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Molecular phylogeny and novel host associations of avian chewing lice (Insecta: Phthiraptera) from South Africa

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Abstract. Compared with Europe and the Americas, the ectoparasites of African birds are poorly understood, despite the avian fauna being relatively well known. Notably, previous studies documenting the host associations and genetic diversity of parasitic chewing lice of southern African birds have been limited in geographic and taxonomic scope. Recent field expeditions exploring the avian diversity in South Africa facilitated an opportunity to obtain louse specimens from a taxonomically diverse host assemblage. This study is the first to investigate avian louse host associations and diversity across a large portion of South Africa encompassing several distinct habitat types, while incorporating molecular genetic data (from portions of the mitochondrial COI and nuclear EF-1α genes) for ectoparasite phylogenetic analyses. From 1105 South African bird individuals and 170 species examined for lice, a total of 105 new louse–host associations were observed. Morphological and genetic examination of lice with these new host associations reveals a maximum of 66 louse species new to science. Results of this study support the observation that examining museum specimens is a useful way to investigate louse diversity and host associations.

Introduction

Chewing lice of the suborders Amblycera and Ischnocera are ectoparasites of birds and mammals across the globe. Members of each of these suborders have distinctive morphologies, and partition the host body according to feeding strategy and host preening avoidance behaviours (Johnson *et al.*, 2012). Amblyceran lice generally show little specialization to any particular part of the host's body. In contrast, most ischnocerans can be roughly divided into one of several 'ecomorphs', in which body shape is adapted to living either on the wings, head, or body of the host (Baum, 1968; Mey, 1982, 1994; Johnson *et al.*, 2012). Different ecomorphs generally differ in their degree of host specificity, and body lice are often more host-specific than wing lice on the same group of hosts (Johnson *et al.*, 2002a). Wing lice vary in host specificity, and morphological studies indicate that some species appear to be widespread (Clayton & Price, 1999; Price *et al.*, 2003; Johnson *et al.*, 2005). However, closely related lice can belong to different ecomorphs such that each ecomorph has evolved multiple times, even on the same group of hosts (Bush *et al.*, 2016).

In total, there are over 3800 species of chewing lice globally, with many species known from Europe, North America, and the Neotropics (Price *et al.*, 2003); however, the number of updated regional checklists of chewing lice remains small (e.g. Mey, 2003; Palma & Jensen, 2005, 2016; Palma & Peck, 2013; Sánchez-Montes *et al.*, 2018; Gustafsson *et al.*, in press). Despite the large diversity of birds in Africa, the diversity and host relationships of their chewing lice are not well known, especially from southern Africa. Studies of chewing lice parasitizing birds in southern Africa have been restricted to small groups of taxa or limited geographic areas (e.g. Złotorzycka *et al.*, 1999; Kopij & Price, 2009; Halajian *et al.*, 2012; Halajian *et al.*, 2014). Furthermore, studies using molecular phylogenetics to explore louse diversity in this region are lacking (Sychra *et al.*, 2014). Molecular studies exploring parasite diversity are necessary because lice have been shown to exhibit cryptic speciation, and

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it is often impossible to tell closely related species apart solely based on morphological traits (Escalante *et al.*, 2016); African lice are no exception. The importance of molecular studies to identify African louse lineages has been highlighted in recent studies based on lice from the Democratic Republic of the Congo (Light *et al.*, 2016) and Benin (Takano *et al.*, 2017). Both studies identified several new louse species, as well as new host associations based on relatively limited host sampling, underscoring how little is actually known about chewing lice in sub-Saharan Africa.

There is substantial faunal turnover from west to east across southern Africa due to a high diversity of habitats, including the Cape Floristic Region, Kalahari Desert, and eastern forests (Linder, 2003; Rutherford *et al.*, 2006). Several recent studies examining speciation in southern African vertebrate taxa (e.g. lizards, elephant-shrews, chameleons, and mice) have found that these taxa show patterns of speciation related to relatively small geographic barriers (as is the case in rock-dwelling species; Matthee & Flemming, 2002; Smit *et al.*, 2007), or across climate-related gradients (Tolley *et al.*, 2008; du Toit *et al.*, 2012). Additionally, Oatley *et al.* (2012) found that the diversification of *Zosterops* white-eyes in South Africa was driven by their association with distinct habitats, in particular speciation between birds of the fynbos (Mediterranean-like shrubland), Karoo (semi-desert), and coastal temperate forests. Outlaw *et al.* (2007) and Voelker *et al.* (2012, 2014) also found habitat relationships specifically in arid-adapted birds, which seem to have speciated in southern Africa as a result of isolation in fragmented ranges during past wet and cold periods when forests expanded and dry grassland areas were reduced. Parasitic invertebrate taxa are often less well studied than their vertebrate hosts, and the chewing lice of southern African birds are no exception (Ledger, 1980; Gustafsson & Bush, 2015). Yet, examining these and other invertebrate taxa may provide additional information about diversification processes and patterns across southern Africa.

Recent field expeditions exploring avian diversification patterns in South Africa (e.g. Oatley *et al.*, 2012; Voelker *et al.*, 2012) allowed an opportunity to obtain louse specimens from diverse habitats. The purpose of this study is threefold: to investigate avian louse diversity, to identify host associations, and to perform phylogenetic analyses of genetic data from avian lice to increase our understanding of South African ectoparasite biodiversity and the potential role habitat types may play in speciation and biogeography of the lice and their bird hosts.

Materials and methods

Louse specimen collection

Ectoparasites were obtained by brushing ornithological museum research specimens housed at the Texas A&M University Biodiversity Research and Teaching Collections (BRTC). These avian specimens were collected over several field excursions from 2009 to 2014 (Texas A&M University Animal Care and Use permits AUP 2009-28 and AUP 2012-6) across 11 localities in five South African provinces: Limpopo (localities 1–3), Mpumalanga (4), Northern Cape (5), Free State (6, 7), and Eastern Cape (8–11; Fig. 1). Localities within 43 km of each other in the same habitat type were combined into a single locality on the map (Table S3). The merged localities consisted of the following: three sites within Venetia Limpopo Nature Reserve, two sites near Munnik in Limpopo Province, three sites near Kimberley in the Northern Cape, and two sites near Graaff-Reinet in Eastern Cape Province (Localities 1, 3, 6, and 8, respectively; Table S3 and Fig. 1). During processing in the field, birds were kept in individual bags to avoid cross-contamination of lice between hosts. In the BRTC, each specimen was meticulously brushed and the collected material was examined for lice using an Olympus SZX10 microscope (Olympus Corporation, Tokyo, Japan; other ectoparasites such as mites, ticks, and hippoboscid flies were saved and not further examined in this study). Lice were identified morphologically to genus or species when possible using published keys and host association checklists (Clay, 1955; Tendeiro, 1961, 1965; Price, 1977; Ledger, 1980; Klockenhoff, 1981; Price *et al.*, 2003; Najer *et al.*, 2012; Halajian *et al.*, 2014; Gustafsson & Bush, 2017) or specimen slides housed in the Price Institute of Phthirapteran Research (PIPeR) collection at the University of Utah. Louse nymphs that could not be identified beyond family level were included in calculations of louse abundance but excluded from further analyses (Table S3).

