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Lack of host-dependent genetic structure in ectoparasites of *Calonectris* shearwaters

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Abstract

We compared patterns of mitochondrial DNA (mtDNA) differentiation in three host-specific lice (*Halipeurus abnormis*, *Austromenopon echinatum* and *Saemundssonia peusi*) and one generalist flea (*Xenopsylla gratiosa*), parasitizing 22 colonies of Cory's and Cape Verde shearwater (*Calonectris*). The shearwater hosts show distinct phylogeographic structure corresponding to the three taxa *Calonectris d. diomedea*, *C. d. borealis*, and *C. edwardsii*. The host-specific lice appeared undifferentiated among the three *Calonectris* taxa, whereas the more generalist flea displayed significant levels of population differentiation. Neither genetic distances among host populations, nor their spatial distribution explained the patterns of genetic variability observed in the ectoparasites. The lack of differentiation among lice is unexpected, given that previous work has found evidence of cospeciation between procellariiform seabirds and their lice, and lice typically have an elevated rate of mtDNA evolution with respect to their hosts. Our results suggest that either rates of evolution in seabird lice are not always as high as previously thought, or that the magnitude of movement of lice between seabird hosts has been substantially underestimated.

Keywords: co-evolution, congruence, fleas, host specificity, lice, population differentiation

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Introduction

Host–parasite cospeciation has mainly been investigated at the interspecific level (e.g. Paterson *et al*. 1993; Hafner *et al*. 1994; Page 1994; Johnson *et al*. 2003a), whereas factors and processes acting at microevolutionary scale have received little attention. At the population level, the dynamics and co-evolution of host–parasite interactions would ultimately depend on the genetic variation and its structure in both interacting species (Thompson *et al*. 1994). Therefore, factors acting at this level can play an important role as the causal factors driving co-evolution (Clayton & Johnson 2003; Clayton *et al*. 2004). The expectation for cospeciating hosts is that any factor promoting the isolation of hosts is likely to result in the concomitant isolation of the parasite, resulting in congruent patterns of genetic structuring for both host and parasite.

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Relative rates of host and parasite dispersal, host geographic distribution, and parasite host specificity can all influence congruence between host and parasite population genetic structures (Blouin 1995; Dybdahl & Lively 1996; Johnson *et al*. 2002; McCoy *et al*. 2003; Weckstein 2005). First, only when host and parasite dispersal are linked is genetic structure expected to be correlated (McCoy *et al*. 2003). The degree of congruence will also depend to some extent on host specificity which in turn is influenced by the ecology and the dispersal ability of the parasite (Clayton *et al*. 1992; Tompkins & Clayton 1999; Hahn *et al*. 2000; Whiteman *et al*. 2004). Finally, ecological factors that affect the distribution and abundance of host and parasites can also affect congruence (Rannala & Michalakis 2003; Clayton *et al*. 2004).

Lice (Insecta: Phthiraptera) have long been seen as useful model organisms for understanding co-evolutionary relationships between hosts and parasites because of their high host specificity (Hafner & Nadler 1988; Paterson *et al*. 1993, 1995, 2000; Hafner *et al*. 1994; Page 1994, 1996). Louse species are often restricted to a single species or genus of host,

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and spend all their entire life cycle on that host (Marshall 1981). Thus opportunities for vertical transmission down through a host lineage are expected to be much more likely than horizontal transmission between different host lineages (Page 1996). Despite of being highly specific parasites, the degree of host specificity can vary among lice genera on the same hosts. For example, pigeon body lice are more host-specific than wing lice (Johnson *et al*. 2002). Conversely, fleas tend to be less host-specific than lice. Fleas are obligatory blood feeders that spend only one stage of their life on the host during the host breeding period. At this time, fleas are closely associated with the host's nest. Whereas similarity in the degree of genetic structure is expected among highly specific species, it may decrease the more generalist the parasite (Clayton *et al*. 2004). Although several studies have investigated congruence among evolutionary trees for lice and their vertebrate hosts, few studies have investigated the degree of congruence among different species of lice on the same host species (Johnson *et al*. 2002), and no study to date explores those patterns including other ectoparasite taxa simultaneously. In this context, fleas offer a great opportunity to investigate the influence of host specificity in the degree of similarity between host and ectoparasite population genetic relationships.

Seabirds, particularly petrels and shearwaters (Procellariiformes), provide an interesting model to investigate the ecological effects of host isolation and geographic distance on the genetic structure of parasite communities. Since shearwaters breed on oceanic islands and spend most of their life in the open ocean, dispersal of parasites is presumably spatially and temporally limited. Furthermore most procellariform species show strong philopatry to natal and breeding sites which would limit dispersal promoting genetic isolation and differentiation among populations (Brooke 2004). Nevertheless, the extent of population genetic and phylogeographic structure can vary extensively among species (Friesen *et al*. 2007), and some seabirds can travel enormous distances (Croxall *et al*. 2005).

Here we chose Cory's shearwater (*Calonectris diomedea*), which includes the Mediterranean (*C. d. diomedea*) and the Atlantic (*C. d. borealis*) Cory's shearwater subspecies, and the Cape Verde (*C. edwardsii*) shearwater, three closely related seabird taxa that host three lice (*Halipeurus abnormis*, *Austromenopon echinatum* and *Saemundssonia peusi*) and one flea species (*Xenopsylla gratiosa*), and assess the influence of the host genetic structure and host specificity on the genetic structure of these ectoparasites. Previous work suggests the existence of phylogeographic and population genetic structure in *Calonectris* shearwaters (Rabouam *et al*. 2000; Gómez-Díaz *et al*. 2006). Hence, we would expect to find congruent genetic structure between the ectoparasite species and their host at two levels; either among host taxa or among populations of each taxon. However, differences in life-history traits (i.e. host specificity and dispersal modes) of the ectoparasite species considered here could lead to differences in the degree of congruence observed (Johnson *et al*. 2002; Clayton *et al*. 2004). Since highly specific ectoparasites such as lice spend their whole life cycle on the host and show vertical transmission (i.e. the three louse species examined), we would expect to find similar patterns of host–ectoparasite genetic structure. Conversely, we would expect the more generalist flea species, with greater ability of dispersal and horizontal transmission, to be less structured.

In the present study, we aim (i) to compare patterns of genetic structure and levels of genetic differentiation among ectoparasites (the three lice and the single flea species) and their host taxa (the Mediterranean and the Atlantic Cory's and the Cape Verde shearwaters), and (ii) to examine whether host specificity is reflected in differences in the degree of similarity between host and ectoparasite population genetic relationships.

Material and methods

Study species and sampling

Cory's shearwater is a colonial and monogamous seabird breeding on islands distributed across the Mediterranean Sea and the northeastern Atlantic archipelagos, and is differentiated into two subspecies: the Mediterranean, breeding mainly from the Iberian coast to the Adriatic and Aegean, and the Atlantic Cory's shearwater breeding mainly in the northeast Atlantic from Canary to Azores archipelagos (Thibault *et al*. 1997; Gómez-Díaz *et al*. 2006). The Cape Verde shearwater, from Cape Verde islands, was previously considered a subspecies of the Cory's shearwater, but it is now regarded as a full species (see Gómez-Díaz *et al*. 2006).

