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# WIDESPREAD AND STRUCTURED DISTRIBUTIONS OF BLOOD PARASITE HAPLOTYPES ACROSS A MIGRATORY DIVIDE OF THE SWAINSON'S THRUSH (*CATHARUS USTULATUS*)

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ABSTRACT: We examined the phylogenetic distribution of cytochrome *b* haplotypes of the avian blood parasite genera *Haemoproteus* and *Plasmodium* across the migratory divide of the Swainson's thrush (*Catharus ustulatus*) in British Columbia, Canada. From 87 host individuals, we identified 8 parasite haplotypes; 4 of *Plasmodium* and 4 of *Haemoproteus*. Six haplotypes were novel; 1 *Haemoproteus* haplotype was identical to *H. majoris* found in the blue tit (*Parus caeruleus*) in Sweden, and another halotype was identical to a *Plasmodium* haplotype found in the white-crowned sparrow (*Zonotrichia leucophrys*) in Oregon. The 2 most abundant parasite haplotypes were widely distributed across the contact zone, whereas 2 other parasite haplotypes seem to have structured distributions. Compared with 74 *Plasmodium* and *Haemoproteus* haplotypes published in GenBank, haplotypes recovered from Swainson's thrushes do not form monophyletic groups, and they are closely related to haplotypes from a variety of other hosts and localities. In addition, we recovered 2 Swainson's thrush *Plasmodium* haplotypes from the nonmigratory orange-billed nightingale thrush (*Catharus aurantiirostris*) in Costa Rica. This study is the first to elucidate avian blood parasite transmission, distribution, and phylogenetic relationships in an avian contact zone in North America.

Studies on spatial distributions of avian blood parasites contribute to the understanding of host switching and expansion into new territories. This knowledge may be useful in predicting future global parasite distributions and have implications for vector-borne parasites affecting other vertebrates, such as humans.

Migratory divides, narrow regions where 2 populations with different migratory routes and typically distinct wintering areas meet on the breeding grounds (Bensch et al., 1999; Ruegg and Smith, 2002), provide opportunities to study the spatial differentiation and dispersal of avian blood parasite communities (Bensch and Åkesson, 2003; Reullier et al., 2006). For parasites to transmit across the divide, competent insect vectors are required. In addition, because blood parasites seem to be largely host specific at least at the family level (Bensch et al., 2000; Ricklefs and Fallon, 2002; Beadell et al., 2004; Ricklefs et al., 2004), transmission across the divide should be enhanced when hosts are closely related to each other. Consequently, in contact zones of closely related hosts, one would expect expansion of parasites to occur freely if the appropriate vectors are present.

Blood parasites can be transmitted both on the breeding grounds (Bennett and Cameron, 1974; Bensch and Åkesson, 2003; Ricklefs et al., 2005) and on the wintering grounds (Waldenström et al., 2002; Pérez-Tris and Bensch, 2005). The latter authors also demonstrated that more widespread parasites exhibit a more general transmission behavior and that they are capable of year-round transmission both on the hosts' breeding and wintering grounds. If parasite lineages across migratory divides are structured and differ between bird populations that come into contact only at breeding grounds, then parasite transmission likely occurs solely on the wintering grounds. Alternatively, if parasite lineages are widespread and identical lineages are distributed across contact zones between populations with different wintering locations, then these lineages are likely transmitted on the breeding grounds. Parasite distribution across migratory divides has been studied in the Europe–Africa migratory system (Bensch and Åkesson, 2003; Reullier et al., 2006), but data on parasite distribution across migratory divides in the Americas were, until the present study, lacking. In a region of British Columbia, 2 groups of Swainson's thrush (coastal and inland) form a migratory divide (Ruegg and Smith, 2002). The coastal group is concordant with the russet-backed Swainson's thrush subspecies group (*Catharus ustulatus ustulatus*), and the inland group is concordant with the olive-backed Swainson's thrush subspecies group (*Catharus ustulatus swainsoni*). The 2 groups exhibit a nearly complete separation of migratory routes and wintering locations (Fig. 1), the mitochondrial control region shows a sequence divergence of 0.7% (Ruegg and Smith, 2002), and the breeding ground habitats are ecologically different (Ruegg et al., 2006). The coastal Swainson's thrush winters in Central America and Mexico, migrates along a Pacific coastal route, and breeds in a climate with more precipitation and less seasonal variation in temperature and precipitation than the inland Swainson's thrush, which winters in Panama and South America and migrates via an eastern route (Ruegg and Smith, 2002; Ruegg et al., 2006).

