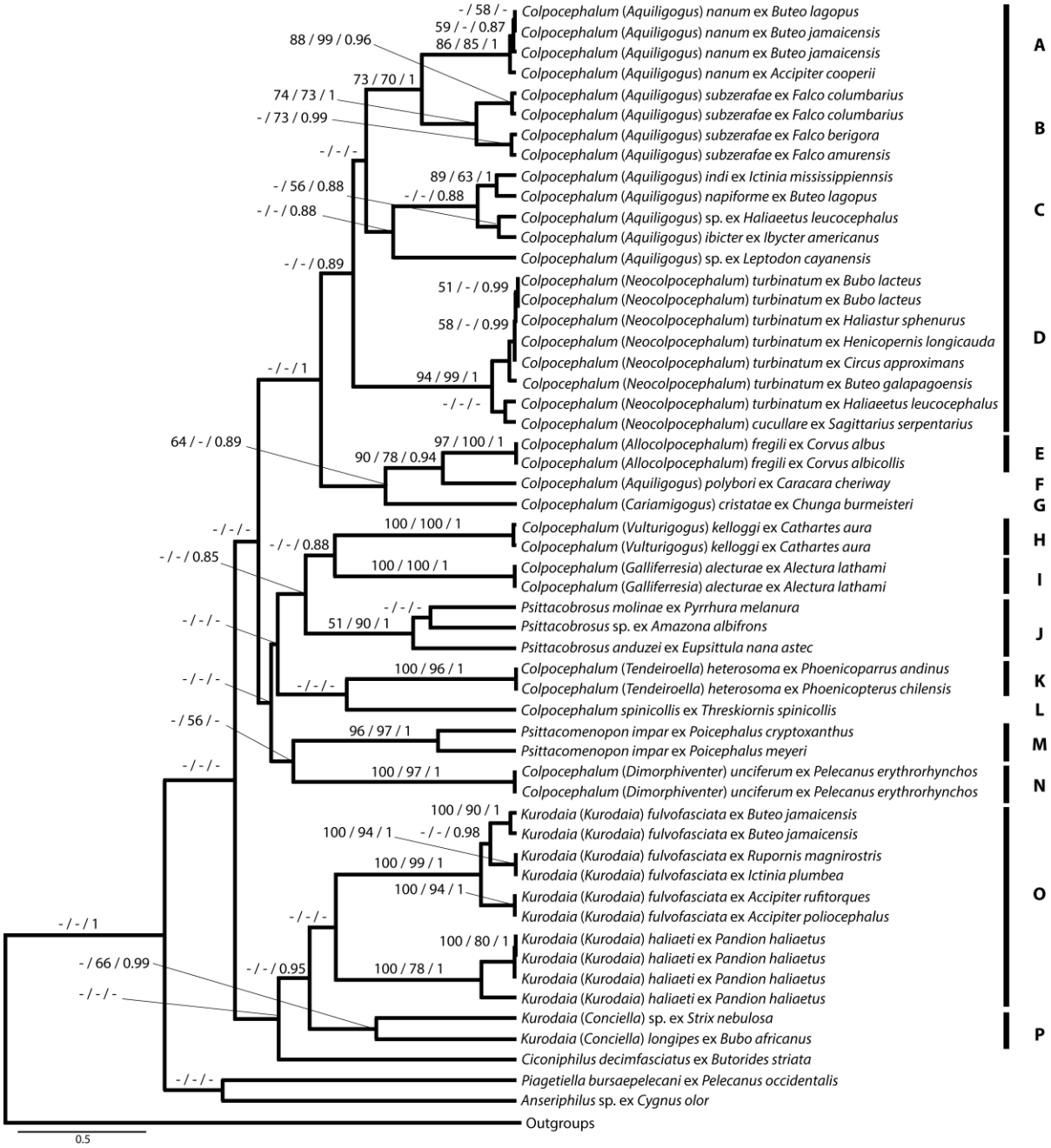

Figure 4.2. Phylogeny of the *Colpocephalum*-complex (with outgroups removed). Base tree is an ultrametric tree generated from BEAST. Numbers on branches represent MP bootstrap values (≥50), ML bootstrap values (≥50) and BI posterior probability values (≥0.85). Letters next to clades identify clades discussed in the text

