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ON THE PHYLOGENETIC RELATIONSHIPS OF HAEMOSPORIDIAN PARASITES FROM RAPTORIAL BIRDS (FALCONIFORMES AND STRIGIFORMES)

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ABSTRACT: Haemosporidian parasite diversity among raptorial birds (hawks and owls), as estimated by DNA sequencing, is proving to be greater than previously anticipated from taxonomic assessments based on parasite morphology. Here, we place raptor parasites in a phylogenetic context, including new parasite cytochrome *b* (cyt *b*) sequences from North America and Europe and from a variety of host species not previously sampled. Mitochondrial DNA sequences reveal raptor-specific parasite clades within *Parahaemoproteus*, but not within *Plasmodium*. We also recovered a strikingly divergent clade of raptor parasites that aligns with neither genus, but groups with both as a sister clade to *Leucocytozoon*. Different cyt *b* primer sets recovered additional sequences from 3 of these samples, which grouped with *Parahaemoproteus* in 2 cases and with *Plasmodium* in 1 case. Possible explanations (after excluding contamination) include multiple infections, alternative cyt *b* copies within the mitochondrial genome, and nuclear copies of mitochondrial genes. We believe the latter 2 explanations are unlikely because these divergent cyt *b* lineages form a single clade and were also recovered with several additional genomic markers.

Molecular phylogenetic approaches continue to uncover unanticipated diversity in avian haemosporidian species of *Plasmodium*, *Parahaemoproteus*, and *Leucocytozoon* (Ricklefs and Fallon, 2002; Bensch et al., 2004; Sehgal et al., 2006; Martinsen et al., 2008). The diversity of these parasites seems to match that of the avian hosts, and the raptorial Falconiformes (hawks and falcons) and Strigiformes (owls) appear to be no exception. Hawks and owls are prone to infection by *Leucocytozoon* (*Leucocytozoon toddi* and *Leucocytozoon danilewski*, respectively), but also by species of *Plasmodium* and *Parahaemoproteus*. The most recent morphologic–taxonomic assessment of avian haemosporidian diversity (Valkiunas, 2005) lists 6 *Parahaemoproteus* species unique to Falconiformes and 2 unique to Strigiformes; within the more species-rich *Plasmodium*, only 3 are unique to Strigiformes, while 3 additional species infect both Strigiformes and Falconiformes as well as many other host taxa. As in the case of most other host taxa (Bensch et al., 2000; Ricklefs et al., 2004), molecular characterizations of the haemosporidian parasites of these groups of raptors reveal many lineages distributed widely within the haemosporidian phylogeny which may or may not be unique to raptorial birds.

Four recent molecular studies highlight patterns of host distribution and phylogenetic relationships among raptor parasites. The morphologically defined species *L. toddi* is unique to Falconiformes and is geographically widespread, yet Sehgal et al. (2006) showed that this ''species'' has distinctive mitochondrial DNA sequences in *Accipiter* and *Buteo* spp. in both the New World and the Old World. Two parasite clades within the morphologically defined *L. toddi* were 10.9% divergent in mitochondrial cytochrome *b* (cyt *b*) and, because this divergence is coupled with host specificity within and among clades, the authors suggest that the cyt *b* lineages represent cryptic species.

Ortego et al. (2007) focused on *Plasmodium* and *Parahaemoproteus* parasite species from 1 host species, *Falco naumanni* (lesser kestrel), and confirmed 3 morphologically described species from both parasite genera, as well as other undescribed lineages identified by unique cyt *b* sequences, particularly of *Parahaemoproteus*. None of the recovered sequence-based lineages was of parasites unique to Falconiformes. In another Old World study having a larger host-taxonomic scope, Krone et al. (2008) identified 1 *Parahaemoproteus* lineage to species, *P. noctuae*, a morphologically defined parasite unique to Strigiformes. Other raptor haemosporidian lineages were interspersed phylogenetically among the parasites of passeriform (perching; passerines) birds, while 1 unidentified lineage on a very long branch was unaffiliated with either *Parahaemoproteus* or *Plasmodium*.

