Unexpected distribution patterns of *Carduiceps* feather lice (Phthiraptera: Ischnocera: Philopteridae) on sandpipers (Aves: Charadriiformes: Scolopacidae)

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Abstract. The louse genus *Carduiceps* Clay & Meinertzhagen, 1939 is widely distributed on sandpipers and stints (Calidrinae). The current taxonomy includes three species on the Calidrinae (*Carduiceps meinertzhageni*, *Carduiceps scalaris*, *Carduiceps zonarius*) and four species on noncalidrine hosts. We estimated a phylogeny of four of the seven species of *Carduiceps* (the three mentioned above and *Carduiceps fulvofasciatus*) from 13 of the 29 hosts based on three mitochondrial loci, and evaluated the relative importance of flyway differentiation (same host species has different lice along different flyways) and flyway homogenization (different host species have the same lice along the same flyway). We found no evidence for either process. Instead, the present, morphology-based, taxonomy of the genus corresponds exactly to the gene-based phylogeny, with all four included species monophyletic. *Carduiceps zonarius* is found both to inhabit a wider range of hosts than wing lice of the genus *Lunaceps* occurring on the same group of birds, and to occur on *Calidris* sandpipers of all sizes, both of which are unexpected for a body louse. The previously proposed family Esthiopteridae is found to be monophyletic with good support. The concatenated dataset suggests that the pigeon louse genus *Columbicola* may be closely related to the auk and diver louse genus *Craspedonirmus*. These two genera share some morphological characters with *Carduiceps*, but no support was obtained for grouping these three genera together. Based on mitochondrial data alone, the relationships among genera within this proposed family cannot be properly assessed, but some previously suggested relationships within this proposed family are confirmed.

Introduction

Influence of flyways on louse distribution

The most frequent opportunities for transfer of lice between two avian host individuals are during mating (Hillgarth, 1996) or from parents to the young in the nest (Clayton & Tompkins, 1994; Lee & Clayton, 1995). However, lice are likely to exploit any opportunity to transfer among hosts that arises during the host’s life cycle. For instance, Brooke & Nakamura (1998) suggested that cuckoos might gain their cuckoo-specific lice when groups of cuckoos gather at caterpillar outbreaks during migration. Communal sand baths, nest holes and theft of nest material have also been proposed as likely opportunities for lateral louse transfer (references in Price et al., 2003).

Gustafsson & Olsson (2012a) suggested that for lice of shorebirds (Charadriiformes), such opportunities may be very frequent outside of mating and nesting, due to the ecology of the host. While host population densities in breeding areas may be low, shorebirds gather into large, dense flocks during migration. These flocks follow specific flyways, which channel different populations of the same species into different wintering areas (e.g. Wilson & Barter, 1998; Tjørve & Tjørve, 2007; Lopes et al., 2008). Migration and wintering flocks often consist of a mixture of shorebirds belonging to different species, genera and even families, and may include shorebird species of very different body sizes. The size difference between two potential...
hosts may impede the success rate of louse dispersal from one host to another (Tompkins et al., 1999; Johnson et al., 2005; Bush & Clayton, 2006). Conversely, the presence of multiple host species of similar size in the same mixed flocks may aid the establishment of lice on novel hosts. Host species like Dunlin (Calidris alpina), Sanderling (Calidris alba) and Curlew Sandpiper (Calidris ferruginea) are of similar size and occur in sympatry along several flyways, with different subpopulations or subspecies restricted to different flyways (Message & Taylor, 2005).

The co-occurrence of potential hosts of similar size in the same wintering area, and the isolation of different populations of the same host species into different flyways may have two different effects on their louse populations, if transfer between hosts happens more frequently during migration than during breeding. Gustafsson & Olsson (2012a) established the term ‘flyway differentiation’ for the scenario in which different populations of the same host species are parasitized by different louse species depending on the flyway along which hosts migrate. They further suggested that if louse populations on wintering hosts encounter a variety of potential host species of similar body size, and there are no other restrictions to movement between hosts, the lice may spread laterally to parasitize all hosts of similar size along one flyway, a scenario they termed ‘flyway homogenization’. Gustafsson & Olsson (2012a) tested these hypotheses for the louse genus Lunaceps of sandpipers (Calidris sensu lato). They found some evidence for flyway homogenization among some, but not all, hosts of similar size along a flyway. However, flyway differentiation was not seen among any of the Lunaceps species sampled from the same host species from multiple flyways.

Most groups of birds are parasitized by multiple genera of chewing lice (Price et al., 2003). In general, co-occurring genera of lice on the same host have differentiated to specialize in different microhabitats of the host. These microhabitat specializations are typically correlated with distinct morphological traits, which are often convergent between distantly related louse genera in the same microhabitat (Johnson et al., 2012). For instance, head lice generally have rounded bodies and large, triangular heads, wing lice (like Lunaceps) usually have elongated, slender bodies, and body lice (such as Carduiceps) typically have broad, rounded or triangular heads. Two species of lice inhabiting different microhabitats of the same host species may have different rates of straggling to novel hosts, with wing lice being more likely to switch hosts than body lice (Johnson et al., 2002; Page et al., 2004; Whitman et al., 2004). As flyway homogenization relies on the lice being able to transfer easily between hosts, flyway homogenization may be more common among wing lice than among body lice. By contrast, the potentially more limited capability of dispersal among body lice than among wing lice may suggest that flyway differentiation is more common among body lice than among wing lice.