DNA extraction and sequencing

Phylogenetic analyses of molecular data were used to confirm morphological identifications and assess genetic diversity of lice. The Omega Bio-Tek E.Z.N.A. Tissue DNA Extraction Kit (Omega Bio-Tek Inc., Norcross, GA, U.S.A.) was used to extract DNA from individual lice according to standard louse protocols (Cruickshank *et al.*, 2001). Photographic vouchers and slide-mounted exoskeletons were retained for each louse specimen. All slide vouchers are housed in PIPeR at the University of Utah. Polymerase chain reactions of 381 bp of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene and 345 bp of the nuclear elongation factor 1-alpha (EF-1α) gene were performed using the primers L6625 and H7005 (Hafner *et al.*, 1994) and EF1-For3 and Cho10 (Danforth & Ji, 1998), respectively. The PCR protocols followed Light *et al.* (2016) and Takano *et al.* (2017). Mitochondrial COI sequences were obtained for all lice, whereas EF-1α was obtained for a subset of lice representing one individual per unique lineage (when possible) based on the COI phylogeny. Prior to sequencing, PCR results were visualized on an agarose gel using electrophoresis, and all positive PCR products were purified using ExoSAP-IT (Affymetrix, Inc., Santa Clara, CA, U.S.A). Samples were sent to Beckman Coulter Genomics (Beckman Coulter, Inc., Danvers, MA, U.S.A., now part of GENEWIZ) for sequencing. SEQUENCHER v. 4.5 (Gene Codes Corp., Ann Arbor, MI, U.S.A) was used to examine raw reads and manually edit base calls. In an effort to identify novel species genetically, each louse sequence was compared to published sequences in GenBank

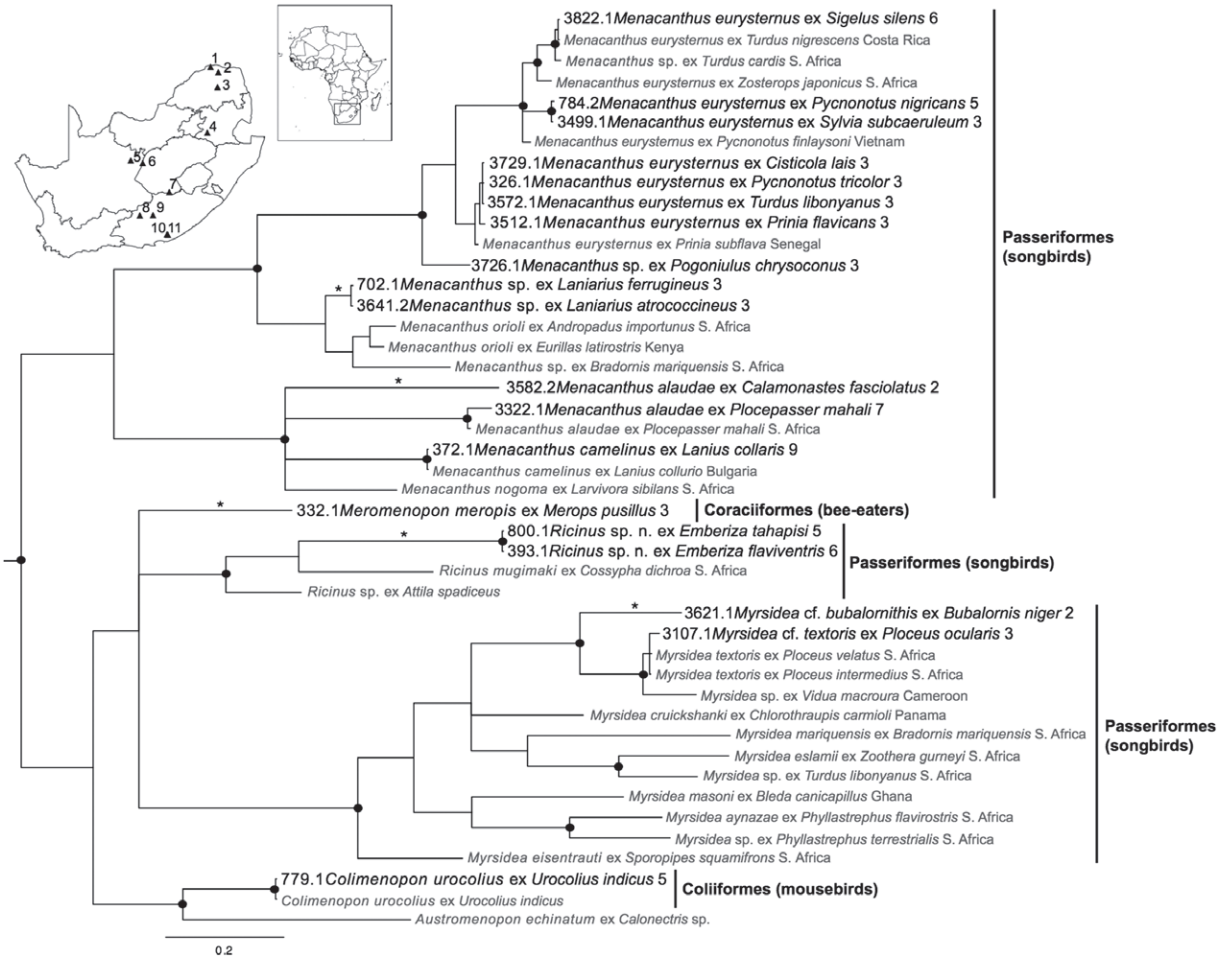

Fig. 1. Bayesian phylogeny of South African amblyceran lice based on analysis of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene. Newly collected specimens as part of this study are in black (grey indicates GenBank specimens; Table S4), with specimens identified by louse voucher number (Table S5). Unique louse lineages identified in this study are indicated by an asterisk (*) on the branches. Posterior probabilities ≥ 0.95 are shown as filled circles at the nodes. Locality numbers (corresponding to Fig. 1) are indicated in the tip labels, and major clades of hosts are indicated. Ingroup taxa were supported as monophyletic with high support relative to the outgroup taxa. Outgroup taxa were removed from this figure for readability. Inset map of South Africa shows louse collection localities (indicated by black triangles) from five provinces: Limpopo (localities 1–3), Mpumalanga (4), Northern Cape (5), Free State (6, 7), and Eastern Cape (8–11). See Table S3 for more information on these collection localities.

using the Basic Local Alignment Search Tool (BLAST), and top hits were included in subsequent phylogenetic analyses. In addition, all available South African louse sequences from GenBank were included in the analysis (Table S4).

Phylogenetic analyses

All sequences were aligned by eye using SE-AL alignment software v.2.0a11 (Rambaut, 1996) and submitted to GenBank (COI, MG682384–MG682442; EF-1α, MG682357–MG682383). All phylogenetic analyses were performed using MRBAYES v.3.2 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Three separate Bayesian analyses were run: (i) Amblycera COI, (ii) Ischnocera COI, and (iii) Amblycera and Ischnocera COI + EF-1α for a subset of taxa based on unique lineages identified from the COI analyses. Prior to each analysis, PARTITIONFINDER v.1.1.1 (Lanfear *et al.*, 2012; Lanfear *et al.*, 2014) was used with the Bayesian information criterion to select the best-fitting partitions and models of evolution. For the Amblycera COI analysis, three optimal partitions (corresponding to each codon position) were selected with the following models of evolution: GTR + I + G for positions 1 and 2, and HKY + G for the third codon position. The same partitioning scheme and models of evolution were identified for the Ischnocera COI dataset. For the COI + EF-1α analysis, six optimal partitions and models of evolution were identified: K80 + I + G for the first and third EF-1α codon positions, GTR + I + G for the second EF-1α codon position, and GTR + I + G for all COI codon positions. Two mammalian sucking louse species (Anoplura: *Fahrenholzia zacatecae* and *Haematopinus eurysternus*; GenBank HM171445 and

HM171422 for COI, respectively) were included as outgroup taxa in the COI analyses, and one Psocopteran bark louse species (Trogiomorpha: *Echmepteryx hageni*; GenBank AY275298 for COI and HQ124319 for EF-1α) was included as an outgroup in the COI + EF-1α analysis. Phylogenetic analyses in MRBAYES were performed using two independent runs with four incrementally heated chains (Metropolis-coupled Markov chain Monte Carlo; Ronquist & Huelsenbeck, 2003), run for 10 million generations, and sampled every 1000 generations. The first 25% of trees from each run were discarded as burn-in. The remaining trees were used to create a 50% majority consensus tree and calculate posterior probabilities. To examine genetic differentiation between and among taxa, average uncorrected *p*-distances were calculated using PAUP* v.4.0 (Swofford, 2002).

Results

Louse specimens from this study were morphologically identified to species when possible, using published descriptions and identification keys (e.g. Price, 1977). In other cases, where detailed illustrations and descriptions are lacking, lice were identified tentatively based on host associations (e.g. Price *et al.*, 2003; Halajian *et al.*, 2014), by comparison with identified voucher specimens, or by genetic similarity to published sequences. In most cases, lice could be identified only to genus level (Table 1), particularly when the specimen represented an undescribed species. Specimens found to differ significantly from known species in diagnostic characters (e.g. setal counts, head shapes, and structure of male genitalia) are listed as likely new species in Table S1.