Molecular techniques are becoming increasingly popular in studies on the evolution and distribution of avian haemosporidia (e.g., Richard et al., 2002; Fallon, Ricklefs et al., 2003; Hellgren et al., 2004). Here, we use molecular analyses to investigate the spatial distribution of blood parasite haplotypes found in the Swainson's thrush in British Columbia and to compare the distribution and phylogenetic relationship of the Swainson's thrush's haemosporidian haplotypes to published haplotypes found in other birds throughout the world, as well as haplotypes found in orange-billed nightingale thrushes that are resident in Central America. Specifically, we set out to determine (1) whether the parasites are specific to either of the 2 groups of Swainson's thrush or whether they are widespread across the migratory divide; (2) whether Swainson's thrush parasites are more closely related to each other than they are to blood parasites in other hosts; and (3) whether the presence of shared parasite lineages across species that reside in distinct regions sheds light on the origin of parasite transmission in migratory birds such as the Swainson's thrush.

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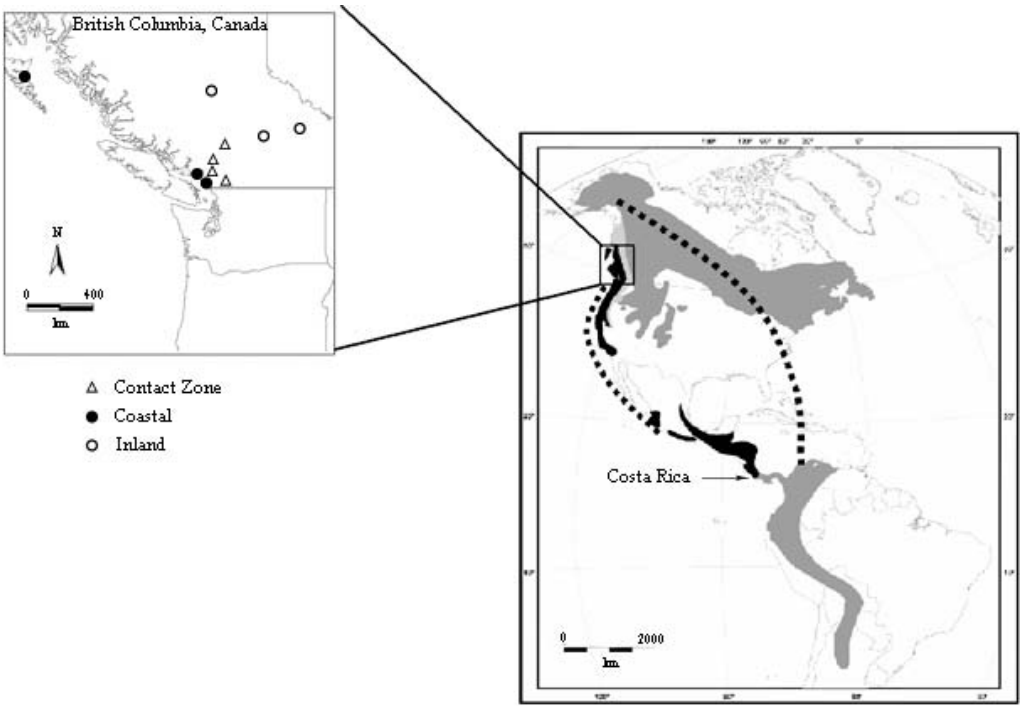


FIGURE 1. A map of the distribution of the coastal and inland Swainson's thrush groups. Black areas represent the range of the coastal group, dark gray areas represent the range of the inland group, and light gray areas represent the location of the contact zone of the 2 groups. The inset shows sampling localities on the breeding grounds in British Columbia. Costa Rica is also shown to indicate the site of collection of the orange-billed nightingale thrush.

## MATERIALS AND METHODS

Swainson's thrushes were caught in mist nets in the beginning of the breeding season (May–June) in 2000, 2003, 2004, and 2005. Blood was extracted via brachial venipuncture and stored in lysis buffer (Seutin et al., 1991). DNA was extracted with a DNeasy (QIAGEN, Valencia, California) extraction kit. The birds were classified as having 1 of 2 mitochondrial haplotypes based on an restriction fragment length polymorphism analysis of the control region that identified 2 reciprocally monophyletic haplotype groups within the Swainson's thrush, i.e., the coastal haplotype group restricted to the Pacific coast of North America and the inland haplotype group found throughout the remainder of the breeding range (Ruegg and Smith, 2002).

Blood from 273 birds from 12 different localities (Fig. 1) in British Columbia was screened for species of *Plasmodium* and *Haemoproteus* using primers 343F (5′GCTCACGCATCGCTTCT3′) and 496 (5′GACCGGTCATTTTCTTTG3′) following the protocol of Fallon, Ricklefs et al. (2003). The PCR product was viewed on 1% agarose gels stained with ethidium bromide. The presence of a 153-bp band scored an individual as positive for *Plasmodium*, *Haemoproteus*, or both.