Lastly, Ishak et al. (2008) found many lineages of *Leucocytozoon*, *Plasmodium*, and *Parahaemoproteus* among a variety of strigiform (owl) host species. Overall, these studies highlight an unsurprising lack of phylogenetic cohesion among raptor parasites. Considering that there is no a priori reason to suspect that parasite lineages would be unique to these highly divergent orders of avian hosts (Hackett et al., 2008), a more wide-ranging assessment of raptor parasite diversity is in order.

In the present study, we include *Parahaemoproteus* and *Plasmodium* cyt *b* ''lineages'' from 43 falconiform and strigiform host samples and evaluate these with previously published data on bird, lizard, and mammal parasites and with data generated in our laboratory for avian parasites from raptors, passeriform, columbiform (doves and pigeons), and anseriform (ducks and geese) birds. We augment these cyt *b* data for many of our samples with sequence data from the mitochondrial cytochrome oxidase 1 (*cox* I), apicoplast caseinolytic protease C (ClPC), and nuclear diacylglycerol-O-acyltransferase (trf) genes. Our main objectives were to characterize the diversity and relationships among raptor parasites and to identify clades unique to raptors as a whole, or to groups or individual species of raptors. We did not attempt to relate lineages defined by DNA sequences to described species of parasites. A significant finding of this study is a new clade of haemosporidian parasites that is differentiated from both *Plasmodium* and *Parahaemoproteus*.

MATERIALS AND METHODS

Samples

About 200 raptor samples were acquired from the Carolina Raptor Center, the University of California Davis Raptor Center, the Riverbanks Zoo, the Florida Audubon Society, and the Spain Raptor Center. Most of these birds had been maintained in captivity for varying periods, generally in outdoor enclosures; we had no information concerning the acquisition of haemosporidian parasite infections, particularly whether infections might have been acquired subsequent to captivity.

Laboratory procedures

DNA extraction and polymerase chain reaction (PCR)-based screening for parasites were conducted as in Fallon et al. (2003). Of those screened, 29 individuals were positive for malaria parasites (Table I). PCR for

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TABLE I. Raptor cytochrome *b* sequences included in phylogenetic analyses. Sample name: the parasite lineage name (Fig. 1); STRI: Strigiformes host, FALC: Falconiformes host. parasite genera identified: parasite lineages clearly associated with either *Plasmodium* or *Parahaemoproteus* were assigned as appropriate from Figure 1, whereas *Unknown* refers to the divergent parasite clade (Clade 7, Fig. 1). Samples with multiple infections are noted. Host species, geographic locality of sample collection, clade (from Fig. 1), and accession number (2, if applicable) are listed for each sample. Accession numbers EU627829 through EU627845 are from Ishak et al. (2008).