A total of 14 bird orders, representing 47 families (19 non-passerine and 28 passerine), 109 genera, and 170 species were examined for lice (Table S3). Of 1105 host individuals examined, 248 (22%) were parasitized by lice; 98 (58%) of the 170 host species were parasitized (Table S3). Seven amblyceran and 17 ischnoceran genera were identified, with 26 and 93 likely species represented, respectively. In total, 141 host associations were observed, with 105 of these representing new associations (Table 1). These new associations included both 68 first records of a louse parasitism for some bird species and 37 cases of parasitism by additional louse species for others (Table 1). Parasitism by a single species of louse per host was most common; however, co-infections of a single host parasitized by multiple louse species were also found: 36 bird species were host to two or more louse species. Of these, co-infection by different suborders was most common with 23 bird species parasitized by both amblyceran and ischnoceran lice. Additionally, 22 bird species were parasitized by more than one louse species of the same suborder: 20 bird species with two or more ischnoceran species, and two bird species with two amblyceran species. It should be noted that most of these co-infections were observed across multiple individuals of the same host species, as only 13 host individuals were actually parasitized by multiple louse species (Table S5).

Mitochondrial COI phylogenetic analyses were performed using sequences from 45 amblyceran and 95 ischnoceran specimens. Of these, 19 amblyceran and 44 ischnoceran sequences were obtained from our samples; the rest were sequences from GenBank (Tables S4, S5; Figs 1, 2; note that the taxonomy of sequences originally published in the phylogeny of Bush *et al.*, 2016 have here been updated based on Gustafsson & Bush, 2017). Within the amblyceran tree (Fig. 1), average uncorrected *p*-distances among genera were large: 20%. There was high support for clades containing the genera *Ricinus* De Geer, *Myrsidea* Waterston, and *Colimenopon* Clay & Meinertzhagen [all with posterior probability (PP) of 1; Fig. 1]. These same three clades were recovered with high support in the COI + EF-1α phylogeny as well, although EF-1α could not be amplified for the South African *Myrsidea* samples and only GenBank *Myrsidea* were included in this analysis (Fig. 3). *Menacanthus* Neumann forms the largest clade of amblyceran lice, but without strong support for monophyly of the genus using only COI data (Fig. 1). However, monophyly for *Menacanthus* was supported in the COI + EF-1α analysis (PP = 1; Fig. 3).

Within the ischnoceran COI tree (Fig. 2), diversity among genera was high (average 26% uncorrected *p*-distances). There was strong support for the clades comprising the genera *Alcedoecus* Clay & Meinertzhagen and *Colilipeurus* Bedford (PP = 1 in both cases). There was also strong support (PP = 1) for the speciose *Brueelia*-complex (represented in this study by the genera *Brueelia* Kéler, *Guimaraesiella* Eichler, *Rostrinirmus* Złotorzycka, and *Sturnidoecus* Eichler; Smith, 2001; Bush *et al.*, 2016; Gustafsson & Bush, 2017; Fig. 2). The ischnoceran COI + EF-1α phylogeny provided high support for an additional genus, *Philopterus* Nitzsch, and also recovered the same highly supported clades as mentioned previously for the COI analysis (Fig. 3). The genus *Penenirmus* Clay & Meinertzhagen does not receive high support for monophyly in either of the COI or COI + EF-1α phylogenies, but there is support for smaller groupings within the genus (Figs 2, 3).

The number of birds examined at each locality varied from 10 (at locality 11) to 279 individuals (locality 3; Table S2). New host associations were found at all localities (Tables 1 and S3). Across the geographic localities, the most commonly encountered louse genera were the ischnoceran *Brueelia* s.l., *Philopterus*, and *Penenirmus*, and the amblyceran *Menacanthus* (35, 14, 12, and 14% rates of parasitism, respectively; Tables S3 and S5).

Discussion

In southern Africa, the diversity and host associations of avian chewing lice are poorly understood (Gustafsson & Bush, 2015), despite the avian fauna being relatively well known. This study represents the first extensive assessment of avian chewing louse diversity and host associations from the region, via the examination of over 1100 avian museum specimens representing 170 species. This sample represents approximately 22% of the bird fauna of South Africa (Chittenden, 2007). Based on the findings reported here, examining museum specimens is a useful way to investigate louse diversity, particularly when considering the large number of new host associations (105) and louse lineages (see later) resulting from this study. Previous studies

Table 1. Bird–louse host associations from South Africa, including the first louse record for a particular bird species (*), as well as new host associations for bird hosts that were previously known to be parasitized by other louse species (†). The numbers of host individuals examined are indicated in parentheses following the host species. Due to a lack of reference material, some louse taxa were not identified to species ('sp.'). New louse species are indicated as 'sp.n.' See Tables S3 and S5 for collection localities and specimen and voucher numbers of hosts and their associated lice.

Host family	Host species (number of individuals examined)	Louse suborder	Louse family	Louse species
Order: Bucerotiformes				
Phoeniculidae	*Rhinopomastus cyanomelas* (5)	Amblycera	Menoponidae	*Odoriphila* sp.
		Ischnocera	Philopteridae	*Hopkinsiella* sp.
				Philopterus solus
Upupidae	*Upupa africana* (6)	Ischnocera	Philopteridae	*Upupicola* sp.
Order: Caprimulgiformes				
Caprimulgidae	*Caprimulgus pectoralis* (3)	Ischnocera	Philopteridae	*Mulcticola pectoralis*
Order: Charadriiformes				
Charadriidae	*Charadrius tricollaris* (6)	Ischnocera	Philopteridae	*Quadraceps bicuspis*
Order: Coliiformes				
Coliidae	*Colius colius* (4)	Amblycera	Menoponidae	*Colimenopon* sp.
		Ischnocera	Philopteridae	*Colilipeurus obscurior*
	Colius striatus (9)	Ischnocera	Philopteridae	*Colilipeurus radiatus*
	Urocolius indicus (5)	Amblycera	Menoponidae	*Colimenopon urocolius*
		Ischnocera	Philopteridae	*Colilipeurus* sp.
Order: Columbiformes				
Columbidae	*Streptopelia senegalensis* (1)	Ischnocera	Philopteridae	*Coloceras* sp.
				Hohorstiella asiatica
Order: Coraciiformes				
Alcedinidae	*Halcyon albiventris* (12)	Ischnocera	Philopteridae	*Alcedoecus mossambicanus*
Coraciidae	*Coracias naevius* (1)	Ischnocera	Philopteridae	*Capraiella* sp.n.*
Meropidae	*Merops pusillus* (2)	Amblycera	Menoponidae	*Meromenopon meropis*
Order: Gruiformes				
Rallidae	*Amaurornis flavirostra* (1)	Ischnocera	Philopteridae	*Fulicoffula* sp.n.*
				Rallicola sp.n.*
Order: Passeriformes				
Alaudidae	*Chersomanes albofasciata* (4)	Ischnocera	Philopteridae	*Penenirmus* sp.n.*
	Eremopterix verticalis (4)	Ischnocera	Philopteridae	*Penenirmus* sp.†
	Mirafra africana (1)	Ischnocera	Philopteridae	*Brueelia* sp.n.*
Cisticolidae	*Apalis flavida* (3)	Amblycera	Menoponidae	*Menacanthus* sp.*
		Ischnocera	Philopteridae	*Brueelia* sp.n.*
	Apalis thoracica (11)	Amblycera	Menoponidae	*Machaerilaemus* sp.*
				Menacanthus sp.*
	Calamonastes fasciolatus (5)	Amblycera	Menoponidae	*Menacanthus alaudae**
		Ischnocera	Philopteridae	*Penenirmus* sp.n.*
				Sturnidoecus sp.n.*
	Camaroptera brachyura (2)	Ischnocera	Philopteridae	*Guimaraesiella* sp.n.†
	Cisticola fulvicapilla (6)	Ischnocera	Philopteridae	*Brueelia* sp.n.*
	Cisticola lais (16)	Amblycera	Menoponidae	*Menacanthus eurysternus**
		Ischnocera	Philopteridae	*Brueelia* sp.n.*
	Prinia flavicans (3)	Amblycera	Menoponidae	*Menacanthus eurysternus*
	Prinia maculosa (4)	Ischnocera	Philopteridae	*Brueelia* sp.n.*
				Philopterus sp.n.*
Dicruridae	*Dicrurus adsimilis* (13)	Ischnocera	Philopteridae	*Philopterus* sp.n.†
Emberizidae	*Emberiza flaviventris* (15)	Amblycera	Ricinidae	*Ricinus* sp.n.*
		Ischnocera	Philopteridae	*Brueelia* sp.n.*
				Penenirmus sp.n.*
				Philopterus sp.n.*
	Emberiza tahapisi (5)	Amblycera	Ricinidae	*Ricinus* sp.n.†
		Ischnocera	Philopteridae	*Brueelia* sp.†

