Parasitology

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Two lineages of kingfisher feather lice exhibit differing degrees of cospeciation with their hosts

Therese A. Catanach[1,*], Kevin P. Johnson[2], Ben D. Marks[3], Robert G. Moyle[4], Michel P. Valim[5] and Jason D. Weckstein[6]

[1]Department of Ornithology, Academy of Natural Sciences of Drexel University, 1900 Benjamin Franklin Parkway, Philadelphia, PA, 19103, USA; [2]Illinois Natural History Survey, Prairie Research Institute, University of Illinois at Urbana-Champaign, 1816 S. Oak St., Champaign, IL, 61820, USA; [3]Field Museum of Natural History, Science and Education, 1400 S. Lake Shore Drive, Chicago, IL 60605, USA; [4]Department of Ecology and Evolutionary Biology and Biodiversity Institute, University of Kansas, 1345 Jayhawk Blvd., Lawrence, KS, 66045, USA; [5]Biotério da Universidade Iguaçu, 2134 Av. Abílio Augusto Távora, Nova Iguaçu, RJ, 26260-045, Brazil and [6]Department of Ornithology, Academy of Natural Sciences of Drexel University, and Department of Biodiversity, Earth, and Environmental Sciences, Drexel University, 1900 Benjamin Franklin Parkway, Philadelphia, PA, 19103, USA

Abstract

Unlike most bird species, individual kingfisher species (Aves: Alcedinidae) are typically parasitized by only a single genus of louse (*Alcedoffula*, *Alcedoecus*, or *Emersoniella*). These louse genera are typically specific to a particular kingfisher subfamily. Specifically, *Alcedoecus* and *Emersoniella* parasitize Halcyoninae, whereas *Alcedoffula* parasitizes Alcedininae and Cerylinae. Although *Emersoniella* is geographically restricted to the Indo-Pacific region, *Alcedoecus* and *Alcedoffula* are geographically widespread. We used DNA sequences from two genes, the mitochondrial COI and nuclear EF-1α genes, to infer phylogenies for the two geographically widespread genera of kingfisher lice, *Alcedoffula* and *Alcedoecus*. These phylogenies included 47 kingfisher lice sampled from 11 of the 19 currently recognized genera of kingfishers. We compared louse phylogenies to host phylogenies to reconstruct their cophylogenetic history. Two distinct clades occur within *Alcedoffula*, one that infests Alcedininae and a second that infests Cerylinae. All species of *Alcedoecus* were found only on host species of the subfamily Halcyoninae. Cophylogenetic analysis indicated that *Alcedoecus*, as well as the clade of *Alcedoffula* occurring on Alcedininae, do not show evidence of cospeciation. In contrast, the clade of *Alcedoffula* occurring on Cerylinae showed strong evidence of cospeciation.

Research Article

*Current Address: Department of Wildlife and Fisheries Sciences, Texas A&M University College Station, 2258 TAMU, College Station, TX 77843-2258

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Author for correspondence:
Therese A. Catanach,
E-mail: tacatanach@tamu.edu

Introduction

Parasitic lice rely on their hosts to complete all life stages, spending their entire life cycle on a host individual (Price *et al*., 2003). Parasitic lice, which have adapted to survive only within the microclimatic conditions provided by their host's body often die within hours or days after becoming separated from the host (Price *et al*., 2003). This obligate association limits dispersal opportunities for lice, which normally occur *via* direct physical contact between individuals during copulation or between parents and offspring during brooding. Over macroevolutionary time scales, the lack of dispersal opportunities also limits the ability of most louse lineages to switch to novel host species. For some chewing lice parasitizing birds, dispersal to a novel host species, which ultimately drives host-switching, could occur *via* phoresy (lice attaching to hippoboscid flies, which are winged generalist parasites), takeover of nest cavities, or physical contact during intraspecific territorial disputes (Clayton, 1990; Harbison and Clayton, 2011). However, survival on novel host species is thought to be low, potentially due to difficulties in escaping host defenses on a novel host (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 species 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 (Clayton and Johnson, 2003; Hughes *et al*., 2007). However, if lice colonized a group of hosts after the hosts diverged, or if host-switching of lice between different host taxa is common, then host and parasite phylogenies would differ (Weckstein, 2004; Banks *et al*., 2006). These two 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* Mjöberg, 1910 lice and their suliform hosts show strong evidence of cospeciation (Hughes *et al*., 2007). Conversely, louse genera within the *Degeeriella*-complex match higher level classifications of their hosts (Johnson *et al*., 2002), but the toucan lice within this complex show no evidence of cospeciation with their hosts (Weckstein, 2004). Different louse genera codistributed on the same host group often show differing patterns of host specificity and

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cophylogenetic history. For example, dove body lice in the New World show evidence of cospeciation whereas dove wing lice do not (Clayton and Johnson, 2003).

In this study we focus on cophylogenetic patterns in the feather lice (Phthiraptera: Ischnocera: Philopteridae) occurring on kingfishers (Aves: Coraciiformes: Alcedinidae). Kingfishers include 117 species divided into three avian subfamilies: Halcyoninae Vigors, 1825 (equivalent to Daceloninae Bonaparte, 1837; as used by Moyle, 2006), Alcedininae Rafinesque, 1815, and Cerylinae Reichenbach, 1851. Halcyoninae and Alcedininae are limited to the Old World, and Cerylinae occurs worldwide. The monophyly of each of these avian subfamilies is strongly supported by morphological and molecular characters with Alcedininae being sister to Cerylinae + Halcyoninae (Maurer and Raikow, 1981; Johansson and Ericson, 2003; Moyle, 2006; Andersen *et al.*, 2017). The cosmopolitan distribution (New and Old Worlds) of Cerylinae is likely the result of two New World invasions (Moyle, 2006; Andersen *et al.*, 2017). Halcyoninae is mainly restricted to Australia and southern Asia, with a single genus, *Halcyon* Swainson, 1821, also occurring in Africa. Alcedininae is widespread across the Old World. Moyle (2006) and Moyle *et al.* (2007) found that the majority of kingfisher genera were not monophyletic, resulting in a substantial taxonomic reorganization. In a phylogeny with almost complete taxon sampling, Andersen *et al.* (2017) identified both *Dacelo* Leach, 1815 and *Actenoides* Bonaparte, 1850 as paraphyletic, suggesting additional taxonomic revision is necessary. Furthermore, species level relationships and species limits within some kingfisher taxa are also in a state of flux. For example, 26 species splits have been recognized within the kingfishers 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 host three louse genera, *Alcedoffula* Clay and Meinertzhagen, 1939, *Alcedoecus* Clay and Meinertzhagen, 1939, and *Emersoniella* Tendeiro, 1965 (Price *et al.*, 2003; Johnson *et al.*, 2012; Gustafsson and Bush, 2014). Although many bird species are host to multiple genera of lice, individual kingfishers are typically only infected with a single louse genus, and each of these genera is specific to one or more kingfisher subfamilies. In most of the small number of cases where a kingfisher species is parasitized by two louse species, one is in the genus *Alcedoecus* and the other is in the genus *Emersoniella*. Both *Alcedoecus* and *Emersoniella* parasitize only Halcyoninae kingfishers, although *Emersoniella* is less common and is one of the least diverse genera of chewing lice with only seven described species. Furthermore, *Emersoniella* is known only from Indo-Pacific kingfishers, whereas both *Alcedoecus* (limited to Halcyoninae) and *Alcedoffula* (found on Alcedininae and Cerylinae) are geographically widespread. Although lice are known from 54 (46%) of the 117 currently recognized kingfisher species (Price *et al.*, 2003 and novel host records published here), there are only two instances where both *Alcedoffula* and *Alcedoecus* have been collected from the same kingfisher species, and two instances where multiple louse species from the same genus are known from the same host species.

Lastly, based on morphological data, species groups have been proposed for both louse genera. For *Alcedoffula*, Tendeiro (1967) proposed two species groups based on head and male genitalic morphology, the *duplicata* and the *alcedinis* groups. Whereas for *Alcedoecus*, Tendeiro (1983) proposed two species groups based exclusively on the length of male genitalia, the *capistratus* and the *alatoclypeatus* groups. Furthermore, Uchida (1948) proposed a new genus *Halcyonicola* based on *Docophorus alatoclypeatus* Piaget, 1885 as its type species. This genus has been subsequently considered a junior synonym of *Alcedoecus* (Hopkins and Clay, 1952; Tendeiro, 1965; Price *et al.*, 2003).

Here we use DNA sequences from two genes (one mitochondrial and one nuclear) to infer phylogenies for both of the widespread genera of kingfisher lice: *Alcedoffula* and *Alcedoecus*. We compare the louse phylogenies with a molecular phylogeny of the kingfishers to reconstruct their cophylogenetic history and we assess the validity of kingfisher louse species groups as proposed by Tendeiro (1967, 1983).