The Atlantic Cory's, the Mediterranean Cory's and the Cape Verde shearwaters share three lice known only from these taxa (Price *et al*. 2003): *Halipeurus abnormis* (Piaget, 1885) (Ischnocera: Philopteridae), *Saemundssonia peusi* (Eichler, 1949b) (Ischnocera: Philopteridae), and *Austromenopon echinatum* (Edwards, 1960) (Amblycera: Menoponidae). Amblyceran and ischnoceran lice are usually referred as body and wing lice, respectively, and typically differ in several life–history traits such as vagility and host specificity (Marshall 1981; Price *et al*. 2003). Cory's and Cape Verde shearwaters also share the flea *Xenopsylla gratiosa* (Jordan & Rothschild, 1923) (Siphonaptera: Pulicidae), which is recorded from the seabird genera: *Calonectris*, *Puffinus*, and *Hydrobates*. These seabird genera, comprised of eight species, are mainly distributed over the Atlantic and the Mediterranean regions (Beaucournu *et al*. 2005).

From 2001 to 2005, we collected blood samples and ectoparasites from adult birds on 22 breeding colonies of Cory's shearwater across the Mediterranean and Atlantic

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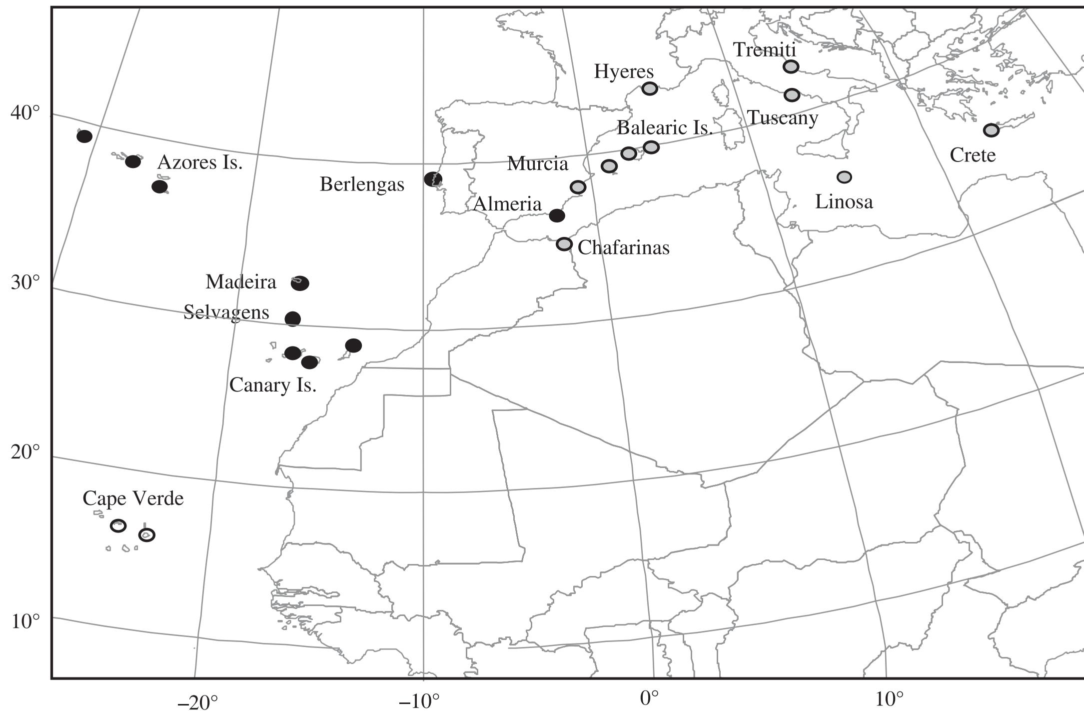

Fig. 1 Breeding colonies of Atlantic (●) and Mediterranean (◍) Cory's shearwaters and Cape Verde shearwaters (○) sampled across their geographic distribution.

regions, and two breeding colonies of the Cape Verde shearwater at Cape Verde islands (Fig. 1). We collected ectoparasites from their hosts by visual examination or by using the dust-ruffling method described by Clayton & Walther (1997). Ectoparasites from individual hosts were kept separated and care was taken to clean all working surfaces between host fumigation. Ectoparasites were stored in absolute ethanol at −20 °C for subsequent genetic analyses. We sequenced one or two individuals of each ectoparasite species per host population. The total number of specimens of each species analysed per host taxa is shown in Table 1. Louse and flea specimens from each locality were taxonomically identified by Ricardo Palma based on morphological characters (representative lice specimens from each *Calonectris* taxa were deposited at the Museum of New Zealand *Te Papa Tongarewa*).

Amplification and sequencing

Host DNA was isolated from ethanol-preserved whole blood using the salting-out extraction protocol from Bruford *et al*. (1998). We amplified the mitochondrial cytochrome *b* gene in two fragments of approximate lengths of 420 base pairs (bp) and 680 bp using the two primer pairs L14987/H15685 and L15562/H16025. We amplified a 293-bp fragment of Domain I of the mitochondrial control region of all three *Calonectris* species using three specific primers previously designed for the species: CAL2H, CAL4H and CAL1L. Reaction conditions and automated sequencing for both genes, mitochondrial control region and cytochrome *b* gene, were described by Gómez-Díaz *et al*. (2006).

For fleas and lice, we extracted DNA from individual specimens using a salting-out protocol for insects (Sunnucks & Hales 1996) and DNeasy Tissue Kit (QIAGEN). To assure that our species identifications were correct, from individual lice we extracted DNA by removing the head from the body of the louse and placing both in the digestion buffer. After extraction, the head and body of each louse were stored for further examination.

For all three louse species, we amplified 360 bp of the cytochrome oxidase I gene (COI) using the primers L6625 and H7005 as described by Hafner *et al*. (1994). We amplified 665 bp of the mitochondrial cytochrome *b* gene of *A. echinatum* using previously published primers for *Dennyus* sp. L11120 and H11823 (Page *et al*. 1998). In both *S. peusi* and *H. abnormis*, we amplified 563 bp and 600 bp, respectively, of the cytochrome *b* using two specific primer pairs that we designed based on a few published sequences of various louse species; (CYB-146-L (5′-CGAGAATC-TTTCTTTCCTTCCATTA-3′) and CYB-825-H (5′-AAAGT-ATCATTCTGGTTGAATGTG-3′) for *S. peusi*, and P3-HaL (5′-TGGGTCTTTGCTGGGAGTAT-3′) and P3-HaH (5′-ATCAGGGTCCATGACCACAT-3′) for *H. abnormis*. For fleas, we amplified COII gene by using the primers AtLeu and BtLys (Maekawa *et al*. 1999) following Dittmar & Whiting (2003) and 359 bp of the cytochrome *b* gene

