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AN ASSESSMENT OF HOST ASSOCIATIONS, GEOGRAPHIC DISTRIBUTIONS, AND GENETIC DIVERSITY OF AVIAN CHEWING LICE (INSECTA: PHTHIRAPTERA) FROM BENIN

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ABSTRACT: Host associations of highly host-specific chewing lice (Insecta: Phthiraptera) across multiple avian species remains fairly undocumented in the West African country of Benin. Two hundred and seventeen bird specimens collected from multiple localities across Benin and housed at the Texas A&M University Biodiversity Research and Teaching Collections were examined for lice. Lice were identified and genetic data (mitochondrial COI and nuclear EF-1α genes) were obtained and phylogenetically analyzed. In total, we found 15 host associations, 7 of which were new to science. Genetically, most lice from Benin were unique and could represent new species. Based on host associations and unique genetic lineages, we estimate we discovered a minimum of 4 and possibly as many as 8 new chewing louse species. Given the lack of current data on chewing louse species distributions in Benin, this study adds to the knowledge of host associations, geographic distribution, and genetic variability of avian chewing louse species in West Africa.

Chewing lice (Insecta: Phthiraptera) are small ectoparasites that exhibit a dorsoventrally compressed body plan, with adults ranging in size from 0.8 to 11 mm (Marshall, 1981). Taxonomically, chewing lice belong to 2 suborders: Amblycera and Ischnocera, which primarily feed on host feathers and dead skin, with some amblycerans also feeding on blood (Waterhouse, 1953). These ectoparasites are known to parasitize virtually all bird and many mammal species and are often highly host-specific (Price et al., 2003). Compared to the information known about the biogeography of their bird hosts, relatively little is known about avian chewing louse distributions. Despite the fact that these chewing lice are permanent ectoparasites that spend their entire life cycle on the hosts, several studies have shown that the geographical distribution of some avian lice does not necessarily coincide with the same distribution of its hosts. Clay (1964) showed that the same species of boobies (*Sula* spp.) were hosts to different species of *Pectinopygus* lice in different parts of their range. Edwards (1965) and Weckstein (2004) have shown the same pattern for grebes and toucans, respectively. Bush et al. (2009) showed that different populations of *Aphelocoma* jays in the American southwest were hosts to different communities of lice, differences they attributed to differential tolerance for aridity in different groups of lice. Additionally, higher taxonomic units sometimes appear to be limited geographically despite not being limited to monophyletic host groups. For instance, Gustafsson and Bush (2015) found that the *Brueelia clara* species group appears to be limited to Africa but occurs on 2 distantly related host families. Several other undescribed *Brueelia* species groups, which are also limited to Africa, are similarly widely distributed over multiple host species (D.R. Gustafsson, pers. obs.). Lastly, the head louse genus *Paraphilopterus* is known from 3 different host families, all of which occur in the Australo-Papuan region (Mey, 2004; Gustafsson and Bush, 2014).

Together, these examples suggest that much remains to be discovered about the biogeography of chewing lice, especially avian lice. However, chewing lice are rarely sampled across the range of the host, such that the distribution of a particular species of louse is typically hard to assess. In addition, many lice have very low infestation rates, and actual absence of a louse species from a part of the host's range may be hard to differentiate from low infestation rates. A possible means to better understand avian louse distributions and biogeographic patterns is to examine scientific research specimens with collection locality information housed in natural history museums. Examining museum specimens might provide an opportunity to uncover unknown diversity of parasites without relying on collecting new host specimens, especially in areas where avian lice are traditionally understudied.

Host associations of avian chewing lice remain largely undocumented throughout West Africa, and the species of chewing lice reported from West African countries are limited (e.g., Ansari, 1957; Klockenhoff, 1975, 1981, 1984; Sychra et al., 2010a, 2010b; Najer et al., 2012; Bush et al., 2016). Only a few ecological bird louse studies in West Africa have been published, focusing primarily on domestically important species such as chickens and turkeys in Nigeria, and the role that humid seasons play in louse geographical distribution patterns (Fabiyi, 1972, 1980, 1986, 1996). No studies to date have been conducted specifically in Benin. Geographically, Benin is unique in that it offers a variety of habitats within a small area ranging from semi-arid in the north, to humid coastal regions consisting of a mosaic of grasslands and forests in the south. The Texas A&M University Biodiversity Research and Teaching Collections (BRTC; College Station, Texas) currently house approximately 200 avian specimens that were collected from multiple localities across northern and southern Benin (Fig. 1). These specimens represent a diverse group of avian host species from which to gather data on louse associations. Given the lack of current data on louse species in Benin, this study adds to the knowledge of host associations, geographic distributions, habitat associations, and genetic variability of avian chewing louse species in West Africa.

