The Condor 113(2):385–394

COMPLEX BIOGEOGRAPHIC HISTORY OF *LANIUS* SHRIKES AND ITS IMPLICATIONS FOR THE EVOLUTION OF DEFENSES AGAINST AVIAN BROOD PARASITISM

Brian D. Peer[1,2,3,4], Carl E. McIntosh[1], Michael J. Kuehn[2], Stephen I. Rothstein[2], and Robert C. Fleischer[1]

[1]*Center for Conservation and Evolutionary Genetics, Smithsonian Conservation Biology Institute, National Zoological Park, Smithsonian Institution, P. O. Box 37012 MRC 5503, Washington, DC 20013-7012*
[2]*Department of Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, CA 93106*
[3]*Department of Biological Sciences, Western Illinois University, Macomb, IL 61455*

Abstract. Using portions of three mitochondrial genes to resolve the uncertain systematic relationships, we constructed a phylogeny of the "gray shrikes" in the genus *Lanius*. We used the tree and estimates of the rate of evolution of passeriform mtDNA genes to project the nodes' ages and to assess the pattern and age of egg-rejection behavior in shrikes. Our results suggest that *Lanius excubitor* and *L. m. meridionalis* are sister taxa and that this clade is sister to *L. ludovicianus*, then *L. sphenocercus* is sister to this clade. *Lanius excubitor* from the Old World was considerably diverged from both New World species and part of a clade also containing four other subspecies of *L. meridionalis*. The paraphyly and sequence divergence between New World and Old World *L. excubitor* suggest that these populations represent distinct species. Mapping egg rejection onto the phylogeny suggests that rejection is deeply rooted in shrikes, as it is present as deep as two species, *L. collurio* and *L. bucephalus*, that are outgroups to the gray shrikes and in the derived *L. ludovicianus*. Rejection in *L. ludovicianus* may have been retained as long as 1.1–1.8 million years, since its clade split from the Old World *L. sphenocercus*, providing more evidence that once hosts evolve rejection they retain it for long periods of time. These results suggest hosts are becoming increasingly resistant to brood parasitism, which will force parasites to specialize on a few host species.

Key words: brood parasitism, coevolution, cuckoo, egg rejection, Lanius, *phylogeny, shrike.*

Historia Biogeográfica Compleja de *Lanius* y sus Implicaciones para la Evolución de las Defensas Contra el Parasitismo de las Nidadas de las Aves

Resumen. Utilizando partes de tres genes mitocondriales para resolver las relaciones filogenéticas inciertas, construimos una filogenia de los "especies grises" dentro del género *Lanius*. Utilizamos el árbol y los estimados de la tasa de evolución de los genes del ADNm de los paseriformes para proyectar las edades de los nodos y determinar el patrón y la edad del comportamiento de rechazo de huevos en esta especie. Nuestros resultados sugieren que *Lanius excubitor* y *L. m. meridionalis* son taxones hermanos y que este clado es hermano de *L. ludovicianus*, por ende *L. sphenocercus* es hermano de este clado. *Lanius excubitor* del Viejo Mundo fue considerablemente divergente de las especies del Nuevo Mundo y de parte de un clado que también contenía otras cuatro subespecies de *L. meridionalis*. La parafilia y la divergencia en las secuencias entre *L. excubitor* del Mundo Nuevo y del Viejo Mundo sugieren que estas poblaciones representan especies diferentes. El mapeo del comportamiento de rechazo de huevos en la filogenia sugiere que este comportamiento está arraigado en los linajes más antiguos de *Lanius*, al estar presente en *L. collurio* y *L. bucephalus*, dos especies que son grupos externos de los *Lanius* grises, y en la especie derivada *L. ludovicianus*. El comportamiento de rechazo en *L. ludovicianus* puede haberse mantenido por 1.1–1.8 millones de años, desde su separación del clado de *L. sphenocercus* del Viejo Mundo, dando más evidencia de que una vez que el hospedero desarrolla el comportamiento de rechazo, éste lo mantiene por periodos de tiempo largos. Estos resultados sugieren que los hospederos se están haciendo cada vez más resistentes a los parásitos de nidada, lo que forzará a los parásitos a especializarse en unas pocas especies hospederas.