We present here a phylogeny of the lice in the genus Carduiceps that parasite sandpipers and allies, based on three mitochondrial loci, testing the hypothesis that flyway homogenization may be less common in body lice than in wing lice. Moreover, flyway differentiation may be more common in body lice than in wing lice, as the lesser propensity for dispersal to novel hosts among body lice than among wing lice would tend to isolate the former along different flyways.

**Taxonomy and relationships of Carduiceps**

Carduiceps was described by Clay & Meinertzhagen (1939) based on head and abdominal characters. The genus mainly parasitizes sandpipers (Calidris sensu lato) and godwits (Limosa spp.), but also the Terek Sandpiper Xenus cinereus and the dowitchers (Limnodromus spp.). Most of the hosts of Carduiceps are also parasitized by the genus Lunaceps, and co-occurrence of lice in these two genera on the same host is common (D. Gustafsson & U. Olsson, Unpublished data). Despite the large overlap in host distribution between these two louse genera (Price et al., 2003; D. Gustafsson & U. Olsson, Unpublished data, 2012b), Carduiceps is considered to consist of fewer species than Lunaceps. This could imply that lice inhabiting different body parts of sandpipers are subject to different mechanisms or opportunities for lateral spread to novel hosts. However, another explanation may be that Carduiceps contains cryptic species and that the current taxonomy of the genus based on Timmermann (1954) is too conservative.

The systematics of ischnoceran chewing lice is poorly known, and all species parasitizing birds are presently placed in one of three families. Of these, the family Heptapso gastridae is limited to Neotropical tinamous (Timiniformes), Goniodidae is largely limited to wittows (Galiformes) and pigeons (Columbiformes), and all other lice are placed in the large and morphologically diverse Philopteridae. The most thorough alternative to this conservative classification was proposed by Eichler (1963), who divided the Ischnocera parasitizing birds into 17 families and 34 subfamilies. Eichler’s (1963) proposed subdivision of the Ischnocera has never been widely used, but molecular evidence suggest that at least some of these groups may be meaningful (Cruickshank et al., 2001). In Eichler’s (1963) proposed classification, Carduiceps is placed in the family Esthiopteridae, which he further subdivided into five subfamilies: Anatoecinae, Aqauiniminae, Columbicolinae, Esthiopterinae and Ibdioecinae. Carduiceps is placed in the subfamily Anatoecinae in Eichler’s (1963) classification scheme. The family Esthiopteridae contains a variety of louse genera occurring on hosts across most of the major divisions of birds (Table 1); however, most of the host groups were placed in the clade Aequorlitorinithes by Prum et al. (2015). Cruickshank et al. (2001) did not find any support for Esthiopteridae, but their analysis included only six of the 15 genera included in the family by Eichler (1963). No species of Carduiceps have hitherto been included in any phylogenetic analysis, and the phylogenetic position of this genus in relation to other shorebird lice is unknown. Eichler (1963) placed most of the other ischnoceran shorebird lice in the family Rallicolidae, which was placed together with Esthiopteridae in his ‘interfamily’ Esthiopteriformia.

We have included representatives of all five of the proposed subfamilies of Esthiopteridae suggested by Eichler (1963), to test whether this family and subfamilies are monophyletic, and
Table 1. Host distribution of the lice in Eichler’s (1963) proposed Esthiopteridae.

<table>
<thead>
<tr>
<th>Louse genus</th>
<th>Host order</th>
<th>Host clade</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaticola*</td>
<td>Anseriformes</td>
<td>Galloanserae</td>
</tr>
<tr>
<td>Anatoecus*</td>
<td>Anseriformes</td>
<td>Galloanserinae</td>
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<td>Aphanopterus*</td>
<td>Podicipediformes</td>
<td>Aequorlitornithes</td>
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<td>Aroicola*</td>
<td>Pelecaniformes</td>
<td>Aequorlitornithes</td>
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<tr>
<td>Ardeiphagus</td>
<td>Pelecaniformes</td>
<td>Aequorlitornithes</td>
</tr>
<tr>
<td>Carduceps*</td>
<td>Charadriiformes</td>
<td>Aequorlitornithes</td>
</tr>
<tr>
<td>Colombicola*</td>
<td>Columbiformes</td>
<td>Columbaves</td>
</tr>
<tr>
<td>Craspedonirmus*</td>
<td>Gaviiformes,</td>
<td>Aequorlitornithes</td>
</tr>
<tr>
<td></td>
<td>Charadriiformes</td>
<td></td>
</tr>
<tr>
<td>Esthiopterum</td>
<td>Gruiformes</td>
<td>Gruiformes</td>
</tr>
<tr>
<td>Falicocula*</td>
<td>Gruiformes</td>
<td>Gruiformes</td>
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<tr>
<td>Ibisoeuc*</td>
<td>Pelecaniformes</td>
<td>Aequorlitornithes</td>
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<td>Neophilocterus</td>
<td>Ciconiformes</td>
<td>Aequorlitornithes</td>
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<td>Pesseoecia</td>
<td>Cuculiformes</td>
<td>Columbaves</td>
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<td>Turnicola</td>
<td>Charadriiformes</td>
<td>Aequorlitornithes</td>
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<tr>
<td>Turturicola</td>
<td>Columbiformes</td>
<td>Columbaves</td>
</tr>
</tbody>
</table>

In addition to the genera listed, Eichler (1963) included Stresemanniella (now Falicocula), Abumarkub (junior synonym of Neophilocterus), Cereopsocus and Flamingobius (both now Anatoecus), and Parasoriccus and Soricella (both now Columbicola). Wilsonia Eichler, 1940, is preoccupied by Wilsonia Khalil, 1939, and is here replaced with Pesaeoecia Guimarães, 1940, following Nemésio (2006). Host systematics follows Clements et al. (2015). The placement of the Hoatzin (Opisthocomus hoazin) in Cuculiformes by Clements et al. (2015) does not correspond to its phylogenetic placement in Prum et al. (2015). The louse genus Craspedonirmus is known mainly from divers (Gaviiformes), but a single species is known from two species of auks (Nelson, 1972). The genera represented in our analyses are marked with an asterisk (*).

where Carduceps is placed in relation to the other genera included in this family by Eichler (1963).