Materials and methods

Specimen acquisition

Lice were collected from avian hosts using ethyl acetate fumigation or dust ruffling (Clayton *et al.*, 1992; Walther and Clayton, 1997) and were stored in 95% ethanol at −80 °C until DNA extraction. The authors and their colleagues sampled avian hosts that were collected and prepared as museum voucher specimens, avian hosts that were captured and banded, and dead hosts salvaged and prepared as specimens for deposition in natural history collections. In total, 47 kingfisher lice were included from 11 of the 19 currently recognized genera of kingfishers (Table 1). When possible, lice were sequenced from multiple individuals from each host species (up to 4 specimens per host taxon), particularly in cases of geographically widespread host species or island populations. Furthermore, 34 lice from 9 genera were used as outgroups (see Table 1).

Louse identification

Slide mounted voucher specimens were identified using available parasite literature for each louse genus. We compared the morphology of these voucher specimens to those described from the same host (*sensu* Price *et al.*, 2003) based on original descriptions or redescriptions published in the taxonomic literature for lice parasitizing kingfishers (Carriker, 1959; Tendeiro, 1965, 1967, 1983; Gustafsson and Bush, 2014). Some specimens used in our dataset could not be positively identified to species based on available literature, reference specimens, or because we only had a specimens of one sex. These individuals are labelled as 'sp.,' regardless of their host association. We did not identify louse species based exclusively on host-parasite associations.

Parasite DNA sequencing

DNA was extracted from specimens by cutting a small incision between the head and thorax and a second incision between two abdominal sclerites and then subsequently placing the specimen in digestion buffer. A QIAamp DNA Micro Kit (Qiagen, Valencia, CA) was used for DNA extractions following a modified version of the protocol for total genomic DNA from tissues. Modifications include lengthening the incubation period (step 4) to 36 h, incubating the sample for 10 min at 70 °C (step 6), and decreasing the amount of elution Buffer AE to 50 μL (step 12), which was used to make two elutions into different 1.5 mL collection tubes. After applying Buffer AE to the filter, we incubated the filter for 5 min at 70 °C prior to centrifugation (step 13). After digestion, louse exoskeletons were retained as a voucher, cleared, and mounted on a microslide in balsam following the protocols of Palma (1978).

After extraction, PCR was performed in 25 μL reactions to amplify portions of two 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 from Smith *et al.* (2004) except an annealing temperature of 50 °C was used for EF-1α amplifications. Sequencing reactions were performed using 1 μL of BigDye and then submitted for sequencing on an ABI 3730xl capillary

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Table 1. Sample data with Genbank numbers

Louse species	Code	Host species	Country	COI	EF1α
Alcedoffula sp.	Afsp.Alsem.7.1.2014.5	*Alcedo semitorquata*	Malawi	MK526917	
Alcedoffula cristata	Alsp.Alcri.1.16.2001.12	*Corythornis cristatus*	Ghana	MK526944	MK570276
Alcedoffula sp.	Alsp.Alleu.1.16.2001.9	*Corythornis leucogaster*	Uganda	MK526945	MK570277
Alcedoffula duplicata	Afdup.Cerud.4.3.2000.4	*Ceryle rudis*	Ghana	MK526942	
Alcedoffula duplicata	Afdup.3.16.2001.10	*Ceryle rudis*	Ghana	JX121669.1	JX121682.1
Alcedoffula sp.	Afsp.Alazu.8.27.2014.7	*Ceyx azureus*	Australia	MK526932	MK570268
Alcedoffula sp.	Afsp.Ceeri.8.27.2014.8	*Ceyx erithaca*	Malaysia (Borneo)	MK526933	MK570273
Alcedoffula ceycis	Afsp.Ceeri.7.1.2014.1	*Ceyx erithaca*	Malaysia (Borneo)		MK570272
Alcedoffula ceycis	Alori.1.16.2001.7	*Ceyx erithaca*	Malaysia (Borneo)		MK570275
Alcedoffula cf. *cristata*	Afsp.Ceruf.7.1.2014.9	*Ceyx rufidorsa*	Malaysia (Borneo)		MK570244
Alcedoffula aeneae	Alae.04.v.2015.6	*Chloroceryle aeneae*	Brazil	MK526968	MK570282
Alcedoffula sp.	Afsp.Chame.8.27.2014.9	*Chloroceryle americana*	Panama	MK526928	MK570265
Alcedoffula sp.	Alsp.Chama.1.16.2001.10	*Chloroceryle amazona*	Peru	MK526946	MK570278
Alcedoffula columbiana	Afsp.Chame.7.18.2014.3	*Chloroceryle americana*	Peru	MK526915	
Alcedoffula columbiana	Alco.04.v.2015.4	*Chloroceryle americana*	Brazil	MK526969	MK570284
Alcedoffula chocoana	Alcoh.10.viii.2015.5	*Chloroceryle inda*	Brazil	MK526967	MK570283
Alcedoffula chocoana	Alsp.Chind.8.12.2014.2	*Chloroceryle inda*	Peru	MK526935	MK570280
Alcedoffula cristata	Afsp.Alcri.7.18.2014.1	*Corythornis cristatus*	Kenya		MK570269
Alcedoffula cristata	Afsp.Alcri.7.1.2014.7	*Corythornis cristatus*	Malawi	MK526916	MK570248
Alcedoffula sp.	Afsp.Alleu.7.18.2014.4	*Corythornis leucogaster*	DR Congo	MK526918	MK570270
Alcedoffula sp.	Afsp.Alleu.3.16.2001.11	*Corythornis leucogaster*	Uganda	MK526943	MK570274
Alcedoffula sp.	Afsp.Coleu.8.27.2014.2	*Corythornis leucogaster*	Uganda	MK526930	MK570252
Alcedoffula sp.	Afsp.Ismad.8.12.2014.3	*Corythornis madagascariensis*	Madagascar	MK526936	MK570251
Alcedoffula elongata	Afsp.Alcri.8.12.2014.4	*Corythornis vintsioides*	Madagascar	MK526937	MK570281
Alcedoffula sp.	Afsp.Ispic.8.27.2014.10	*Ispidina picta ferrugina*	DR Congo	MK526931	MK570267
Alcedoffula sp.	Afsp.Ispic.7.18.2014.2	*Ispidina picta picta*	Kenya	MK526920	MK570266
Alcedoffula sp.	Afsp.Ispic.7.1.2014.13	*Ispidina picta natalensis*	Malawi	MK526919	
Alcedoffula alcyonae	Alsp.Cealc.8.27.2014.1	*Megaceryle alcyon*	USA	MK526929	MK570255
Alcedoffula alcyonae	Afsp.Cealc.7.1.2014.3	*Megaceryle alcyon*	USA	MK526921	MK570245
Alcedoffula alcyonae	Mealc.1.16.2001.8	*Megaceryle alcyon*	USA	MK526947	MK570279
Alcedoffula alcyonae	Afalc.Mealc.8.12.2014.7	*Megaceryle alcyon*	Canada	MK526938	MK570254
Alcedoffula theresae	Alsp.Cetor.8.12.2014.1	*Megaceryle torquata*	Peru	MK526934	MK570253
Alcedoecus sp.	Issp.Dalea.10.16.2002.11	*Dacelo leachii*	Australia	MK526940	MK570271
Alcedoecus delphax	Alsp.Danov.8.27.2014.3	*Dacelo novaeguineae*	Australia	MK526927	MK570258
Alcedoecus mossambicanus	Alsp.Haalb.7.1.2014.6	*Halcyon albiventris*	Malawi		MK570247
Alcedoecus mossambicanus	Alsp.Haalb.7.1.2014.12	*Halcyon albiventris orientalis*	Malawi	MK526922	MK570257
Alcedoecus sp.	Alsp.Habad.8.27.2014.5	*Halcyon badia*	Ghana	MK526925	MK570261
Alcedoecus chelicutii	Alsp.Hache.7.1.2014.16	*Halcyon chelicuti*	Malawi	MK526914	MK570262
Alcedoecus mystacinus	Alsp.Hacor.7.1.2014.11	*Halcyon coromanda*	Malaysia	MK526910	MK570256
Alcedoecus mystacinus	Alsp.Hacor.7.1.2014.10	*Halcyon coromanda*	Philippines	MK526911	MK570246
Alcedoecus alatoclypeatus	Alsp.Hamal.4.3.2000.3	*Halcyon malimbica*	Ghana	AY314807.1	AY314825.1
Alcedoecus alatoclypeatus	Alsp.Hamal.1.16.2001.11	*Halcyon malimbica*	Ghana	KT892064.1	KT892356.1
Alcedoecus senegalensis	Alsp.Hasen.8.27.2014.6	*Halcyon senegalensis*	Ghana	MK526924	MK570263