Subsequent to the prevalence screening, a 542-bp fragment of the parasite cytochrome *b* gene from 98 infected individuals across the contact zone was amplified using the primers L15183 (5′GTGCAACYGTTATTACTAATTTATTATA3′) and H15730 (5′CATCCAATCCATAATAAAGCAT3′) following the protocol of Szymanski and Lovette (2005). Sequencing of this fragment was done on an ABI 377 sequencer or an ABI 3730 DNA analyzer (ABI PRISM®, Applied Biosystems, Foster City, California). Sequences (498 bp) were cleaned and aligned using Sequencher™ (Gene Codes Corporation, Ann Arbor, Michigan). One sequence had multiple peaks in both the forward and reverse direction, suggesting multiple infections. In addition, we were not able to obtain sequence data from 10 individuals, perhaps due to low parasitemia, or else degraded DNA. In total, 87 good sequences were obtained. We used the likelihood ratio values from chi-square tests to determine the distribution of parasite haplotypes across the migratory divide.

We performed BLAST searchers in the National Library of Medicine (NLM; http://www.ncbi.nlm.nih.gov/blast/) using each haplotype recovered from the Swainson's thrushes, and we downloaded avian malaria sequences posted as of 15 December 2006, of the first 50 BLAST hits that were overlapping our sequences. In total, 84 sequences (including the 8 haplotypes found in the Swainson's thrush and 2 outgroups; see Appendix) were aligned in Clustal X (Thompson, 1997) using default alignment parameters.

In addition, to determine whether Swainson's thrushes share parasite lineages with resident Central American birds, orange-billed nightingale thrushes (*Catharus aurantiirostris*) were mist netted in southern Costa Rica (site details in Sekercioglu et al., 2007) in the beginning of the 2004 and 2005 breeding seasons (March–August). Blood from 20 individuals was screened for *Plasmodium*/*Haemoproteus*, and cytochrome *b* sequences from the 7 positively scored individuals were obtained following the same protocols as for the Swainson's thrush samples.

To estimate the topology of the avian malaria parasite haplotypes recovered from the Swainson's thrushes in British Columbia and the sequences downloaded from GenBank, we first used MrModeltest, version 2.2 (Nylander, 2004) to determine the most appropriate evolutionary model for our 2 data sets partitioned by codon positions. We used the models recommended by the hierarchical likelihood ratio test. The results from the MrModeltest 2.2 runs were combined in MrSecretary 1.0 (Lin and Bonett, 2006) and implemented in MrBayes, version 3.0b4 (Huelsenbeck and Ronquist, 2001). Simulations were run with 4 chains for 10,000,000 steps with a 4,000,000-step burn-in period and sampling every 500 steps. The program Are We There Yet (Wilgenbusch et al., 2004) was used to select the burn-in number. In addition, we ran a maximum likelihood heuristic search with the tree-bisection-reconnection branch-swapping algorithm in PAUP*4.0b10 (Swofford, 2002) with 10 replicates of random stepwise additions, followed by a heuristic bootstrap with 100 replicates. For this analysis, we considered models recommended by the hierarchical likelihood ratio test in Modeltest, version 3.7 (Posada and Crandall, 1998). We consider nodes with bootstrap values equal to or greater than 70% and posterior probabilities greater than or equal to 0.95 strongly supported.

## RESULTS

Of the 273 Swainson's thrushes screened for *Plasmodium*/*Haemoproteus*, 148 (54%) were infected. Of the 87 sequences

TABLE I. Eight parasite lineages recovered from Swainson's thrushes (SWTH) in British Columbia and numbers of individuals and localities in which they were found. Novel lineages begin with SWTH for Swainson's thrush. *P.* sp. 49 is GenBank AF465549, and *H. majoris* is GenBank AY099045.

| Region | SWTH.*P.*1 | SWTH.*P.*2 | SWTH.*P.*3 | *P.* sp. 49 | *H. majoris* | SWTH.*H.*1 | SWTH.*H.*2 | SWTH.*H.*3 |
|---|---|---|---|---|---|---|---|---|
| Coastal | 4 | 0 | 0 | 2 | 0 | 19 | 0 | 0 |
| Hybrid | 1 | 1 | 1 | 6 | 1 | 35 | 0 | 1 |
| Inland | 0 | 0 | 0 | 1 | 2 | 11 | 2 | 0 |
| Total | 5 | 1 | 1 | 9 | 3 | 65 | 2 | 1 |

recovered from the Swainson's thrush, we detected 8 different haplotypes, whereof 1 halotype was identical to *H. majoris* (GenBank AY099045) found in a blue tit (*Parus caeruleus*) in Europe, and another halotype was identical to *Plasmodium* sp. haplotype 49 (GenBank AF465549) found in white-crowned sparrows (*Zonotrichia leucophrys*) in North America. Six sequences were novel, and they have been deposited in GenBank with the accession numbers DQ490060–DQ490065.