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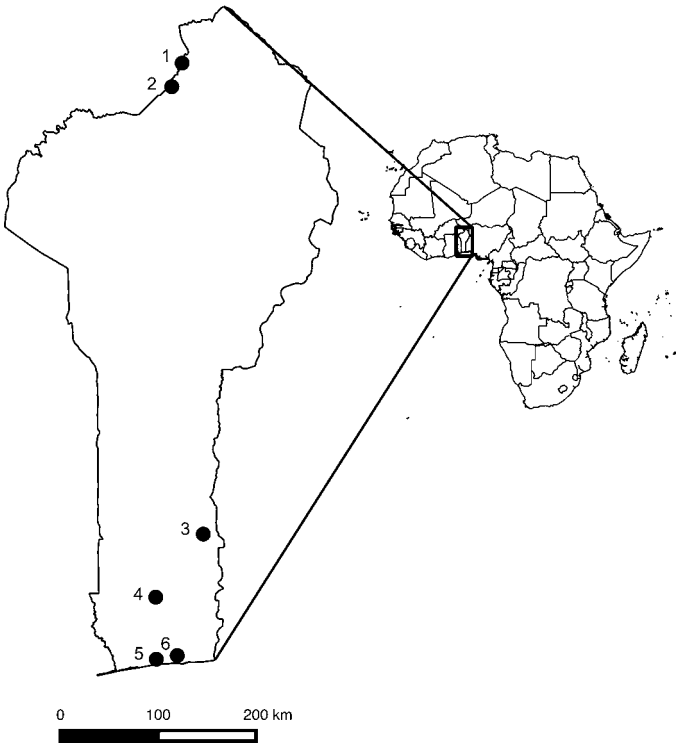

FIGURE 1. Map of Benin in western Africa. Sampling localities are numbered and indicated by closed circles. Locality information is as follows: 1) Alibori: Park W, Point Triple, 2) Alibori: Park W, Chutes du Koudou, 3) Plateau: Dogo Forest and Village, 4) Kou: Lama Forest, 5) Atlantique: Ouidah, 10 km E Lake Toho, and 6) Atlantique: Abomey-Calavi, 2 km N Univ. d'Abomey-Calavi and latitudes and longitudes are available in Table S1.

MATERIALS AND METHODS

Louse specimens collected and examined

Lice were obtained by vigorously brushing the feathers of avian research specimens collected in the West African country of Benin (Fig. 1) and housed in the research collection at the BRTC. An Olympus SZX10 microscope (Olympus Corporation, Tokyo, Japan) was used to sort through the ruffling byproduct, and lice and other ectoparasites such as fleas or mites were retained. Tentative morphological identifications of the lice were made using Price et al. (2003) and compared with existing host association checklists from the region (e.g., Najer et al., 2012; Halajian et al., 2014) or previously mounted specimens located in the Price Institute for Parasitological Research (PIPeR, University of Utah, Salt Lake City, Utah). Lice that could not be identified because they were nymphs, damaged specimens, or likely accidental host associations were omitted from the study. Lice were photographed with an Olympus SZX10 microscope, Intralux 6000 light source (Volpi, Schlieren, Switzerland), and SPOT v4.6 software (2009 Diagnostic Instruments) prior to DNA extraction and retained as digital vouchers. Collected ectoparasites not used in the molecular work described below are housed in PIPeR.

Louse DNA extraction, amplification, and sequencing

Only 1 louse species per host species was examined molecularly. DNA was extracted from individual lice using an Omega Bio-Tek E.Z.N.A. Tissue Kit (Omega Bio-Tek Inc., Norcross, Georgia) following the manufacturer's instructions and louse-specific protocols (Cruickshank et al., 2001). Prior to extraction, louse specimens were soaked in 1× PBS buffer to remove any potential contaminants and to soften the exoskeleton. A sterile scalpel blade was used to lacerate the louse abdomen for extractions. Extractions were performed with a final elution volume of 70 µl. Louse exoskeletons were preserved as slide voucher specimens, housed in PIPeR, and identified to genus or species level using the keys of Clay (1969), Tendeiro (1965, 1967), Price (1974), and Ledger (1980).

Polymerase chain reaction (PCR) amplification and sequencing of a portion of the mitochondrial cytochrome *c* oxidase subunit I (COI) and the nuclear elongation factor-1 alpha (EF-1α) genes was performed using the primers H7005 and L6625 (Hafner et al., 1994) and EF1-For3 and Cho10 (Danforth and Ji, 1998), respectively, following the protocols described in Light et al. (2016). Amplification of the EF-1α gene was performed for a subset of taxa that were identified as unique lineages in the COI phylogeny (see below). PCR success or failure was visualized using electrophoresis on an agarose gel. Successfully amplified PCR products were purified using ExoSAP-IT (USB Corporation, Cleveland, Ohio) prior to sequencing. The cleaned products were sent to Beckman-Coulter Genomics (Danvers, Massachusetts) for cycle sequencing in both forward and reverse directions. Sequencher v.4.2.2 (Gene Codes Corporation, Ann Arbor, Michigan) was used to manually edit base calls and Se-Al v.2.0a11 (Rambaut, 1996) was used to align sequences by eye. All sequences are available on GenBank (COI KY359395–KY349405; EF-1α KY359390–KY359394).

Data analysis

Two separate analyses were conducted: (1) COI only and (2) a combined analysis using both the COI and EF-1α genes for a subset of taxa. Both analyses included sequences from 3 mammalian sucking lice (suborder Anoplura) as outgroup taxa (*Fahrenholzia zacatecae*, *Pedicinus hamadryas*, and *Pediculus humanus*; GenBank numbers HM171445, AY696006, and AY695989 for COI and DQ683190, EF152562, and AY316803 for EF-1α, respectively). Additionally, prior to phylogenetic analysis, each COI and EF-1α sequence was compared to published sequences using the Basic Local Alignment Search Tool (BLAST) in GenBank. If the BLAST search resulted in hits to sequences representing the same louse genus, those sequences were downloaded and included in the phylogenetic analyses. If top hits were not sequences belonging to the same louse genus, a separate nucleotide search was conducted for that genus; if congeneric sequences were available, they were downloaded and included in the analysis.