Material and methods
To avoid confusion, the shorebird genus Calidris is here abbreviated to Cal., whereas the louse genus Carduceps is abbreviated Car. Host taxonomy follows Clements et al. (2015).

Sampling
Fresh material of Carduceps was collected from birds following three major flyways (Table 2; East Atlantic, East Asian/Australasian, Pacific Americas) in Sweden during 2007–2008, in Japan and Australia during 2008, and in Canada during 2009. Material from Cal. ferruginea, Cal. canutus and different subspecies of Cal. alpina was collected from two flyways (East Atlantic and East Asian/Australasian). The Cal. alpina and Cal. canutus samples were collected from host populations considered divergent enough to belong to different host subspecies (Message & Taylor, 2005; Clements et al., 2015; see Table 2). Details about collection of material are the same as in Gustafsson & Olsson (2012a).

All Carduceps species used in this study are listed in Table 2. In addition, representatives of several louse genera belonging to Eichler’s (1963) Esthiopteridae and Rallicolidae were included to test the monophyly of Esthiopteridae. Sequences for these lice were obtained from either GenBank or from our own collections (see Table 2). Carduceps lice were assigned to species initially based on the host they were collected from, but later compared with Timmermann (1954).

Extraction and sequencing
Prior to DNA extraction, the head and prothorax were cut off from the posterior part of the body, and extractions were performed on both parts using DNeasy Blood and Tissue Kit (Qiagen, Sollentuna, Sweden), following the manufacturer’s instructions, with the following exceptions: extraction was allowed to continue in a water bath for 36h, and only one elution (with 100 mL elution fluid) was carried out. The exoskeletons were mounted on slides in Canada balsam as vouchers after extraction. All vouchers were deposited at the Natural History Museum, Stockholm (NRM; Swedish material), the Price Institute for Parasitological Research (University of Utah, Salt Lake City, U.S.A.; Canadian and Australian material), or the Yamashina Institute for Ornithology (Chiba, Japan; Japanese Material).

Amplification and sequencing of cytochrome c oxidase subunit I (COI) used the primers L6625 and H7005 (Hafner et al., 1994), 12S was sequenced using the primers 12SAI and 12SBI (Simon et al., 1994), and 16S was sequenced using the primers 16SAR and 16SBR (Simon et al., 1994). Polymerase chain reactions (PCRs) were performed using GE Healthcare’s Ready-To-Go beads. PCR protocols followed Yoshizawa & Johnson (2003) for 12S and 16S, and Hafnet et al. (1994) for COI. A small sample from each PCR product was visualized on an ethidium bromide gel, and samples showing satisfactory bands were purified using the EZNA Cycle Pure Kit (Omega) or Exonuclease I (Fermentas Life Sciences, Helsingborg, Sweden) following the manufacturer’s instructions. Sequencing of purified DNA, using the same primers as during PCR, was performed in both the forward and reverse directions at Macrogen Inc., South Korea.

In addition to these mitochondrial markers, three nuclear and one mitochondrial primer sets were examined: elongation factor 1-α (EF-1-α; EF1-For3 and EF1-Cho10; Danforth & II, 1998), long-wavelength opsin (LWRHF and LWRHR; Mardulyn & Cameron, 1999), NADH dehydrogenase subunit 5 (F6999 or F7081, and R7495; Yoshizawa, 2004), and LepWG1 and LepWG2a (Brower & DeSalle, 1998). None of these primer sets produced any products visible on ethidium bromide gels. The PCRs using nuclear primer sets were performed in standard, touch-down (Don et al., 1991) and touch-up (Meusnier et al., 2008) mode for all primer sets, with no results. All further analyses were therefore limited to mitochondrial data.

Data treatment
DNA sequences were assembled in seqman II (DNASTar, Inc., Madison, WI, USA) individually for each locus. The 12S and
Table 2. Taxa used in this study.