Table 1. Continued

Host family	Host species (number of individuals examined)	Louse suborder	Louse family	Louse species
Estrildidae	*Estrilda erythronotus* (1)	Ischnocera	Philopteridae	*Brueelia* s. lat.*
	Granatina granatina (8)	Ischnocera	Philopteridae	*Brueelia* sp.n.*
	Lagonosticta rhodopareia (8)	Ischnocera	Philopteridae	*Brueelia* sp.n.*
	Lagonosticta rubricata (6)	Amblycera	Menoponidae	*Myrsidea* sp.n.*
	Pytilia melba (15)	Ischnocera	Philopteridae	*Brueelia* sp.n.†
Fringillidae	*Crithagra atrogularis* (5)	Ischnocera	Philopteridae	*Philopterus* sp.n.†
	Crithagra canicollis (1)	Ischnocera	Philopteridae	*Brueelia* sp.n.*
	Crithagra flaviventris (2)	Ischnocera	Philopteridae	*Penenirmus* sp.n.†
	Crithagra gularis (5)	Ischnocera	Philopteridae	*Brueelia* sp.n.*
				Philopterus sp.n.*
	Crithagra mozambica (8)	Ischnocera	Philopteridae	*Brueelia* sp.n.*
Laniidae	*Eurocephalus anguitimens* (3)	Amblycera	Menoponidae	*Menacanthus* sp.†
	Lanius collaris (3)	Amblycera	Menoponidae	*Menacanthus camelinus*
		Ischnocera	Philopteridae	*Philopterus* sp.n.†
Leiothrichidae	*Turdoides bicolor* (2)	Amblycera	Menoponidae	*Myrsidea* sp.n.†
Macrosphenidae	*Sylvietta rufescens* (17)	Ischnocera	Philopteridae	*Brueelia* sp.n.*
Malaconotidae	*Dryoscopus cubla* (9)	Ischnocera	Philopteridae	*Philopterus* sp.n.†
	Laniarius atrococcineus (15)	Amblycera	Menoponidae	*Menacanthus* sp.*
	Laniarius ferrugineus (15)	Amblycera	Menoponidae	*Menacanthus* sp.*
		Ischnocera	Philopteridae	*Guimaraesiella* sp.*
				Philopterus sp.n.*
	Nilaus afer (6)	Ischnocera	Philopteridae	*Brueelia* sp.*
				Philopterus sp.n.*
	Tchagra australis (3)	Ischnocera	Philopteridae	*Sturnidoecus wittei*
Monarchidae	*Terpsiphone viridis* (8)	Ischnocera	Philopteridae	*Brueelia* s. lat.
				Sturnidoecus sp.†
Motacillidae	*Anthus* sp. (30)	Amblycera	Ricinidae	*Ricinus* sp.
		Ischnocera	Philopteridae	*Brueelia* sp.
	Macronyx capensis (3)	Ischnocera	Philopteridae	*Brueelia* sp.n.*
				Philopterus sp.n.*
	Motacilla capensis (17)	Ischnocera	Philopteridae	*Brueelia* sp.n.*
Muscicapidae	*Bradornis mariquensis* (3)	Ischnocera	Philopteridae	*Philopterus* sp.n.†
	Cercotrichas coryphaeus (5)	Ischnocera	Philopteridae	*Philopterus* sp.n.*
	Cercotrichas leucophrys (5)	Amblycera	Menoponidae	*Myrsidea* sp.n.†
			Ricinidae	*Ricinus* sp.
	Cercotrichas paena (12)	Ischnocera	Philopteridae	*Penenirmus* sp.n.†
	Cossypha caffra (39)	Ischnocera	Philopteridae	*Philopterus* sp.n.†
	Cossypha humeralis (7)	Amblycera	Menoponidae	*Menacanthus* sp.*
	Muscicapa striata (2)	Ischnocera	Philopteridae	*Guimaraesiella* sp.†
	Myrmecocichla formicivora (16)	Ischnocera	Philopteridae	*Penenirmus* sp.n.*
				Philopterus sp.n.*
	Sigelus silens (28)	Amblycera	Menoponidae	*Menacanthus eurysternus**
		Ischnocera	Philopteridae	*Brueelia* sp.*
	Stenostira scita (7)	Ischnocera	Philopteridae	*Philopterus* sp.n.*
Nectariniidae	*Chalcomitra amethystina* (14)	Ischnocera	Philopteridae	*Philopterus* sp.n.*
	Cinnyris chalybeus (5)	Amblycera	Menoponidae	*Menacanthus* sp.*
		Ischnocera	Philopteridae	*Philopterus* sp.*
				Sturnidoecus sp.*
	Nectarinia famosa (3)	Ischnocera	Philopteridae	*Brueelia* sp.n.†
Oriolidae	*Oriolus larvatus* (7)	Ischnocera	Philopteridae	*Philopterus* sp.
Paridae	*Parus cinerascens* (4)	Ischnocera	Philopteridae	*Philopterus* sp.*
	Parus niger (5)	Ischnocera	Philopteridae	*Brueelia* sp.n.*

Table 1. Continued

Host family	Host species (number of individuals examined)	Louse suborder	Louse family	Louse species
Passeridae	*Passer diffusus* (20)	Amblycera	Menoponidae	*Menacanthus* sp.*
		Ischnocera	Philopteridae	*Brueelia* sp.n.*
				Guimaraesiella sp.*
	Passer melanurus (14)	Ischnocera	Philopteridae	*Brueelia* sp.n.†
				Rostrinirmus sp.†
	Plocepasser mahali (9)	Amblycera	Menoponidae	*Menacanthus alaudae*
		Ischnocera	Philopteridae	*Brueelia* sp.n.†
	Sporopipes squamifrons (4)	Ischnocera	Philopteridae	*Brueelia* sp.n.†
Platysteiridae	*Batis pririt* (9)	Ischnocera	Philopteridae	*Philopterus* sp.n.*
Ploceidae	*Anaplectes melanotis* (6)	Amblycera	Menoponidae	*Menacanthus* sp.*
	Bubalornis niger (2)	Amblycera	Menoponidae	*Myrsidea* cf. *bubalornithis**
	Euplectes capensis (5)	Ischnocera	Philopteridae	*Philopterus* sp.n.*
	Ploceus capensis (11)	Ischnocera	Philopteridae	*Brueelia* sp.n.†
	Ploceus cucullatus (4)	Ischnocera	Philopteridae	*Brueelia* sp.n.†
	Ploceus ocularis (6)	Amblycera	Menoponidae	*Myrsidea* cf. *textoris**
	Ploceus velatus (27)	Ischnocera	Philopteridae	*Brueelia* sp.n.†
				Sturnidoecus sp.
	Quelea quelea (4)	Ischnocera	Philopteridae	*Brueelia quelea*
Prionopidae	*Prionops plumatus* (4)	Ischnocera	Philopteridae	*Guimaraesiella* sp.n.†
Pycnonotidae	*Chlorocicla flaviventris* (5)	Ischnocera	Philopteridae	*Brueelia* sp.n.†
	Pycnonotus nigricans (28)	Amblycera	Menoponidae	*Menacanthus eurysternus*†
		Ischnocera	Philopteridae	*Brueelia pseudognatha*
				Philopterus sp.n.†
	Pycnonotus tricolor (21)	Amblycera	Menoponidae	*Menacanthus eurysternus**
		Ischnocera	Philopteridae	*Brueelia* s. lat*
Sturnidae	*Creatophora cinerea* (2)	Amblycera	Menoponidae	*Menacanthus* sp.
	Lamprotornis nitens (7)	Ischnocera	Philopteridae	*Brueelia* sp.
				Sturnidoecus senegalensis
	Onychognathus nabouroup (1)	Ischnocera	Philopteridae	*Philopterus* sp.n.*
Sylviidae	*Sylvia subcaeruleum* (33)	Amblycera	Menoponidae	*Menacanthus eurysternus*
		Ischnocera	Philopteridae	*Brueelia* sp.n.†
Turdidae	*Turdus libonyanus* (8)	Amblycera	Menoponidae	*Menacanthus eurysternus*†
		Ischnocera	Philopteridae	*Philopterus* sp.n.
	Turdus smithi (9)	Ischnocera	Philopteridae	*Brueelia* sp.n.†
Viduidae	*Vidua macroura* (2)	Ischnocera	Philopteridae	*Brueelia* sp.n.†
Zosteropidae	*Zosterops capensis* (27)	Amblycera	Menoponidae	*Menacanthus* sp.*
		Ischnocera	Philopteridae	*Penenirmus* sp.*
	Zosterops pallidus (21)	Amblycera	Menoponidae	*Menacanthus* sp.
Order: Piciformes				
Lybiidae	*Lybius torquatus* (8)	Amblycera	Menoponidae	*Menacanthus* sp.
		Ischnocera	Philopteridae	*Penenirmus* sp.
	Pogoniulus chrysoconus (5)	Amblycera	Menoponidae	*Menacanthus* sp.*
	Trachyphonus vaillantii (8)	Ischnocera	Philopteridae	*Penenirmus* sp.*
	Tricholaema leucomelas (37)	Amblycera	Menoponidae	*Menacanthus* sp.†
		Ischnocera	Philopteridae	*Brueelia* sp.n.†
				Penenirmus leucomelan