For each data set, Partition Finder v1.1.1 (Lanfear et al., 2012, 2014) was used with the Bayesian Information Criterion to select the best fitting partitioning scheme and model of evolution for each partition, with each codon position considered as a possible partition. Three optimal partitions were determined for the COI data set, with the GTR+I+G, GTR+I+G, and HKY+G models of evolution selected as the best models for the COI first, second, and third codon positions, respectively. Five optimal partitions were determined for the COI + EF-1α data set, and the best models of evolution were GTR+I+G, GTR+G, and HKY+I+G

TABLE I. Louse–host associations of birds from Benin. Superscripts next to host names indicate collection localities (See Fig. 1, Suppl. Table 1). Asterisks (*) indicate novel host associations, and daggers (†) indicate species with no molecular data.

Host order	Host family	Host species (common name)	Louse species (suborder; voucher number)
Charadriiformes	Glareolidae	*Rhinoptilus chalcopterus* (Bronze-winged courser)[2]	*Quadraceps schusteri* (Ischnocera)
	Jacanidae	*Actophilornis africana* (African jacana)[6]	*Pseudomenopon lanceolatum* (Amblycera)
			Rallicola africana (Ischnocera)
Columbiformes	Columbidae	*Turtur abyssinicus* (Black-billed wood dove)[1]	*Coloceras* sp. (Ischnocera)†
Coraciiformes	Alcedinidae	*Alcedo quadribrachys* (Shining-blue kingfisher)[2]	*Alcedoffula brachialis* (Ischnocera)
		Ispidina picta (African pygmy kingfisher)[1,2,3]	*Alcedoffula* cf. *carvalhoi* (Ischnocera)*
		Halcyon leucocephala (Grey-headed kingfisher)[2]	*Alcedoecus* cf. *alatoclypeatus* (Ischnocera)*
			Alcedoecus cf. *capistratus* (Ischnocera)†
		Halcyon malimbica (Blue-breasted kingfisher)[2]	*Alcedoecus* cf. *capistratus* (Ischnocera)*
Passeriformes	Estrildidae	*Lonchura cucullata* (Bronze mannikin)[6]	*Brueelia lonchurae* (Ischnocera)
	Monarchidae	*Terpsiphone viridis* (African paradise flycatcher)[2]	*Ricinus* sp. (Amblycera)
	Ploceidae	*Amblyospiza albifrons* (Thick-billed weaver)[6]	*Sturnidoecus* cf. *basilewskyi* (Ischnocera)*
		Euplectes ardens (Red-collared widowbird)[3]	*Sturnidoecus* sp. (Ischnocera)*†
		Ploceus nigricollis (Black-necked weaver)[5]	*Myrsidea* sp. (Amblycera)*
	Pycnonotidae	*Pycnonotus barbatus* (Common bulbul)[5]	*Myrsidea* sp. (Amblycera)*†

for the COI first, second, and third codon positions, and K80+I for the EF-1α first and second codon positions, and K80+G for the third codon position, respectively. MrBayes 3.2 (Ronquist et al., 2011) was used to perform a Bayesian phylogenetic analysis on each data set in a partitioned (by codon position) framework. The model parameters were estimated as part of the analysis and treated as unknown variables with uniform priors. Bayesian analyses were run for 10 million generations (initiated with random starting trees), with 4 incrementally heated chains (Metropolis-coupled Markov chain Monte Carlo; Ronquist and Huelsenbeck, 2003), and sampled at 1,000 generation intervals. Two simultaneous and independent runs were conducted, after which the first 25% of the sampled trees were discarded as burn-in. Convergence of independent runs was assessed using the potential scale reduction factor (convergence was obtained). A 50% majority rule consensus tree was constructed using the retained trees, with the sample percentage recovering any particular clade representing that clade's posterior probability (PP; Huelsenbeck and Ronquist, 2001). Average uncorrected *P*-distances were calculated using PAUP* v. 4.0 (Swofford, 2002) to examine genetic differentiation between and among taxa.

RESULTS

Overall, a total of 217 avian research specimens collected from 6 sampling sites across Benin (2 sites in the arid north and 4 sites in the humid south; Fig. 1) were ruffled for ectoparasites (Suppl. Table S1). These specimens provided a diverse sampling set with 78 host species, representing a total of 28 families and 7 orders. Within these taxonomic categories, 17.9%, 28.6%, and 57.1% were parasitized, respectively (Table S1). Five of the 6 different localities sampled had at least 1 avian specimen that was parasitized by chewing lice (Table S1). In total, 8.3% of the host individuals (18 birds) examined were parasitized by lice (Table S1), and 7 new host associations were discovered (out of 15; Table I). Notably, some louse specimens could not be identified to species due to a lack of reference material, absence of adult specimens, or poor condition of the available specimens, and not all specimens were assessed molecularly due to small size or poor condition.