<table>
<thead>
<tr>
<th>Louse species</th>
<th>Host species</th>
<th>Flyway (location)</th>
<th>Voucher no.</th>
<th>GenBank accession numbers</th>
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<td>860</td>
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<td><strong>Other Esthiopteridae sensu Eichler (1963)</strong></td>
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<td>--</td>
<td>--</td>
<td>AF380005 -- --</td>
</tr>
<tr>
<td><em>Ibidocerus bicornatus</em></td>
<td><em>Plagidias chibi</em></td>
<td>--</td>
<td>--</td>
<td>AY314817 -- --</td>
</tr>
<tr>
<td><strong>Outgroups</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Degeeriella fulva</em></td>
<td><em>Buteo lagopus</em></td>
<td>--</td>
<td>471</td>
<td>KX865181 KX865253 --</td>
</tr>
<tr>
<td><em>Degeeriella nisus</em></td>
<td><em>Accipiter nisus</em></td>
<td>--</td>
<td>350</td>
<td>KX865178 KX865249 --</td>
</tr>
</tbody>
</table>
16S sequences were aligned using CLUSTALW as implemented in GENEIOUS (Biomatters Ltd, Auckland, New Zealand), followed by manual adjustment to ensure that similar sequences in difficult sections were aligned with each other. The COI sequences were aligned in MEALIGN (DNA Star, Inc.) and manually inspected and adjusted in mEGA5 (http://tree.bio.ed.ac.uk/software/mega5/). As useful sequences were obtained for fewer specimens using the 12S and 16S primer sets, these datasets are smaller than the COI dataset. For the combined dataset, a single louse individual from each host species was selected and its individual sequences for the three loci were concatenated in DNA STAR, INC. and manually inspected and adjusted to ensure that similar sequences in difficult sections were aligned with each other. The COI sequences were aligned in MEALIGN (DNA Star, Inc.) and manually inspected and adjusted in mEGA5 (http://tree.bio.ed.ac.uk/software/mega5/).

Data were phylogenetically analysed using Bayesian inference (BI). The choice of model for the partitions in BI was determined based on the Akaike information criterion (Akaike, 1973) calculated in MRMODELTEST 2 (Nylander, 2004). In COI, first, second and third positions were modelled separately.

Gene trees were estimated by BI using MRBAYES 3.1.2 (Huelsenbeck & Ronquist, 2001, 2005) according to the following: (i) all loci were analysed separately (single-locus analyses, SLAs); (ii) sequences were concatenated all loci together (multilocus analysis). In the multilocus analysis, the data were partitioned by locus and by codon position, using rate multipliers to allow different rates for the different partitions and codon positions (Ronquist & Huelsenbeck, 2003; Nylander et al., 2004). Four Metropolis-coupled Markov chain Monte Carlo chains were run with incremental heating temperature 0.1 for 100 × 10⁶ generations and sampled every 10000 generations, except the 12S dataset, which was run for 50 × 10⁶ generations before convergence occurred. The first 10% of the generations were discarded as "burn-in", well after the chain likelihood values had become stationary, and the posterior probability (PP) was estimated for the remaining generations. The model fit between an analysis with monophly constrained to conform with the flyways within each species was compared with the unconstrained model by differences in log Bayes factors as implemented in TRACER v1.6 (http://tree.bio.ed.ac.uk/software/tracer/).

### Results

The alignment of the 12s and 16s sequences revealed some highly incompatible sections, which had to be readjusted manually. For all loci (COI, 12S, and 16S), PPs were calculated under the general time-reversible (GTR) model (Lanave et al., 1984; Tavaré, 1986; Rodríguez et al., 1990), assuming rate variation across sites according to an inverse gamma distribution with six rate categories for all models except COI third positions, in which a discrete gamma (G) distribution with six rate categories was assumed (Yang, 1994). Results of the BI analysis of the combined and COI datasets are shown in Figs 1 and 2, respectively. Results from the analyses of the smaller 12S and 16S datasets are shown in Figures S1 and S2, respectively. Uncorrected p-distances within the Esthiopteridae are shown in Table 3, and distances within Carduiceps are shown in Table 4. Uncorrected p-distances within each genus are similar to those reported from other groups (summarized in Gustafsson & Olsson, 2012a). Uncorrected p-distances within each Carduiceps species are between 0.0% and 1.2%, which is also similar to that observed in other louse genera (Gustafsson & Olsson, 2012a). The matrices used for this study can be found at http://purl.org/phylo/treebase/phylows/study/TB2:S20287.

### Influence of flyways

All four included species of Carduiceps are monophyletic in all analyses, typically with high support (Figs 1, 2; Figures S1, S2). Moreover, none of the Carduiceps species samples from more than one host flyway unambiguously separated into distinct clades comprising the material from each flyway. Comparisons between unconstrained trees and trees constrained to conform to the flyways resulted in much lower log Bayes
Fig. 1. Majority rule (50%) consensus tree of Esthiopteridae sensu Eichler (1963) based on the combined cytochrome c oxidase subunit I (COI), 12S and 16S dataset, inferred by Bayesian inference under the GTR + I + G model, except for third codon positions of COI, which used the GTR + G model. Posterior probabilities (≥50%) are indicated at the nodes. The specific identity of the host is given directly after the name of each individual louse sample. Numbers before names are sample identifiers (see Table 2). Abbreviations after taxon names correspond to flyway affiliation (PAm, Pacific Americas Flyway; EAtl, East Atlantic Flyway; EAs, East Asian/Australasian Flyway), as outlined in the inset, where arrows denote approximate collection localities for migrating birds, and ‘W’ approximate collection localities for wintering birds. [Colour figure can be viewed at wileyonlinelibrary.com].
Fig. 2. Majority rule (50%) consensus tree of Esthiopteridae sensu Eichler (1963) based on mitochondrial cytochrome c oxidase subunit I (COI) sequences, inferred by Bayesian inference under the GTR+G+I model, except for third codon positions of COI, which used the GTR+G model. Posterior probabilities (≥50%) are indicated at the nodes. Numbers before names are sample identifiers (see Table 2). Flyway abbreviations at the end of terminals are: PAm, Pacific Americas; EAtl, East Atlantic; EAs, East Asian/Australasian. [Colour figure can be viewed at wileyonlinelibrary.com].