Species of *Haemoproteus* are more prevalent than those of *Plasmodium* (Table I). At least 1 *Plasmodium* haplotype (AF465549) and 1 *Haemoproteus* haplotype (SWTH.*H.*sp.1) are equally distributed across the contact zone ($\chi^2 = 0.89$, $P = 0.64$ for *P.* sp haplotype 49 and $\chi^2 = 0.51$, $P = 0.78$ for SWTH.*H.*sp.1). SWTH.*H.*sp.1 was recovered from 65 individual Swainson's thrushes (72%), and it is thus, by far, the most common haplotype. Two haplotypes seem structured; SWTH.*P.*sp.1 was detected only in the coastal Swainson's thrush, and SWTH.*H.*sp.2 was detected only in the inland Swainson's thrush.

The phylogenetic analysis of 82 haplotypes in the ingroup split the data into a *Haemoproteus* clade and a *Plasmodium* clade (Fig. 2). The 8 haplotypes found within the Swainson's thrush are not always more closely related to each other than they are to parasite haplotypes recovered from a variety of other hosts and localities (Fig. 2). SWTH.*P.*sp.1 seems distinct from the other Swainson's thrush *Plasmodium* haplotypes, all 3 of which form a strongly supported monophyletic clade (SWTH.*P.*sp.2, SWTH.*P.*sp.3, and *P.* sp. haplotype 49; Fig. 2). SWTH.*P.*sp.1, which was also recovered from the orange-billed nightingale thrush, groups with a *Plasmodium* haplotype (GenBank AY099036) recovered from an Inca tern (*Larosterna inca*, Laridae) in the Washington D.C. National Zoo (Perkins and Schall, 2002). The node connecting SWTH.*P* sp.1 and the Inca tern *Plasmodium* is strongly supported by the maximum likelihood analysis (bootstrap value of 81%), but not by the Bayesian analysis (posterior probability of 0.89). The pairwise distance between SWTH.*P.*sp.1 and the Inca tern *Plasmodium* is 0.44%. All Swainson's thrush *Haemoproteus* haplotypes (except for *H. majoris*) form an unresolved clade with haplotypes recovered from both other Muscicapidae and non-Muscicapidae species from Africa, Asia, Europe, and North America. The pairwise distances between SWTH.*H.*sp.1, SWTH.*H.*sp.2, and SWTH.*H.*sp.3 range between 0.2% and 0.8%.

Two *Plasmodium* haplotypes were recovered from the 20 orange-billed nightingale thrushes in Costa Rica. One haplotype, recovered from 2 individuals, was identical to SWTH.*P.*sp.1, and 1 haplotype, recovered from 5 individuals, was identical to SWTH.*P.*sp.2. No orange-billed nightingale thrushes were infected with *Haemoproteus*.

## DISCUSSION

### Distribution across the divide

The widespread distribution of SWTH.*H.*sp.1 and *P.* sp. haplotype 49 could reflect either, or both, of the following 2 processes. First, the cytochrome *b* marker may not evolve fast enough to detect differentiation between parasite haplotypes shared by very closely related hosts, i.e., the 2 subspecies that constitute our study system. Ricklefs and Fallon (2002) proposed a slower rate of nucleotide substitution in avian malaria mitochondrial DNA to be accountable for patterns of low host specificity in avian malaria parasites. Second, the results could reflect 3 different possibilities for the location of transmission. That is, the parasite haplotypes recovered from the Swainson's thrushes could be widespread in South and Central America and transmitted on the wintering grounds of both the inland Swainson's thrush (which winters predominantly in South America) and the coastal Swainson's thrush (which winters predominantly in Central America), they could be transmitted solely on the breeding grounds across the migratory divide, or they could be transmitted year-round transcontinentally. We have no reason to believe that transmission occurs preferentially on either the wintering or breeding grounds, because previous studies have indicated that parasites can be transmitted in tropical and temperate regions alike (Waldenström et al., 2002; Bensch and Åkesson, 2003; Fallon, Bermingham et al., 2003; Pérez-Tris and Bensch, 2005; Ricklefs et al., 2005). However, a recent study has shown that blood parasites rarely are transmitted at both breeding and wintering grounds and that *Plasmodium* parasites more often infect both resident and migratory bird species than *Haemoproteus* (Hellgren et al., 2007). Due to the lack of avian malaria sampling in South America, we cannot compare our