INTRODUCTION

Avian brood parasites lay their eggs in the nests of other birds and exploit these hosts to raise their offspring. Brood parasitism is costly to hosts, and hosts evolve defenses to counter parasitism (Rothstein 1990, Peer and Sealy 2004a), making the interactions between parasites and hosts among the clearest examples of coevolution (Rothstein 1990). The interactions between shrikes and avian brood parasites are of interest, particularly in North America, where the Loggerhead

Manuscript received 2 April 2010; accepted 14 October 2010.
[4]E-mail: BD-Peer@wiu.edu

 DOI: 10.1525/cond.2011.100066

Shrike (*Lanius ludovicianus*) rejects 100% of Brown-headed Cowbird (*Molothrus ater*) eggs yet is an unlikely host of the cowbird because it breeds earlier than the cowbird and cowbirds risk injury or death if they approach nests of predatory shrikes (see Rothstein 2001). Because of this, Rothstein (2001) suggested that in *L. ludovicianus* rejection is likely a trait retained from ancestors parasitized by cuckoos (Cuculinae) in the Old World. In Europe and Asia, shrikes are common hosts of brood-parasitic cuckoos, which are often larger than shrike hosts and therefore do not face the same risk as cowbirds when parasitizing nests. Thus it is possible egg rejection evolved in the palearctic shrikes and was inherited by their nearctic descendants (see Rothstein 2001, Peer et al. 2005, 2007).

The shrikes (*Lanius*, *Corvinella* spp.) are primarily an Old World group; only two of the 28 known species occur in the New World. *L. excubitor* has a holarctic distribution with at least seven described palearctic subspecies and two described nearctic ones. It, along with *L. ludovicianus*, *L. meridionalis*, and *L. sphenocercus*, form what has sometimes been considered a superspecies, the gray shrikes (Lefranc 1997).

In the New World, *L. excubitor* occurs in the taiga and taiga–tundra ecotone from Alaska to Labrador (Cade and Atkinson 2002). In the Old World, it occurs in the same habitat from Russia and Siberia to northwestern China in the east to Norway and France in the west (Cade and Atkinson 2002). *L. meridionalis* occurs in North Africa, the Middle East, and parts of Asia, with the nominate race in southern France and the Iberian Peninsula (Lefranc 1997). *L. sphenocercus* occurs primarily in northeastern and central China and occupies habitat very similar to that of the Old World *L. excubitor* (Lefranc 1997). *L. ludovicianus* breeds widely in North America from the prairie provinces of Canada south to southern Mexico and from Maryland to Florida west to Washington and Baja California (Yosef 1996).

Mayr (1946) suggested that the New World *L. excubitor* was derived from Siberian stock via the Bering Strait land bridge formed during the Pleistocene. The relationship between *L. excubitor* and *L. ludovicianus* in North America is unclear. *L. ludovicianus* may have evolved earlier from Asian stock or it could have evolved from New World *L. excubitor* by isolation south of glaciation during the Pleistocene or Pliocene (Cade and Atkinson 2002). On the basis of a small sample (128 bp) of the control region of mtDNA, Mundy and Helbig (2004) suggested that the *L. meridionalis* is in the same clade as *L. excubitor*, while *L. ludovicianus* is sister to this clade. Gonzalez et al. (2008) and Klassert et al. (2008) have detailed the evolutionary relationships and genetic variability of *L. meridionalis*, *L. minor*, and *L. excubitor*, along with other outgroup taxa of *Lanius*. These studies independently discovered patterns of paraphyly within two of these taxa. Klassert et al. (2008) assessed only a single mitochondrial DNA locus, while Gonzalez et al. (2008) provided sequence data for two nuclear loci. Particularly unexpected was that subspecies of *L. meridionalis* were split among at least three other clades of *Lanius*. From Genbank, Klassert et al. (2008) and Gonzalez et al. (2008) included only one to three individual sequences of cytochrome *b* of New World *Lanius*, but these taxa provided tantalizing hints that additional cases of paraphyly might arise. One of the goals of our paper is to add further resolution to the phylogeny of *L. excubitor* and *L. ludovicianus*.

Here we provide complementary data on the New World *L. excubitor* and *L. ludovicianus* that are mostly lacking from Gonzalez et al. (2008) and Klassert et al. (2008) and additional geographic coverage of *L. excubitor* in Asia (all previous samples originated from Europe). We combine our larger North American dataset of mitochondrial DNA sequences with the Eurasian sequences from the above studies and find additional support for most of their conclusions, including support for the paraphyly of North American *L. excubitor* with respect to most Eurasian members of the species. We also provide taxonomic recommendations based on these results and use the phylogeny to explain the occurrence of egg rejection behavior in the shrikes and discuss its implications for avian brood parasite–host coevolution.