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Table 1 Genetic statistics for each ectoparasite species and host taxa. Number of sequence, percentage of variable sites, number of haplotypes and both haplotypic and nucleotide diversity are shown

Species	*n* sequences	Variable sites		*n* haplotypes		Haplotypic diversity		Nucleotide diversity	
Lice		COI	CYB	COI	CYB	COI	CYB	COI	CYB
H. abnormis	20	2/359	5/600	3	5	0.195 ± 0.115	0.368 ± 0.135	0.0056 ± 0.0034	0.0083 ± 0.0024
A. echinatum	18	1/360	3/665	2	3	0.471 ± 0.082	0.307 ± 0.132	0.0013 ± 0.0002	0.0013 ± 0.0003
S. peusi	16	3/332	8/563	4	7	0.442 ± 0.145	0.817 ± 0.033	0.0014 ± 0.0005	0.0028 ± 0.0006
Flea		COII	CYB	COII	CYB	COII	CYB	COII	CYB
X. gratiosa	15	108/702	75/359	14	13	0.971 ± 0.032	0.963 ± 0.033	0.055 ± 0.003	0.076 ± 0.005
Host		CR	CYB	CR	CYB	CR	CYB	CR	CYB
C. d. diomedea	24	29/293	14/957	20	9	0.975 ± 0.024	0.812 ± 0.058	0.024 ± 0.002	0.0037 ± 0.006
C. d. borealis	21	30/293	13/957	19	9	0.986 ± 0.022	0.824 ± 0.060	0.024 ± 0.002	0.0023 ± 0.004
C. edwardsii	11	16/293	8/957	7	5	0.909 ± 0.066	0.836 ± 0.080	0.016 ± 0.003	0.0030 ± 0.001

using primers A5 and B 1.1 (Dittmar de la Cruz & Whiting 2003). Polymerase chain reactions (PCRs) were carried out in a total volume of 25 μL containing 40 mM Tris (pH 8.0), 200 mM KCl, 8 mM $MgCl_2$, 0.01% gelatin, 0.4 mM of each primer, 0.15 mM of each dNTP, 0.5 U Bio*Taq* DNA polymerase (Bio-Rad Laboratories) and 10–20 ng of DNA template. For both COI and COII, amplification procedures followed those outlined in Hafner *et al*. (1994) and Dittmar de la Cruz & Whiting (2003), respectively. Amplification conditions for the cytochrome *b* gene were adjusted in each louse and flea species separately. Each reaction started with 4 min at 94 °C, then the amplification was carried out for 40 cycles of denaturation at 94 °C for 45 s, annealing at 52 °C (for L11120/H11823, *A. echinatum*; and CYBL/CYBH, *S. peusi*), 56 °C (for A5/B1.1, *X. gratiosa*) or 58 °C (for P3-HaL/P3-HaH, *H. abnormis*) for 45 s, and extension at 72 °C for 1 min 30 s. A final extension step at 72 °C for 5 min was performed.

Amplification products were separated by electrophoresis in 6% polyacrylamide stained using ethidium bromide and visualized under UV light. PCR products were purified using the JETquick PCR Product Purification Spin Kit (Genomed). PCR products were sequenced with the same amplification primers on an automated ABI-301 DNA Sequencer (Applied Biosystems) using the BigDye Terminator Cycle Sequencing Kit version 3.1 (Applied Biosystems). We used BIOEDIT version 5.0.1 (Hall 1999) to assemble, edit and align sequences and all variable sites were confirmed by visual inspections of the chromatograms. To assess the reliability of the data, we compared COI and CYB sequences with previously published data on various seabird louse species. Sequences reported in this study have been placed in GenBank and Treebase under Accession nos (DQ372022–DQ372047, DQ372047, DQ371968–DQ372018 and EU088135–EU088185).

Genetic analyses

Genetic analyses of the *Calonectris* species (Fig. 3A) were based on Domain I of the mitochondrial control region and the cytochrome *b* gene as described by Gómez-Díaz *et al*. (2006). Analyses of louse and fleas (Fig. 3) were based on the mitochondrial COI (louse) or COII (fleas) genes, and the cytochrome *b* gene (*Calonectris* and ectoparasite individuals analysed and geographic locations of populations sampled are listed in Table 1).

We used the partition homogeneity test (Farris *et al*. 1994; Swofford 2003) to examine whether there was evidence for different phylogenetic signals between cytochrome *b* and the cytochrome oxidase I and II genes. No significant differences were found between mitochondrial markers for all species except for *X. gratiosa* ($P = 0.001$). Thus, for the lice, we combined the sequences and analysed them together, whereas for the flea, we performed both a combined analysis of the two markers, and separate analyses of each gene. We tested for neutrality for each ectoparasite species using the Tajima's *D* test included in the DNASP package (Rozas & Rozas 1999). We calculated genetic statistics at the intraspecific level as the gene diversity index, the number of haplotypes and the number of polymorphic sites using DNASP. Genetic distances at the intraspecific level were calculated using MEGA version 3.0 (Kumar *et al*. 2005). To visualize genealogical relationships of host and ectoparasite species, we constructed a haplotype tree using the split decomposition algorithm implemented in SPLITSTREE version 4.6 (Huson & Bryant 2006).

Both host genetic structure and geographic distribution of populations can be responsible for the observed patterns of genetic differentiation among ectoparasite species populations. We first tested isolation by distance by measuring correlation between ectoparasite genetic distances, measured

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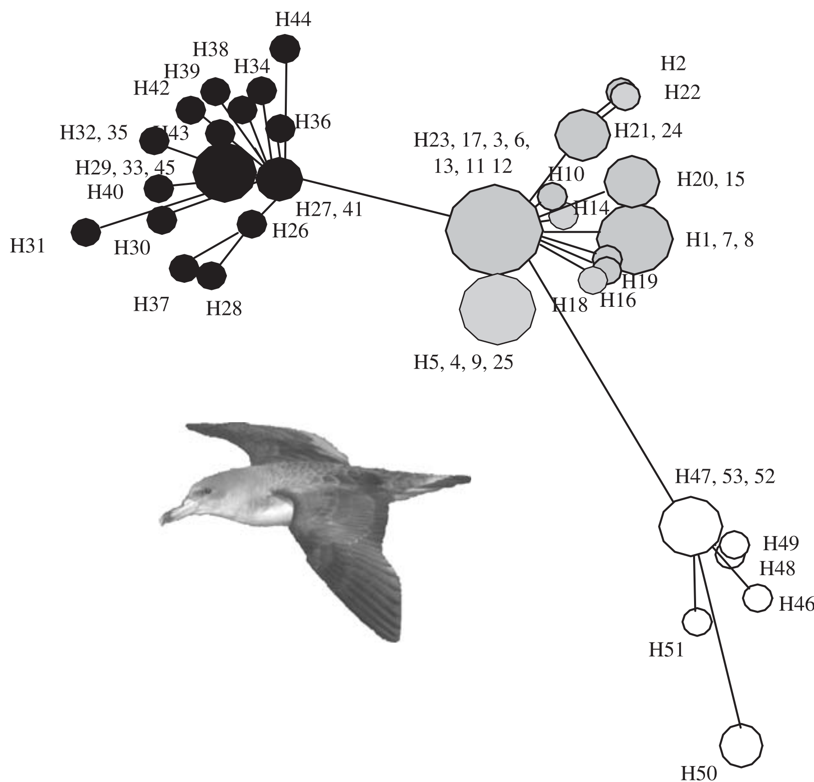