Sample name	Parasite genera identified	Host species	Geographic locality	Clade	Accession nos.
STRI1	*Plasmodium*	*Strix varia*	USA	6	EU627845
STRI2	*Parahaemoproteus*	*Asio otus*	Lithuania	8	EU627844
STRI3	*Plasmodium*	*Strix occidentalis*	USA	3	EU627827
STRI4	*Plasmodium*	*Bubo virginianus*	USA	4	EU627831
STRI5	*Plasmodium*	*S. varia*	USA	4	EU627835
STRI6	*Parahaemoproteus*	*A. otus*	USA	8	EU627836
STRI7	*Parahaemoproteus*	*Strix woodfordii*	Cameroon	8	EU627832
STRI8	*Parahaemoproteus*	*Strix occidentalis occidentalis*	USA	8	EU627839
STRI9	*Parahaemoproteus*	*S. varia*	USA	9	EU627834
STRI10	*Parahaemoproteus*	*S. o. occidentalis*	USA	9	EU627833
STRI11	*Plasmodium*	*Strix occidentalis caurina*	USA	9	EU627837
STRI12	*Parahaemoproteus*	*S. varia*	USA	9	EU627840
STRI13	*Parahaemoproteus*	*Tyto alba*	USA	13	EU627838
STRI14	*Parahaemoproteus*	*T. alba*	USA	13	EU627829
FALC1	*Unknown* (*Plasmodium*?)	*Buteo jamaicensis*	USA	7	GQ141614
FALC2	*Unknown* (*Plasmodium*?)	*B. jamaicensis*	USA	7	GQ141615
FALC3	*Plasmodium*/*unknown* (*Plasmodium*?)	*B. jamaicensis*	USA	4/7	GQ141622/ GQ141607
STRI15	*Plasmodium*	*B. virginianus*	USA	9	GQ141604/ GQ141612
STRI16	*Parahaemoproteus*	*S. varia*	USA	9	GQ141608
FALC4	*Unknown* (*Plasmodium*?)	*B. jamaicensis*	USA	7	GQ141611
STRI17	*Parahaemoproteus*	*B. virginianus*	USA	9	GQ141609
STRI18	*Parahaemoproteus*	*B. virginianus*	USA	9	GQ141610
FALC5	*Unknown* (*Plasmodium*?)	*Buteo platypterus*	USA	7	GQ141605
FALC6	*Parahaemoproteus*/*unknown* (*Plasmodium*?)	*B. jamaicensis*	USA	7/12	GQ141606/ GQ141613
FALC7	*Parahaemoproteus*	*Falco sparverius*	USA	13	GQ141621
FALC8	*Unknown* (*Plasmodium*?)	*B. jamaicensis*	USA	7	GQ141628
FALC9	*Unknown* (*Plasmodium*?)	*B. jamaicensis*	USA	7	GQ141629
STRI19	*Parahaemoproteus*	*S. varia*	USA	8	GQ141623
STRI20	*Parahaemoproteus*	*Otus asio*	USA	8	GQ141626
STRI21	*Parahaemoproteus*	*O. asio*	USA	8	GQ141624
FALC10	*Parahaemoproteus*	*B. jamaicensis*	USA	9	GQ141625
STRI22	*Parahaemoproteus*	*S. varia*	USA	9	GQ141627
FALC11	*Parahaemoproteus*	*Falco sparverius*	USA	13	GQ141558
STRI23	*Plasmodium*	*B. virginianus*	USA	3	GQ141560
FALC12	*Plasmodium*	*B. jamaicensis*	USA	App. 2	GQ141612
FALC13	*Unknown* (*Plasmodium*?)	*B. jamaicensis*	USA	7	GQ141616
FALC14	*Unknown* (*Plasmodium*?)	*B. jamaicensis*	USA	7	GQ141617
STRI24	*Parahaemoproteus*	*B. virginianus*	USA	9	GQ141618
STRI25	*Parahaemoproteus*	*S. varia*	USA	9	GQ141619
STRI26	*Parahaemoproteus*	*S. varia*	USA	9	GQ141620
FALC15	*Unknown* (*Plasmodium*?)	*Circus aeroginosus*	Spain	7	GQ141602
FALC16	*Unknown* (*Plasmodium*?)	*Buteo buteo*	Spain	7	GQ141603
STRI27	*Parahaemoproteus*	*A. otus*	Spain	8	GQ141601

partial cyt *b* sequence data was performed on positive samples using primers 543F and 926R (~300 base pair [bp]) as in Fallon et al. (2004); these products were purified and then sequenced with the same primers on an ABI 3100 (Applied Biosystems, Foster City, California) using standard protocols. For a subset of these samples (n = 14), full-length cyt *b* sequences (~1,100 bp) were obtained using primers and protocols from Perkins et al. (2007; see Fig. S1, available online). Additional sequences from the *cox* I (~1,100 bp), ClPC (~600 bp), and trf (~300 bp) genes were generated with primers from Perkins et al. (2007; *cox* I and ClPC) and Beadell et al. (2009; trf); for 12 samples (3 raptors and 9 passerines), we amplified partial cyt *b* using primers HaemF and Haem R1 (Hellgren et al., 2004).

Mitochondrial sequences were edited and aligned unambiguously using BioEdit (Hall, 1999). All sequences translated into appropriate codons, as compared to the *Plasmodium falciparum* cyt *b* gene (Gardner et al., 2002), and no stop codons were detected. We combined our sequences with a larger dataset, including additional avian parasite samples from our own laboratory and a diverse sample of avian, mammal, and lizard parasites from GenBank (Appendix 1, available online); including these diverse parasites is necessary given the non-monophyly of avian malaria parasites (Martinsen et al., 2008). With 3 *Leucocytozoon* outgroup species, the cyt *b* dataset comprised 131 samples; additional *Leucocytozoon* sequences did not alter the results in preliminary analyses and, therefore, only 3 samples were used to reduce computation time. Limited data were included from previous studies due to non-overlap of sequences from those studies with sequences obtained from 1 primer set (543–926) in this study which highlighted an important, novel clade (see Clade 7 below, and cyt *b* data map in Fig. S1, Perkins et al. 2007).