Table 3. Uncorrected p-distances for cytochrome c oxidase subunit I (COI) within Esthiopteridae.

<table>
<thead>
<tr>
<th></th>
<th>Ac</th>
<th>Ae</th>
<th>Aq</th>
<th>Ar</th>
<th>Ca</th>
<th>Co</th>
<th>Cr</th>
<th>Fu</th>
<th>Ib</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ac</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Ae</td>
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<td>18.5</td>
<td>18.5</td>
<td>18.5</td>
<td>18.5</td>
<td>18.5</td>
<td>18.5</td>
<td>18.5</td>
</tr>
<tr>
<td>Aq</td>
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<td>23.4</td>
<td>14.4</td>
<td></td>
<td></td>
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<tr>
<td>Ar</td>
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<td>24.0</td>
<td>24.0</td>
<td>24.0</td>
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<td>24.0</td>
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<tr>
<td>Ca</td>
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<td>27.0</td>
<td>28.6</td>
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<td>Co</td>
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<td>29.1</td>
<td>26.9</td>
<td>28.4</td>
<td>19.5</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Cr</td>
<td>27.5</td>
<td>28.6</td>
<td>29.4</td>
<td>30.3</td>
<td>29.9</td>
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<td>29.9</td>
<td>29.9</td>
<td>29.9</td>
</tr>
<tr>
<td>Fu</td>
<td>23.7</td>
<td>22.4</td>
<td>25.6</td>
<td>22.9</td>
<td>25.6</td>
<td>29.0</td>
<td>25.8</td>
<td>17.8</td>
<td></td>
</tr>
<tr>
<td>Ib</td>
<td>25.2</td>
<td>22.2</td>
<td>26.1</td>
<td>23.9</td>
<td>26.5</td>
<td>28.9</td>
<td>27.8</td>
<td>23.9</td>
<td>23.9</td>
</tr>
</tbody>
</table>

Ac, Anaticola; Ae, Anatococcus; Aq, Aquanirmus; Ar, Ardeicola; Ca, Carduiceps; Co, Columbicola; Cr, Craspedonirmus; Fu, Fulicoffula; Ib, Ibidoecus. All numbers are expressed as percentages. Highest and lowest between-genus distances have been bolded.

Table 4. Uncorrected p-distances for the COI dataset within Carduiceps.

<table>
<thead>
<tr>
<th></th>
<th>C. fulvofasciatus</th>
<th>C. meinertzjægeni</th>
<th>C. scalaris</th>
<th>C. zonarius</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ac</td>
<td>0.5</td>
<td>21.8</td>
<td>24.2</td>
<td>24.3</td>
</tr>
<tr>
<td>Ae</td>
<td></td>
<td>0.0</td>
<td>18.3</td>
<td>16.0</td>
</tr>
<tr>
<td>Aq</td>
<td></td>
<td></td>
<td>4.0</td>
<td>18.0</td>
</tr>
<tr>
<td>Ar</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Ca</td>
<td></td>
<td></td>
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<tr>
<td>Co</td>
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<td>Cr</td>
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</tr>
<tr>
<td>Fu</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ib</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All numbers expressed as percentages.

Discussion

Taxonomic and systematic issues within Carduiceps

The phylogeny reconstructed for Carduiceps based on three mitochondrial genes corresponds perfectly with the current taxonomy of the genus (Timmermann, 1954; Table 5), and no changes in the taxonomy of Carduiceps are implied by this study. As several of the Carduiceps species treated by Timmermann (1954) were not included in these analyses, its division of the genus into three species groups cannot be tested presently. The four included species of Carduiceps are all reciprocally monophyletic (Figs 1, 2; Figures S1, S2), but apart from the placement of Carduiceps as sister to the three other species, there is no supported structure among Carduiceps in the combined dataset (Fig. 1). Many of the samples included here were only successfully sequenced for one or two of the three genes. This has probably affected the resolution of the trees, especially the 12S and 16S datasets, which contain the least amount of specimens.

Influence of flyways on host distribution of Carduiceps

We recovered no support for either flyway homogenization or flyway differentiation in Carduiceps. Carduiceps scalaris and Carduiceps fulvofasciatus are both restricted to a single host species, and do not occur on other hosts species samples in the same localities at the same time (data not shown). Xenus cinereus, the host of Carduiceps, also occur along the West Palearctic flyway, but no samples were obtained from this host population, and its potential division into populations following different flyways could therefore not be tested.

Carduiceps meinertzjægeni was sampled from three morphologically distinct host subspecies that migrate along two different flyways (Wenink et al., 1996; Message & Taylor, 2005; but see Marthinsen et al., 2007). Despite this broad sampling range,
Table 5. Taxonomy and host relationships of Carduiceps.