METHODS

SAMPLES

We sequenced portions of three mitochondrial gene regions, NADH dehydrogenase subunit 2 (ND2), cytochrome *b* (cyt *b*), and ATP synthase subunits 6 and 8 (ATP6/8), for 11 individuals of New World *L. excubitor*, seven of Old World *L. excubitor*, 18 of *L. ludovicianus*, one of *L. sphenocercus*, and one of *L. collurio* and one of *L. schach* as outgroups (Appendix 1). We also used partial cyt *b* sequences from GenBank (Fig. 1) for 37 individuals of *L. meridionalis*, two of *L. sphenocercus*, eight of *L. schach*, five of *L. collurio*, six of *L. bucephalus*, one of *L. nubicus*, one of *L. collaris*, four of *L. senator*, three of *L. minor*, one of *L. tigrinus*, five of *L. isabellinus*, two of *L. tephronotus*, and three of *L. cristatus*, representing 15 of the 28 species recognized in the genus (Clements et al. 2009). Because we have only cyt *b* for these taxa (we were unable to obtain tissues for them), we analyzed two sets of data: one with all taxa but only cyt *b* sequences, the other with all three mtDNA regions but fewer taxa. We obtained tissue samples from much of the breeding range of *L. ludovicianus* (Appendix 1), but all of our North American samples of *L. excubitor* came from wintering birds, collected from Washington State east to Wisconsin and Minnesota. If the birds migrate directly south from their breeding range, this distribution suggests broad coverage of this species also. Our seven Old World samples of *L. excubitor* were collected from Mongolia and Russia (samples used in previous studies are all of European provenance).

DNA EXTRACTION AND SEQUENCING

We extracted tissues or blood samples for genomic DNA by following the Dneasy Tissue Kit protocol (Qiagen, Inc., Valencia, CA) but extended the proteinase-K soak at 50 °C until