(*Continued*)

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Table 1. (*Continued.*)

Louse species	Code	Host species	Country	COI	EF1α
Alcedoecus senegalensis	Alsp.Hasen.8.27.2014.11	*Halcyon senegalensis*	Ghana	MK526923	MK570264
Alcedoecus senegalensis	Alsp.Hasen.7.1.2014.14	*Halcyon senegalensis cyanoleuca*	Malawi	MK526912	
Alcedoecus annulatus	Alann.Hasmy.CT091	*Halcyon smyrnensis*	Vietnam	KF385882.1	
Alcedoecus sp.	Alsp.Hachl.7.1.2014.4	*Todiramphus sacer*	Solomon Islands	MK526913	MK570259
Alcedoecus sp.	Alsp.Tochl.8.12.2014.6	*Todiramphus sordidus*	Australia	MK526941	MK570250
Alcedoecus sp.	Alsp.Tosan.8.27.2014.4	*Todiramphus sanctus*	Australia	MK526926	MK570260
Cirrophthirius testudinarius	Issp.Reame.4.11.2000.10	*Recurvirostra americana*	USA	MK526948	MK570227
Cirrophthirus testudinarius	Zites.1.15.2003.3	*Recurvirostra americana*	USA	AF545685.1	AF545778.1
Cirrophthirius recurvirostrae	Zirec.3.15.2001.12	*Recurvirostra novaehollandiae*	Australia	MK526966	MK570243
Craspedorhynchus hirsutus	Cfhir.1.15.2000.6	*Buteo regalis*	USA	AF545690.1	AF545780.1
Emersoniella braeteata	Embra.2.4.2002.11	*Dacelo novaeguinnea*	Australia	KT892333.1	KT892623.1
Emersoniella galateae *	Alsp.Tasyl.8.12.2014.5	*Tanysiptera sylvia*	Australia	MK526939	MK570249
Incidifrons sp.*	Rasp.Arcaj.3.29.1999.2	*Aramides cajanea*	Mexico	AF545760.1	MK570238
Incidifrons transpositus	Intra.1.15.2000.9	*Fulica americana*	USA	AF545719.1	AF545790.1
Lunaceps actophilus	Issp.Caalb.1.15.2000.7	*Calidris alba*	USA	DQ314498.1	DQ314508.1
Lunaceps rothkoi	Issp.Trsub.9.27.2000.7	*Tryngites subruficollis*	USA	MK526949	MK570228
Quadraceps aethereus	Qusp.Aecri.11.22.2001.2	*Aethia cristatella*	USA	MK526952	MK570231
Quadraceps aethereus	Quaet.11.22.2001.4	*Aethia pusilla*	USA	MK526950	MK570229
Quadraceps strepsilaris	Qustr.3.16.2001.13	*Arenaria interpes*	Australia	MK526959	MK570237
Quadraceps sp.	Qusp.Esmag.1.9.2001.6	*Esacus magnirostris*	Australia	MK526953	MK570232
Quadraceps impar	Quimp.3.16.2001.7	*Heteroscelus brevipes*	Australia	MK526951	MK570230
Quadraceps sp.	Qusp.Haful.3.16.2001.8	*Haematopus fuliginosus*	Australia	MK526954	MK570233
Quadraceps sp.	Qusp.Hihim.3.24.2001.6	*Himantopus himantopus*	Australia	MK526955	MK570234
Quadraceps sp.	Qusp.Himex.3.16.2001.9	*Himantopus mexicanus*	USA	MK526956	MK570235
Quadraceps punctatus	Qupun.3.24.2001.8	*Larus californica*	USA		AF320457.1
Quadraceps punctatus	Qupun.2.3.1999.2	*Larus cirrocephalus*	South Africa	AY149405.1	JX121692.1
Quadraceps zephyra	Quzep.4.11.2000.11	*Recurvirostra americana*	USA	AF545759.1	AF545803.1
Quadraceps sp.	Qusp.Renov.3.24.2001.5	*Recurvirostra novaehollandiae*	Australia	MK526957	
Quadraceps quadrisetaceus	Ququa.4.11.2000.5	*Rostratula benghalensis*	Ghana	AF545758.1	AF545802.1
Quadraceps sp.	Qusp.Stisa.10.16.2002.12	*Stiltia isabella*	Australia	MK526958	MK570236
Rallicola (*Rallicola*) *advenus*	Raad.1.3.2011.11	*Fulica americana*	USA	JQ717183.1	JQ717191.1
Rallicola (*Aptericola*) sp.	Rasp.Apsp.3.3.2011.4	*Apteryx* sp.	New Zealand	JQ717186.1	JQ717194.1
Saemundssonia wumisuzume	Sawum.11.22.2001.3	*Aethia cristatella*	USA	MK526964	MK570242
Saemundssonia wumisuzume	Sawum.11.22.2001.7	*Aethia cristatella*	USA	MK526965	
Saemundssonia wumisuzume	Sasp.Aepus.11.22.2001.5	*Aethia pusilla*	USA	MK526961	MK570240
Saemundssonia wumisuzume	Sasp.Aepyg.2.4.2002.8	*Aethia pygmaea*	USA	MK526962	
Saemundssonia haematopi	Sahae.1.9.2001.7	*Haematopus ostralegus*	Australia	MK526960	MK570239
Saemundssonia lari	Salar.4.7.1999.12	*Larus cirrocephalus*	South Africa	AY149406.1	AY149435.1
Saemundssonia sp.	Sasp.Scsp.7.14.1999.8	*Scolopax* sp.	Philippines	MK526963	MK570241
Strigiphilus sp.	Stcru.1.27.1999.10	*Otus guatemalae*	Mexico	AF545767.1	AF320467.1

New host records are denoted by an '*'.

machine at the University of Illinois Keck Center for Comparative and Functional Genomics. 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

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4.3.0 where they were checked and adjusted by eye (Edgar, 2004; Gouy *et al.*, 2010).

Host DNA sequencing

For four host taxa, *Halcyon chelicuti* (Stanley, 1814), *H. albiventris orientalis* Peters, 1868, *H. senegalensis cyanoleuca* (Vieillot, 1818), and *Ispidina picta natalensis* (Smith, 1831) we extracted DNA from tissues, amplified ND2 and RAG-1 genes, and then sequenced the resulting PCR products. Host DNA was extracted from tissues using a Qiagen DNeasy Blood & Tissue Kit (Qiagen, Valencia, CA) following the manufactures protocols for tissue samples. After extraction, PCR was performed in 25 μL reactions to amplify the ND2 and RAG-1 genes. For ND2 amplifications we used primers L5215 (Hackett, 1996) and H6313 (Johnson and Sorenson, 1998) following the protocol described in Weckstein (2005). For sequencing, we also used internal primers ND2Hal and ND2Alc (Moyle, 2006). Two initial PCRs for RAG-1 were performed using the PCR protocol described in (Groth and Barrowclough, 1999). One RAG-1 PCR 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 (5′- TGGCTCCTGGTTATGGAGTGG-3′; designed by R. G. Moyle) were used to sequence the fragment between R7 and R4B. With the exception of RagB, all RAG-1 primers are from Groth and Barrowclough (1999). PCR products were submitted to Functional Biosciences (Madison, Wisconsin) for Sanger sequencing on an ABI 3730xl machine. 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). Resulting sequences were deposited in GenBank (MK579322-MK579329).

Phylogenetic analysis of kingfisher lice

The two genes were first analyzed separately to evaluate the extent of conflict (if any) between gene trees for the ingroup (i.e. conflicts with posterior probability >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 and Rambaut, 2007; Lanfear *et al.*, 2012). PartitionFinder selected the models GTR + I + G for COI codon positions 1 and 2, GTR + G for COI codon position 3, TrN + G for EF-1α codon positions 1 and 2, and K80 + G for EF-1α codon position 3 for *Alcedoffula* and SYM + I + G for COI codon position 1, GTR + I + G for COI codon position 2, GTR + G for COI codon position 3, TrN + G for EF-1α codon position 1, HKY + I for EF-1α codon position 2, and TrN + I + G for EF-1α codon position 3 for *Alcedoecus*. Although some nodes were in conflict at the 0.98 level between the two gene trees, the conflict was typically limited to relationships among outgroups (potentially due to long branches and sparse outgroup taxon sampling) and the placement of two ingroup taxa [*Alcedoffula duplicata* (Piaget, 1890) from *Ceryle rudis* (Linnaeus, 1758) and *Alcedoffula chocoana* Carriker, 1959 from *Chloroceryle inda* (Linnaeus, 1766)], both of which were placed on long branches sister to a given clade in the COI gene tree but within this clade in the EF-1α gene tree. Since conflict was limited, we concatenated genes for further analysis.