Fig. 2 Haplotype network for *Calonectris*. Haplotypes corresponding to the Atlantic and the Mediterranean Cory's and Cape Verde shearwaters are indicated in black, grey and white circles, respectively. The size of the circles is proportional to the number of birds sharing that haplotype.

as $\Phi_{ST}/(1 - \Phi_{ST})$, and geographic distances, calculated as ln (by-sea geographic distance) of colony pairs (Rousset 1997). Then, we tested correlation between ectoparasite and host genetic distances, both measured as $\Phi_{ST}/(1 - \Phi_{ST})$. In both cases, we applied Mantel test analysis using ZT (Bonnet & Van de Peer 2002). The significance test of the *r* statistic was determined by employing a randomization procedure in which the original value of the statistic is compared with the distribution found by randomly reallocating the order of the elements in one of the matrices. To control for the effect of a third matrix *C*-testing on the correlation between matrices A and B, we applied a partial Mantel test (Smouse *et al*. 1986). First, we performed the partial Mantel test between ectoparasites genetic and by-sea geographic distances while controlling host genetic distances. Then, we tested correlation between ectoparasite and host genetic distances while controlling by-sea geographic distances among colonies. In this case, the permutation approach applied was that developed by Anderson & Legendre (1999).

Results

For *Calonectris* sequences, the results of the Tajima's *D* test were not significant considering both cytochrome *b* and the control region (Tajima's $D = -1.02$; $P > 0.10$ and $D = -0.89$; $P > 0.10$, respectively). The neutrality test scores were not significant for any of the ectoparasites three genes analysed (COI, COII and CYB) (all $P > 0.10$), except for cytochrome *b* data of *Halipeurus abnormis* which deviates from neutrality expectations (Tajima's $D = -1.97$; $P < 0.05$). Genetic statistics (polymorphic sites, number of haplotypes, haplotype diversity and nucleotide diversity indices) of *Calonectris* and the four ectoparasites species are shown in Table 1.

Average pairwise sequence divergence (uncorrected pairwise distance) in the control region among *Calonectris* populations was 4.4% (SE 0.007; bootstrap 1000 replicates). The sequence divergence estimated between the Atlantic and Mediterranean shearwaters was 2.7% (SE 0.8%), similar to the divergence between the Mediterranean subspecies and the Cape Verde shearwater (3.3%; SE 1.0%; Appendix 1). The divergence between the Atlantic subspecies and the Cape Verde shearwater was slightly greater (4.3%; SE 0.9%). In the cytochrome *b* gene (CYB), uncorrected per cent sequence divergence among all *Calonectris* populations was 0.8% (SE 0.002; bootstrap 10 000 replicates). For the CYB, sequence divergence estimated between the Atlantic and Mediterranean shearwaters was 0.8% (SE 0.3%) similar to the divergence from the two Cory's shearwater subspecies to the Cape Verde shearwater (0.8%; SE 0.3%; Appendix 1).

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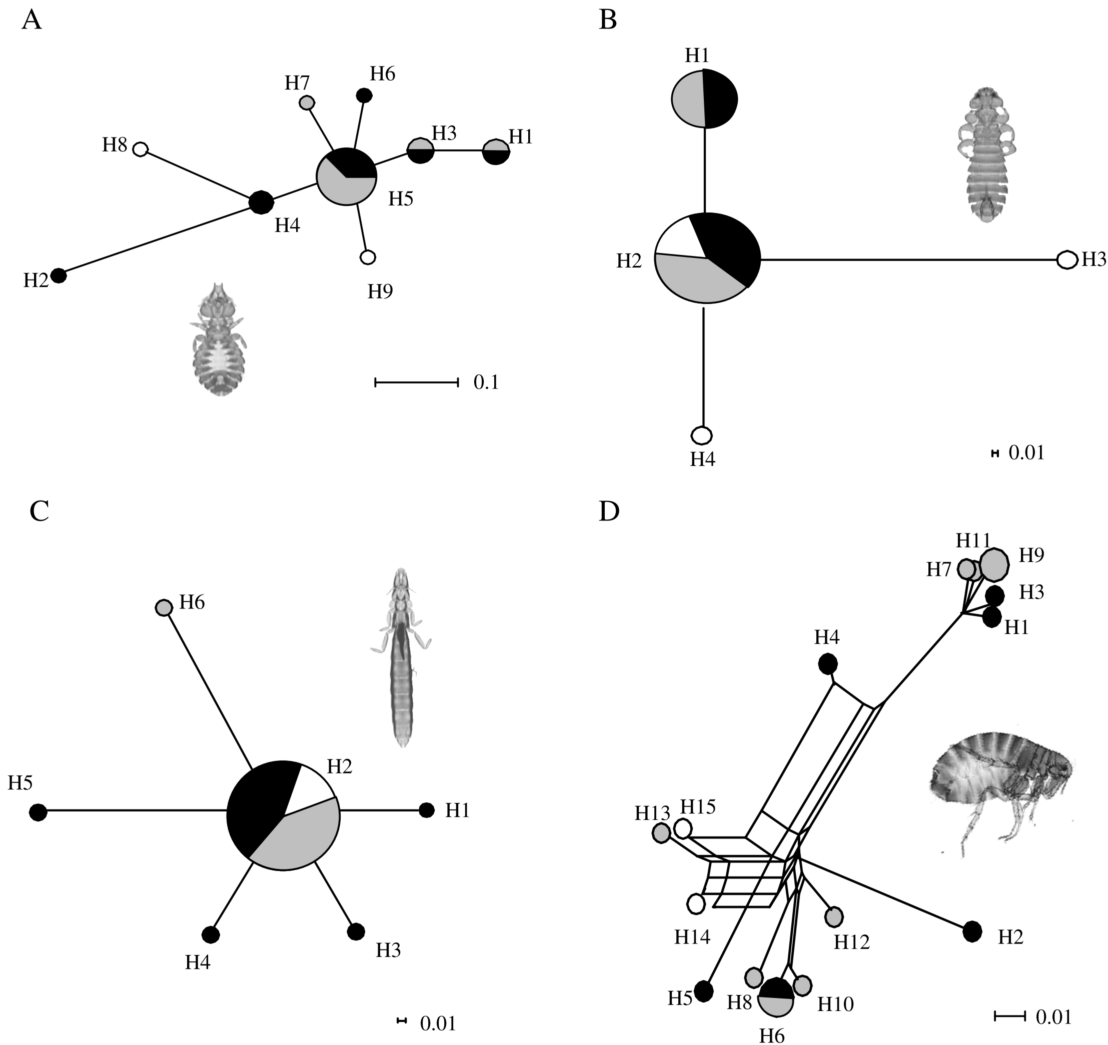

Fig. 3 Haplotype networks for *Calonectris* ectoparasite *Saemundssonia peusi* (A), *Austromenopon echinatum* (B), *Halipeurus abnormis* (C) and *Xenopsylla gratiosa* (D). Haplotypes corresponding to the Atlantic and the Mediterranean Cory's and Cape Verde shearwaters are indicated in black, grey and white circles, respectively. The size of the circles is proportional to the numbers of individuals sharing that haplotype.