A total of 52 louse individuals were included in the mitochondrial COI phylogenetic analysis, representing 11 new sequences from this study and 41 GenBank sequences (Table II). Average uncorrected *P*-distances within the suborders Amblycera and Ischnocera were high: 23.6% and 26.6%, respectively; genetic distance between the suborders was 30.8%. Compared to GenBank sequences, 8 of the Benin louse samples represent unique genetic lineages, being at least 15% genetically divergent (uncorrected *P*-distance) from their closest relatives (with 1 exception within the ischnoceran genus *Alcedoecus*; see below, Fig. 2).

Phylogenetic analysis strongly supported a monophyletic Amblycera (Bayesian posterior probability [PP] = 1) nested within Ischnocera (Fig. 2). The amblyceran genera *Myrsidea* and *Ricinus* received high support (both with PP = 1), and *Pseudomenopon* was not recovered as monophyletic. Although support for a monophyletic Ischnocera was lacking, there was strong support for several smaller clades within the suborder (Fig. 2). The clade representing the *Brueelia*-complex (Smith, 2001), in this study consisting of the genera *Brueelia* and *Sturnidoecus*, had high support (PP = 1) as did the ischnoceran genus *Alcedoecus* (PP = 1).

The combined COI + EF-1α analysis included 27 samples: 5 new sequences from this study and 22 GenBank sequences (Table II). Results were similar to the COI tree, although support values were generally higher (Fig. 3). Notably, although Ischnocera was recovered as monophyletic, support for the suborder was low (PP = 0.55).

DISCUSSION

Although chewing louse associations have been documented in several other countries in Africa (e.g., Ledger, 1980; Sychra et al., 2010a, 2010b; Najer et al., 2012; Halajian et al., 2014), chewing louse diversity within the West African country of Benin has remained unexplored. Our examination of 217 museum study skins yielded 15 host associations, 7 of which were new to science. Furthermore, 8 of the 11 lice examined in the genetic analysis

TABLE II. Louse GenBank sequences included in the phylogenetic analyses. Host species and collection locality are also given, if known.

Louse species	Host species	Collection locality	COI GenBank number	EF-1α GenBank number
Suborder Amblycera				
Myrsidea cecilae	*Ramphastos* sp.	Brazil	KF048126	—
Myrsidea cruickshanki	*Chlorothraupis carmioli*	Panama	GQ454449	—
Myrsidea masoni	*Bleda canicapilla*	Ghana	DQ366670	FJ171306
Myrsidea sp.	*Xanthomixis zosterops*	Madagascar	KT314064	—
Pseudomenopon rostratulae	*Rostratula benghalensis*	—	AF545754	AF545798
Pseudomenopon carrikeri	*Heliornis fulica*	—	AF545753	AF320456
Ricinus sp.	*Attila spadiceus*	—	AF545762	—
Ricinus sp.	*Ficedula hyperythra*	—	AF545764	—
Ricinus sp.	*Terpsiphone batesi*	DRC*	KU187310	KU187343
Suborder Ischnocera				
Alcedoecus sp.	*Halcyon badia*	DRC	KU187341	—
Alcedoecus alatoclypeatus	*Halcyon malimbica*	—	AY314807	AF545775
Alcedoecus sp.	*Halcyon malimbica*	Ghana	KT892064	—
Alcedoecus annulatus	*Halcyon smyrnensis*	Vietnam	KF385882	—
Alcedoffula brachialis	*Criniger calurus*	DRC	KU187333	—
Alcedoffula brachialis	*Ispidina lecontei*	DRC	KU187332	—
Alcedoffula brachialis	*Alcedo quadribrachys*	DRC	KU187334	—
Alcedoffula duplicata	*Halcyon malimbica*	—	JX121669	JX121682
Alcedoffula sp.	*Alcedo leucogaster*	DRC	KU187330	—
Alcedoffula sp.	*Alcedo leucogaster*	DRC	KU187331	KU187361
Brueelia semiannulata	*Cracticus argenteus*	Australia	KT892143	KT892435
Brueelia sp.	*Lagonosticta rhodopareia*	Mozambique	KT892187	KT892479
Brueelia sp.	*Lonchura striata*	China	KT892191	KT892594
Brueelia sp.	*Randia pseudozosterops*	Madagascar	KT892334	KT892624
Palmaellus inexpectatus	*Psophia dextralis*	Brazil	JQ717180	JQ717188
Quadraceps punctatus	*Larus cirrocephalus*	South Africa	AF444874	AF447209
Quadraceps fissus	*Charadrius semipalmatus*	Canada	JN900158	—
Quadraceps obscrurus	*Tringa stagnatilis*	Australia	JN900144	—
Quadraceps connexus	*Phalaropus lobatus*	Japan	JN900134	—
Quadraceps auratus	*Haematopus ostralegus*	Sweden	JN900109	—
Rallicola irediparrae	*Irediparra gallinacea*	Australia	JQ717185	JQ717193
Rallicola foedus	*Psophia leucoptera*	Brazil	JQ717182	JQ717190
Rallicola advenus	*Fulica americana*	United States	JQ717183	JQ717191
Rallicola kelloggi	*Rallus limicola*	United States	JQ717184	—
Rallicola gadowi	*Apteryx* sp.	New Zealand	JQ717186	—
Sturnidoecus sp.	*Ploceus ocularis*	Mozambique	KT892350	KT892640
Sturnidoecus sp.	*Ploceus velatus*	Malawi	KT892352	—
Sturnidoecus sp.	*Quelea quelea*	Malawi	KT892353	KT892643
Sturnidoecus xanthops	*Ploceus xanthops*	Malawi	KT892355	KT892645
Outgroups				
Fahrenholzia zacatecae	*Chaetodipus eremicus*	—	HM171445	DQ683190
Pedicinus hamadryas	*Papio hamadryas*	—	AY696006	EF152562
Pediculus humanus	*Homo sapiens*	—	AY695989	AY316803