In the concatenated analysis, each codon position for a given protein coding gene was treated as a separate partition, and each model parameter as above. Phylogenies based on the combined analysis were inferred using Bayesian inference as implemented in BEAST (Drummond and Rambaut, 2007; 40 million generations, sampled every 1000 generations, burnin = 10 000 samples), Maximum Likelihood (ML) as implemented in Garli (Zwickl, 2006; 10 independent runs, default settings, automated stop criterion = 50 000), and Maximum Parsimony (MP) as implemented in PAUP* (Swofford, 2003; 1000 random addition sequences with TBR branch swapping). To evaluate branch support, we used posterior probabilities as implemented in BEAST, parsimony bootstrap values as implemented in PAUP* with 1000 replicates and 100 random addition sequences per replicate with maxtrees set at 500 due to computational constraints, and ML bootstrap values as implemented in Garli (2.0) with 500 bootstrap replicates on default settings with automated stop criterion = 5000).

Phylogenetic analysis of kingfishers

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 RAG1 third positions, for which SYM + G was the best model. We evaluated branch support using posterior probabilities (generated by BEAST) and parsimony bootstrap values generated by PAUP* using 100 replicates with 100 random addition sequences per replicate and maxtrees allowed to automatically increase by 100.

Cophylogenetic history of kingfishers and their lice

We 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 as noted above (for the other two subfamilies) to conduct statistical tests of cospeciation using Jane4 (Conow *et al.*, 2010). We used Moyle *et al.* (2007) because *Ceyx rufidorsa* Strickland, 1847, a species for which we had a louse sample, was treated as a synonym of *Ceyx erithaca* (Linnaeus, 1758) in Andersen *et al.* (2017) and thus was not included in their tree. The topology of both the Moyle *et al.* (2007) and Anderson *et al.* (2017) phylogenies agreed with regard to all taxa in our study. Parasite tips were collapsed to insure that each tree topology only included a single representative of each putative louse species, and terminals from the kingfisher phylogeny that did not include a louse association were removed. Because we found that *Alcedoffula* contained two distinct monophyletic lineages, which do not parasitize sister kingfisher subfamilies, these two louse lineages and their hosts were analyzed separately in cophylogenetic analyses. These analyses were run using default costs. To assess whether there were more cospeciation events than expected by chance, we generated 1000 random tip mappings and 1000 randomly generated parasite trees in Stats Mode.

Reconstruction of biogeographic history on kingfisher louse trees

Using BioGeoBEARS (Matzke, 2013), we reconstructed the biogeographic history of both *Alcedoffula* and *Alcedoecus*. Within BioGeoBEARS, we 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 the lice, we removed outgroup taxa and collapsed tips if COI divergence between terminal taxa was less than 2.5%. We coded geographic range to represent the six major

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biogeographic regions for which we had sampled kingfisher lice (Ethiopian, Australia, Nearctic, Neotropics, Southeast Asia, and Madagascar). Louse biogeographic region was coded based on where each specimen was collected, not the entire range of the host species as other studies have shown birds with large ranges do not always share the same species of louse across the entire geographic distribution (Catanach and Johnson, 2015). Lice collected from hosts on Indo-Pacific Islands were placed in either the Southeast Asia or Australian regions based on whether they were north or south of Wallace's Line, respectively. In all instances, maxareas was set to two. Results from each method were compared using AIC scores.

Results

Phylogenetic analysis of kingfisher lice

The phylogenies resulting from combined analysis of COI and EF-1α were well resolved and were supported by posterior probability of at least 0.95 at most nodes (Figs 1, 2, and Supplementary Fig. 1). *Alcedoffula* and *Alcedoecus* were recovered as reciprocally monophyletic (posterior probability = 1.0 for both clades).

Within *Alcedoffula* (Fig. 1), two well-supported clades (posterior probability = 1.0 for both clades) were recovered and each parasitizes a different kingfisher subfamily. These clades match the two louse species groups, *duplicata* and *alcedinis*, proposed by Tendeiro (1967) based on shapes of frontal heads and the mesosome of the male genitalia. The *duplicata*-group of *Alcedoffula* parasitizes only Cerylinae and contains two well-supported subclades (both with posterior probability = 1.0). One of these subclades infects New World *Megaceryle* kingfishers and the other infects both New World *Chloroceryle* kingfishers and Old World *Ceryle rudis* (pied kingfisher). Within the subclade infecting New World *Chloroceryle* kingfishers and Old World *Ceryle rudis* (Pied Kingfisher), *Alcedoffula duplicata*, from pied kingfisher, is sister to lice from the New World genus *Chloroceryle*. Also, two individual *Alcedoffula* parasitizing green kingfisher [*Chloroceryle americana* (Gmelin, 1788)] are not each other's closest relatives with respect to the lice infecting the two other *Chloroceryle* species. One of the green kingfisher lice is sister to a louse collected from Amazon kingfisher [*Chloroceryle amazona* (Latham, 1790)].

The other clade of *Alcedoffula*, the *alcedinis*-group, is found exclusively on the Old World kingfisher subfamily Alcedininae. This clade comprises two subclades, one of which is well supported (posterior probability = 0.95) and includes lice collected from three African kingfisher species, including *Corythornis madagascariensis* (Linnaeus, 1766), *C. leucogaster* (Fraser, 1843), and *Ispidina picta* (Boddaert, 1783). The second subclade is also well supported (posterior probability = 0.99) and comprises lice from African and Asian kingfishers including *Corythornis cristatus* Pallas, 1764, *C. vintsioides* Eydoux and Gervais, 1836, *Ceyx rufidorsa* Strickland, 1847, *Ceyx erithaca* (Linnaeus, 1758), *Ceyx azurenus* Latham, 1801, and *Alcedo semitorquata* Swainson, 1823.

We sampled multiple louse individuals from across the ranges of the African *Corythornis* (*C. madagascariensis* and *C. leucogaster*) and *Ispidina picta* kingfishers. Lice collected from the same host species were each other's closest relatives in each case (posterior probability = 1.0). For example, we included louse samples collected from all three recognized *Ispidina picta* subspecies. Although all three were members of a single clade, the louse from *Ispidina picta natalensis* (Smith 1832) was highly divergent (COI uncorrected *p*-distance = 3.0%) from lice collected from the other two subspecies, whereas the lice from *I. p. picta* (Boddaert, 1783) and *I. p. ferrugina* (Clancey, 1984) were identical to one another. Lice from the host genus *Corythornis* as a whole are not monophyletic, each species being more closely related to lice from other alcedinine kingfishers.

Within *Alcedoecus* (Fig. 2), members of which only parasitize the Halcyoninae, the most basal node unites *A. annulatus* Ansari, 1955 collected from *Halcyon smyrnensis* Linnaeus, 1758 with all other species of *Alcedoecus*. The remaining members of the louse genus *Alcedoecus* form a well-supported clade (posterior probability = 0.99). Within this louse clade, lice from two species of Kookaburra (*Dacelo* spp.) are sister to a well-supported clade (posterior probability = 0.99) containing lice collected from six species of *Halcyon* and three species of *Todiramphus* Lesson, 1827. Lice from *Todiramphus* form a well-supported clade (posterior probability = 1.0). In all instances where we have sampled *Alcedoecus* from multiple individual hosts from a single host species, they fall out as sisters in the phylogeny, although not all of these sister relationships were well supported.

Our results (Fig. 2) show that the two species groups proposed by Tendeiro (1983) including those with long genitalia (i.e., *A. chelicutii* Tendeiro, 1965, *A. annulatus*, *A. mossambicanus* Tendeiro, 1979) and those with short genitalia (i.e., *A. alatoclypeatus*, *A. senegalensis* Tendeiro, 1965, *A. mystacinus*) do not form reciprocally monophyletic groups in our tree.

Phylogenetic analysis of kingfishers

The kingfisher phylogeny recovered the three well-supported subfamilies and all genera with multiple representatives as monophyletic with high levels of statistical support assessed *via* both bootstrap values and posterior probability (Fig. 3).

Cophylogenetic analysis of kingfishers and their lice

The results of the Jane4 cophylogenetic analyses varied across groups of lice and hosts (Table 2 and Figs 4 and 5). The cophylogenetic analysis of *Alcedoffula* from cerylinine kingfishers and their hosts consistently recovered four cospeciation events which were significantly more than expected by chance $p = 0.01$. In contrast, cophylogenetic reconstructions of both *Alcedoffula* with Alcedininae and *Alcedoecus* with Halcyoninae showed no evidence for cospeciation between the louse and host phylogenies (both $p > 0.21$).