Phylogenetic analyses

All cyt *b* sequences were included in analyses, and shorter sequences were padded with dashes to the length of the longest sequence (~1,100 bp); dashes were treated as missing data. Using PAUP (Swofford, 2003), we first reconstructed distance-based phylogenetic trees (HKY85+I+Γ; step-wise addition, 500,000 rearrangements). From 1 of these trees (chosen at random), we estimated parameters of the HKY substitution model (transition:transversion ratio), the proportion of invariable sites (I), and the alpha-shape parameter of the gamma distribution (Γ; rate heterogeneity across nucleotide sites) and reconstructed a maximum likelihood (ML) tree using the distance tree as a starting tree. We stopped this analysis after the likelihood score had not changed for 5,000 rearrangements for a total of 11,500 rearrangements. Support for nodes was determined using the ML bootstrap (500 pseudoreplicates; TREEFINDER, Jobb, 2008) and Bayesian posterior probabilities (in Beast [Drummond and Rambaut, 2007]); HKY+I+ Γ). In Bayesian analyses, we conducted 2 runs using a relaxed clock method (uncorrelated lognormal) to account for discrepancies in branch lengths between lineages. We calculated a (50% majority rule) consensus tree from the 20,000 trees. We considered posterior probabilities above 0.90 to be significant, although we report all values at or above 0.75.

RESULTS

For 3 samples (Table I), the 543–926 and full-length cyt *b* primers amplified different sequences. In BLAST searches (Altschul et al., 1990), the 543–926 sequences obtained for 1 clade were no more than 82% similar to known haemosporidian cyt *b* sequences from *Leucocytozoon*, *Parahaemoproteus*, and *Plasmodium*. These sequences are hereafter referred to as raptor cyt *b* Clade 7 (Fig. 1). Other 543–926 sequences, as well as the full-length cyt *b* sequences, were 99% similar to either *Parahaemoproteus* or *Plasmodium* sequences (not shown). Inclusion of data from both 543–926 and full-length cyt *b* primer sets indicates that Clade 7 is not closely related to other haemosporidian parasite clades, i.e., it is no more closely related to *Leucocytozoon* lineages than it is to *Parahaemoproteus* or *Plasmodium* lineages (Fig. 1).

Ignoring the placement of Clade 7 for now, the remaining raptor parasite lineages fall into either *Parahaemoproteus* or *Plasmodium* (Fig. 1; Table I); most of the sequences from this study, and from previously published raptor parasite lineages (Ishak et al., 2008), are closely related within raptor-specific clades. Within *Parahaemoproteus*, all but 2 raptor parasites fall into 2 raptor-specific, monophyletic clades. One of these, Clade 13, has very high support and is composed of parasites recovered from *Falco sparverius* (American kestrel) and *Tyto alba* (barn owl), while the other, Clade 9, almost exclusively includes owl parasites, with the exception of 1 *Buteo jamaicensis* (red-tailed hawk) parasite. The latter clade is closely related to Clade 8, with 7 raptor sequences, but also contains a duck parasite, *Parahaemoproteus enucleator*, and a parasite associated with Coraciiformes (kingfishers). The remaining 2 raptor *Parahaemoproteus* parasites, both from *Buteo jamaicensis*, are members of a clade including passerine parasites (Clade 12; Fig. 1). Within *Plasmodium*, raptor parasites are not monophyletic, but instead are distributed across several clades (Clades 3, 4, and 6; Fig. 1).