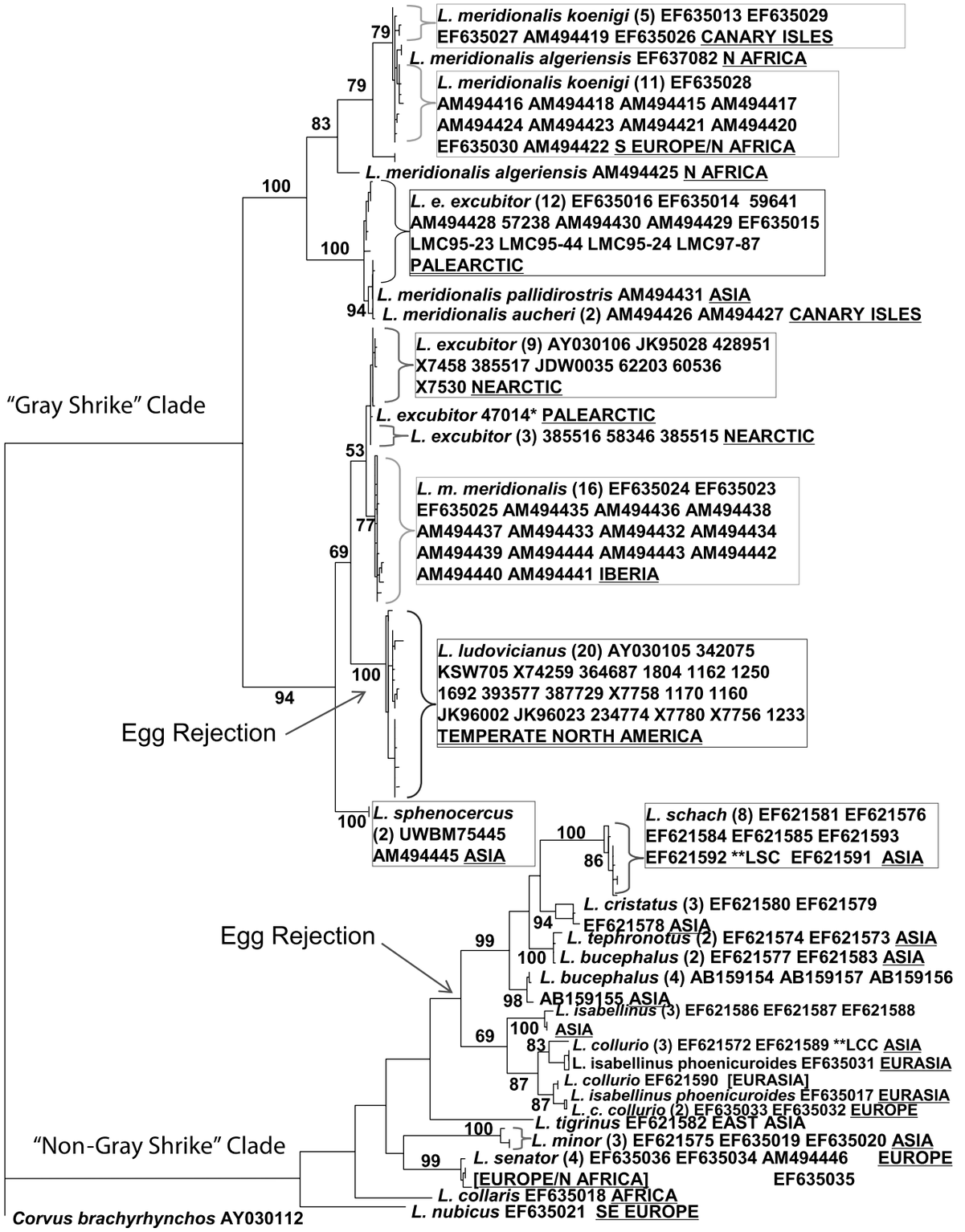

FIGURE 1. Best maximum-likelihood tree produced by RAxML from cyt *b* sequences of shrikes. Names with Genbank acquisition numbers are from prior studies. Bootstrap-support values from a 100-replicate bootstrap are included at the nodes, if above 50%. Clades in which rejection is known are indicated.

samples were fully dissolved in solution. We ran a 1.0% agarose gel (Amresco, Inc., Solon, OH) to check the quantity and quality of isolated DNA for PCR.

We amplified from DNA samples with 0.5 units of AmpliTaq DNA polymerase (Applied Biosystems, Foster City, CA) in a 25-μL PCR reaction containing 2-mM $[Mg]^{++}$ buffer and 200 μM each of the deoxynucleotide triphosphates (dNTPs) Primers used were CYTB-H16065 (Helm-Bychowski and Cracraft 1993), CYTB1 (Kocher et al. 1989), ND2-5215, ND2-6113 (Slikas et al. 2000), ATP6-R1 (Fleischer et al. 2006), and A6-GQL (Lovette et al. 1998). All amplifications ran under the following conditions: 30 cycles of denaturation at 94 °C for 30 sec, annealing at 50 °C for 30 sec, and extension at 72 °C for 60 sec. We ran a 2.0% agarose gel to check the yield of PCR product. PCR products were cleaned of excess primers and dNTPs with Qiaquick PCR purification kit (Qiagen). We did standard Sanger dideoxy sequencing, cleaned the sequencing reaction's products with Sephadex, and analyzed them on a 3100 Genetic Analyzer (Applied BioSystems).

DATA ANALYSIS

We aligned and verified sequences with Sequencher 3.1.1 (Gene Codes Corporation) and exported them into Nexus files. Cyt *b* sequence alone was used for a second analysis, which included our sequences and others obtained from GenBank (as described above; see Appendix 1, Fig. 1). We checked sequences for abnormalities such as gaps, double sequences, or unexpected stop codons, potentially indicating nuclear pseudogenes (Sorenson and Fleischer 1996). Use of multiple fragments and genes reduces the likelihood of amplification of nuclear copies and increases probability of detection. We estimated variation at the three loci within the three taxa for which we had adequate samples: nearctic *L. excubitor* and *L. ludovicianus* and palearctic *L. excubitor*. We used Arlequin version 2.000 (Schneider et al. 2000) to calculate haplotype diversity (*h*), nucleotide diversity, mismatch distributions, and Tajima and Fu's "neutrality tests" to assess the possibility of population change.

We applied two different optimality criteria to reconstruct phylogenies—maximum parsimony (using PAUP*; Swofford 2003) and maximum likelihood (using RAxML; Stamatakis et al. 2008). Trees were subjected to 1000 replicate bootstraps for parsimony and 100 replicate bootstraps for maximum likelihood. As noted above, we ran two separate tree analyses: a total-evidence analysis for the three gene regions combined for a smaller set of taxa and an analysis of the cyt *b* sequences only including the taxa obtained from earlier studies downloaded from Genbank.