Reconstruction of biogeographic history on kingfisher louse trees

Biogeographic reconstructions using the DIVALIKE + J model had the highest likelihood scores for *Alcedoffula* whereas both the DIVALIKE + J and BAYAREALIKE + J models had equal likelihood scores for *Alcedoecus*. Within *Alcedoffula*, no single geographic region (or pair of regions) was favored in the ancestral state reconstruction of the basal nodes (Fig. 1). We recovered a single South American origin for lice parasitizing Cerylinae, with subsequent colonization of Africa. An African origin was inferred for lice parasitizing Alcedininae, but it is unclear if a single or multiple origins scenario best explain the distribution of lice in Southeast Asia. Lastly, two origins of *Alcedoffula* from Malagasy host species were also inferred. For *Alcedoecus*, biogeographic reconstruction inferred an Australian + southeast Asian origin (Fig. 2). However, the other deep nodes in this clade were relatively equivocal, although Australian origins had some support.

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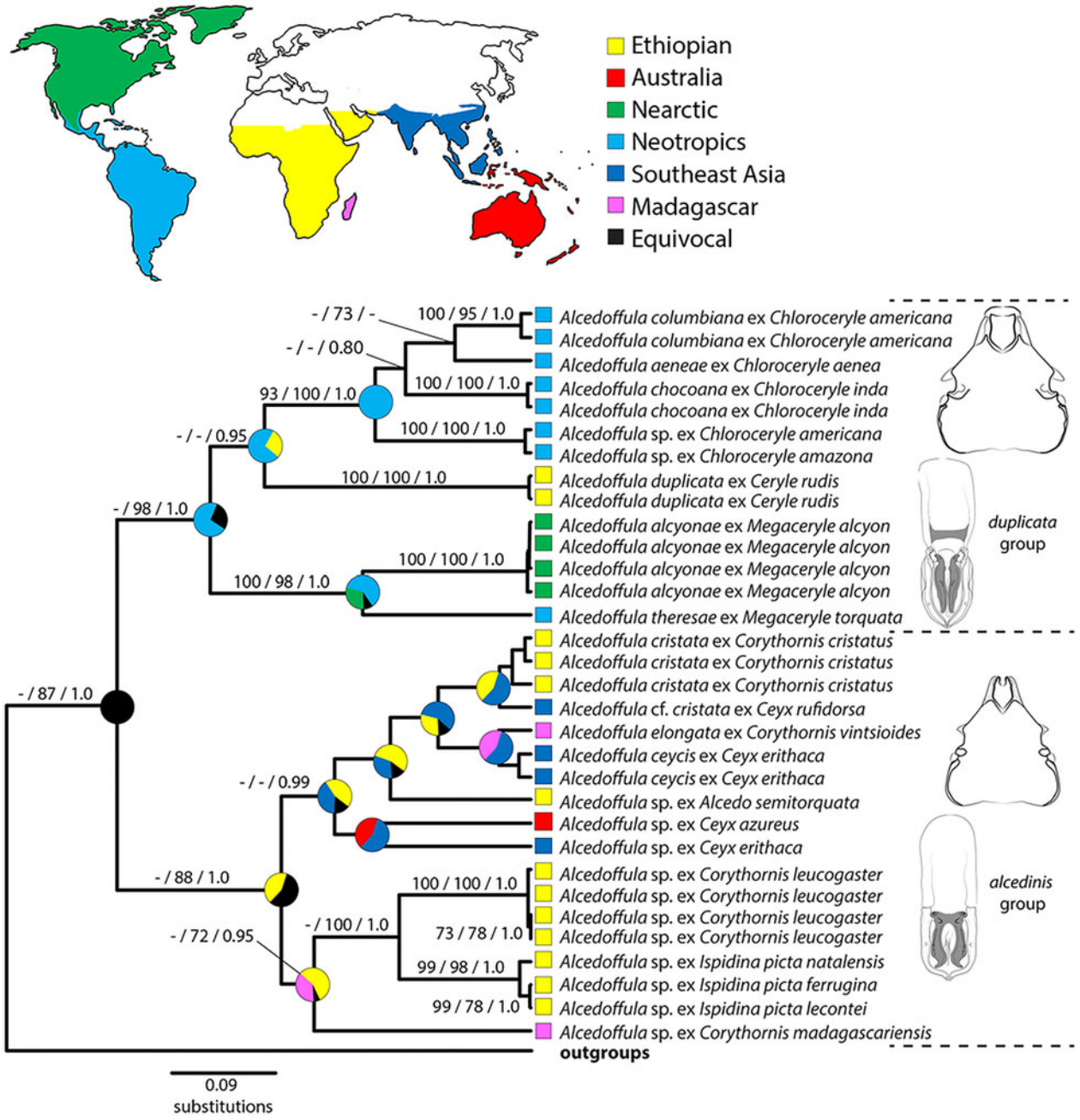

Fig. 1. *Alcedoffula* phylogeny resulting from Bayesian Analysis of EF-1α and COI. Numbers on branches are MP bootstrap values/ML bootstrap values/posterior probabilities. Bootstrap values below 70 and posterior probabilities below 0.80 not shown. Piecharts at nodes show biogeographic reconstruction and tips are coded based on geographic area in which each louse specimen was collected. Head drawings represent the two species groupings proposed by Tendeiro (1967).

Discussion

Phylogenetic analysis of kingfisher lice

Phylogenies for two louse genera, *Alcedoffula* and *Alcedoecus*, which broadly parasitize kingfishers provided insights into the evolutionary history and taxonomy of these parasites. A two gene phylogeny recovered both *Alcedoffula* and *Alcedoecus* as strongly supported, reciprocally monophyletic clades. Representatives of the *Alcedoffula duplicata* and the *alcedinis* species groups form strongly supported reciprocally monophyletic clades in our phylogenetic reconstructions (Fig. 1) and therefore support these species groups proposed by Tendeiro (1967). However, our phylogenetic reconstructions do not support the species groups proposed by Tendeiro (1983) for *Alcedoecus*, because representatives of the *capistratus* species group (including *A. chelicutii*, *A. annulatus*, *A. mossambicanus*) and the *alatoclypeatus* species group (including *A. alatoclypeatus*, *A. senegalensis*, *A. mystacinus*) do not form well-supported reciprocally monophyletic clades.

Although many groups of birds are parasitized by multiple species of lice, typically from different louse genera or subgenera, individual kingfishers are typically parasitized by only a single louse species. The two kingfisher louse genera in this study are generally restricted to particular kingfisher subfamilies, with *Alcedoffula* parasitizing Cerylinae and Alcedininae and *Alcedoecus* parasitizing only Halcyoninae. These generic host associations do not exactly mirror the higher level phylogeny of kingfishers (Moyle, 2006; Andersen *et al.*, 2017) because Alcedininae is sister to a clade made up of Halcyoninae and Cerylinae. Thus, one way to explain this host-parasite association pattern is that *Alcedoffula* was lost from the ancestor of Halcyoninae and subsequently 'replaced' by *Alcedoecus*.

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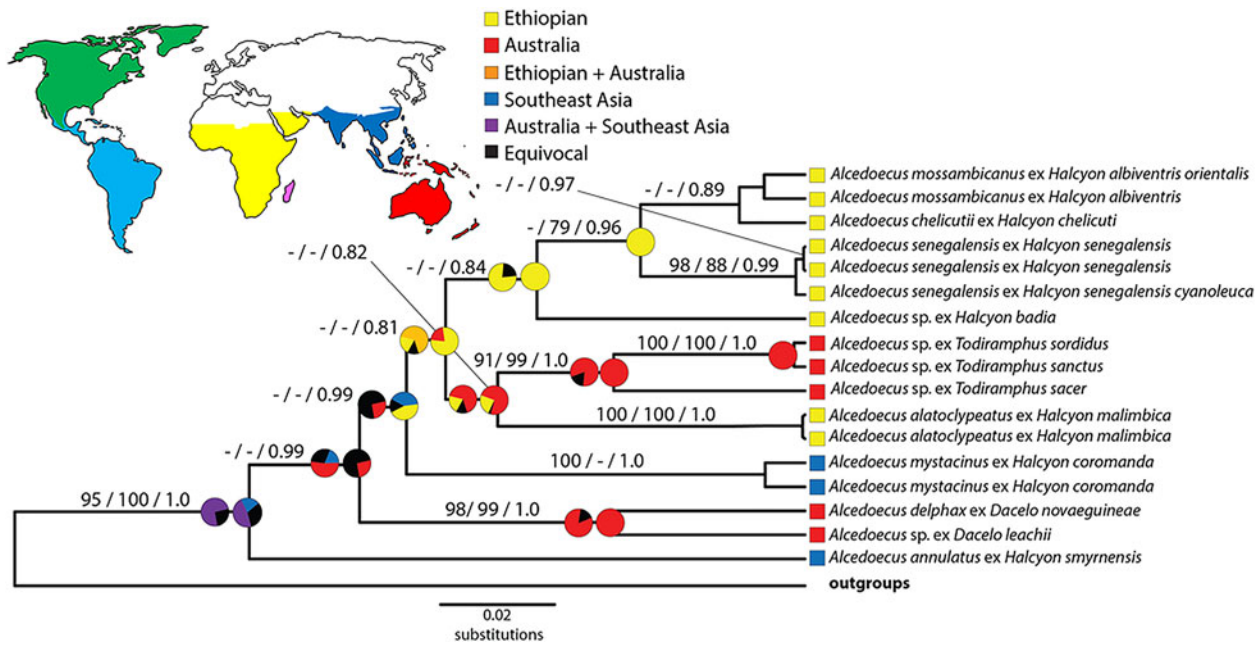