Intraspecific diversity (levels of polymorphism, haplotype and nucleotide diversity) was smaller in lice than for *Calonectris* shearwaters and most louse populations shared identical haplotypes (Appendix 2A–C). Genetic divergence among populations in all three lice species for both gene partitions analysed, cytochrome oxidase I (COI) and cytochrome *b* (CYB) genes, was low (Table 1). In the COI gene, uncorrected per cent sequence divergence for all species was almost 0% (0.1%, SE 0.001; bootstrap 10 000 replicates). In the CYB gene, average pairwise sequence divergences of the body lice *Saemundssonia peusi* was slightly greater (0.3%, SE 0.001; bootstrap 10 000 replicates), whereas values for either *Austromenopon echinatum* or *H. abnormis* did not significantly differ from 0% (0.1%, SE 0.001; bootstrap 10 000 replicates). In contrast, the flea's genetic structure appears more diverse and complex and displayed higher levels of genetic variability in both gene partitions (Appendix 2D). In the CYB gene, the average pairwise sequence divergence was 7.6% (SE 0.008; bootstrap 10 000 replicates), whereas it was 5.5% in the COII (cytochrome oxidase II) gene (SE 0.006; bootstrap 10 000 replicates).

Network analysis on *Calonectris* mitochondrial DNA (mtDNA) indicated a clear geographic pattern with three groups of haplotypes corresponding to each of the two geographically isolated Cory's shearwater subspecies and the Cape Verde species (Fig. 2). In contrast, phylogenetic networks of lice (*A. echinatum*, *S. peusi* and *H. abnormis*) suggested negligible genetic structure within each louse species and confirmed the low levels of intraspecific genetic variability observed for lice. Although differentiation appeared slightly greater in *S. peusi* (Fig. 3A), in any case no obvious spatial pattern was apparent (Fig. 3A–C).

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In contrast, the network for the flea *X. gratiosa* obtained from the two gene (COII and CYB) partitions separately, or the combined data, displayed intraspecific genetic structure, but genealogical relationships among haplotypes did not correspond to their geographic distributions. Furthermore, there was no evidence of differentiation on different host taxa (Fig. 3D). Split decomposition analyses suggest conflicting phylogenetic relationships among some haplotypes (H4 and H13–15, Fig. 3D). Partition homogeneity test (see methods) indicated different and incompatible phylogenetic signals between the two gene partitions (COII and CYB), explaining the unresolved network for fleas.

We applied simple and partial Mantel test analyses to examine correlation among genetic distances of host and ectoparasite species as well as to test the existence of spatial patterns of variation in the genetic structure of populations. For all four ectoparasite species analysed, simple Mantel test analyses revealed no significant correlation between genetic and geographic distances among populations except for the louse species *S. peusi* (*X. g*: $r = 0.117$, $P = 0.105$; *A. e*: $r = –0.146$, $P = 0.132$; *H. a*: $r = –0.133$, $P = 0.177$; *S. p*: $r = 0.302$, $P = 0.045$). We did not find any correlation in genetic distances between *Calonectris* and its ectoparasites (*X. g*: $r = 0.007$, $P = 0.197$; *H. a*: $r = –0.105$, $P = 0.202$; *S. p*: $r = 0.109$, $P = 0.205$), except for the louse species *A. echinatum*, which showed a significant negative correlation ($r = –0.189$, $P < 0.05$). In contrast, a spatial pattern of variation in genetic structure was apparent for *Calonectris* as genetic and geographic distances were significantly correlated ($r = 0.607$, $P < 0.001$). Partial Mantel test analyses showed similar results, although in all four ectoparasite species, genetic and geographic distances among populations were not correlated (*X. g*: $r = 0.097$, $P = 0.141$; *A. e*: $r = 0.062$, $P = 0.367$; *H. a*: $r = –0.091$, $P = 0.290$; *S. p*: $r = 0.286$, $P = 0.069$) neither were ectoparasite and host genetic distances (*X. g*: $r = 0.0065$, $P = 0.4060$; *A. e*: $r = –0.136$, $P = 0.138$; *H. a*: $r = –0.041$, $P = 0.394$; *S. p*: $r = –0.043$, $P = 0.384$).

Discussion

Lice have long been seen as useful models in co-evolutionary studies (Paterson *et al*. 1993, 2000; Page 1994; Paterson & Gray 1997; Page *et al*. 2004; Banks *et al*. 2006) and some ecological characteristics of seabirds such as a strong philopatry and nesting fidelity may be expected to promote cospeciation between seabirds and their lice. Since the three *Calonectris* taxa (the Atlantic and the Mediterranean Cory's and the Cape Verde shearwaters) show distinct phylogeographic structure (Gómez-Díaz *et al*. 2006), we could expect congruent patterns of genetic structuring among host and parasites. However, all three lice species analysed here exhibited no significant population genetic structure and appeared genetically undifferentiated compared to their seabird host taxa.

Under a cospeciation scenario, approximately synchronous divergence between host and parasites is expected (Page 1996), although lice may demonstrate '*failure to speciate*' or '*cophylogenetic inertia*' (Paterson & Banks 2001; Johnson *et al*. 2003a). If lice evolve at lower rates than the host, insufficient time of isolation for lice could explain the low levels of genetic divergence observed (Rannala & Michalakis 2003). The negligible sequence divergence we found in *Calonectris* lice would imply that the rate of louse mtDNA evolution lags behind that of their avian hosts, in marked contrast with previous work reporting a substantially higher rate of evolution in louse mtDNA, both with respect to other insects (Johnson *et al*. 2003b), and with respect to vertebrate hosts (Hafner *et al*. 1994; Page *et al*. 1998), including in seabirds (Paterson *et al*. 2000; Page *et al*. 2004). Our results also contrast with previous studies that suggested a close association between genetic differentiation in pocket gophers and their lice (Nadler *et al*. 1990). There are, however, several examples of louse species showing very little differentiation compared to their seabird hosts, i.e. *Paraclisis hyalina* on albatrosses or *Austromenopon waterstoni* on little penguins (Page *et al*. 2004; Banks *et al*. 2006). In all those cases, parasite species appeared to be genetically undifferentiated, despite their occurring on genetically divergent hosts. Thus, either our previous knowledge about host specificity and dispersal ability of seabird lice is erroneous, or that other factors are over-riding these characteristics.