We estimated timing of cladogenic events with a relaxed clock analysis in BEAST (Drummond and Rambaut 2007). We selected a Yule tree prior (appropriate for evaluating phylogenies of distinct species). We used a empirical nucleotide frequencies and a general time reversible plus invariant sites plus gamma parameter (GTR + I + G) model of sequence evolution. We ran one chain with 10 000 000 iterations, and discarded a 10% burn-in to ensure that parameter estimates and posterior probabilities had converged (in Tracer version 1.4.1, Drummond and Rambaut 2007). We used a rate of cyt *b* sequence evolution of 2% per million years (MY) as a prior, in accordance with the rate estimated from multiple avian taxa in the meta-analysis of Weir and Schluter (2008). We assumed a normally distributed prior for the rate and applied a standard error that resulted in 95% of the rates falling between about 1.5 and 2.5% divergence per MY. For the combined analysis, we applied a calibration based on ages of the Hawaiian Islands that included all three genes (cyt *b*, ND2, ATP6/8) from Hawaiian honeycreepers (Fleischer et al., unpubl. data). The combined rates were averaged from penalized-likelihood and nonparametric rate-smoothing estimates and resulted in a mean rate of about 3.4% sequence divergence per MY, with a range from about 3.0 to 4.1% per MY. Thus we used in BEAST a normally distributed prior of 3.4% per MY and a standard error that resulted in a 95% credibility interval from about 2.9 to 3.9% per MY.

RESULTS

DNA SEQUENCES

We obtained up to 2781 bp of sequence (ND2, 871 bp; cyt *b*, 1143 bp; ATP6/8, 767 bp) from New and Old World *L. excubitor*, *L. ludovicianus*, *L. sphenocercus*, *L. collurio*, and *L. schach*, but we trimmed regions with missing data to total concatenated sequence of up to 2502 bp for most taxa. These sequences have been submitted to Genbank.

Estimates of genetic variability within each of the well-sampled taxa (New and Old World *L. excubitor*, *L. ludovicianus*) are presented in Table 1, including the number of haplotypes, haplotype diversity (*H*), and nucleotide diversity (π). Nucleotide diversity was highest for the Old World *L. excubitor*, reflecting what appears to be a relatively wide divergence between the Russian and Mongolian samples. In addition, values of Tajima's *D* and Fu's F_S are significantly negative for the New World *L. excubitor* and *L. ludovicianus*, likely indicating a relatively recent population expansion. There was very little sequence variation or evidence of genetic structuring in all the three mtDNA regions of either *L. ludovicianus* or *L. excubitor* across their North American ranges.

PHYLOGENY RECONSTRUCTION

We ran two phylogenetic analyses, first of the cyt *b* dataset with the broad taxonomic sample that includes sequences from other studies (Fig. 1), and second of the limited taxonomic sample (six species) for which we have sequences of all three genes (Fig. 2). Analyses based on cyt *b* sequence (Fig. 1) reveal a complicated picture but generally support the relationships suggested by Klassert et al. (2008) and Gonzalez et al. (2008). The cyt *b* dataset analysis produces a maximum-likelihood

TABLE 1. Statistics of genetic variation in the three mtDNA loci (2502 bp) and tests of neutrality, by analysis with the program Arlequin version 2.000 (Schneider et al. 2000). NA, North America; Eur, Eurasia; *n*, the number of individuals sequenced; no. haps, number of unique haplotypes found; *H*, haplotype or gene diversity; π, nucleotide diversity. Tajima's *D* and Fu's F_S are interpreted in terms of population change.

Species	Region	*n*	No. haps	*H* (SE)	π (SE)	Tajima's *D*	Fu's F_S
L. excubitor	NA	11	11	1.00 (0.04)	0.0014 (0.0009)	−2.29	−4.17**
L. excubitor	Eur	7	7	1.00 (0.08)	0.0170 (0.0097)	−1.84	−0.49
L. ludovicianus	NA	18	18	0.0017 (0.0010)	0.0017 (0.0010)	−2.22	−1.89