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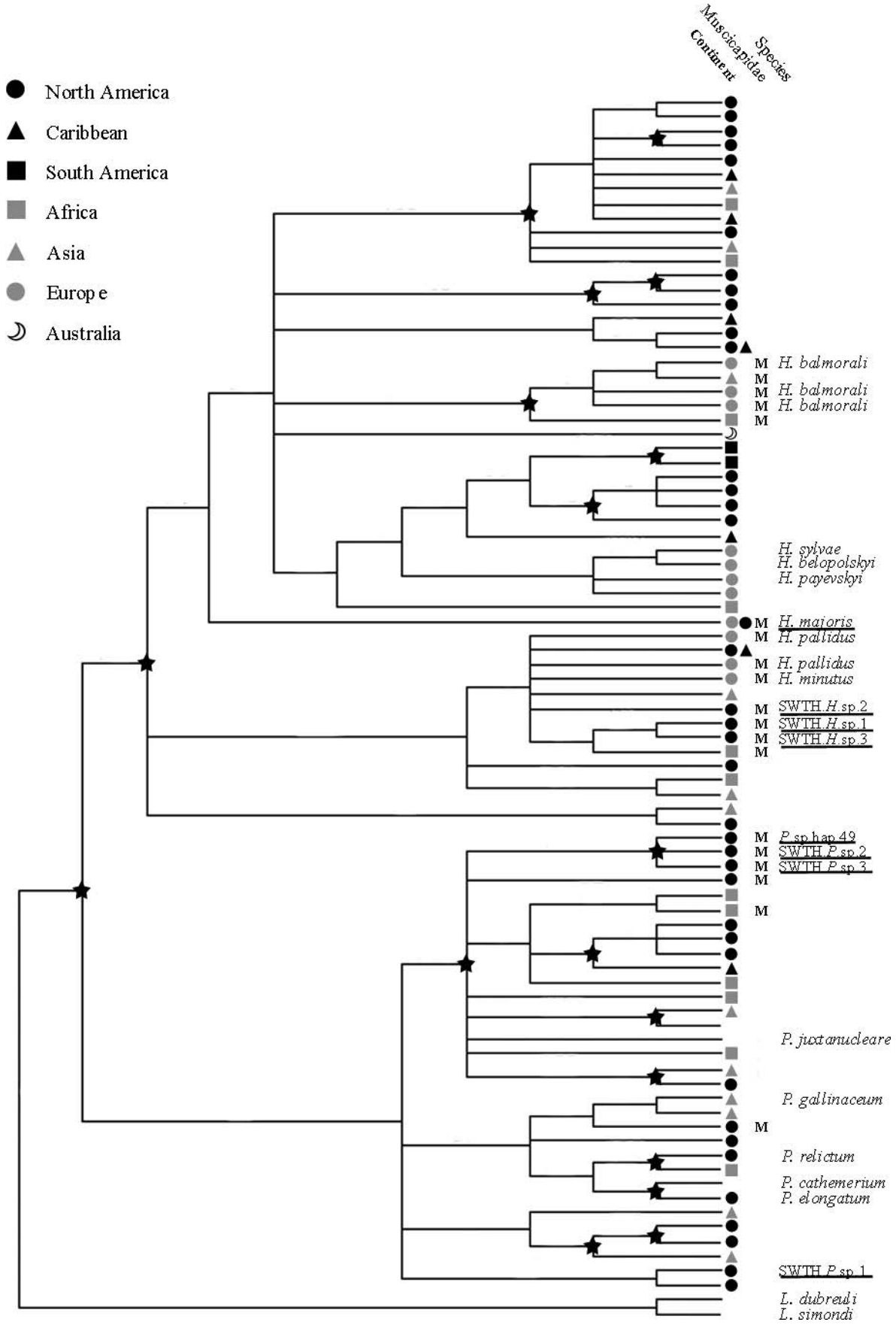
FIGURE 2. A Bayesian consensus rooted with *Leucocytozoon dubreuli* and *L. simondi*. Solid stars represent nodes where the posterior probabilities from the Bayesian analysis are greater than 0.95 and where bootstrap values from the maximum likelihood analysis are greater than 70%. Sequences recovered from the Swainson's thrush are underlined. The continent where a lineage was found, when known, is shown as different symbols. Haplotypes recovered from Muscicapidae species are marked with a bold ''M.'' If a parasite haplotype was documented as a species in the GenBank record, it is noted to the right.