Failure to speciate is likely to occur when gene flow among parasite populations is much higher than that of their hosts (Johnson *et al*. 2003a). Since *Calonectris* speciation is allopatric (Gómez-Díaz *et al*. 2006) and lice are relatively immobile, ongoing gene flow among lice from all three *Calonectris* taxa seems implausible. Furthermore, for a seabird species living on islands and spending most of its life in the open ocean, louse dispersal is both spatially and temporally limited. Nonetheless, despite the strong breeding-site fidelity behaviour of *Calonectris*, recent work indicates a substantial degree of mixing among breeding populations in the wintering areas (González-Solís *et al*. 2007). Indeed, the wintering areas of all three *Calonectris* taxa do not appear to be geographically exclusive (Lima *et al*. 2002; González-Solís *et al*. 2007), suggesting a potential for ectoparasite dispersal among host populations, and ultimately among host taxa. However, horizontal transmission of lice requires direct physical contact among hosts. Shearwaters are strictly pelagic during winter, but at sea feedings, flocks may provide opportunities for lice to switch among individual hosts. Alternatively, in some locations up to four species of seabirds breed sympatrically with *Calonectris*, which may provide opportunities for parasite dispersal among different localities by switching through different host taxa. However, no records of straggling

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Calonectris lice parasitizing other seabird species have been previously documented (see Price *et al*. 2003). In cases where parasite gene flow is independent of host dispersal, geographic distances among localities could better explain the spatial genetic structure of parasite populations (Blouin 1995; McCoy *et al*. 2005; Weckstein 2005). But in the present study, genetic variation in lice was not correlated with either host or geographic distances among localities. Apart from louse gene flow, alternative hypotheses, such as frequent metapopulation extinction and recolonization events, combined with low effective population sizes, could also act to reduce parasite genetic structure (Nadler 1995; Criscione & Blouin 2005). Indeed, ecological data of the ectoparasite community of *Calonectris* shearwaters indicates a strong temporal variation in the patterns of abundance of lice, with frequent extinction and recolonization events synchronized with the breeding cycle of their host (Gómez-Díaz, Navarro, González-Solís, unpublished).

In contrast to our expectation, the generalist flea shows considerably more intraspecific genetic variation than the host-specific lice. The genetic variation of the flea populations may result from local adaptation to different host species. For a generalist parasite, greater levels of genetic variability can provide evolutionary potential for local host race formation. Previous examples have been reported for ticks and lice parasitizing sympatric hosts (McCoy *et al*. 2001, 2002, 2003; Johnson *et al*. 2002). *Xenopsylla gratiosa* parasitizes seabird species that share habitat and even nest sites with *Calonectris* shearwaters (Beaucournu *et al*. 2005; personal observation). Parasitizing multiple host species may reflect an ecological specialization of fleas on tracking a specific resource which is shared by many host species using the same habitat (Brooks *et al*. 2006). According to this hypothesis, we would expect a greater genetic similarity between fleas parasitizing different host species in the same breeding grounds (habitat-specific), than between fleas parasitizing different host populations of the same or phylogenetically close host species (host-specific). But whether fleas can be considered as habitat-specific rather than host-specific parasites remains poorly understood. Further genetic studies in fleas parasitizing different sympatric seabird hosts across the distribution range of *Calonectris* shearwaters (i.e. *Puffinus*, *Hydrobates*, *Oceanodroma* and *Bulweria* sp.) are needed.

In conclusion, neither genetic distances among host populations, nor their spatial distribution explained the patterns of genetic variability observed in the ectoparasites. The flea species displayed high levels of intraspecific variability. In contrast, mitochondrial genetic differentiation among lice populations was less than in their hosts. The three louse species show almost no variation despite being distributed over three taxa of *Calonectris* shearwaters and thousands of kilometres of ocean. This is not what would be expected for highly specific parasites with a history of codivergence. This result suggests that either rates of evolution in seabird lice are not always as high as previously thought, or that the magnitude of dispersal of lice between seabird hosts has been substantially underestimated. Many seabird species are long-distance migrants and show substantial population mixing in wintering areas, which may provide opportunities for parasite exchange among different breeding populations.

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Appendix I The site matrix shows variable positions on a composite sequence of 1250 bp of the cytochrome *b* gene (1–956 bp) and the control region (957–1250 bp) in the 53 haplotypes found in all three *Calonectris* taxa. Dots indicate identity with the most common genotypes