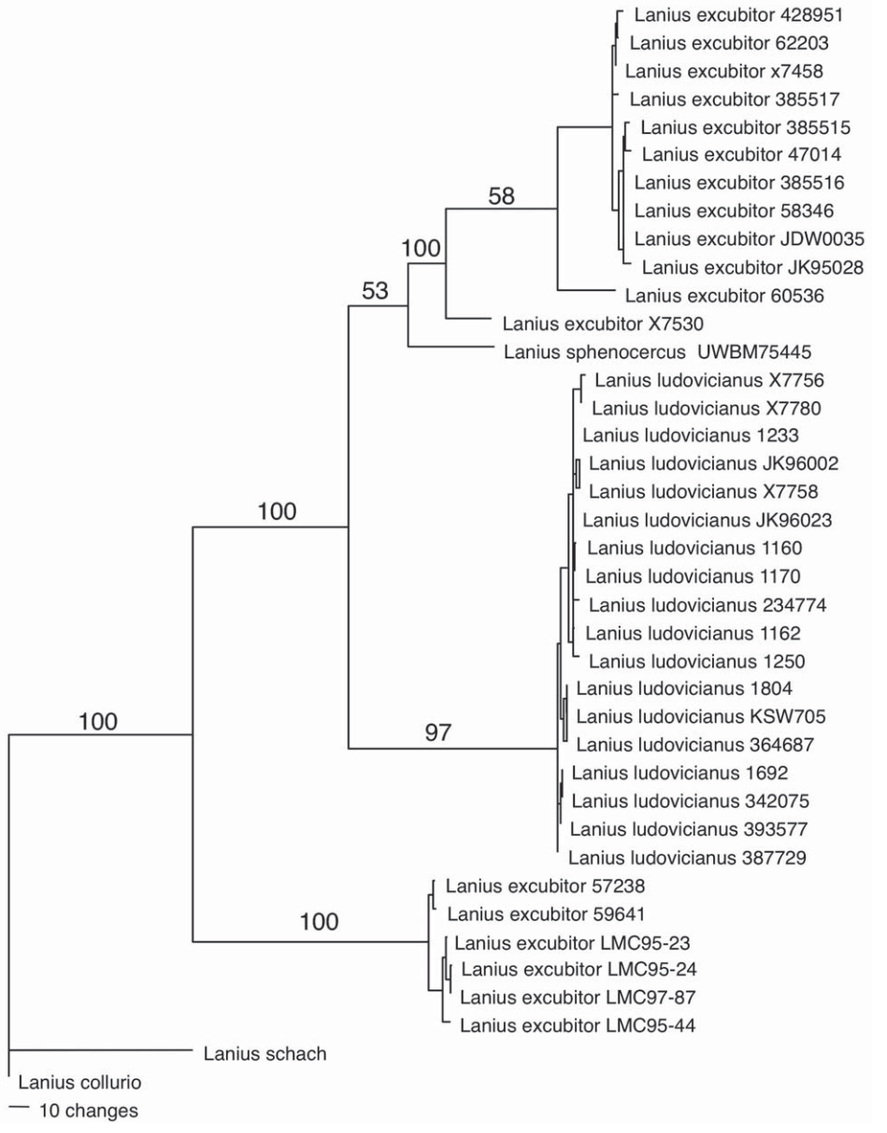

FIGURE 2. Best maximum-likelihood tree produced by RAxML from cyt *b*, ND2, and ATP6/8 sequences for six shrike taxa. Bootstrap-support values from a 100-replicate bootstrap are included at the nodes, if above 50%.

TABLE 2. Times (in MYA) to a most recent common ancestor, with 95% credibility intervals, for selected nodes in the cyt *b* and total-evidence trees, estimated in BEAST (Drummond and Rambaut 2007).

Comparison	Average time to a most recent common ancestor	95% credibility interval[a]
Cyt *b* only		
Gray clade vs. others	3.61	—
Old World vs. New World *L. excubitor*	2.53	1.32–3.15
New World *L. excubitor* vs. *L. m. meridionalis* (Iberia)	1.24	0.45–1.38
L. ludovicianus vs. *New World L. excubitor/L. meridionalis clade*	1.76	0.70–1.91
Old World *L. excubitor vs. L. meridionalis* clades	2.32	0.84–2.26
L. sphenocercus vs. New World *Lanius*	2.09	0.85–2.34
L. collurio vs. *L. bucephalus* clades	1.76	1.29–3.41
L. minor vs. "African" clade	2.21	0.92–.88
Cyt *b*, ND2, ATP6/8		
Gray clade vs. others	3.49	—
Old World vs. New World *L. excubitor*	1.96	0.84–2.15
L. sphenocercus vs. New World *Lanius*	1.15	0. 43–1.25

[a]Dashes indicate BEAST was unable to resolve a confidence interval for this node.

tree (Fig. 1) with good bootstrap support for two major clades: one containing the "gray shrikes" (largely Northern Hemisphere in distribution), and a second "non-gray shrike" clade containing all of the other, strictly Old World taxa. The four "species" of gray shrikes are split into two well-differentiated subclades with a high degree of paraphyly. One subclade contains sequences from the New World *L. excubitor* and one *L. m. meridionalis* found on the Iberian peninsula. *L. ludovicianus* is a poorly supported sister taxon to this clade, and *L. sphenocercus* is the basal sister to the rest within this subclade. The other subclade is markedly paraphyletic, with the two subspecies *L. m. pallidirostris* and *L. m. aucheri* nested within the clade of Old World *L. excubitor*. In addition, a single sample (UWM 47014) of *L. excubitor* from Sakhalin Island was not in this clade but in the clade containing New World *L. excubitor*. We reanalyzed this sample to confirm that no mistakes were made during processing, and all three genes were in concordance. Additional samples from this region are needed to determine if this New World haplotype is representative of the *L. excubitor* in this region or if the sample came from a vagrant.