Returning to Clade 7, all members are parasites of *Buteo* species in both North America and Europe, with the exception of 1 sample from a *Circus aeroginosus* (marsh harrier) of Eurasia. Full-length cyt *b* sequences from 3 of these samples differed from the Clade 7 sequences obtained with the 543–926 primers; the full-length sequences align closely with lineages from either *Parahaemoproteus* (Clade 12, 2 cases) or *Plasmodium* (Clade 4, 1 case; Table I; Appendix S1). There is nothing unusual about these primer sequences when comparing them to all raptor parasite sequences. Primer 543F exhibits 2 polymorphic sites; in both cases, the polymorphism is restricted to *Parahaemoproteus*, and both *Plasmodium* and Clade 7 lineages are identical in this region. Primer 926R contains no polymorphic sites across sequences that overlap. In addition, sequences from 3 raptor samples generated with primers HaemF and HaemR1 also closely aligned with *Plasmodium* (Clade 3, 1 case; Appendix S1) and *Parahaemoproteus* (Clades 2 and 13, 2 cases; Appendix S1). All passerine sequences generated with full-length cyt *b* primers (Perkins et al., 2007) and HaemF–HaemR1 (Hellgren et al., 2004) were identical (data not shown).

Contamination of these samples, whether at extraction or during PCR, seems unlikely; the samples in Clade 7 were extracted at different times and, when they were extracted, other samples clearly held either *Plasmodium* or *Parahaemoproteus* parasites. The unusual Clade 7 sequences were repeatable with the 543–926 primers, but other primer sets (from our laboratory and from the literature) were unable to amplify additional, i.e., longer, cyt *b* sequence data from this unusual "lineage." Moreover, *cox* I, ClPC, and trf data all suggest that this clade is divergent from other malaria parasite lineages; Clade 7 samples did not group with either *Plasmodium* or *Parahaemoproteus* in phylogenetic analyses based on data from these 3 other markers (see Fig. S2a, b, c, available online).

DISCUSSION

Our broad sample of raptor haemosporidian parasites has revealed 2 raptor-specific clades (9 and 13) and 1 raptor-dominated clade (8) within *Parahaemoproteus*, several parasite lineages scattered within the *Plasmodium* phylogeny (Clades 3, 4, and 6), plus an additional, previously unrecognized clade that forms a polytomy with *Plasmodium* and *Parahaemoproteus* (Clade 7). Two clades exclusively contain raptor parasites. Even the *Parahaemoproteus* clades of raptor parasites (9 and 13) exhibit host specificity to some degree, i.e., Clade 9 includes exclusively owl parasites (with 1 exception) while Clade 13 includes only American kestrel and barn owl parasites. In addition, 2 *Parahaemoproteus* parasites recovered from red-tailed hawk were from Clade 12, which contains a wide variety of parasites of passerines. Within *Plasmodium*, parasites of raptors do not form exclusive clades, but are distributed in Clades 3, 4, and 6 with the parasites of a wide variety of other bird groups (Fig. 1). Thus, several *Parahaemoproteus* lineages might be raptor specialists. The exclusively raptor Clade 7 is not clearly associated with either *Plasmodium* or with *Parahaemoproteus*, but rather forms a polytomy with these genera.

The ML phylogenetic analysis does not clearly associate Clade 7 with either *Plasmodium* or *Parahaemoproteus*. Krone et al. (2008) found a similarly unplaced parasite lineage that belongs with our Clade 7 (see below). Clade 7 is notable for several reasons. First, in our study, sequences for this clade were generated only by a single set of cyt *b* primers (543–926). These are the only cases from more than 100 samples in which 2 primer sets for cyt *b* have not produced identical sequence (this study; D. Outlaw and R. Ricklefs, unpubl. obs.). Second, the branch leading to this clade is longer than any other branch in the