Fig. 2. *Alcedoecus* phylogeny resulting from Bayesian Analysis of EF-1α and COI. Numbers on branches are MP bootstrap values/ML bootstrap values/posterior probabilities. Bootstrap values below 70 and posterior probabilities below 0.80 not shown. Piecharts at nodes show biogeographic reconstruction and tips are coded based on geographic area in which each louse specimen was collected. Piecharts on the nodes represent DIVALIKE + J reconstructions and pie charts to the left of the nodes represent BAYAREALIKE + J reconstructions.

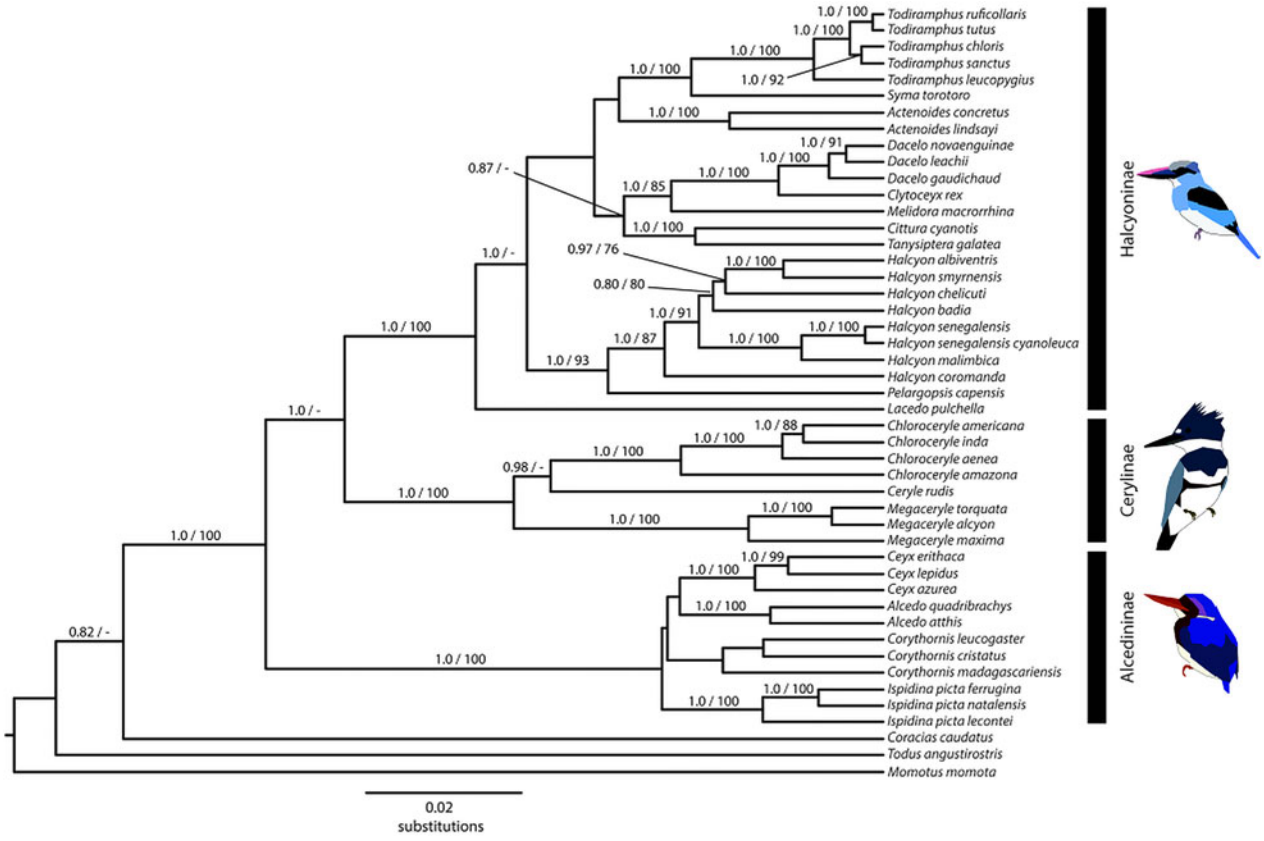

Fig. 3. Kingfisher phylogeny resulting from Bayesian Analysis of RAG1 and ND2. Numbers on branches are posterior probabilities/maximum parsimony bootstrap values. Bootstrap values below 50 and posterior probabilities below 0.80 are not shown. Thick black bars denote the three kingfisher subfamilies.

There are only a few examples in the literature of kingfishers parasitized by a louse from a genus not typical of that kingfisher subfamily, and it is possible that these are examples based on erroneous generic identifications or incorrect host associations because of field contamination. For example, Price *et al* (2003) list two louse species associated with *Ceyx erithaca*: *Alcedoecus orientalis* and *Alcedoffula ceycis* Tendeiro, 1967. We sequenced three lice from this host species, which were all identified

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Table 2. Results of Jane analysis by host subfamily (upper) and using the statistical solutions option based on 1000 random samples (lower)

Host family	Actual solutions						
	# of isometric solutions	# of inferred cospeciation	# of inferred duplications	# of inferred duplications + host switches	# of inferred host losses	# of inferred failures to diverge	Total cost
Alcedininae solution 1	20	5	0	4	2	0	10
Alcedininae solution 2	15	5	0	4	2	0	10
Cerylinae	4	4	0	2	1	1	6
Halcyoninae	5	4	0	1	1	0	3

Host family	Statistical solutions						
	Random parasite tree			Random tip mapping			
	Mean cost	Standard deviation	% Sample with lower cost than actual solution	Mean cost	Standard deviation	% Sample with lower cost than actual solution	
Alcedininae	12.41	1.39	8.80%	12.59	1.42	8.00%	
Cerylinae	10	1.84	3.10%	9.81	1.85	4.40%	
Halcyoninae	5.86	1.46	8.40%	5.71	1.31	11.50%	

morphologically as *Alcedoffula ceycis*. The resulting topology included two distinct lineages of *Alcedoffula* from *Ceyx erithaca*; however the relationship between these two louse lineages lacked statistical support. Additional sampling of *Ceyx erithaca* is needed to determine whether this host species is parasitized by multiple lineages of *Alcedoffula* (i.e. whether *Alcedoffula ceycis* comprises multiple cryptic species). Although we sampled multiple *Ceyx erithaca* host individuals we did not collect any individuals of *Alcedoecus orientalis*, the original description of which was based on six specimens sampled from a single host individual (Tendeiro, 1965). This kingfisher is distributed across the Indian subcontinent, Southeast Asia, and nearby islands. Additional sampling from across the geographic range of this host species may help to determine whether *Alcedoecus orientalis* has a narrow geographic range or is perhaps a rarer louse species than *Alcedoffula ceycis*. Secondly, the only record of *Alcedoecus* on Cerylinae is *Alcedoecus nepalensis* Tendeiro, 1983 on *Megaceryle lugubris* (Temminck, 1834), an Old World kingfisher species. *Megaceryle* occurs in both the New and Old Worlds. Tendeiro (1983) suggested that lice from New World *Megaceryle* kingfishers are both morphologically distinct from other *Alcedoffula* and more similar to the species within the louse genus *Quadraceps* Clay and Meinertzhagen, 1939 and thus are erroneously placed in the genus *Alcedoffula*. However, we found that lice from New World *Megaceryle* are embedded within *Alcedoffula* (Fig. 1). The presence of an *Alcedoecus* species on an Old World *Megaceryle* kingfisher could be the result of a host switch because numerous kingfisher taxa, which traditionally host *Alcedoecus*, overlap geographically with *Megaceryle lugubris*. Alternatively, this *Alcedoecus* record might have resulted from field or laboratory contamination, because *Megaceryle maxima* (Pallas, 1769), the other Old World *Megaceryle* species, is parasitized by an *Alcedoffula* species (Tendeiro, 1967; Price *et al*., 2003), although different from the species found on New World *Megaceryle*.