* DRC = Democratic Republic of the Congo.

represent unique genetic lineages (Fig. 2). Four of these unique lineages correspond to novel host associations (*Myrsidea* sp. from *Ploceus nigricollis*, *Alcedoffula* cf. *carvalhoi* from *Ispidina picta*, *Alcedoecus* cf. *alatoclypeatus* from *Halcyon leucocephala*, and *Alcedoecus* cf. *capistratus* from *Halcyon malimbica*; Table I) and likely represent louse species new to science. We could not obtain genetic data from 2 of the new host associations (*Sturnidoecus* sp. from *Euplectes ardens*, and *Myrsidea* sp. from *Pycnonotus barbatus*; Table I), and future work may reveal that these lice also represent new genetic lineages. Based on host associations and unique genetic lineages, we estimate we discovered a minimum of 4 and possibly as many as 8 new species as part of this research. In other words, 26.7–53.3% of the 15 host associations may represent species new to science. Examination of additional avian museum specimens (or specimens newly collected in the field) will almost certainly result in the discovery of additional, unique louse lineages from Benin.

Examining the phylogenies broadly, the lack of support for a monophyletic Ischnocera is not necessarily surprising; other studies have also been unable to support monophyly of this louse suborder (e.g., Cruickshank et al., 2001; Johnson and Whiting, 2002; Yoshizawa and Johnson, 2010; Light et al., 2016). Lack of

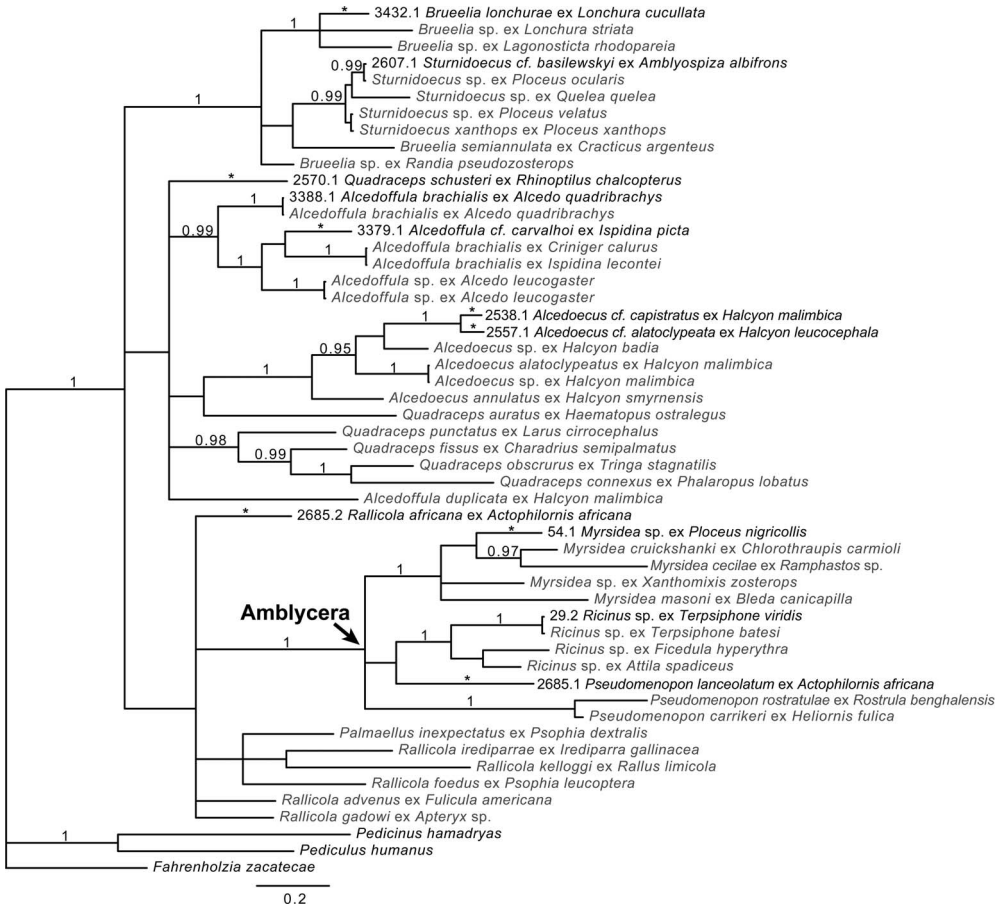

FIGURE 2. Bayesian phylogeny of Benin lice based on the mitochondrial COI gene. Posterior probabilities ≥0.95 are indicated at nodes and sequences in gray are from GenBank (Table II). Louse voucher numbers, species identification, and host species are indicated for all Benin lice (Table I; Table S1). The suborder Amblycera is shown, and asterisks indicate unique genetic lineages.

ischnoceran monophyly in this study is likely the result of taxon sampling; lice examined were collected from taxonomically diverse hosts, representing distantly related birds. Furthermore, overall louse sample size was small. These 2 factors combined could result in a phylogeny that does not accurately reflect higher-level relationships and (with results from other studies) suggests that sampling a substantial diversity of birds will be required to establish ischnoceran monophyly. Additionally, continued use of slowly evolving molecular markers may also help to resolve higher-level relationships.