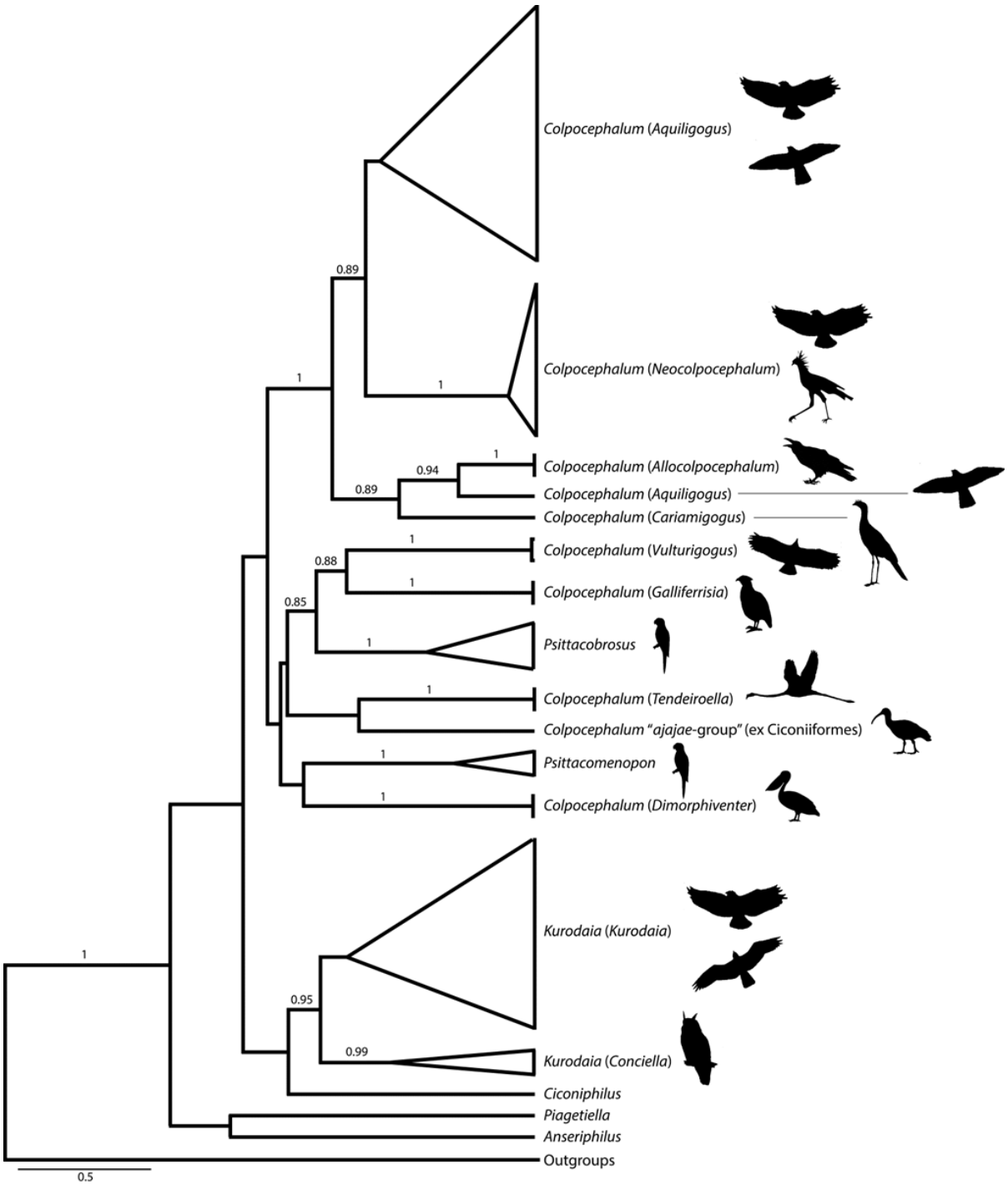

Figure 4.3. Phylogeny of the *Colpocephalum*-complex (with outgroups removed) showing subgenera of *Colpocephalum* and *Kurodaia*. Silhouettes represent host orders. Numbers on branches represent BI posterior probability values (≥ 0.85).