DATING ESTIMATES

We analyzed the cyt *b* and total-evidence datasets in BEAST (Drummond and Rambaut 2007), producing a large set of parameter and tree estimates. We then analyzed the estimates of model parameters in Tracer 1.4.1 (Drummond and Rambaut 2007) and generally obtained effective sample sizes >100, indicating that we had sampled model parameters adequately. We assessed the average ages of nodes and their 95% credibility intervals with Tree Annotator V1.5beta (Drummond and Rambaut 2007). This analysis calculates the age of the most recent common ancestor (MRCA) of two taxa. Results for both cyt *b* and total-evidence datasets revealed that the radiation of *Lanius* shrikes is largely a Pliocene and Pleistocene phenomenon. The split from an outgroup including *Corvus* was estimated at about 5.1 MYA, and the earliest coalescence within *Lanius* occurred about 3.6 MYA (Table 2).

DISCUSSION

BIOGEOGRAPHY AND SPECIES-LEVEL SYSTEMATICS

Our cyt *b* tree supports the previous finding of paraphyly in *L. meridionalis* and *L. excubitor*. In this case New World *L. excubitor* is sister to *L. sphenocercus* and then *L. ludovicianus*. The Old World samples of *L. excubitor*, with the exception of one bird from Sakhalin Island (47014), form a basal clade, and bootstrap support for these relationships is generally strong. The paraphyly and level of sequence divergence between New World and Old World *L. excubitor* suggest that they represent distinct species. These results indicate one of two possible scenarios for shrikes colonizing North America. The first is that an ancestral shrike from Eurasia colonized North America possibly from the current range of *L. meridionalis* and diverged to become *L. ludovicianus*. A second colonization occurred relatively recently, again from the Eurasian *L. meridionalis*, to northern North America, and the population diverged to become the New World population of *L. excubitor*. However, this would require two independent colonizations of North America from Eurasia. The second and perhaps more parsimonious scenario is that an ancestral shrike colonized North America, possibly from the current range of *L. sphenocercus*, split through vicariance from glaciation or other geological processes into *L. ludovicianus* and *L. excubitor*, and then a subsequent colonization of Europe across the Atlantic by *L. excubitor* gave rise to *L. meridionalis*.

Our results, in combination with those of Gonzalez et al. (2008) and Klassert et al. (2008), suggest that the taxonomy of the gray shrikes requires revision, although we caution that additional data (e.g., nuclear DNA, morphology, behavior)

should be obtained before such changes are made. Nonetheless, the paraphyly and divergence we found suggest the following changes may be warranted: (1) *Lanius meridionalis meridionalis*, resident of the Iberian Peninsula, should be elevated to species status. Our data indicate that it is most closely related to the North American form (*borealis*) of *L. excubitor* and not a member of the clade comprising the other populations that have been classified as subspecies of *L. meridionalis*. (2) The New World and Old World forms of *L. excubitor* are not each other's closest relatives. Each of these clades deserves species status (pending greater resolution of relationships of *L. excubitor* across Eurasia, and with certain subspecies of *L. meridionalis*). (3) *L. m. pallidirostris*, *L. m. algeriensis*, *L. m. aucheri*, and *L. m. koenigi*, currently included in *L. meridionalis*, should be reviewed and perhaps assigned to different species. According to these results, it is likely that the *Lanius* [*excubitor*] superspecies proposed by Lefranc (1993) and Panov (1995) should also be modified.

IMPLICATIONS FOR BROOD PARASITE–HOST COEVOLUTION

To our knowledge, all shrikes that have been tested reject foreign eggs, and mapping rejection onto the phylogeny (Fig. 1) suggests that rejection is deeply rooted in the shrikes. Not only does *L. ludovicianus* reject 100% of experimental parasitic eggs (Rothstein 2001), but *L. collurio* and *L. bucephalus*, outside the group of gray shrikes, reject 93% and 41% of cuckoo eggs, respectively (Nakamura et al. 1998, Lovászi and Moskát 2004). Given the results of similar studies indicating that rejection may evolve once in a lineage and then be retained in descendent species (Rothstein 2001, Peer and Sealy 2004b, Peer et al. 2005, 2007), it is possible that New and Old World *L. excubitor*, *L. sphenocercus*, *L. meridionalis*, *L. schach*, and others in these lineages are also rejecters (see Fig. 1). Experimental parasitism of additional species of *Lanius* is warranted to determine whether they too demonstrate rejection.