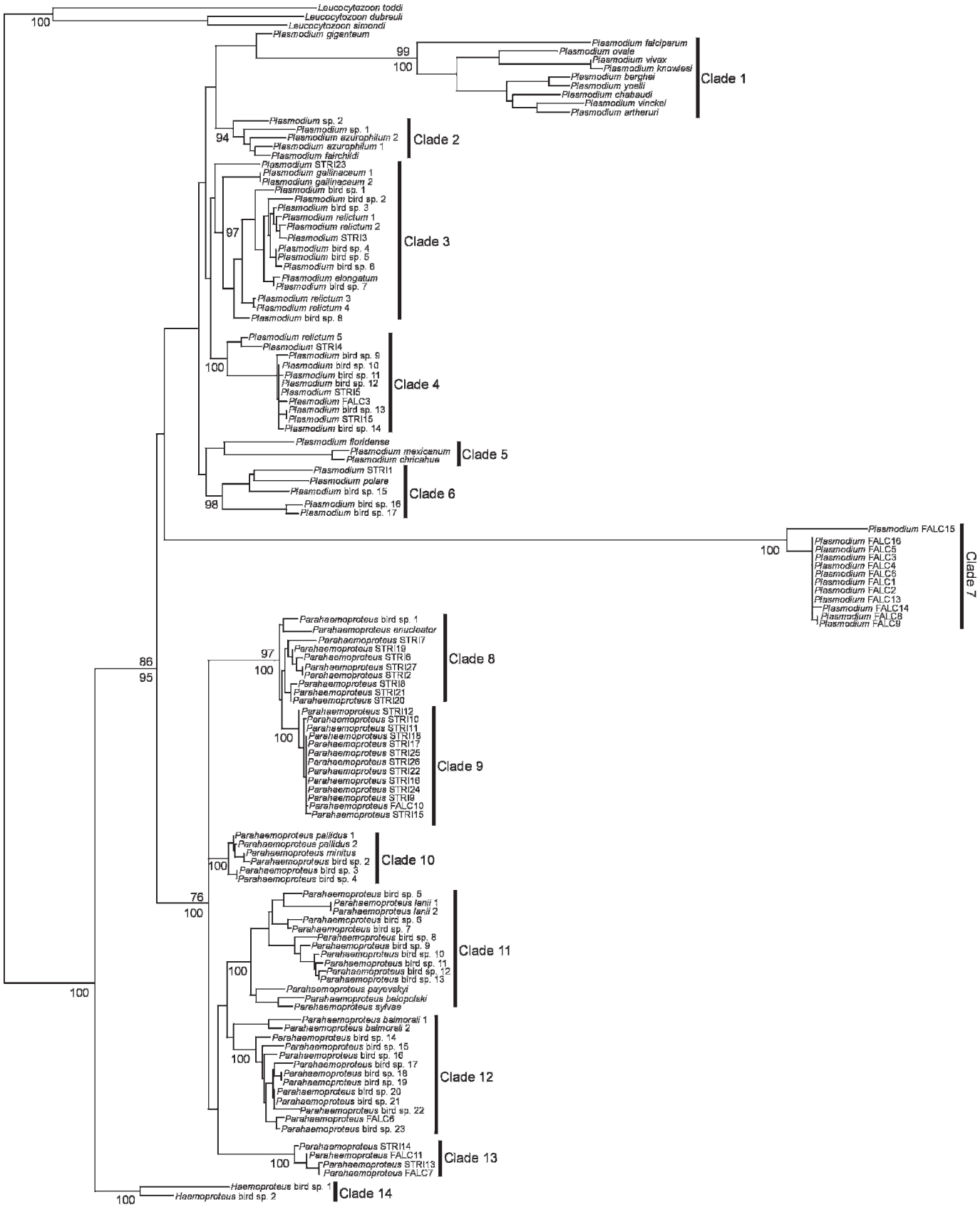

FIGURE 1. Maximum likelihood cytochrome *b* phylogeny of haemosporidian parasite lineages. Maximum likelihood bootstrap values above 0.75 are listed above nodes and Bayesian support values above 0.75 are listed below nodes.

phylogeny, owing to a higher proportion of amino acid changes (Fig. 1; 13 synonymous and 17 nonsynonymous nucleotide substitutions; see also Appendix S2 for amino acid differences). Third, with the exception of 1 *Circus aeroginosus* sample and 1 *Buteo buteo* (common buzzard) sample from Spain, the remaining parasite sequences were obtained from North American *Buteo* samples. It is common for raptor parasite lineages to be found in both the New and Old Worlds (Sehgal et al., 2006), but the host specificity in North America is striking; further sampling of Old World raptors may reveal additional raptor hosts that harbor this parasite lineage. In the case of the divergent *B. buteo* parasite from Germany reported by Krone et al. (2008), within the small region of overlap, Clade 7 *Buteo* sequence data and the Krone et al. (2008) sample are identical in many cases and no more than 2% divergent, whereas across all lineages, divergence values range from 3 to 28%. We suspect that additional data will confirm that this parasite belongs to Clade 7, as does a parasite recovered from *B. buteo* in Spain (FALC16; Table I, Fig. 1).