Continent
Muscicapidae
Species
North America
Caribbean
South America
Africa
Asia
Europe
Australia
M H. balmorali
M
M H. balmorali
M H. balmorali
M
H. sylvae
H. belopolskyi
H. payevskyi
M H. majoris
M H. pallidus
M H. pallidus
M H. minutus
M SWTH H. sp. 2
M SWTH H. sp. 1
M SWTH H. sp. 3
M
M P. sp. hap. 49
M SWTH P. sp. 2
M SWTH P. sp. 3
M
M
P. juxtanucleare
P. gallinaceum
M
P. relictum
P. cathemerium
P. elongatum
SWTH P. sp. 1
L. dubreuli
L. simondi

sequences to other sequences from the Swainson's thrush's wintering grounds. However, we found neither the common SWTH.*H.*sp.1 nor *P.* sp. haplotype 49 in the congeneric orange-billed nightingale thrush in Costa Rica, near to where the coastal Swainson's thrush is known to winter and the inland Swainson's thrush stops over during migration. Further support for breeding ground transmission of *P.* sp. haplotype 49 is that it is shared with the white-crowned sparrow in the Pacific Northwest (Ricklefs and Fallon, 2002). More intensive sampling of the wintering grounds alongside sampling of juvenile Swainson's thrushes on the breeding grounds is necessary before determining place of transmission. We sampled early in the breeding season when no juvenile Swainson's thrushes were present. We cannot reject the possibility that transmission also occurs on the wintering grounds or that this haplotype is widespread in the Americas. Nevertheless, this wide distribution of *Plasmodium* and *Haemoproteus* contrasts with patterns of blood parasite distributions in European migratory divides, which exhibit a highly structured parasite distribution with occasional host sharing across the contact zone (Reullier et al., 2005).

### Phylogenetic relationships

That the parasite haplotypes recovered from the Swainson's thrush group with haplotypes recovered from several different host species and localities indicates low host specificity. Szymanski and Lovette (2005) found a similar lack of host specificity in sympatric hosts belonging to 4 families (Hirundinidae, Parulidae, Emberizidae, and Fringillidae). Their phylogenetic analysis of 29 unique parasite haplotypes produced 5 strongly supported clades of which 2 clades contained parasite haplotypes infecting all 4 host families. Our results, which placed haplotypes from the Swainson's thrush within 4 strongly supported clades containing haplotypes from multiple host families, were, therefore, not surprising.

The small pairwise distances between the *Haemoproteus* haplotypes (expept *H. majoris*) recovered from the Swainson's thrush suggest that they are the same lineage if we follow the definition of a lineage as described by Szymanski and Lovette (2005), who consider a lineage to be composed of haplotypes less than 1.4% divergent. However, included in that lineage would be 2 already described species (*H. pallidus* and *H. minutus*; Hellgren et al., 2006), suggesting that rather than reflecting low host specificity, this particular clade shows that more sequence data are necessary to resolve it.

### Evidence for transmission locality

The presence of SWTH.*P.*sp.1 and SWTH.*P.*sp.2 in the nonmigratory orange-billed nightingale thrush shows that these lineages are transmitted in Central America. Following the reasoning of Bensch et al. (2007), the fact that these 2 parasite haplotypes are rarely detected in breeding Swainson's thrush populations supports the idea of them being transmitted on the wintering grounds, because fitness costs are highest after primary infections; fewer birds infected with parasites on the wintering grounds would make it all the way to the breeding grounds.

It is interesting that the majority of the range of the orange-billed nightingale thrush in Central America overlaps with the wintering distribution of the coastal Swainson's thrush, the only Swainson's thrush subspecies known to carry the SWTH.*P.*sp.1 haplotype. No Swainson's thrushes were found wintering in the sampling location of the orange-billed nightingale thrushes in this study, but if future work were to reveal that SWTH.*P.*sp.1 is found to be widespread among orange-billed nightingale thrush populations that co-occur with wintering coastal Swainson's thrushes, then this may help explain the structured distribution of the SWTH.*P.*sp.1 haplotype across the migratory divide.

In conclusion, despite a significant difference in migratory behavior and wintering locations in the Swainson's thrush, 1 highly abundant *Haemoproteus* haplotype and 1 *Plasmodium* haplotype are distributed across the migratory divide, whereas 2 parasite haplotypes provide preliminary evidence for a structured distribution across the migratory divide. The presence of 2 of the *Plasmodium* haplotypes specific to the coastal Swainson's thrush in the nonmigratory orange-billed nightingale thrush from Central America suggests that these lineages are transmitted on the wintering grounds. Future work should use more markers, such as nuclear genes, and sampling of juveniles, vectors, and the entire host community on both the wintering and breeding grounds in South and North America, respectively. Our results add to the understanding of avian malaria dynamics by showing that within a single host species system parasites can be diverse and have both widespread and structured distributions, by demonstrating that differences in cytochrome *b* are not great across the world and several host families, and by suggesting that a greater emphasis should be placed on studying vector ecology to clarify host–parasite interactions.