Within Amblycera (Fig. 2), there are 2 genetically unique lineages from Benin: *Myrsidea* sp. parasitizing *Ploceus nigricollis* and *Pseudomenopon lanceolatum* parasitizing *Actophilornis africana* (Fig. 2). The *Myrsidea* specimen is an average of 22.2% genetically divergent from other *Myrsidea* specimens (uncorrected *P*-distance), likely indicating that this specimen represents a new species (to GenBank or to science). Although there are sequences from 2 *Pseudomenopon* species on GenBank, the Benin sample is quite distinct (26.2% divergent), so much so that our BLAST search did not result in either of those species as a top hit. This indicates that there may be substantial, previously unrecognized genetic diversity within this louse genus. Notably, the *Pseudomenopon* of jacanas (e.g., *Actophilornis*) do not key out to *Pseudomenopon* in the key of Clay (1969); rather, they appear more closely aligned to *Actornithophilus* (based on characters alveoli 26 and 27 not being closely associated). This suggests that the *Pseudomenopon* of jacanas may need to be recognized as a separate genus, or at least that Clay's (1969) key needs to be used with caution. Although we were unable to morphologically identify the *Myrsidea* specimens parasitizing *Ploceus nigricollis* and *Pycnonotus barbatus* (no genetic data were available for the *Myrsidea* specimen on this host; Table I), they may be *Myrsidea textoris* and *Myrsidea pycnonoti*, respectively, based on louse associations for other host species belonging to these genera. Notably, 3 sequences of *M. textoris* are available on GenBank; however, none of these were in the top BLAST hits, supporting that this *Myrsidea* specimen is likely new to science (average uncorrected *P*-distance between the GenBank *M. textoris* and the Benin specimen = 20.5%). Additional collections of these host species will be necessary to determine whether lice parasitizing these birds represent new species. Also within the Amblycera clade is a sequence from 1 Benin louse that is similar to another African louse sequence on GenBank: *Ricinus* sp. (parasitizing *Terpsiphone viridis*) is 0.8% divergent from a *Ricinus* sp. parasitizing *Terpsiphone batesi* from the Democratic Republic of the Congo (DRC). Based on genetic divergence, the 2 *Ricinus* specimens are likely the same species parasitizing a range of *Terpsiphone* spp. over a wide geographic area; additional morphological work, however, is necessary to determine the species identity of these lice.

Within Ischnocera (Fig. 2), several Benin specimens (*Sturnidoecus* and *Alcedoffula*) were genetically similar, or identical, to GenBank sequences, likely representing the same species. *Sturnidoecus* found on the host genera *Ploceus* and *Amblyospiza*

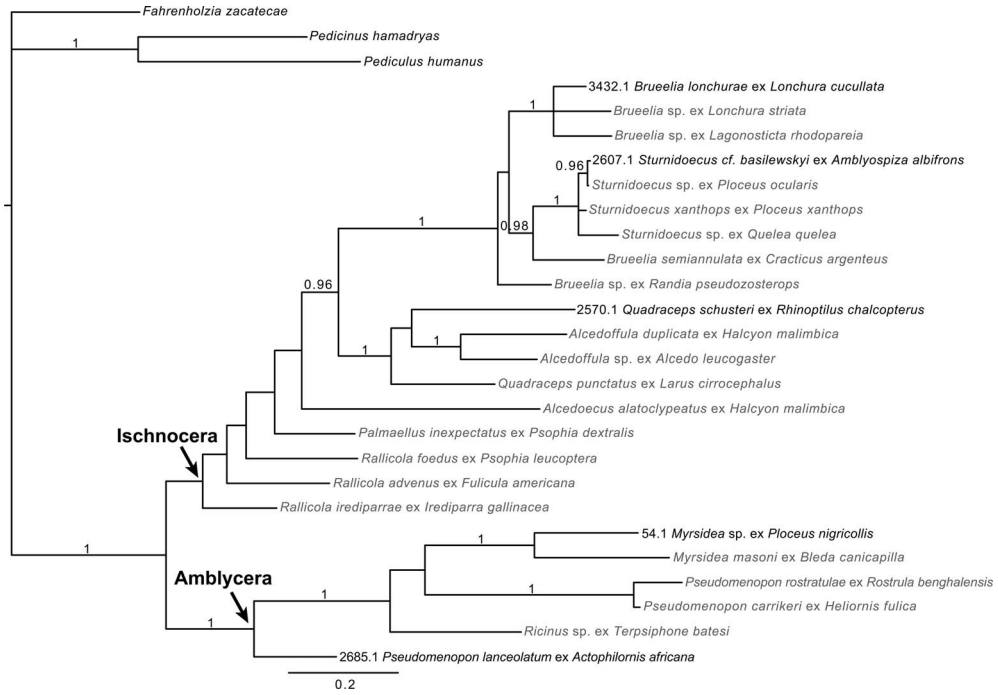

FIGURE 3. Bayesian phylogeny of lice based on the combined mitochondrial COI and nuclear EF-1α gene datasets. Posterior probabilities ≥0.95 are shown, and sequences in gray are from GenBank (Table II). Louse voucher numbers, species identification, and host species are indicated for all Benin lice (Table I; Table S1). The suborders Amblycera and Ischnocera are shown.