	Nucleotide positions									
					1111111111	1111111111	1111111111	1111111111	1111111111	11
	1112	333334444	5555567778	8888889999	0000000000	0000000011	1111111111	1111111112	2222222222	22
	13468994588	568992367	3568981671	2225993455	1223455666	8888999900	0022222233	3347778990	1111222344	45
Haplotype	27078177039	892574032	8980125981	0695287325	9391767345	3456012712	8912467801	6721474480	3456489101	60
Hap_1	TGAGATACAG	GTGGTAAAGA	ATTATTGGAT	ATAGATAAAT	GAGAAGGGGA	AGGAGGAGAG	GTGGTCGAGG	TGCTTTAGAA	GAGAAGGTGA	AG
Hap_2	.......T.A	..A.G..GT.	G.......G.	.....C...C	..AG......	..A....A..	..A.......	....C..A..	A.....A...	G.
Hap_3	.........A	..A.....T.	G.......G.	.....C...C	...G.A....	...G...A..	..A.......	......GA..	......A...	..
Hap_4	...A.....A	..A.....T.	G.......G.	.....C...C	...G......	.......A..	..A.C.....	.......A..	......A...	..
Hap_5	...A.....A	..A.....T.	G.......G.	.....C...C	...G......	.......A..	..A.C.....	.......A..	......A...	G.
Hap_6	.........A	..A.....T.	G.......G.	.....C...C	...G.A....	...G...A..	..A.......	......GA..	......A...	G.
Hap_7	....	....	....	....	....	....	....	....	....	G.
Hap_8	....	....	....	....	....	...G......	....	....	....	..
Hap_9	...A.....A	..A.....T.	G.......G.	.....C...C	...G......	.......A..	..A.C....A	.......A..	....	..
Hap_10	.........A	..A..G..T.	G.......G.	.....C...C	....	...G.A....	..A.......	......GA..	......AC..	G.
Hap_11	.........A	..A.....T.	G.......G.	.....C...C	..AG......	...G...A..	..A.......	.A....GA..	......A...	G.
Hap_12	...A.....A	..A.....T.	G.......G.	.....C...C	...G......	.......A..	..A.......	....	......A...	G.
Hap_13	.........A	..A.....T.	G.......G.	.....C...C	...G....A.	.A...AGA..	..A.......	.......A..	......AC..	G.
Hap_14	C........A	..A.....T.	G.......G.	.....C...C	...G....A.	G..G.A.A..	..A.....A.	....C.GA..	....	G.
Hap_15	.........A	..A.....T.	G.......G.	.....C...C	A..G......	...G...A..	..A.......	......GA..	.G....A...	G.
Hap_16	......G..A	..A.......	....	....	...G....A.	.A...A.A..	..A.......	.....C.A..	......A...	G.
Hap_17	.........A	..A.....T.	G.......G.	.....C...C	...G.A..A.	.....A.A..	..A.......	.......A..	......A...	G.
Hap_18	....	....	....	....	...G...A..	GA........	..A.......	......G...	....	G.
Hap_19	.........A	..A.....T.	G.......G.	.....C...C	.....A..AG	...G...AG.	..A.......	......GA..	......A...	G.
Hap_20	....	..A.....T.	G.......G.	.....C...C	A..G......	...G...A..	..A.......	......GA..	.G....A...	G.
Hap_21	.........A	..A....GT.	G.......G.	.....C...C	..AG......	.......A..	..A.......	....C..A..	A.....A...	G.
Hap_22	.........A	..A....GT.	G.......G.	.C...C...C	...G....A.	.......A..	..A.......	....C..A..	A.....A...	G.
Hap_23	.........A	..A.....T.	G.......G.	.....C...C	...G......	...G...A..	..A.......	......GA..	......A...	G.
Hap_24	.........A	..A....GT.	G.......G.	.....C...C	..AG......	..A....A..	..A.......	....C..A..	A.....A...	G.
Hap_25	...A.....A	..A.....T.	G.......G.	.....C...C	...G......	.......A..	..A.C.....	....	......A...	G.
Hap_26	........G.	A.A......G	G.CG..A.G.	.....C..GC	...G...T..	.....AG...	..CA.....A	.A....G...	.....T.C..	GA
Hap_27	........G.	A.A......G	G.CG....G.	.....C..GC	...G...T..	.A.G......	..CA......	......G...	.....T.C..	G.
Hap_28	........G.	A.A......G	G.CG....G.	.....C..GC	...G...T..	.....AG...	..CA.....A	.A.C..G...	..A.GT.C..	GA
Hap_29	.A......G.	A.A......G	G.CG....G.	..G..C..GC	..AG...T..	.....AG...	..CA.....A	......GA.G	.....T.CA.	G.
Hap_30	........G.	A.A.G....G	G.CG..A.G.	.....C..GC	.......T.G	.....AG...	..CA.....A	.A..C.G...	.....T.C..	G.
Hap_31	........G.	A.A......G	G.CG....G.	.....C..GC	A.AG...T..	.A.G...AG.	..CA.....A	.AT...GA..	.....C.C.G	G.
Hap_32	.A......G.	A.A......G	G.CG....G.	..G..C..GC	.G.G...T..	.....AG...	..CA.....A	......GA.G	.....T.CA.	G.
Hap_33	.A......G.	A.A......G	G.CG....G.	..G..C..GC	.......T..	.....AG...	..CA.....A	......GA.G	.....T.CA.	G.
Hap_34	........G.	A.A......G	G.CG..A.G.	.....C..GC	..AG...T..	.....AG...	..CA.....A	.A....G.G.	.....T.C..	G.
Hap_35	.A......G.	A.A......G	G.CG....G.	..G..C..GC	.G.G...T..	.....AG...	..CA.....A	......GA.G	.....T.C..	G.
Hap_36	........G.	A.A......G	G.CG....G.	..C..C..GC	.......T..	.A.GA.GA..	..CA.....A	.A....G...	.....T.C..	G.
Hap_37	........G.	A.A......G	G.CG....G.	.....C..GC	...G...T..	.....AG...	..CA.....A	.A.C..G...	...G.T.C..	GA
Hap_38	........G.	A.A......G	G.CG.C....	.....C..G.	.......T..	.A.GA.G...	..CA.....A	.A....G...	.....T.C..	G.
Hap_39	....	A.A......G	G.CGC..TG.	.....C..GC	...G...T..	.A.GA.GA..	..CA.....A	.A....G...	.....T.C..	G.
Hap_40	.A......G.	A.A......G	GCCG....G.	..G..C..GC	...G...T..	.....AG...	..CA.....A	......GA.G	.....T.CA.	G.
Hap_41	........G.	A.A......G	G.CG....G.	.....C..GC	...G...T..	.A.G......	..CA.....A	......GA.G	.....T.CA.	G.
Hap_42	........G.	A.A......G	G.CG....G.	...A.C.GGC	.......T..	.....AG...	..CA.....A	......G...	.....T.C..	G.
Hap_43	........G.	A.A......G	G.CG....G.	.....C..GC	.....A.T..	.A.G..GA..	..CA.....A	.A....G...	....GT.C..	G.
Hap_44	........G.	A.A......G	G.CG....G.	.....C..GC	..AG...T..	.....AG..A	..CA.....A	CA....G...	.....T.C..	G.
Hap_45	.A......G.	A.A......G	G.CG....G.	..G..C..GC	...G...T..	.....AG...	..CA.....A	......GA.G	.....T.CA.	G.
Hap_46	..G.....G.	ACAA..G...	G.......G.	....GC...C	...GG..T..	...G.A....	AC....AGA.	......GA..	.....A....	G.
Hap_47	..G.....G.	A.A...G...	G.......GG	....GC...C	...GG..T..	...G.A....	AC...TAGA.	......GA..	.....A....	G.
Hap_48	..G.....G.	A.A...G...	G.......GG	....GCG..C	...GG..T..	...G...A..	AC...TAGA.	......GA..	.....A....	G.
Hap_49	..G.....G.	A.A...G...	G.......GG	....GC...C	...GG.AT..	.....A....	AC....AGA.	......GA..	.....A....	G.
Hap_50	..G.GC..G.	A.A...G...	G.......G.	G...G....C	..AG...T.G	..AG.A....	AC.....GA.	..TC..GA..	.....A....	G.
Hap_51	..G.GC..G.	A.A...G...	G.......G.	....GC...C	...GG..TAG	.A.G......	AC....AGA.	....C.GA..	.....A....	G.
Hap_52	..G.GC..G.	A.A...G...	G.......G.	G...G....C	...GG..T..	...G...A..	AC...TAGA.	......GA..	.....A....	G.
Hap_53	..G.....G.	A.A...G...	G.......GG	....GC...C	...GG..T..	...G......	AC....AGA.	.A....GA..	.....A....	G.

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Appendix IIA The site matrix shows variable positions on a composite sequence of 1025 bp of the cytochrome *b* (1–665 bp) and the cytochrome oxidase I gene (666–1024 bp) in the four haplotypes found in *A. echinatum*. Dots indicate identity with the most common genotypes

Haplotype	Nucleotide positions 2269 3645 9973
Hap_1	TACA
Hap_2	...G
Hap_3	C.TG
Hap_4	.G.G

Appendix IIB The site matrix shows variable positions on a composite sequence of 959 bp of the cytochrome *b* gene (1–600 bp) and the cytochrome oxidase I gene (601–959 bp) in the six haplotypes found in *H. abnormis*. Dots indicate identity with the most common genotypes

Haplotype	Nucleotide positions 1145578 7863787 0385090
Hap_1	CAGTATT
Hap_2	...G...
Hap_3	T..G...
Hap_4	...G..C
Hap_5	.G.GG..
Hap_6	..AG.C.