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APPENDIX. GenBank identification number, species of parasite, host in which the parasite was found, and country in which the host was caught. *P.* is Plasmodium, *H.* is *Haemoproteus*, and *L.* is *Leucocytozoon*. Host taxonomy is based on information from the Integrated Taxonomic Information System (http://www.itis.gov/index.html).

| GenBank | Parasite species | Host species | Host family | Country |
|---|---|---|---|---|
| AB250415 | *P. juxtanucleare* | Unknown | | Unknown |
| AF069611 | *P. elongatum* | *Passer domesticus* | Passeridae | N. America |
| AF465548 | *P.* sp. haplotype 48 | *Turdus migratorius* | Muscicapidae | N. America |
| AF465549 | *P.* sp. haplotype 49 | *Zonotrichia leucophrys* | Fringillidae | N. America |
| AF465550 | *P.* sp. haplotype 50 | *Andropadus latirostris* | Pycnonotidae | Cameroon |
| AF465551 | *P.* sp. haplotype 51 | *Nectarinia olivacea* | Nectariniidae | Cameroon |
| AF465552 | *P.* sp. haplotype 52 | *Alethe diademata* | Muscicapidae | Cameroon |
| AF465553 | *P.* sp. haplotype 53 | *Cinclocerthia ruficauda* | Sturnidae | Caribbean |
| AF465555 | *P.* sp. haplotype 55 | *Baeolophus bicolor* | Paridae | N. America |
| AF465559 | *P.* sp. haplotype 64 | *Vireo griseus* | Vireonidae | N. America |
| AF465565 | *H.* sp. haplotype 4 | *Margarops fuscus* | Sturnidae | Caribbean |
| AF465567 | *H.* sp. haplotype 6 | *Coereba flaveola* | Fringillidae | Trinidad |
| AF465568 | *H.* sp. haplotype 7 | *Tiaris bicolor* | Fringillidae | Venezuela |
| AF465571 | *H.* sp. haplotype 19 | *Alophoixus bres* | Pycnonotidae | East Asia |
| AF465572 | *H.* sp. haplotype 20 | *Margarops fuscatus, Margarops fuscus,* and *Dumetella carolinensis* | Sturnidae | N. America/ Caribbean |
| AF465573 | *H.* sp. haplotype 21 | *Corvus brachyrhynchos* | Corvidae | N. America |
| AF465574 | *H.* sp. haplotype 22 | *Neocossyphus poensis* and *Neocossyphus fraseri* | Muscicapidae | Cameroon |
| AF465575 | *H.* sp. haplotype 2 | *Vireo altiloquus* | Vireonidae | Caribbean |
| AF465576 | *H.* sp. haplotype 28 | *Vireo olivaceus* | Vireonidae | N. America |
| AF465577 | *H.* sp. haplotype 29 | *Vireo griseus* | Vireonidae | N. America |
| AF465578 | *H.* sp. haplotype 30 | *Erythrura prasina* | Passeridae | Asia |
| AF465579 | *H.* sp. haplotype 31 | *Coereba flaveola* and *Loxigilla noctis* | Fringillidae | Caribbean |
| AF465580 | *H.* sp. haplotype 32 | *Dendroica pensylvanica* | Fringillidae | N. America |
| AF465581 | *H.* sp. haplotype 33 | *Junco hyemalis* | Fringillidae | N. America |
| AF465582 | *H.* sp. haplotype 34 | *Piranga rubra* | Fringillidae | N. America |
| AF465583 | *H.* sp. haplotype 35 | *Piranga olivacea* and *Piranga rubra* | Fringillidae | N. America |
| AF465584 | *H.* sp. haplotype 36 | *Margarops fuscatus* | Sturnidae | Caribbean |
| AF465585 | *H.* sp. haplotype 37 | *Motacilla clara* | Passeridae | Cameroon |
| AF465586 | *H.* sp. haplotype 38 | *V. griseus* | Vireonidae | N. America |
| AF465587 | *H.* sp. haplotype 39 | *Copsychus malabaricus* | Muscicapidae | Asia |
| AF465588 | *H.* sp. haplotype 40 | *Alethe poliocephala* | Muscicapidae | Cameroon |
| AF465594 | *H.* sp. haplotype 46 | *Polyborus plancus* | Falconidae | N. America |
| AY099029 | *P. gallinaceum* | *Gallus gallus* | Phasianidae | Vietnam |
| AY099033 | *P.* sp. | *T. migratorius* | Muscicapidae | N. America |
| AY099034 | *H.* sp. | *Vireo olivaceus* | Vireonidae | N. America |
| AY099035 | *P.* sp. | *Ninox scutulata* | Strigidae | Singapore |
| AY099036 | *P.* sp. | *Larosterna inca* | Laridae | N. America (zoo) |
| AY099040 | *H. sylvae* haplotype GRW1 | *A. arundinaceus* | Sylviidae | Sweden |
| AY099041 | *P.* sp. haplotype GRW4 | *Acrocephalus arundinaceus* | Sylviidae | Kenya |
| AY099042 | *H.* sp. haplotype HLW1 | *Phylloscopus humei* | Sylviidae | India |
| AY099043 | *H.* sp. haplotype LCLW1B19 | *Phylloscopus occipitalis* | Sylviidae | Europe |
| AY099044 | *P.* sp. haplotype ORW1G278 | *Acrocephalus orientalis* | Sylviidae | Japan |
| AY099045 | *H. majoris* | *Parus caeruleus* | Paridae | Sweden |
| AY099063 | *L. dubreuli* | Unknown | | Unknown |
| AY099064 | *L. simondi* | *Anas platyrhyncos* | Anatidae | Canada |
| AY377128 | *P. cathemerium* | Unknown | | Unknown |
| AY540202 | *H.* sp. strain NA10 | *Vireo olivaceus* | Vireonidae | N. America/ Caribbean |
| AY540203 | *H.* sp. strain NA12 | *Cardinalis cardinalis* | Fringillidae | N. America |
| AY640126 | *H.* sp. A12 | *Tachycineta bicolor* | Hirundinidae | N. America |
| AY640129 | *H.* sp. A3 | *Dendroica petechia* | Fringillidae | N. America |
| AY640134 | *P.* sp. C3 | *D. petechia* | Fringillidae | N. America |
| AY640135 | *P.* sp. G1 | *Melospiza melodia* | Fringillidae | N. America |
| AY640136 | *P.* sp. D1 | *M. melodia* | Fringillidae | N. America |
| AY640137 | *P.* sp. H1 | *Dendroica petechia* | Fringillidae | N. America |
| AY640139 | *H.* sp. A11 | *Tachycineta bicolor* | Hirundinidae | N. America |
| AY640141 | *H.* sp. A6 | *T. bicolor* | Hirundinidae | N. America |