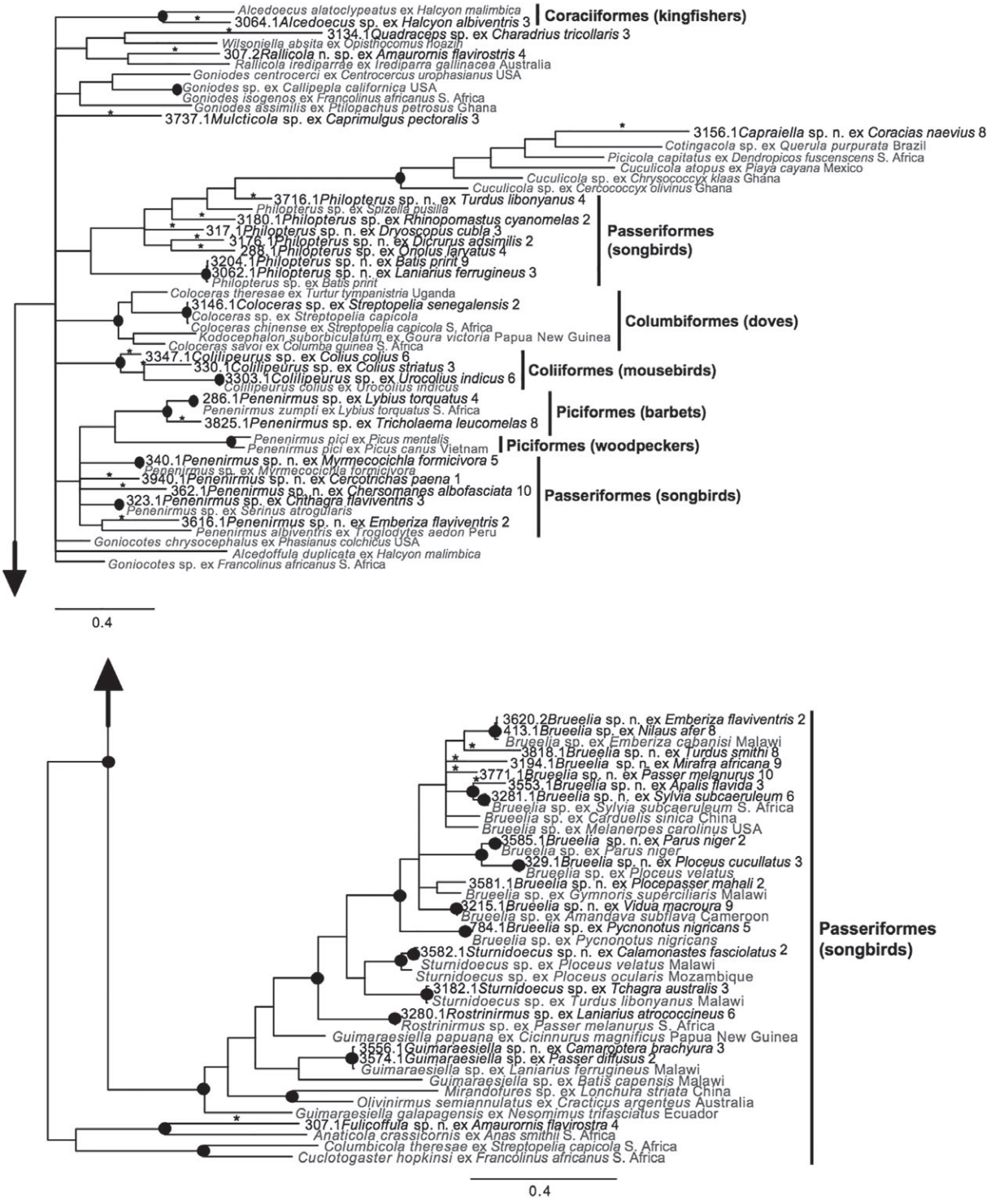

Fig. 2. Bayesian phylogeny of South African ischnoceran lice based on analysis of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene. Newly collected specimens as part of this study are in black (grey indicates GenBank specimens; Table S4), with specimens identified by louse voucher number (Table S5). Unique louse lineages identified in this study are indicated by an asterisk (*) on the branches. Posterior probabilities ≥0.95 are shown as filled circles at the nodes. Locality numbers (corresponding to Fig. 1) are indicated in the tip labels and major clades of hosts are indicated. Ingroup taxa were supported as monophyletic with high support relative to the outgroup taxa. Outgroup taxa were removed from this figure for readability.