The phylogeny of *Alcedoffula* collected from Cerylinae broadly resembles the Cerylinae portion of the kingfisher phylogeny published by Andersen *et al.* (2017). *Alcedoffula* from *Megaceryle* kingfishers are sister to a clade of lice from the Neotropical *Chloroceryle* and the African/southern Asian kingfisher *Ceryle rudis*. Andersen *et al.* (2017) placed *Ceryle rudis* as sister to the *Chloroceryle* radiation, which matches our louse phylogeny. However, the branching pattern of *Alcedoffula* on *Chloroceryle* kingfishers does not closely match the published *Chloroceryle* portion of the host tree, although these relationships in the louse tree are not well supported. Lice collected from *Chloroceryle americana* are paraphyletic, with one louse from Panama placed as sister to lice from *Chloroceryle amazona* sampled from Peru. All other *Chloroceryle americana* lice included in our phylogeny are from South America and identified as *Alcedoffula columbiana* Carriker, 1959. This paraphyly among *Chloroceryle americana* lice could be the result of geographically specific louse species infecting a single host species (Catanach and Johnson, 2015). Catanach and Johnson (2015) found that Old World and New World *Buteo lagopus* Pontoppidan, 1763 (Accipitriformes, Accipitridae) were parasitized by distantly related lice. Alternatively, a louse straggling event (when a parasite ends up on a non-typical host) from *Chloroceryle amazona* to *Chloroceryle americana* could cause this paraphyletic pattern. Additional samples from Central American kingfishers will help determine the origin of this louse paraphyly.

Andersen *et al.* (2017) found evidence for a clade of kingfishers containing *Corythornis* and *Ispidina*. Our data set included *Alcedoffula* from five of the six host species currently placed within these host genera and we found close affinities between lice from *Corythornis leucogaster, Corythornis madagascariensis*, and *Ispidina picta*. However, not all lice collected from

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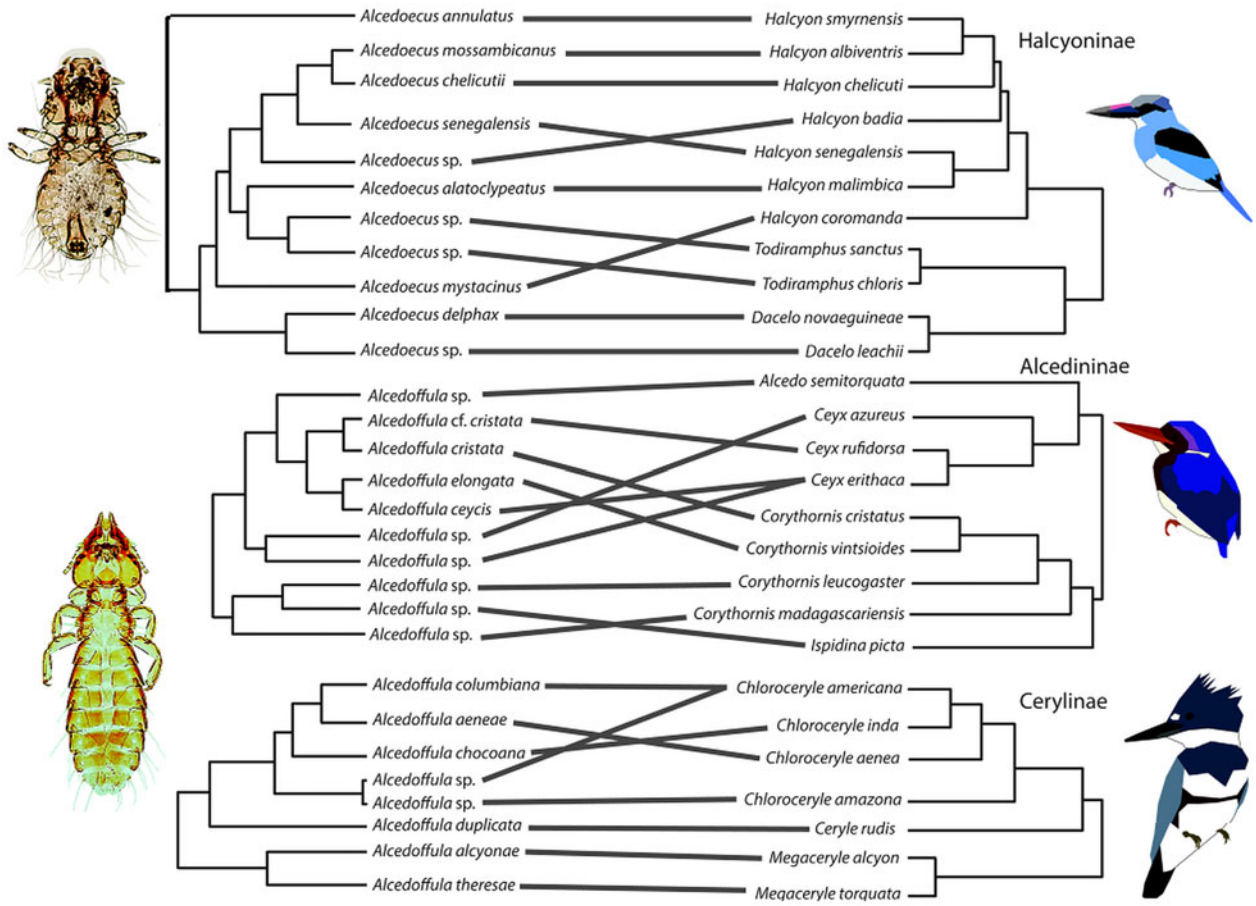

Fig. 4. Tanglegrams of three host subfamilies and their lice showing links between lice (left) and host (right).

Corythornis fall out in the same clade. For example, *Corythornis cristatus* and *Corythornis vintsioides*, which are sister host species (Andersen *et al.*, 2017), are parasitized by lice that fall into different louse clades. Furthermore, lice from these host sister species are not recovered as louse sister species. This suggests that something other than host relationships is driving patterns of *Alcedoffula* louse distribution, although statistical support for relationships within this louse clade are lacking. Although these louse species appear to be host species specific, there is no evidence of codivergence. Instead, host-switching and sorting events were likely more important in structuring the host-parasite associations between *Alcedoffula* and Alcedininae. It is possible that *Alcedoffula* colonized Alcedininae after the kingfishers themselves radiated. Furthermore, lice from both *Corythornis vintsioides* and *Corythornis madagascariensis* were collected in Madagascar, whereas lice from *Corythornis cristatus* were sampled from regions overlapping with the sampling of *Corythornis leucogaster* and *Ispidina picta*. Thus, the lack of geographically structured clades suggests that recent host-switching among geographically proximate hosts is not the primary cause of the incongruence between host and parasite trees.

In the *Alcedoecus* tree (Fig. 2), lice from kookaburras form a clade that is sister to all other sampled *Alcedoecus* with the exception of *Alcedoecus annulatus* ex *Halcyon smyrnensis*. This specimen is placed on a long branch and is sister to all other *Alcedoecus*. Although lice from *Halcyon* do not form a monophyletic group, the placement of the specimen from *Halcyon smyrnensis* is surprising. However, we only have COI sequence data for this louse species and thus its placement requires further study.

Where specimens were available, we included samples from multiple individuals of the same host species to determine whether louse lineages are host specific. Within this dataset we included two to four representatives from 11 of the 27 sampled host species. Lice from all but two hosts (*Ceyx erithaca* and *Chloroceryle americana*) were each other's closest relatives (posterior probability = 0.95 or greater). Both of these host species are geographically widespread. *Ceyx erithaca* is widely distributed in Southeast Asia and many surrounding oceanic islands, whereas *Chloroceryle americana* is a geographically widespread host species that breeds from the southern United States to Argentina (Clements *et al.*, 2017).