<table>
<thead>
<tr>
<th>Louse name</th>
<th>Host name</th>
<th>Common name</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carduiceps cingulatus</td>
<td>Limnodromus griseus</td>
<td>Short-billed Dowitcher</td>
</tr>
<tr>
<td>(Denny, 1842)</td>
<td>Limnodromus scolopaceus</td>
<td>Long-billed Dowitcher</td>
</tr>
<tr>
<td></td>
<td>Limosa limosa</td>
<td>Black-tailed Godwit</td>
</tr>
<tr>
<td>Carduiceps clavata</td>
<td>Lymnocryptes minimus</td>
<td>Jack Snipe</td>
</tr>
<tr>
<td>Timmermann, 1954</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carduiceps fulvofasciatus</td>
<td>Xenus cinerarius</td>
<td>Terek Sandpiper</td>
</tr>
<tr>
<td>(Grube, 1851)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carduiceps lapponicus</td>
<td>Limosa lapponica</td>
<td>Bar-tailed Godwit</td>
</tr>
<tr>
<td>Emerson, 1953</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carduiceps meinertzhageni</td>
<td>Calidris alpina alpina</td>
<td>Dunlin</td>
</tr>
<tr>
<td>Timmermann, 1954</td>
<td>Calidris alpina sakkalina</td>
<td>Dunlin</td>
</tr>
<tr>
<td></td>
<td>Calidris alpina schinzii</td>
<td>Purple Sandpiper</td>
</tr>
<tr>
<td></td>
<td>Calidris maritima</td>
<td>Rock Sandpiper</td>
</tr>
<tr>
<td></td>
<td>Calidris pilocnemis</td>
<td></td>
</tr>
<tr>
<td>Carduiceps scalaris</td>
<td>Calidris mugri</td>
<td></td>
</tr>
<tr>
<td>(Piaget, 1880)</td>
<td>Calidris himantopas</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calidris minutata</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Calidris melanotos</td>
<td></td>
</tr>
<tr>
<td>Carduiceps subscalaris</td>
<td>Calidris acuminata</td>
<td>Sharp-tailed Sandpiper</td>
</tr>
<tr>
<td>(Piaget, 1880)</td>
<td>Calidris alba</td>
<td>Sanderling</td>
</tr>
<tr>
<td>Carduiceps zonarius</td>
<td>Calidris albus</td>
<td></td>
</tr>
<tr>
<td>(Nitzsch [in Giebel], 1866)</td>
<td>Calidris bairdi</td>
<td>Baird's Sandpiper</td>
</tr>
<tr>
<td></td>
<td>Calidris canus canusus</td>
<td>Red Knot</td>
</tr>
<tr>
<td></td>
<td>Calidris canus rogersi</td>
<td>Red Knot</td>
</tr>
<tr>
<td></td>
<td>Calidris ferruginea</td>
<td>Curlie Sandpiper</td>
</tr>
<tr>
<td></td>
<td>Calidris fascicollis</td>
<td>White-rumped Sandpiper</td>
</tr>
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<td>Calidris muri</td>
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<td></td>
<td>Calidris himantopas</td>
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<td></td>
<td>Calidris melanotos</td>
<td>Pectoral Sandpiper</td>
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<tr>
<td></td>
<td>Calidris minutula</td>
<td>Little Stint</td>
</tr>
<tr>
<td></td>
<td>Calidris pusilla</td>
<td>Least Sandpiper</td>
</tr>
<tr>
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<td>Calidris pycnoeus</td>
<td>Semipalmated Sandpiper</td>
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<tr>
<td></td>
<td>Calidris rubricollis</td>
<td>Spoon-billed Sandpiper</td>
</tr>
<tr>
<td></td>
<td>Calidris subminuta</td>
<td>Long-toed Stint</td>
</tr>
<tr>
<td></td>
<td>Calidris subfuscicolis</td>
<td>Buff-breasted Sandpiper</td>
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<td>Calidris temmincki</td>
<td>Temminck's Sandpiper</td>
</tr>
<tr>
<td></td>
<td>Lymnocryptes minimus</td>
<td></td>
</tr>
</tbody>
</table>

Taxa marked with an asterisk (*) are new host records in this paper. All other host relationships follow Price et al. (2003).

the sequences from these lice are genetically identical (Table 4), and there is no division between louse populations sampled from the different flyways. Moreover, we have found no specimens of Car. meinertzhageni on other host species sampled at the same localities at the same time (data not shown).

The homogeneity of the Car. meinertzhageni material across host subspecies may be an effect of recent divergence in these host subspecies (Wenink et al., 1996), with differentiation in Carduiceps being slower than in their hosts. This is surprising, as base substitution rates are generally much faster in lice than in their host animals (Johnson et al., 2003a). Alternatively, as different host subspecies may be found in the same flocks during migration and wintering (e.g. Wenink & Baker, 1996), the occurrence of the same Carduiceps haplotype on birds sampled from different subspecies may indicate that the lice are capable of dispersal to other subspecies of Cal. alpina, but not to other Calidris species. No American populations of Cal. alpina were sampled, so it is impossible to tell whether there is a split between Old and New World populations of Car. meinertzhageni. In addition, two recorded hosts of Car. meinertzhageni (Price et al., 2003) with more limited distributions (Message & Taylor, 2005), Cal. maritima and Cal. pilocnemis, were not sampled. Johnson et al. (2003b) suggested that a very small amount of gene flow, even through an intermediary host, may be enough for speciation to fail even in allopatric species. Small numbers of sandpipers from one flyway regularly visit other flyways, which could potentially be sufficient to stifle speciation. In either case, among the host species studied, dispersing individuals of Car. meinertzhageni only seem to have become successfully established on Cal. alpina.