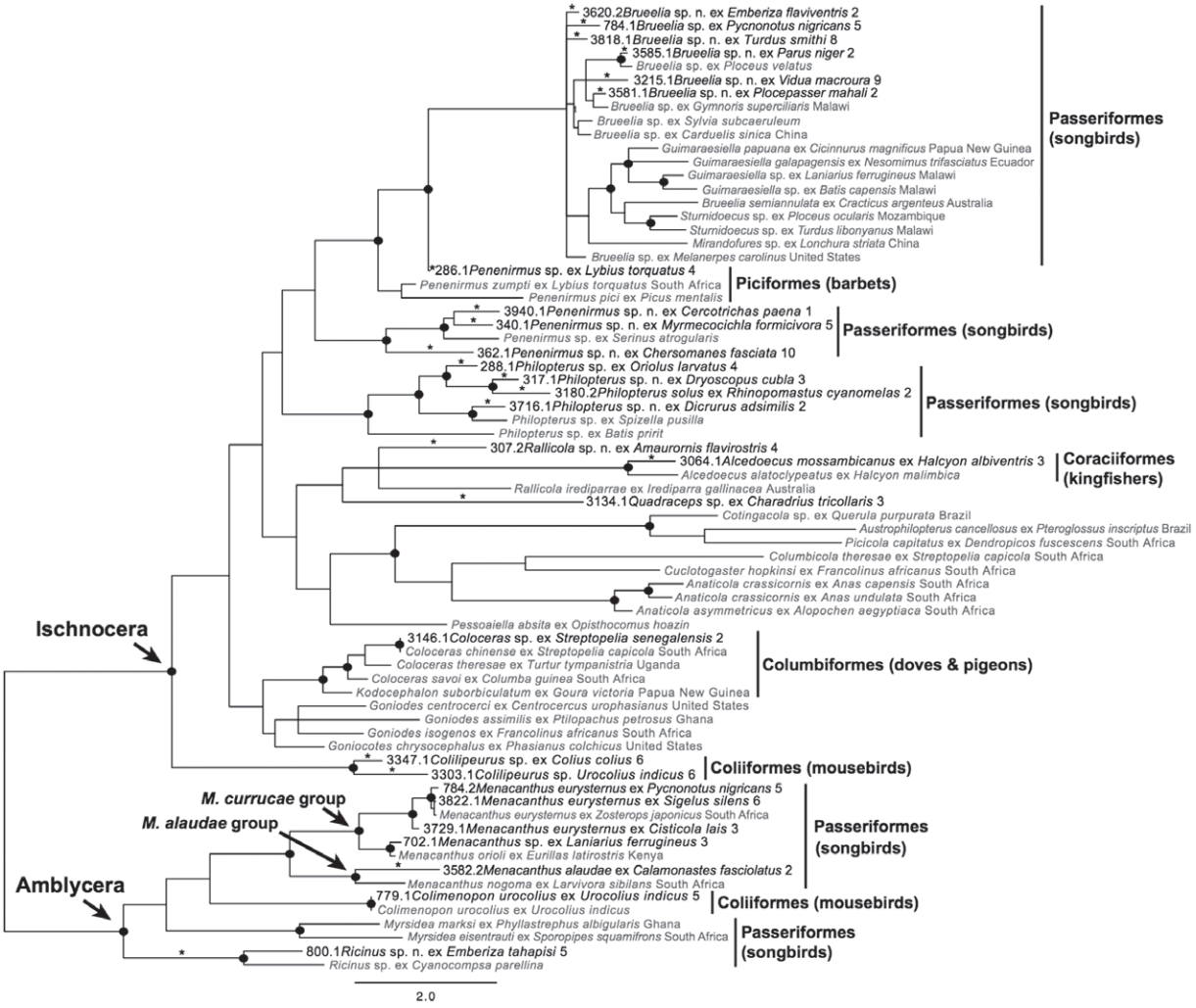

Fig. 3. Bayesian phylogeny of South African amblyceran and ischnoceran lice based on analysis of the mitochondrial cytochrome *c* oxidase subunit I (COI) and nuclear elongation factor 1-alpha (EF-1α) genes. Newly collected specimens as part of this study are in black (grey indicates GenBank specimens; Table S4), with specimens identified by louse voucher number (Table S5). Unique louse lineages identified in this study are indicated by an asterisk (*) on the branches. Posterior probabilities ≥ 0.95 are shown as filled circles at the nodes. Locality numbers (corresponding to Fig. 1) are indicated in the tip labels and major clades of hosts are indicated. The outgroup taxon was removed from this figure for readability.

have also found museum skins to be useful for exploring parasite diversity (e.g. Mey, 2002; Valim *et al.*, 2006; Light *et al.*, 2016; Takano *et al.*, 2017). The new host associations found in our study included 70 louse records for bird species not previously known to be parasitized by lice (based on comparison with host association checklists in Ledger, 1980; Price *et al.*, 2003; Kopij & Price, 2009; Sychra *et al.*, 2014; Gustafsson & Bush, 2017; Table 1). Compared with field studies on birds in Europe and South America where the louse parasitism rates of individuals are typically 40–60% (Clayton *et al.*, 1992; Sychra *et al.*, 2011; Enout *et al.*, 2012; Girisgin *et al.*, 2013), the rates of parasitism in this study were relatively low (22% of individuals and 58% of species). This difference in louse prevalence rates may be due to the high percentage (86% of individuals) of passerines in our study, as passerines usually have lower infestation rates than nonpasserines (Rózsa, 1997; Enout *et al.*, 2012). Alternatively, lice may have been lost during the bird specimen collection and preparation process in our study (see later).

As many louse specimens in our samples represent undescribed species (Table S1), the 105 new host associations comprise a minimum estimate based on the assumption that all lice belonging to the same genus and parasitizing the same host species represent the same species. It is possible that multiple congeneric species of lice may exist on a single host species (Price *et al.*, 2003), especially given that previous studies have shown that different host populations can show high levels of genetic differentiation in their respective lice, resulting in the discovery of cryptic lineages (Voelker *et al.*, 2013; Escalante *et al.*, 2016). For instance, our *Brueelia* specimens from *Plocepasser mahali mahali* represent a different species

than material from the same host species (subspecies *P. mahali melanorhynchus*) we have seen from Ethiopia (D. Gustafsson, personal observation). More detailed taxonomic studies are needed to establish whether this is the case in other louse taxa included here. Additionally, studies such as ours are often hampered by the lack of detailed, modern descriptions of many species of lice. It is likely that there are more South African louse–host associations than what we report here, especially considering that we had 16 instances of unidentified nymphal lice found on the birds we examined (Table S3). These may represent novel host associations; none of these nymphs could be identified to species level due to the lack of published nymphal characters for most African louse species, and in some cases it was impossible to identify these nymphs correctly even to genus level. Nymphal lice that could only be identified to family level were excluded from the phylogenetic analyses (Table S3).

The careful morphological examination of each louse specimen as well as the phylogenetic analyses in our study lend insight as to whether these new host associations represent new species. High sequence divergence (average uncorrected *p*-distance $\geq 15\%$ for the COI gene), especially when combined with unique morphology based on comparisons to the literature and reference material, indicated to us the possibility of new species. Many of the newly collected lice have COI and EF-1α sequences that are highly differentiated from louse sequences available on GenBank (Figs 1–3). Genetically unique lineages were identified for five amblyceran and 21 ischnoceran specimens; some of these unique lineages may represent species new to science (Table S1). An additional three amblyceran and 37 ischnoceran lice were identified morphologically as new host associations, but did not have associated molecular data (Table S3 and Tables 1 and S1). These lice may also represent additional new species, for a total maximum number of 66 potential new louse species from this study (Table S1). Further evidence for new louse species was obtained by examining the geographic ranges of their hosts. Lice from southern Africa are poorly known, and have most likely not been previously described, especially those lice parasitizing hosts with restricted ranges (defined as southern African endemic species, or near endemics with 85% or more of the range within southern Africa; Chittenden, 2007; Table S1). Lice from widespread hosts, on the other hand, could have been described from other geographic locations, even in cases where genetic data are not available (Table S1).

Examining broader phylogenetic relationships based on the COI data, relationships among genera and species within each suborder are not always clear, although there is strong support for several smaller groupings (Figs 1, 2). The difficulty in resolving species relationships may be explained by the high variability in the COI gene (Johnson *et al.*, 2002a; Smith *et al.*, 2004). The combined COI + EF-1α analysis included both Amblycera and Ischnocera, and consistently supported the same clades as the COI-only analysis while yielding higher support values overall than the analyses of the COI gene alone (Fig. 3). The hosts and lice examined were highly diverse, making the phylogenetic results difficult to interpret for higher-level relationships of lice. The dataset for Ischnocera was larger and more diverse than Amblycera overall, as is expected based on the species diversity of the two suborders (2737 and 1172 known ischnoceran and amblyceran species, respectively; Price *et al.*, 2003).

Amblycera

Within Amblycera, the majority of *Menacanthus* specimens were identified as *M. eurysternus* Burmeister, while others were identified as *M. alaudae* Schrank and *M. camelinus* Giebel (Fig. 1). A single lineage (from two species of *Laniarius*) showed contradictory characters in the key of Price (1977), and could not be placed morphologically; these specimens may represent an undescribed species. A specimen from *Calamonastes fasciolatus* keyed out to *M. alaudae* in the Price (1977) key, but is genetically very distinct from other representatives of the same species. This may indicate that the specimen from *C. fasciolatus* also represents a new species of *Menacanthus*. While our sampling of *Menacanthus* is not as extensive as that of Martinů *et al.* (2015), some findings of that study are corroborated by our dataset. In both datasets, *Menacanthus* is basally divided into two main lineages, the *currucae* group (including *M. eurysternus*, *M. orioli* Blagoveshtchensky, and some potentially new species from *Laniarius* spp. and *Pogoniulus chrysoconus*) and the *alaudae* group (including *M. alaudae*, *M. nogoma* Uchida, *M. camelinus*, and the potentially new species from *C. fasciolatus*); this division is not supported by our COI data alone (Fig. 1), but is supported in the combined EF-1α and COI dataset (Fig. 3). Notably, Martinů *et al.* (2015) found that *M. eurysternus* is genetically diverse, but that genetically similar specimens were found in very different areas of the world. This finding is replicated in our study, as *M. eurysternus* from South African *Sigelus silens* are virtually identical to *M. eurysternus* from Costa Rican *Turdus nigrescens*. Similar to our study, Martinů *et al.* (2015) also found that genetic variation within some species of *Menacanthus* is high; therefore, more detailed taxonomic studies of this genus are needed to establish morphological and genetic species limits.