Although the owl louse clade, which contains 2 owls, one from Africa and one from North America was well supported in BI, it was not supported in MP or ML (PP = 0.99, ML = 66). Within clade O were all *Kurodaia* (*Kurodaia*) *haliaeti* (Denny 1842) sampled from the osprey (*Pandion haliaetus*; Fig 4.2; PP = 1.0, MP = 100, ML = 78). Within the rest of clade O were lice from Accipitridae including a clade of red-tailed hawk lice (*Buteo jamaicensis*), a clade containing lice from roadside hawk (*Rupornis magnirostris*) and plumbeous kite (*Ictinia plumbea*), and a clade containing lice from Fiji goshawk (*Accipiter rufitorques*) and gray-headed goshawk (*Accipiter poliocephalus*; Fig 4.2). Each of these lineages has a posterior probability of 1.0 and bootstrap values over 90 in both MP and ML. The currently recognized subgenera *Kurodaia* (*Kurodaia*) from diurnal birds of prey and *Kurodaia* (*Conciella*) from owls form reciprocally monophyletic groups in the tree.

Two genera of lice (*Psittacomenopon*; Bedford 1930 and *Psittacobrosus;* Carriker 1954) from parrots fell within the genus *Colpocephalum*, although the placement of these genera was not strongly supported (Fig 4.2, clades J and M). Within *Colpocephalum*, one major clade consisted primarily of lice from diurnal and nocturnal birds of prey (clades A-G) whereas a second includes *Colpocephalum* from a variety of other birds and the 2 genera of parrot lice (clades H-N). There are 6 main lineages within this second major clade, all of them restricted to distinct host groups; however, the relationships among them were not well-resolved. This group also includes two genera of parrot lice, *Psittacobrosus* (Fig. 4.2, clade J) and *Psittacomenopon* (Fig. 4.2, clade M). The remaining lineages of *Colpocephalum* correspond to groups some authors recognize as subgenera of *Colpocephalum*: *Vulturigogus* Eichler & Złotorzycka, 1963 (on New World Vultures; Fig. 1, clade H), *Dimorphiventer* Eichler, 1944 (on pelicans and frigate birds; Fig. 4.2, clade N), *Tendeiroella* Eichler, 1982

(on flamingos; Fig. 4.2, clade K), and *Galliferrisia* Ansari, 1951 on Australian Brushturkey (Galliformes: *Alectura lathami*; Fig. 1, clade I).

Although the first major clade (Fig. 4.2 clades A-G, exclusively containing *Colpocephalum* from diurnal birds of prey (Accipitridae and Falconidae), nocturnal birds of prey (Strigidae), corvids, and seriemas (Cariamidae)) was well supported in BI (PP = 1.0), it was not supported in MP or ML. This clade is divided into 2 lineages. One is comprised of lice from 2 African corvids (Fig 4.2, clade E), black-legged seriema (Cariamiformes: *Chunga burmeisteri*; Fig 4.2, clade G), and crested caracara (Falconidae: *Caracara cheriway*; Fig 4.2, clade F), but has weak statistical support (PP = 0.89, MP = 64). This clade includes two taxa here considered subgenera within *Colpocephalum*: *Allocolpocephalum* Qadri, 1939, from corvids, and *Cariamigogus* Eichler, 1952, from seriemas. The other (Fig. 4.2, clade A–D) (PP = 0.89) contains lice from only birds of prey, including owls, hawks, and falcons. Within this clade, lice placed in the subgenera *Neocolpocephalum* Ewing, 1933 (on Accipitriformes and Strigiformes; Fig. 4.2, clade D) and *Aquiligogus* Eichler & Złotorzycka, 1971 (on hawks and falcons; Fig. 4.2 clades A, B, and C) fall into two distinct groups, though monophyly of *Aquiligogus* is not well supported and the monophyly of *Neocolpocephalum* is only supported in BI.

Cophylogenetic Analysis

All 3 Maddison-Slatkin (1991) tests (for host order, host family, and host geography) revealed significant evidence of phylogenetic signal ($P < 0.05$ in all cases). However, host taxonomy was highly correlated ($P < 0.001$) and biogeography was less strongly correlated with the louse tree ($P = 0.039$).

PARAFIT indicated congruence between host and parasite trees for both *Degeeriella* and *Colpocephalum* (global test $P = 0.001$ for both genera). Although 5 links within *Degeeriella* were significant (F1 and F2 statistics were identical for each pair) and 3 links in *Colpocephalum* were significant, no links were shared between the 2 genera. Thus, the results of a Fisher's Exact test on the contingency table were not significant ($P = 0.264$), indicating that branching patterns of *Colpocephalum* and *Degeeriella* were independent of each other.