Amplification of different sequences with different primer sets has several possible explanations including contamination, the amplification of divergent copies of cyt *b* within the parasite's concatenated mitochondrial genome, the presence of nuclear copies of cyt *b*, and multiple infections (which we found in 1 case, i.e., STRI15; Table I). Because these parasites were consistently amplified in many independent trials, we believe we can discount contamination. Morphological identification of these parasites might provide some clarification, but blood smears were not available from these samples.

With regard to amplification of different cyt *b* copies, it is pertinent that the mitochondrial genome of haemosporidian parasites, which contains only 3 genes, i.e., cyt *b*, *cox* I, and *cox* III as well as several remnant tRNAs and rRNA fragments (Vaidya and Arasu, 1987; Vaidya and Mather, 2005), is present in multiple, concatenated copies. Accordingly, different primer sets might amplify cyt *b* copies from different copies of the mitochondrial genome that have undergone divergent evolution. However, this is unlikely simply because, while Clade 7 lineages are monophyletic, i.e., have shared ancestry, sequences generated for the same samples with other primer sets are not—they fall into either *Plasmodium* or *Parahaemoproteus* (Table I; Fig. 1). If Clade 7 lineages represent alternative cyt *b* copies, then we would expect these lineages to have been derived from, i.e., evolved from, the functional copies of the respective lineages to which the parasites seem to belong (from other primer sets), or to have been equally divergent from each other as they are from *Parahaemoproteus* and *Plasmodium* sequences.

Alternatively, the divergent cyt *b* sequence may be a non-transcribed nuclear insert, or numt (Richly and Leister, 2004). However, this partial cyt *b* sequence contains no stop codons, which suggests that the integrity of the protein is being maintained. Moreover, the "alternative cyt *b* copy" argument articulated above also makes numts unlikely as well.

Lacking plausible alternatives, multiple infections seem the most likely explanation for the presence of widely divergent, alternative cyt *b* sequences in several of the samples. However, none of our sequences revealed double peaks; thus, single primer sets did not amplify sequences from more than 1 lineage within the same host. Although Clade 7 differs substantially from the other lineages, other primer sets amplify cyt *b* sequences across the entire range of *Parahaemoproteus* and *Plasmodium*. Multiple infections are commonly detected across avian malaria parasite groups (e.g., Perez-Tris and Bensch 2005; D. Outlaw and R. Ricklefs, unpubl. obs.; D. Santiago-Alarcon et al., unpubl. obs.). Additional data from other markers obtained from Clade 7 cyt *b* lineages are likewise highly divergent from other parasite data, but this does not help to clarify the affiliation of this parasite group (see Fig. S2a, b, c).

Even with the current sampling of *Leucocytozoon*, *Plasmodium*, *Haemoproteus*, and *Parahaemoproteus*, we cannot place Clade 7 within any of these other parasite genera. It is clear that Clade 7 is associated with *Plasmodium* and *Parahaemoproteus* from data for cyt *b* (Fig. 1), *cox* I, and ClPC (see Fig. S2a, b). Lacking outgroup taxa for trf data (see Fig. S2c), we cannot determine the clade's affiliation with this marker. It is surprising that all 3 other markers, i.e., *cox* I, ClPC, and trf, seem to amplify the same novel lineage as the 543–926 cyt *b* primer set. More *cox* I, ClPc, and trf data from raptor parasites, as well as a broad screening of raptor parasites using alternative primer sets of these markers, may shed some light on the phylogenetic position of this clade.

Raptor parasites are extremely diverse, belong to raptor host-specific clades in *Parahaemoproteus* but not in *Plasmodium*, and include a highly divergent, novel parasite lineage (Clade 7). An intriguing result was the dual amplification of cyt *b* sequences belonging to this divergent lineage and to conventional lineages within the same host sample. The highly divergent cyt *b* sequences are supported by equally divergent *cox* I lineages in the mitochondrial genome that place Clade 7 outside of *Parahaemoproteus* and *Plasmodium* and by an apicoplast marker and a nuclear marker that are consistent with the existence of a highly divergent parasite lineage. Although we offer several possible explanations for the dual amplification of the cyt *b* gene, we argue that multiple infection is the most likely explanation. Further investigation of raptor parasites and complete sequencing of mitochondrial genomes may offer insight into the patterns we present here and help to elucidate the taxonomic identity of lineages within Clade 7.

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