(both in the family Ploceidae) from Benin and Mozambique are 0.8% divergent (uncorrected *P*-distance), likely representing the same species. The *Sturnidoecus* of many ploceid hosts (mainly *Ploceus* and *Amblyospiza* species) belong to a morphologically homogeneous group that includes the Congolese species *Sturnidoecus textoris* and the Mozambican species *Sturnidoecus galbula*, *Sturnidoecus neointermedius*, *Sturnidoecus sexualis*, and *Sturnidoecus xanthops*. It is likely that this group of *Sturnidoecus* parasitizing *Ploceus* and *Amblyospiza* hosts is really only 2 species, each of which may have very large geographical ranges and occur on several closely related hosts (D.R. Gustafsson, pers. obs.). Additionally, *Sturnidoecus* from the genus *Euplectes* are similar genetically (Balakrishnan and Sorenson, 2007; Bush et al., 2016) but are genetically distinct from the *Sturnidoecus* on *Ploceus* and *Amblyospiza*. A third lineage of *Sturnidoecus* occurs on hosts in the ploceid genus *Quelea* (Bush et al., 2016). All of these groups are also morphologically distinct from each other (D.R. Gustafsson and S.E. Bush, pers. comm.). However, the exact species limits within the group on *Ploceus* and *Amblyospiza* are not certain, and more taxonomic work is needed based on sampling a larger geographical range and more host species.

The kingfisher louse genus *Alcedoffula* was not recovered as monophyletic in our phylogeny, as the GenBank sample of *Alcedoffula duplicata* falls well outside the clade comprising the rest of the *Alcedoffula* (average uncorrected *P*-distance = 27.5%). *Alcedoffula duplicata* belongs to a group of lice with broad-fronted dorsal anterior plates and hyaline margins that lack the typical deep median indentation found in most other *Alcedoffula*. Tendeiro (1967) placed *A. duplicata*, *Alcedoffula extumida*, and *Alcedoffula theresae* in a separate species group based on these characters; this group also includes *Alcedoffula aeneae*, *Alcedoffula alcyonae*, *Alcedoffula chocoana*, *Alcedoffula columbiana*, and *Alcedoffula mahigir*. Notably, all of these species except *A. mahigir* parasitize hosts in the subfamily Cerylinae, whereas the narrow-fronted *Alcedoffula* species with an indented hyaline margin parasitize host species in the Alcedininae. The 2 host subfamilies are well separated genetically (Moyle, 2006), with Alcedininae being restricted to the Old World and Cerylinae being largely tropical and having distinct Old World and New World clades. This genetic and largely geographic separation of the lice from different host subfamilies is thus supported by morphological data and may indicate that *Alcedoffula* as presently circumscribed constitutes 2 distinct genera, each mainly occurring on a separate subfamily of the Alcedinidae.

Within *Alcedoffula* sensu stricto, 4 distinct lineages are represented, each likely comprising a separate species. *Alcedoffula brachialis* is not supported as monophyletic in our tree but is split into 2 different lineages, 1 occurring on *Alcedo quadribrachys* from Benin and the DRC, and 1 occurring on *Ispidina lecontei* and *Criniger calurus* from the DRC (although the latter is likely a straggler; Light et al., 2016). The 2 remaining lineages occur on *I. picta* (Benin) and *Alcedo leucogaster* (DRC). Each of these represents undescribed species. Notably, the *Alcedoffula* species occurring on *Alcedo* spp. and *Ispidina* spp. do not form monophyletic groups, perhaps reflecting the paraphyly of *Alcedo* spp. (Moyle, 2006). The genetic similarity between the 2 louse sequences from *Alcedo quadribrachys* from different areas representing the 2 extremes of the species' range (Benin and DRC) may indicate that *Alcedoffula brachialis* occurs throughout the range of *Alcedo quadribrachys* (this louse species was originally described from Cameroon; Tendeiro, 1967).

All *Alcedoecus* specimens included in this study parasitize the kingfisher genus *Halcyon* (subfamily Halcyoninae). The 2 Benin *Alcedoecus* specimens included in this study are 8.5% genetically divergent and an average of 17.1% divergent from other *Alcedoecus* lice parasitizing *Halcyon* from the DRC. Interestingly, the 2 host species (*Halcyon leucocephala* and *Halcyon malimbica*) were collected from the same locality in arid, northern Benin (locality 2; Fig. 1; Table I). The morphology and host relationships of both of these Benin *Alcedoecus* species are confusing. *Halcyon*