DISCUSSION

Phylogenetic analyses of a mitochondrial and nuclear gene from a diversity of *Colpocephalum*-complex members produced the first molecular phylogeny for this complex of avian lice. Although *Colpocephalum* is not monophyletic in my analysis, its monophyly cannot be ruled out completely because of weakly supported nodes along the backbone of the tree. However, I did find a number of strongly supported clades within the complex, most which correspond to existing genera or subgenera (Fig 4.3). Our work suggests that either *Psittacomenopon* and *Psittacobrosus* should be treated as subgenera of *Colpocephalum* or many subgenera within *Colpocephalum* should be returned to full generic status. However, without a detailed morphological study of the group nomenclatural recommendations would be premature. Further analysis, including more nuclear gene sequences, is required to determine whether the genus *Colpocephalum* is monophyletic. Further taxon sampling of this complex also is needed because my sampling lacked several currently synonymized subgenera, including the type species, *Colpocephalum zebra*, which is recorded from White Stork (*Ciconia ciconia*).

Several genera have been previously synonymized with *Colpocephalum* (Hopkins and Clay 1952; Price and Emerson 1966; Price et al. 2003) and are currently treated as subgenera. These include *Allocolpocephalum* (Fig. 4.2, clade E; on crows), *Cariamigogus* (Fig. 4.2, clade G; on seriemas), *Vulturigogus* (Fig. 4.2, clade H; on New World Vultures), and *Galliferrisia* (Fig. 4.2, clade I; on Australian brushturkey), *Tendeiroella* (Fig. 4.2, clade K; on flamingos), *Dimorphiventer* (Fig. 4.2, clade N; on pelicans and frigatebirds). Each of these subgenera form highly supported, monophyletic clades. There are 2 lineages currently treated as subgenera of *Colpocephalum* that are widely distributed on diurnal and nocturnal birds of prey: *Aquiligogus* and *Neocolpocephalum*. Each of these forms a monophyletic clade and the branch lengths on these lineages are similar to those seen in the lineages currently treated as genera within the *Colpocephalum* complex (uncorrected p values over 15%). Although monophyly of *Neocolpocephalum* is well supported (PP = 1, MP = 94), support is weak for monophyly of *Aquiligogus*. The presence of these well supported lineages corresponding to subgenera suggests these lineages are distinct evolutionary units and further research may warrant them being returned to generic status.

Two main lineages (*Kurodaia* and *Colpocephalum* [in part]) of lice within the *Colpocephalum*-complex parasitize Accipitriformes and Strigiformes. Our data suggests that there are at least three distinct lineages of *Colpocephalum*-complex lice on raptors: *Kurodaia* (comprised of two subgenera: *Kurodaia* and *Conciella* which parasitize diurnal birds of prey and nocturnal owls respectively), *Colpocephalum* from the accipitriform family Accipitridae and the strigiform family Strigidae (comprising two subgenera, *Aquiligogus* and *Neocolpocephalum*), and *Colpocephalum* (*Vulturigogus*) from New World Vultures (Cathartidae).

Two species of *Colpocephalum* and one species of the subgenus *Kurodaia* have been recorded from Osprey (*Pandion haliaetus*), all four sequenced individuals form a single lineage that was placed within the *Kurodaia* clade. Although three of these specimens were from North America, the fourth was from an Osprey sampled in Australia. The Australian Osprey louse was sister to all of the North American Osprey lice. Both the Osprey louse clade as a whole, and the North American Osprey louse clade had high support values (PP=1.0 MP=100, ML= 78 and 80 respectively; Fig 4.2 part of clade O). As Osprey are found on portions of every continent, additional sampling from other portions of the geographic distribution could shed light on the population structure and movement patterns of this cosmopolitan species, and additional sampling could be used to test the findings of Monti et. al (2015) who suggested Osprey originated in the New World and then later colonized the Old World.

Lice from owls fell in 2 different clades in the tree. *Colpocephalum turbinatum*, from Verreaux's eagle owl (*Bubo lacteus*), was deeply embedded within the *Colpocephalum* (*Neocolpocephalum*) clade (Fig. 4.2 clade D), which included a number of *Colpocephalum turbinatum* specimens from diurnal birds of prey. A clade of lice from 2 other owls (great grey owl, *Strix nebulosa*, and spotted eagle-owl, *Bubo africanus*) form the subgenus *Kurodaia* (Fig. 4.2 clade P; *Conciella*) which was sister to the nominal subgenus *Kurodaia* (Fig. 4.2 clade O; *Kurodaia*).