In the Car. zonarius material there seems to be a slight difference between haplotypes collected from the Pacific Americas flyway and those collected from the two Palearctic flyways. However, there is no support for a geographic divergence in the phylogenetic analyses, and the genetic distances within this species are comparable to those of the other three Carduiceps species, and similar to those reported for other chewing lice (Gustafsson & Olsson 2012a). In two cases, Carduiceps zonarius was sampled from the same host species along different flyways (Cal. canusus and Cal. ferruginea). These samples show no evidence of flyway differentiation between the different flyways. In Car. zonarius, the capacity for establishment on different host species seems to be higher than in Car. meinertzhageni, but intense sampling efforts have not recovered Car. zonarius on
any of the hosts of Car. fulvofasciatus, Car. meinertzhageni or Car. scalaris (data not shown).

Both Car. meinertzhageni and Car. zonarius thus exhibit host distribution patterns that are structured more by host species than by host biogeography. Palaeoflyways (Kraaijeveld & Nieboer, 2000; Buehler et al., 2006) could perhaps explain some of the patterns, as the present distribution of Carduiceps on the calidrines may have been established before or during the last ice age when the hosts may have followed different flyways than they presently do.

Possible limitations for host range in Carduiceps

As the hosts of all four species of Carduiceps often occur in mixed flocks in wintering sites, it is difficult to explain why each host species is only parasitized by a single species of Cardui-

ceps, and why Car. zonarius has not been found on the hosts of the other species of Carduiceps. The known hosts of Car. mein-

ertzhageni form a monophyletic clade within the hippoboscid swimmers, but the hosts of Car. zonarius do not (Gibson & Baker, 2012). There is some evidence that wing lice generally cannot successfully colonize new hosts that are much larger or smaller (Tompkins et al., 1999; Johnson et al., 2005; Bush & Clayton, 2006). Whether this is generally true for generalist lice, such as Carduiceps, is unknown. The size range of the hosts of Carduiceps is large, but Car. zonarius occurs on both the smallest sampled hosts (Cal. ruflcollis and Cal. minaitilla) and the largest sampled hosts (Cal. canatus). Calidris alpina, the host of Car. meinertzhageni, falls in between these extremes and is similar in size to several of the hosts of Car. zonarius (Message & Taylor, 2005). Host size alone may therefore not be a factor in the host distribution of Carduiceps lice.

An alternative explanation may be host pigmentation differ-

ences (Bush et al., 2010). All the hosts of Car. meinertzhageni (including unsampled hosts; Price et al., 2003) are either black-bellied or have mainly dark-grey feathers in at least one plumage (Message & Taylor, 2005), whereas the hosts of Car. zonarius are generally white-bellied in all plumages. Lice of the genus Machaerillaenus have been found to prefer white parts of feathers over black parts (Kose & Møller, 1999; Kose et al., 1999), suggesting that melanin in bird feathers may deter lice. If Car. meinertzhageni has a greater ability to digest melanin, this could give it an advantage over host-switching Car. zonarius, and could explain why Car. meinertzhageni occurs only on black-bellied or dark-grey hosts. However, Cal. tennirostris is densely black-spotted, but is nevertheless parasitized by Car. zonarius. Moreover, all three hosts of Car. meinertzhageni also have areas of white body feathers. Bush et al. (2006) found no correlation between the amount of melanin in feathers and the abundance of pigeon lice (Columbicola and Campanulotes), suggesting that the distribution of Car. meinertzhageni on black-bellied or dark-grey hosts may be unrelated to host pigmentation patterns.

The most curious aspect of Carduiceps distribution lies in comparison with the Lunaceps wing lice of the same hosts. In pigeons and doves, wing lice are less species-specific and less geographically structured than body lice (Johnson et al., 2002a; Clayton & Johnson, 2003), which could be related to the greater ability of wing lice to disperse by phoresy on hippoboscid flies (Keirans, 1975; Harbison et al., 2008, 2009; Bartlow et al., 2016). Similar patterns were found in seabird lice (Page et al., 2004). Even in the absence of a host biogeographic structuring according to host flyways, Carduiceps would therefore still be expected to be more species-specific than Lunaceps. No cases of phoresy involving shorebird lice are known (Keirans, 1975; Bartlow et al., 2016), but Lunaceps wing lice would be better placed on its host, topologically, to take advantage of opportunities for spread to new hosts than Carduiceps body lice, even in the absence of phoresy. Despite this, Carduiceps is both much less geographically structured and less host-specific than Lunaceps (Gustafsson & Olsson, 2012a). This implies that some other set of dispersal mechanisms may be available to shorebird lice than to pigeon lice. Continued studies on shorebird louse genera such as Saemundssonia and Quadraceps may be most instructive in this regard.

Esthiopteridae sensu Eichler, 1963

While neither Cruickshank et al. (2001) nor Johnson et al. (2006) recovered monophyly of the Esthiopteridae, it is sug-

gested to be monophyletic in all of our datasets (PP = 1.00; Figs 1, 2; Figures S1, S2). However, relationships within Esthiopteridae remain obscure, and relationships above the genus level generally have no support in either of our analyses. As only a few species each of the proposed esthiopterids genera were included, few conclusions can be drawn. Aquanirmus has been grouped quite consistently with the duck lice Anaticola and Anatoecus in previous molecular studies (Cruickshank et al., 2001; Johnson et al., 2006), but with Ibi-

doeus in morphological studies (Smith, 2001). In this study, Aquanirmus groups with the duck lice Anaticola and Anatoecus in all datasets where all three genera are included.