Only two *Myrsidea* specimens were genetically examined in this study, one of which is highly similar (average uncorrected *p*-distance = 1.7%) to the GenBank *M. textoris* Klockenhoff sequences, while the other was tentatively identified morphologically as *M. bubalornithis* Klockenhoff (Fig. 1). *Myrsidea* is a speciose genus with a worldwide distribution (Price *et al.*, 2006; Price & Johnson, 2006; Valim & Weckstein, 2013; Sychra *et al.*, 2016), making it somewhat surprising that so few *Myrsidea* were found in this study, especially given that typical hosts for *Myrsidea* (primarily passerines; Price *et al.*, 2003) were well represented in our sampling (Table S3). Our sampling of museum specimens as well as a preference by *Myrsidea* for humid habitats may explain the small sample of this genus in our study (see further discussion in 'Geographic patterns' latler).

The two South African *Ricinus* specimens examined here (both found parasitizing the host genus *Emberiza*; Fig. 1) are genetically identical and represent a new genetic lineage, making this a likely new species, especially given that both of

these lice represent new host associations and did not morphologically match known species (Rheinwald, 1968; Nelson, 1972; Tables 1 and S1). Lastly, the *Meromenopon* Clay & Meinertzhagen specimen in this study was identified morphologically as *M. meropis* Clay & Meinertzhagen, which has not previously been sequenced. In total, 26 new amblyceran host associations were found and there appear to be at least five likely candidates for new amblyceran louse species from South Africa (Tables 1 and S1 and Fig. 1).

Ischnocera

In total, 78 new ischnoceran host associations were discovered, in addition to as many as 61 new species (Tables 1 and S1). Each species of mousebird (Coliiformes: Coliidae) is parasitized by a unique species of *Colilipeurus*; however, the original descriptions of the species of *Colilipeurus* are inadequate to allow for actual species identification. Our *Colilipeurus* specimens all differ markedly in morphology, and we consider them to represent three different species. We have tentatively identified two of these species as the *Colilipeurus* species normally found on their respective host species, but a taxonomic revision of the genus is sorely needed. For the genus *Coloceras* Taschenberg, our South African specimen is probably *Coloceras chinense* Kellogg & Chapman, based on host associations (genus *Streptopelia*) and closely related GenBank sequences (0.15% average uncorrected *p*-distance, PP = 1; Fig. 2).

The genus *Penenirmus* parasitizes both the host orders Passeriformes (songbirds) and Piciformes (woodpeckers, barbets, etc.; Price *et al.*, 2003). Previous research by Johnson *et al.* (2002b) found that *Penenirmus* on passeriform hosts form a monophyletic group, while the *Penenirmus* on piciform hosts did not. Our results are somewhat in agreement. In the ischnoceran COI phylogeny (Fig. 2), there are two strongly supported (PP = 1), highly divergent (average uncorrected *p*-distance between clades = 20%) clades of *Penenirmus* parasitizing Piciformes. The first of these clades represents GenBank *Penenirmus pici* from *Picus* (a woodpecker genus), and may represent the proposed genus *Picophilopterus* (Ansari, 1947; Carriker, 1963). The second clade consists of two lineages of *Penenirmus* from *Tricholaema* and *Lybius* (two barbet genera). The COI sequences from the *Penenirmus* parasitizing *Tricholaema* represent a genetic lineage with no previously published sequences. These lice are probably *P. leucomelan* Tendeiro, a known parasite of *Tricholaema* barbets (Price *et al.*, 2003). Although the COI data alone do not support a monophyletic *Penenirmus* for passerine hosts (Fig. 2), inclusion of the EF-1α data provides strong support for a passeriform *Penenirmus* clade (PP = 1; Fig. 3). Given the distinct Passeriformes and Piciformes host associations, the previous findings of Johnson *et al.* (2002b), and the large genetic divergence among generally host-restricted clades, *Penenirmus* may represent a complex of cryptic louse genera. A more comprehensive dataset, as well as morphological studies are needed to evaluate whether *Penenirmus* is monophyletic, and whether *Picophilopterus* warrants recognition as a separate genus or subgenus.

The *Philopterus* complex is one of the most widely distributed groups of lice on passeriform birds (Price *et al.*, 2003). In the COI dataset (Fig. 2), *Philopterus* is paraphyletic with regard to the *Picicola* complex; however, this relationship receives no support and is probably spurious. The relationships within this complex are not clear, and many groups traditionally placed within *Philopterus* s.l. are probably best considered separate genera (Mey, 2004). No thorough revision of the *Philopterus* complex has yet been performed; however, all specimens included here are likely to fall within *Philopterus* s.s. and the higher systematics of this complex could therefore not be addressed. The *Philopterus* specimens included in this study were highly divergent from each other (average uncorrected *p*-distance = 24.8%; Fig. 2), which is consistent with the *Philopterus* specimens being found on four different passerine host families (Turdidae, Malaconotidae, Dicruridae, and Oriolidae). Two louse specimens (3204.1 and 3062.1) were nearly genetically identical (average uncorrected *p*-distance = 0.4%, PP = 1) to an unidentified *Philopterus* species from GenBank and as such may represent the same, novel louse species, or a previously unsequenced species. These *Philopterus* specimens were collected from different host species from localities 3 and 9, which are geographically distant from each other, suggesting that this species is relatively common within southern Africa (Fig. 1). It is also possible that the *Philopterus* on *Laniarius ferrugineus* (3062.1) was a straggler (rare occurrence of a louse on an atypical host via horizontal transfer; Rózsa, 1993), as *Philopterus* species are usually specific to a single host family (Price *et al.*, 2003; Fig. 2).

The *Brueelia* complex (in this study containing the genera *Brueelia*, *Guimaraesiella*, *Rostrinirmus*, *Sturnidoecus*, and GenBank specimens of *Mirandofures* Gustafsson & Bush and *Olivinirmus* Złotorzycka) received high support in the COI phylogeny. The relationships among these genera in the COI-only tree do not reflect those of Bush *et al.*'s (2016) more comprehensive sampling; with the exception of *Olivinirmus* and *Mirandofures*, no relationships between any two genera within the *Brueelia* complex are supported in our data. The relationship between *Olivinirmus* and *Mirandofures* is highly supported, but based on a single species from each genus. These two genera were widely separated in the phylogeny of Bush *et al.* (2016), and their relationship is not supported by any morphological characters (Gustafsson & Bush, 2017). Morphological data support a closer relationship between *Mirandofures* and *Brueelia* s.s., and between *Olivinirmus* and *Guimaraesiella*, as found by Bush *et al.* (2016). The relationship suggested by our data is thus probably the result of too few specimens from each genus being included in the analysis. Only two *Brueelia*-complex genera represented by more than one specimen are monophyletic with high support in our COI data: *Brueelia* s.s. and *Rostrinirmus*. By contrast, *Guimaraesiella* is paraphyletic in our COI analysis with regard to the rest of the *Brueelia* complex; however, this has no support (Fig. 2) and may be an artifact of the high genetic diversity within this genus (Bush *et al.*, 2016). Most specimens included from *Brueelia* s.s. form distinct genetic lineages, or small clades incorporating material from the same or closely related host species. This is not surprising, as species of *Brueelia* are generally host-specific. In a few cases (e.g. sample

413.1 parasitizing *Nilaus*), the occurrence of a louse species on a given host may be the result of straggling or contamination in the field or in collections, as the louse is morphologically distinct from the *Brueelia* lice normally found on that host family. In other cases (e.g. samples 3182.1 and the GenBank specimen from *Turdus libonyanus*), the occurrence of the same louse lineage on hosts belonging to multiple host families may be due to relaxed host specificity. This is known for some groups within the *Brueelia* complex (Bush *et al.*, 2016), particularly for hosts that occur in mixed-species feeding flocks, and for lice that are capable of phoresy.