APPENDIX. Continued.

| GenBank | Parasite species | Host species | Host family | Country |
|---|---|---|---|---|
| AY640143 | *P.* sp. C2 | *Geothlypis trichas* | Fringillidae | N. America |
| AY733087 | *H.* sp. jb2.SEW5141 | *Lichenostomus frenatus* | Meliphagidae | Australia |
| AY733090 | *P. relictum* isolate jb5.NAN015 | *Hemignathus virens* | Fringillidae | N. America |
| AY762059 | *H.* sp. DW5384 | Unknown | | Madagascar |
| AY762061 | *P.* sp. DW5422 | Unknown | | Madagascar |
| AY762062 | *H.* sp. DW5416 | Unknown | | Madagascar |
| AY762066 | *H.* sp. DW5335 | Unknown | | Madagascar |
| AY762078 | *P.* sp. RB61 | Unknown | | Madagascar |
| DQ212192 | *H.* sp. C033 | *Dendrocygna javanica* | Dendrocygnidae | Asia |
| DQ212193 | *P.* sp. C028 | *D. javanica* | Dendrocygnidae | Asia |
| DQ212194 | *P.* sp. C113 | *Gyps tenuirostris* | Accipitridae | |
| DQ212195 | *P.* sp. C114 | *Gyps bengalensis* | Accipitridae | Asia |
| DQ212196 | *P.* sp. C175 | *G. tenuirostris* | Accipitridae | Asia |
| DQ212197 | *H.* sp. C187 | *Passer montanus* | Passeridae | |
| DQ630004 | *H. pallidus* isolate L-PFC1 | *Ficedula hypoleuca* | Muscicapidae | Europe |
| DQ630005 | *H. pallidus* isolate L-COLL2 | *F. hypoleuca* | Muscicapidae | Europe |
| DQ630006 | *H. belopolskyi* isolate L-HIICT1 | *Hippolais icterina* | Sylviidae | Europe |
| DQ630007 | *H. balmorali isolate* L-LULU1 | *Luscinia luscinia* | Muscicapidae | Europe |
| DQ630008 | *H. balmorali isolate* L-SFC1 | *Muscicapa striata* | Muscicapidae | Europe |
| DQ630009 | *H. payevskyi* isolate L-RW1 | *Acrocephalus scirpaceus* | Sylviidae | Europe |
| DQ630013 | *H. minutus isolate* L-TURDUS2 | *Turdus merula* | Muscicapidae | Europe |
| DQ630014 | *H. balmorali isolate* L-COLL3 | *M. striata* | Muscicapidae | Europe |