Appendix IIC The site matrix shows variable positions on a composite sequence of 898 bp of the cytochrome *b* gene (1–563 bp) and the cytochrome oxidase I gene (564–898 bp) in the nine haplotypes found in *S. peusi*. Dots indicate identity with the most common genotypes

Haplotype	Nucleotide positions 111223456 8 1246783276 7 0320798452 2
Hap_1	TTGGGTGAGA A
Hap_2	G.A...AGA. G
Hap_3	........A. .
Hap_4	G.A.....A. .
Hap_5	G.......A. .
Hap_6	GC......A. .
Hap_7	G..A....A. .
Hap_8	G.A.AC..A. .
Hap_9	G.......AG .

Appendix IID The site matrix shows variable positions on a composite sequence of 1061 bp of the cytochrome *b* gene (1–359 bp) and the cytochrome oxidase II gene (360–1061 bp) in the 15 haplotypes found in *X. gratiosa*. Dots indicate identity with the most common genotypes

	Nucleotide positions																		
																		111111111	111
		111111	1111111111	1122222222	2222222222	3333333333	3444444444	4444444555	5555555555	5555556666	6666666677	7777777777	7777777888	8888888888	8888888999	9999999999	9999999999	9000000000	000
	1122233456	6779112233	5555677788	9911222334	4447777899	2446667899	9012344455	6677999001	1123556677	7788990122	3445667701	2223444556	6788899000	0223344445	556799901	1225555566	6666778889	9001122333	344
Haplotype	4878936843	9293283628	0369501409	2306235781	3670349214	4184698203	4247215739	0247259570	3657581403	6928149229	4588015995	4576058172	6214706025	8492912570	8981235406	9270236802	3467014581	4692814067	928
Hap_1	AAAAAAAAAT	CTATTAGGTA	TTAATACTTA	TAATTCTATT	ATTAAATCTA	ACTATCCATT	TTATATGTTA	GATTCCAATT	TCTTAATACG	ATACCACAAG	TCCAACTTTT	TGTACAAATA	CTTTTATCTA	TTTATATTTT	AACTCAATTA	TCCATTATTA	TCTTATTTTC	CCATTCACAG	CAT
Hap_2	G.TGG.T...	T..C..TA..	AGG...T.CT	C..CCTA..C	GC.T..CT..	T..T.TT.CA	C..AG.ACCC	T....T...C	ATGCG.G.TA	...T..TG.C	..T.C.AGCC	....T.....	T.AC...TA.	......C.CC	C...T.T...	CTTT..T.C.	.AC.TA..CA	.TCC.T.T.A	TCC
Hap_3	....	....	....	....	....	....	....	....	....G.....	....	...G......	....	....	....	....	....	....	......TT..	...
Hap_4	.....G....	....	....	....	....	....	C.........	....TTG...	......C...	..C.......	...G......	CA..TG..A.	TAA...CTA.	CC........	C.T.T.T...	CTTT..T..T	.A..TA.CC.	TT..C..TGA	TT.
Hap_5	..T...T...	TC...GAA..	AG...GT.C.	CC..CTG...	...T.GC...	T..TCTT.CA	C..A..A.C.	T...TT..A.	AT...GA.T.	G...TGT..C	A.T.TTAA..	CA..T.G.AG	.AA...CTAG	.CCG.....C	CTT.T.T...	CTTTAAT...	.AC.TA..CT	T...CT.T.A	TTC
Hap_6	.GT..GT..C	T.G.C.AAC.	A.G.C.TC..	..GC.TG.C.	..CT..C...	.T.T..T..A	C.GA..A...	T..CTT.G..	AT...GGGTA	.C....T.G.	..T.T.AG..	..C.T..GA.	..G....TA.	A......C.C	C.TCT.TC.T	CTTT.ATA..	.T.ATA...T	TTT.CT.T.A	TT.
Hap_7	....	....	....	....	....	....	C....C....	....	....	..C.......	...G......	....T.....	....CG....	....	.....G..CT	....	.........T	....	T..
Hap_8	.GT..GT..C	T.G.C.AA..	G.G.C.T...	..GC.TG.C.	..CT..C...	.TCT..T..A	C.GA..A...	T..CTT.G..	AT...GGGTC	.C....T.G.	..T.T.AA..	..C.T..GA.	..G....TA.	A......C.C	C.TCT.TC.T	CTTT.ATA..	.T.ATA...T	TTT.CT.T.A	TT.
Hap_9	....	....	....	....	....	....	C....C....	....	....	..C.......	...G......	....T.....	.....G....	....	.....G..CT	....	.........T	....	T..
Hap_10	.GT..GT..C	T.G...AAC.	G.G.C.T...	..GC.TG.C.	..CTG.....	.TCT..T..A	C.GA..A...	TGC.TT.G..	AT...GGGTA	.C....T.G.	..T.T.AGC.	.ACGTG..A.	..A....TA.	A........C	C.TCT.TC.T	CTTT.ATA..	.T.ATAC...	TTT.CT.T.A	TT.
Hap_11	.T........	....	...G......	....	....	....	C....C....	....	....	..C.......	...G......	....T.....	.....G....	....	.....G..CT	....	.........T	.....A....	T..
Hap_12	..T...T...	T.....AA..	G.....T...	C..C..A.C.	..C...C..G	.T.T..TG.A	C..A..A...	T.C.TT.G..	AT...GA.TA	.C....T...	..T.T.AAC.	.A..TG..A.	..A....TAG	A...CG...C	C.T.T.TC.T	CTTT.CTA..	CT.ATA....	TTT....T.A	TT.
Hap_13	..T..GTGG.	.A..C.AA.G	AA..C.T.C.	CC...TA...	...T..C...	T..T.TT.CA	C..A..AC..	T...TT....	AT.C.GG.TA	...TT.TG.C	.TT.T.AA..	CA..TG..A.	TAA...CTA.	CC........	C.T.T.T...	CTTT..T..T	.A..TA.CC.	TT..C..T.A	TT.
Hap_14	..T..GTGG.	.A..C.AA.G	AA..C.T.C.	CC...TA.C.	...T..C.C.	T..T.TT.CA	CC.A..AC..	T...TT....	AT.C.GA.TA	...TT.TG.C	.TT.T.AG..	....T..GA.	..G....TA.	A........C	C.TCT.TC.T	CTTT.ATA..	.T.ATA...T	TTT.CT.T.A	TT.
Hap_15	..T..GTGG.	.A..C.AA.G	AA..C.T.C.	CC...TAGC.	...T..C.C.	T..T.TT.CA	CC.A..AC..	T...TT....	AT.C.GA.TA	...TT.TG.C	.TT.T.AG..	CA..TG..A.	TAA...CTA.	CC........	C.T.T.T...	CTTT..T..T	.A..TA.CC.	TT..C..TGA	TT.

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