Geographic patterns

Examination of the louse genera across the 11 sampling localities showed, not surprisingly, that increased sampling effort yielded higher diversity of lice. The geographic localities examined in this study represented diverse habitats: localities 1–3 have the highest precipitation overall and consist of mopane woodland (taller woodland with mopane trees) and bushveld (lower, shrubby woodland), localities 4 and 7 are grasslands, localities 5 and 6 are the most arid localities in acacia thornveld habitat (semi-arid savanna with acacia and other thorny trees and shrubs), and the southernmost localities 8–11 were Nama Karoo (relatively dry shrubland) and coastal habitats (van Rensburg *et al.*, 2004; du Toit *et al.*, 2012; Barlow *et al.*, 2013; Table S2). Some studies have indicated that in arid environments, birds have fewer lice than in humid regions (Chandra *et al.*, 1990; Moyer *et al.*, 2002), although ischnoceran lice are less affected by arid conditions than amblycerans due to physiological traits related to ability to uptake water vapour (Rudolph, 1983; Carrillo *et al.*, 2007; Bush *et al.*, 2009). Louse load was not quantified in this study as lice were obtained from museum specimens; examination of newly collected hosts would be necessary to provide an accurate estimate of louse load (Clayton & Drown, 2001).

When considering the number of louse infections observed across our sampling localities, there do not appear to be patterns associated with aridity in southern Africa. In fact, the locality with the lowest observed rate of parasitism (locality 1 at 11.6%; Table S2) was the most humid. The more arid regions (localities 5 and 6) had rates of parasitism that fell within the range of parasitism across all localities (11.6–35%), with locality 5 having a rather high rate (25.6%; Table S2). There also was no difference in the proportion of Amblycera to Ischnocera found across the regions (Table S2). Importantly, the observed rates of louse parasitism are probably underestimates due to our sampling methods of examining museum bird specimens for lice versus examining the bird hosts in the field.

The relative proportions of the most common genera of lice vary across the geographic regions of South Africa. Lice of the *Brueelia* complex (*sensu* Gustafsson & Bush, 2017) were the most common group by far (35% of all louse individuals collected) and were the most common louse group encountered at eight out of 11 localities (Table S5). The frequent occurrence of *Brueelia*-complex lice across sampling localities is unsurprising given that this ischnoceran group is cosmopolitan and common (Johnson *et al.*, 2002c; Bush *et al.*, 2016). It is likely that the *Brueelia* complex contains both genera that are adapted to arid environments and genera that are adapted to more humid environments. Specifically, passerine birds in more humid areas appear to be parasitized mainly by lice in the genus *Guimaraesiella* and its close relative (e.g. all the *Brueelia* complex samples from the more humid Congo area are *Guimaraesiella*; Light *et al.*, 2016), whereas *Brueelia* s.s. and its close relatives are generally found in drier areas. Notably, both of the *Guimaraesiella* specimens in this study were collected in the localities with the highest precipitation, whereas the *Brueelia* specimens collected during this study were distributed across most collection localities, including those with the lowest precipitation (Fig. 1 and Table S2). A similar pattern is found across the world (D. Gustafsson, personal observation); however, detailed studies of large-scale patterns in host relations and biogeography are lacking for the *Brueelia* complex.

The genus *Penenirmus* (12% of collected ischnoceran lice) was the second most common ischnoceran genus after lice of the *Brueelia* complex at most localities, except in the southern region, where *Philopterus* (14% of ischnocerans) was more common. There is an apparent replacement of *Penenirmus* by *Philopterus* in the southern region (Nama Karoo, localities 8–11; Fig. 1 and Table S5); these two ischnoceran genera are similar morphologically and are both considered head lice. Both of these genera are found broadly across the Passeriformes, as well as in some of the Piciformes (Price *et al.*, 2003). *Philopterus* may be more common than *Penenirmus* in the southern region due to habitat restrictions related to aridity, as is *Myrsidea* (see earlier).

The most common amblyceran genus collected across the localities was *Menacanthus* (14% of amblyceran lice). This louse genus is incredibly widespread across both geography and hosts, as it is found on multiple continents and host orders (Price *et al.*, 2003; Martinů *et al.*, 2015). *Menacanthus* specimens that were probably the globally distributed *M. eurysternus* were found from localities 3, 5, and 6, indicating that this species, in particular, has a broad distribution in South Africa (Fig. 1; Table S5). This species is known to be an extreme host generalist (Martinů *et al.*, 2015), and is known from a range of hosts from various environments. The presence of this species from both the most arid and the most humid localities sampled is thus not surprising. However, it is interesting to note that most of the samples from the more humid localities group together in one of the large subclades of *M. eurysternus*, and all the samples from the driest locality are grouped in one clade. It is possible that different subgroups of *M. eurysternus* are adapted to different humidity levels, which may be so recent that this has not yet translated into distinct morphologies.

Myrsidea is the most speciose amblyceran genus with a worldwide distribution (Price *et al.*, 2006; Price & Johnson, 2006; Valim & Weckstein, 2013; Sychra *et al.*, 2016); however, we found few *Myrsidea* in this study. Halajian *et al.* (2012, 2014) also found few *Myrsidea* infesting birds in South Africa, as did Najer *et al.* (2012) in Senegal. Bush *et al.* (2009) similarly

reported fewer *Myrsidea* from dry areas in North America and Mexico, suggesting that this louse genus may be adapted to more humid habitats, and thus may be uncommon in South Africa because of the dry environment. Although *Myrsidea* is known from most parts of the world, many recent descriptions have highlighted the very high diversity of *Myrsidea* present in the wetter tropics (e.g. Dalgleish & Price, 2003, 2005; Hellenthal & Price, 2003; Johnson & Price, 2006; Price & Johnson, 2006, 2009; Sychra *et al.*, 2006, 2007; Kounek *et al.*, 2011, 2013; Valim *et al.*, 2011; Halajian *et al.*, 2012). Compared with the relatively well-known *Myrsidea* fauna of the Holarctic, the fauna of the tropical areas of the world are most likely highly undersampled.

The louse sampling in this study was limited by the availability of bird museum specimens. The set of avian hosts that provided lice were captured with mist nets, leading to a biased sampling of hosts consisting primarily of small and medium passerines (of 1105 individuals examined, 955 were passerines). Increased sampling from both field studies and museum specimens would result in a better estimate of the diversity of lice from South African birds. Although this study makes important strides forward in reducing the knowledge gap about the diversity of parasitic chewing lice in South Africa, both diversity and avian louse associations overall still remain underexplored in southern Africa (not to mention sub-Saharan Africa) as a whole. Additional sampling across southern Africa as well as examining additional host taxa will almost certainly lead to discovery of new host associations and species. This study forms a basis for future studies to investigate co-speciation of avian hosts and louse parasites in southern Africa, which may also be used to infer host biogeographic patterns.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Likely new louse species ('sp.n.') from South Africa based on genetic data and morphological comparisons. Louse species indicated with an asterisk (*) denote likely new species determined solely by morphology. Host distribution is indicated as primarily southern Africa (endemics and near-endemics) or extending beyond southern Africa (African).

Table S2. Number of bird–louse associations found at localities in South Africa. Locality numbers correspond to Fig. 1.

Table S3. South African bird specimens examined for lice in this study from the Texas A&M University Biodiversity Research and Teaching Collections (BRTC) and the Museum of Vertebrate Zoology, University of California, Berkeley (MVZ; specimen numbers pending). Locality numbers match those in Fig. 1. Asterisks (*) indicate specimens parasitized by lice, and crosses (†) indicate unidentified nymphal specimens that were excluded from analyses.

Table S4. Louse GenBank sequences included in the South African phylogenetic analyses. Host species and collection locality are also given, if known. Ischnoceran louse genera follow recent taxonomy published by Gustafsson & Bush (2017).

Table S5. South African lice identified in this study. Due to a lack of reference material, some louse taxa were not identified to species. All host specimens are accessioned into the Texas A&M University Biodiversity Research and Teaching Collections unless otherwise mentioned [MVZ, Museum of Vertebrate Zoology, University of California, Berkeley; specimen numbers pending). Lice are organized by host taxonomy. Louse suborders are denoted by A (Amblycera) and I (Ischnocera)]. Louse specimens not included in the phylogenetic analyses are indicated by an asterisk (*). Louse specimen collection localities correspond to locality numbers in Fig. 1 and Table S3.

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