Overall, host taxonomy at both the order and family level is highly correlated with the louse phylogeny. Host geography also explains louse phylogeny, although with less statistical support. A more formalized cophylogenetic analysis, PARAFIT, showed significant congruence between the *Colpocephalum*-complex phylogeny and host phylogeny

($P = 0.001$). Comparing significant links between 2 louse genera in 2 different suborders (*Colpocephalum* and *Degeeriella*) on the same group of hosts did not reveal any correlation between the 2. Although these two genera infect many of the same species of hosts they differ in dispersal methods. *Degeeriella* is known to disperse via phoresy (hitchhiking on more mobile species, in this case winged hippoboscid flies), whereas *Colpocephalum* does not. This limits *Colpocephalum's* ability to colonize novel host species. Phoresis by *Degeeriella* has the potential to result in regional populations of lice that freely move between different host species, and could explain the lack of correlation in cophylogenetic patterns between these 2 genera of lice.

The *Colpocephalum*-complex includes lice parasitizing a wide array of host species. Here, I identified monophyletic lineages within this complex that parasitize individual host orders. These lineages are treated as either subgenera within the large *Colpocephalum* genus, or as full, but closely related genera. Although our analysis found support for these clades, backbone support to determine how lineages are related to each other was lacking a problem that has been solved with genomic data in other insect taxa (Misof et al. 2014). Additionally, I lacked molecular grade specimens for many of the type species for the various lineages. Future studies should include these species so recommendations regarding the nomenclatural status of these genera/subgenera can be made.

CHAPTER V

CONCLUSIONS

Here, I inferred phylogenies for 2 pairs of feather lice genera infesting 2 groups of birds. While all 4 louse genera feed on feather and skin debris they differ in distribution patterns and dispersal methods. The first pair of genera, *Degeeriella* and *Colpocephalum*, infest a wide variety of diurnal birds of prey, including many of the same host species (and on occasion the same individual bird). However, *Degeeriella* is known to attach to hippoboscid flies, a generalist parasite, potentially allowing these lice to colonize novel host species. Conversely, *Colpocephalum*relies on direct contact to colonize new hosts (Keirans 1975). Thus, opportunities for *Colpocephalum* to colonize novel host species are limited because this genus relies on direct contact (such as while feeding young or during copulation), which tends to occur between conspecifics hosts. Although these lice occur on many of the same host species, I found the phylogenies inferred for these 2 genera are not congruent, suggesting these lineages have different evolutionary histories. This lack of congruence could be driven by phoresy, as *Degeeriella* have more opportunities to colonize novel hosts, which is reflected in the finding that many inferred clades are geographically restricted.

The monophyly of *Degeeriella* and *Colpocephalum* had not been investigated using modern techniques. The phylogeny of *Degeeriella* and related genera inferred that *Capraiella*, a louse genus occurring on rollers, is within *Degeeriella* from hawks. Additionally, *Degeeriella* from hawks is distantly related to *Degeeriella* from falcons. Similarly, *Colpocephalum* has traditionally been comprised of many morphologically distinct

groups. However, due to limitations of taxon sampling and the lack of backbone support no nomenclatural recommendations can be made.

The second pair of lice, *Alcedoffula* and *Alcedoecus*, were restricted to kingfishers. Although most bird species host a number of feather lice, kingfishers are unusual in that each species is usually infested with a single louse species. Additionally, in the 2 genera partition based on higher-level host relationships I found *Alcedoffula* comprises 2 distinct clades, each of which is limited to either Alcedininae kingfishers or Cerylinae kingfishers. These two subfamilies are not sisters so simple cospeciation does not explain this distribution. The third kingfisher subfamily, Daceloninae is parasized by both *Alcedoecus* and *Emersoniella*. Ancestral state reconstruction revealed that a single clade within Daceloniane is parasizied by *Emersoniella* (with two small lineages within this clade parsized by *Alcedoecus*, suggesting multiple host switches have occurred). . I tested the lice on each lineage for evidence of cospeciation and found that while the lice on Daceloninae and Alcedininae did not show evidence of cophylogeny with their hosts, *Alcedoffula* parasitizing Cerylinae showed strong evidence of cophylogeny.

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