malimbica is typically parasitized by *Alcedoecus alatoclypeatus* (Price et al., 2003), but our specimen from this host is morphologically more similar to *Alcedoecus capistratus* (which typically parasitizes *H. leucocephala*; see below). However, this specimen differs morphologically from *Alcedoecus capistratus* as described by Tendeiro (1965) from material from Senegal and Mozambique and may represent an undescribed species. Whether our specimen is conspecific with the Senegalese material studied by Tendeiro (1965) is unknown. *Alcedoecus capistratus* is typically found on *H. leucocephala*, but our sample from this host is not *Alcedoecus capistratus*. The specimen cannot be keyed out using the key of Tendeiro (1965), but it is morphologically similar to Tendeiro's photos on *Alcedoecus alatoclypeatus*, normally found on *H. malimbica*. Thus, both *Alcedoecus* specimens appear to be from the "wrong" host. Although we cannot rule out the possibility of straggling, neither *Alcedoecus* specimen can be reliably identified as the species normally occurring on that host using Tendeiro's (1965) key. They are both genetically different from *Alcedoecus alatoclypeatus* from GenBank, and both are genetically and morphologically distinct from each other. The type locality of *Alcedoecus capistratus* is Ethiopia, and while the host (*H. leucocephala*) populations in Ethiopia and Benin are considered the same subspecies (Fry et al., 1999), this large geographical separation between the 2 collection localities may indicate that the range of *Alcedoecus* on *Halcyon* spp. is influenced by external factors, such as ambient humidity, elevation, or habitat. Notably, several other species of *Halcyon* are parasitized by different species of *Alcedoecus* in different parts of their range (Price et al., 2003). A thorough review of *Alcedoecus* is needed before the specimens included in this study can be identified correctly.

Closely related to the kingfisher louse genera *Alcedoffula* and *Alcedoecus* is the louse genus *Quadraceps*. This louse genus broadly parasitizes shorebird hosts in the order Charadriiformes. There are nearly 130 recognized species of *Quadraceps* (Price et al., 2003), but the genus is not supported as monophyletic in either of our phylogenies (Figs. 2, 3) or in past research (Gustafsson and Olsson, 2012). Genetic divergence within *Quadraceps* is quite high, an average of 26% uncorrected *P*-distance, and additional work on this genus will be necessary to determine whether cryptic genera should be recognized. A preliminary analysis (D.R. Gustafsson, pers. obs.) suggests that *Quadraceps* as currently circumscribed is highly paraphyletic, and the genera *Saemundssonia*, *Lunaceps*, *Cummingsiella*, *Incidifrons*, and parts of *Rallicola* may be nested inside *Quadraceps* sensu lato. A large number of genera have previously been recognized for various groups within *Quadraceps* (e.g., Złotorzycka, 1967), and future studies of the genus may indicate that several of these form good genera. *Quadraceps schusteri*, which in our analysis is placed away from the other *Quadraceps* species, may belong to the genus *Glareolites* following a thorough revision of the *Quadraceps*-complex (D.R. Gustafsson, pers. obs.).

Only 1 *Brueelia* specimen was found in this study, *Brueelia lonchurae* from *Lonchura cucullata*. This species is known only from a single female collected on Sao Tome (Tendeiro and Mendes, 1994), and the present material, a single male, is thus identified tentatively. One hundred and sixty-seven passerine birds were examined for this study, and the low prevalence of *Brueelia* is in line with the overall low prevalence of Benin birds with lice in this study (8.3%). Thus, louse parasitism of Benin birds may be low, even for normally common and widespread lice such as *Brueelia*. Notably, the Benin *Brueelia* specimen is highly divergent (average uncorrected *P*-distance = 14.8%) from its closest relatives, GenBank sequences from specimens parasitizing *Lonchura* (China) and *Lagonosticta* (Mozambique), potentially supporting a new genetic lineage of *Brueelia* (Fig. 2). Notably, the GenBank specimens belong to a separate lineage within the *Brueelia*-complex that is restricted to estrildid finches (clade J in the phylogeny of Bush et al., 2016). Overall, species in this group appear to be host-specific (D.R. Gustafsson and S.E. Bush, pers. comm.), and louse species from closely related host species tend to be morphologically similar.

The *Rallicola* taxa in this study are not monophyletic, and their position is unresolved within both phylogenies (Figs. 2, 3); average genetic divergence within this genus is 22.2% (uncorrected *P*-distance). It is likely that this genus is not monophyletic, and future studies examining the genus *Rallicola* as a whole will be necessary to better understand the geographic range, genetic diversity, and taxonomy of this genus. Lastly, we were unable to identify or collect molecular data from the *Coloceras* specimen parasitizing *Turtur abyssinicus*. Many *Turtur* species are known to be parasitized by *Coloceras* (and *T. abyssinicus* is specifically known to be parasitized by several different *Coloceras* species; Price et al., 2003). Future studies will be necessary to determine whether the Benin host association is novel or harbors a unique genetic lineage.

At least 1 bird from 5 of the 6 Benin localities was parasitized by lice (Table S1). New host associations and genetically unique lineages were discovered at all 5 of these localities (localities 1, 2, 3, 5, and 6; Fig. 1). One new host association and genetically unique lineage (*Alcedoffula* on the African pygmy kingfisher, *I. picta*) was spread across 3 localities spanning the length of the country (localities 1, 2, and 3; Table I). The majority of parasitized birds were collected from locality 2, in arid northern Benin (Table S1; Table I). This is not surprising since over 50% of the birds examined were collected from this locality. Only 6 avian specimens were examined from locality 6, yet half of these were parasitized (Table S1; Table 1). Thus, even though only 8.3% of the birds examined in this study were parasitized by lice, it is likely that increased sampling from across the country (and other West African countries) will yield additional lice, novel host associations, and new species.

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