Ardeicola has a chequered history of having been grouped with the duck lice (Johnson et al., 2003a), the Philoceus complex (Smith, 2001), Multicola (placed in Rallilidae by Eichler, 1963; Cruickshank et al., 2001) or even the Amblycera (Cruick-

shank et al., 2001). In the most inclusive dataset (morphol-
ogy + genetic data) of Smith et al. (2004), Ardeicola appears to have no close relatives, but when molecular data are considered alone, they either group with Falcolipeurus (parsimony), with the mammal lice (likelihood), or are placed as sister to most of the other genera (Bayesian). This study does not resolve the relationships of Ardeicola, except that all datasets where this genus is represented place it inside Esthiopteridae. This placement may be supported by morphology, as aspects of the preantennal area and the male genitalia are similar to those seen in other genera Eichler (1963) placed in Esthiopteridae, but this family has never been satisfactorily circumscribed morphologically.

Columbicola has been separated from other esthiopterids in many previous studies (Cruickshank et al., 2001; Smith, 2001; Johnson et al., 2003a; Smith et al., 2004), but has been placed as sister to Craspedonirmus (Smith et al., 2004, 2006).
fig. 6a, b) and close to Fulicoffula (Johnson & Whiting, 2002) or Anatoecus + Neophilopterus + Fulicoffula + Cirrophthirius [the latter placed in Rallicolidae by Eichler (1963)] (Barker et al., 2003). None of these studies have included any Carduiceps, and the sister-group relationship between Columbicola and Craspedonirmus suggested in the combined dataset, and the close relationship between these two and Carduiceps suggested by the 12S dataset are novel. Columbicola is restricted to pigeons and doves, Craspedonirmus to loons and auks, and Carduiceps to sandpipers and allies. These host groups do not form a monophyletic group together (e.g. Hackett et al., 2008), suggesting that these relationships are either spurious or not explainable through a simple application of Fahrenholz’s rule (i.e. that louse relationships should mirror host relationships; Klassen, 1982).

Carduiceps, Craspedonirmus and Columbicola are not very similar in gross morphology. However, all three genera share at least two morphological characters: the presence of an arched, transversally continuous preantennal carina arising at the pre-antennal nodi and the presence of a transversally continuous dorsal postantennal suture immediately posterior to this carina. In all three genera, the suture is extended posteriorly across at least part of each temple, and the post-nodal seta (sensu Clay, 1951) and sensilla 2–3 (sensu Valim & Silveira, 2014) are generally associated with this suture. This head structure is, to our knowledge, not known from any other genus of ischnoceran lice. However, a similar, medially interrupted carina is found in some members of the Quadraceps complex (e.g. Quadraceps semi-fissus; see Timmermann, 1953).

Leaving aside Columbicola and Craspedonirmus, Carduiceps appears to have no close relatives and is not related to any other louse genus on the shorebirds (D. Gustafsson, unpublished data), but seems to represent a separate, very localized, colonization of the Scolopacidae. However, the louse genera included in this study were selected based on their placement in Esthiopteridae by Eichler (1963), and close relatives of Carduiceps outside this group may well have been overlooked in the process of outgroup selection.

In short, the relationships within Eichler’s (1963) Esthiopteridae are in need of further clarification, requiring greater sampling of genera other than Columbicola and Carduiceps, and the use of additional unlinked molecular markers, particularly nuclear markers, as well as a morphological revision. In addition, several of the genera included here were represented only in the COI analysis, as data were not available for the other two markers used. Suitable sister groups should also be identified and sampled, to test the phylogenetic position and possible sister-group relationship of Carduiceps and Columbicola + Craspedonirmus. If this sister-group relationship is found to be an artifact of sampling or analysis, on present knowledge this leaves Carduiceps with no known close relatives.

Summary

There is no evidence of either flyway homogenization or flyway differentiation in Carduiceps. Two host species were sampled from more than one flyway, and in both cases there were no significant differences between louse material from different flyways. The large host range of Car. zonarius may be the result of flyway homogenization in the past, but if so, this homogenization is incomplete, as the hosts of the other three Carduiceps species sampled migrate along the same flyways and winter in the same areas. Possibly, other features of the hosts’ ecology, such as plumage patterns, may explain the structuring of Carduiceps.

Eichler’s (1963) proposed Esthiopteridae may be monophyletic, as indicated by high Bayesian support across all datasets. However, resolution within this group is poor. One reason may be that appropriate outgroups or sister groups may be lacking, as the phylogeny of lice is incompletely known. Another reason may be that the absence of nuclear markers in this analysis, as well as the few available sequences for most of the genera in this group limit our present understanding of the evolution of this group. In the analysis of the 12S dataset, the dove louse genus Columbicola and the loon and auk louse genus Craspedonirmus are suggested as the closest relatives to Carduiceps. This relationship does not receive any support in the combined analysis, and may be spurious. Nevertheless, the genus Columbicola is a widely used model group for many aspects of louse and parasite evolution, and this novel relationship with Craspedonirmus and Carduiceps requires further study.

Supporting Information

Additional Supporting Information may be found in the online version of this article under the DOI reference: 10.1111/syen.12227

Figure S1. Majority rule (50%) consensus tree of Esthiopteridae sensu Eichler (1963) based on mitochondrial 12S sequences, inferred by Bayesian inference under the GTR+G+I model. Posterior probabilities are indicated at the nodes. Numbers before names are sample identifiers (see Table 2).

Figure S2. Majority rule (50%) consensus tree of Esthiopteridae sensu Eichler (1963) based on mitochondrial 16S sequences, inferred by Bayesian inference under the GTR + G + I model. Posterior probabilities are indicated at the nodes. Numbers before names are sample identifiers (see Table 2).

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