In several cases, our louse phylogeny also mirrors recently proposed host species splits. For example, *Todiramphus chloris* (Boddaert, 1783) was recently split into six species (Andersen *et al.*, 2015) and our dataset includes parasite data from two of these host taxa, *Todiramphus sacer* (Gmelin, 1788) and *Todiramphus sordidus* (Gould, 1842). These two lice from *T. sacer* and *T. sordidus* are 16% divergent from one another in COI sequences and are not each other's closest relatives in the phylogeny. A second example includes *Alcedoffula* from *Corythornis vintsioides* and *Corythornis cristatus,* two kingfisher species which are sometimes treated as conspecific (Moyle *et al.*, 2007). Our study examined lice from both host taxa, including multiple representatives from *Corythornis cristatus* from multiple localities in Africa. Our phylogeny recovered a clade containing all lice from *Corythornis cristatus* but this clade excluded the louse from *Corythornis vintisioides*. The louse from *Corythornis vintisioides* had an uncorrected COI *p*-distance of ~15% from members of the *cristatus* louse clade, suggesting that these louse taxa may be sufficiently divergent to support Tendeiro's (1967) description of different species of lice from *Corythornis vintisioides* and *Corythornis cristatus*. These

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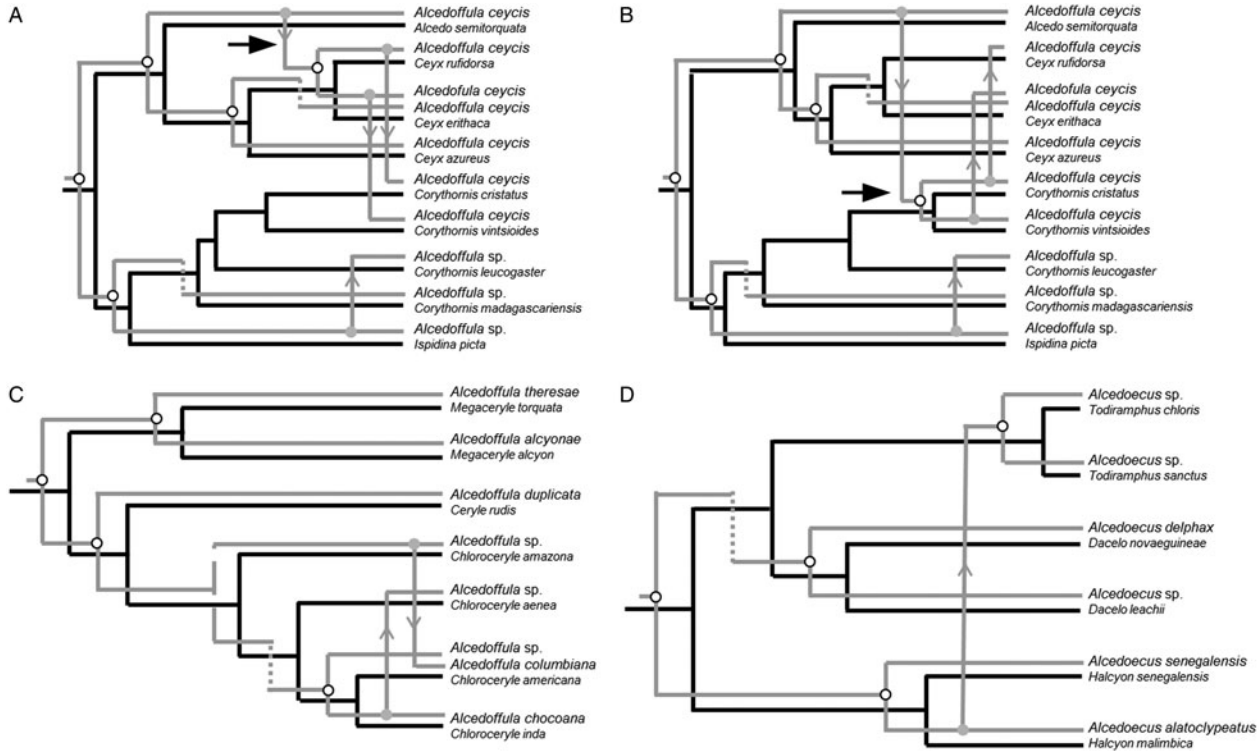

Fig. 5. Inferred patterns of cospeciation for Alcedininae (A and B), Cerylinae (C), and Daceloninae (D) and their lice. Dashed lines represent losses, open circles mark nodes of cospeciation and the filled circle indicates duplication coupled with host switching. Large arrows in A and B denote where two equally costly solutions differ in their reconstructions.

Alcedoffula species have been retained by Price *et al.* (2003), although our limited samples were morphologically identical, suggesting that the key provided by Tendeiro is of limited use. A revision of the lice from these hosts (and *Alcedoffula* as a whole) would better define species limits among these taxa and diagnostic morphological features for specimen identification.

Cophylogenetic analysis of kingfishers and their lice

Varying degrees of cospeciation have been found in comparisons of bird and louse phylogenetic trees, ranging from associations which show strong congruence (Hughes *et al.*, 2007) to virtually no similarity between host and parasite trees (Weckstein, 2004). Here we found the degree of congruence varied not only between the two kingfisher louse genera but also when comparing cophylogenetic histories among the two distinct clades of *Alcedoffula* and their hosts (Figs 4 and 5). Within *Alcedoffula*, lice occurring on Cerylinae showed strong evidence of cospeciation with their hosts. In contrast, *Alcedoffula* from Alcedininae and *Alcedoecus* from Halcyoninae do not show evidence of cospeciation with their hosts. However, our sampling of louse diversity among the three subfamilies is somewhat uneven. In particular, Cerylinae includes ten kingfisher species distributed in both the New and Old Worlds and we sampled lice from all but two Old World species. On the other hand, the other two subfamilies of kingfishers are more diverse, including many with extremely limited distributions, and therefore lice were only available from a smaller fraction of host species in these subfamilies. Further taxon sampling could result in increased evidence of codivergence between kingfishers and their lice, especially among closely related kingfisher species. This is particularly the case in island archipelagos where one kingfisher lineage invaded an island and then diversified down the island chain, a common pattern in Old World kingfishers (Andersen *et al.*, 2017).

A second potential explanation for the pattern observed in *Alcedoffula* is that this genus radiated by extensive host-switching on kingfishers after their hosts already radiated (similar to an escape-and-radiate hypothesis, Ehrlich and Raven, 1964). This hypothesis is consistent with the fact that this louse genus does not occur on a monophyletic host group (the Alcedininae and the Cerylinae are not each other's closest relatives; rather Cerylinae is sister to Halcyoninae). Thus one possibility is that the ancestor of *Alcedoffula* may have switched onto Cerylinae kingfishers early in this host groups diversification, allowing the lice to cospeciate with the hosts. Three species of Cerylinae kingfisher have overlapping ranges with Alcedininae kingfishers creating the potential for louse transfer between unrelated hosts. If this host switch from cerylinine kingfishers occurred later in alcedinine diversification then this would explain the lack of cospeciation between the alcedinine kingfishers and their *Alcedoffula*. Divergence time estimation of the lice could further refine this hypothesis.

Reconstruction of biogeographic history on kingfisher louse trees

Andersen *et al.* (2017) inferred an Indomalayan origin of kingfishers as a whole. This finding somewhat conflicts with the biogeographic patterns found in the louse phylogenies inferred here, because no biogeographic reconstruction for the lice favored a strictly Southeast Asian origin, although this region was reconstructed as part of the ancestral range of *Alcedoecus*.

An Australian + southeast Asian origin was favored for *Alcedoecus*, the louse genus parasitizing Halcyoninae; however a number of other potential histories were also inferred. Australian lice from *Alcedoecus* were embedded within large African louse clades. However, one clade of Australian lice from kookaburras is sister to the majority of *Alcedoecus*, causing the

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biogeographic reconstruction of *Alcedoecus* to favor an Australian + southeast Asian origin. This contrasts with relationships in the host phylogeny in which kookaburras are deeply embedded within Halcyoninae, suggesting the kingfishers of Australia originated from Asian stock.

Although the ancestral range reconstructed at the *Alcedoffula* basal node was equivocal, within lice parasitizing Cerylinae, we inferred a single South American origin. This clade subsequently spread into North America (belted kingfisher lice) and Africa (pied kingfisher lice). This contrasts with the host biogeography inferred by Andersen *et al.* (2017), which is consistent with an Old World origin and two subsequent New World invasions as inferred for Cerylinae. Moyle (2006) and Andersen *et al.* (2017) both recovered Australian alcedinine kingfishers embedded deeply within African and Asian alcedinine kingfishers. This arrangement is similar to our biogeographic reconstruction of *Alcedoffula* from Alcedininae which inferred an African origin of the clade, followed by potentially two southeast Asian invasions. One of these lineages later colonized Australia.

Similar to their kingfisher hosts, biogeographic reconstruction revealed multiple biogeographic transitions across Wallace's Line suggesting that this typically strong biogeographic barrier was not extremely important for kingfishers or their associated chewing lice. Although other examples of avian clades dispersing across Wallace's Line exist (i.e. Cuckooshrikes in the family Campephagidae (Jønsson *et al.*, 2008), this is the first formal analysis of the impact of Wallace's Line on chewing lice.

Author ORCIDs. Therese Catanach, 0000-0003-3850-1196.

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