The shrikes are primarily an Old World family and are regularly parasitized by cuckoos. *L. schach*, *L. collaris*, *L. cristatus*, *L. bucephalus*, *L. tigrinus*, *L. collurio*, *L. minor*, and *Corvinella corvina* have been recorded as hosts of nine species of cuckoos (see Lowther 2010). In contrast, New World shrikes are rarely if ever parasitized by cowbirds. Indeed, only three cases of parasitism have been recorded, all from a single study of *L. ludovicianus* (DeGeus and Best 1991). *L. excubitor* breeds in allopatry from the cowbird. As Rothstein (2001) has suggested, rejection in New World *Lanius* likely originated from cuckoo parasitism. Only one other known selection pressure could result in the evolution of rejection, albeit rarely, and that is conspecific brood parasitism (reviewed in Peer and Sealy 2000). Shrikes lack the characteristics of conspecific brood parasites (e.g., colonial, cavity nesters, waterfowl) and they are not known to practice conspecific brood parasitism (LeFranc 1997), which suggests that this behavior cannot account for the expression of egg rejection in this family. Furthermore, a DNA fingerprinting study of *L. bucephalus* found no evidence of conspecific brood parasitism (Yamagishi et al. 1992).

Our data also suggest that *L. ludovicianus* has maintained rejection for a relatively long time in the absence of parasitism, since it colonized the New World. Our combined gene and cyt *b* BEAST analyses suggest that *L. ludovicianus* and *L. sphenocercus* diverged 1.1–1.8 MYA (Table 2), raising the possibility that rejection has been maintained for this period in the absence of cuckoo parasitism in the New World. However, New World shrikes could have been parasitized during this period by a brood parasite that has gone extinct. Also, the Brown-headed Cowbird was likely much more common during the Pleistocene when mammalian megafauna were present (Peer et al. 2005, Rothstein and Peer 2005). Nevertheless, our data clearly suggest that rejection can be retained for a long time in the absence of parasitism.

These data and those from similar studies (Nakamura et al. 1998, Rothstein 2001, Peer and Sealy 2004b, Lahti 2006, Peer et al. 2005, 2007) indicate that a host's egg rejection is maintained for long periods in the absence of selection pressure from brood parasitism, presumably because its cost is minimal or even zero. This maintenance of rejection has significant implications for brood parasite–host coevolution because it suggests that hosts are becoming increasingly resistant to brood parasitism. This "single trajectory" model predicts that parasites will have fewer options as more and more hosts evolve and retain defenses and will eventually be forced to evolve specific adaptations for one or a few host species (e.g., mimetic eggs; Rothstein 2001). An alternative to this "single trajectory" model, is that parasites follow a "coevolutionary cycles" model and alternate from currently well-defended hosts to old hosts that have lost defenses, provided that hosts tend to lose rejection in the absence of parasitism because of costs or genetic drift (Rothstein 2001). Possible costs to hosts include rejecting their own oddly colored egg, for hosts whose eggs vary widely, but this appears to be rare in North American hosts (but see Peer and Bollinger 1997, Peer and Sealy 2004b, Peer and Rothstein 2010). Overall, most evidence indicates that rejection has low costs in the absence of brood parasitism and supports the long-term maintenance of rejection rather than its loss (summarized in Rothstein 2001, Peer et al. 2005, 2007).

ACKNOWLEDGMENTS

We thank the following institutions and individuals for providing tissue samples used in this study: Burke Museum, University of Washington; American Museum of Natural History; University of Minnesota Bell Museum; Field Museum of Natural History; Natural History Museum, University of Kansas; University of Michigan Museum of Zoology, and Lori Eggert and Nick Mundy. Two anonymous reviewers provided comments that improved the manuscript. This research was supported by National Science Foundation grant 0078139 awarded to SIR, RCF, and BDP, and BDP and RCF were supported by a Faculty Mentoring grant from Western Illinois University while preparing the manuscript.

LITERATURE CITED

CADE, T. J., AND E. C. ATKINSON. 2002. Northern Shrike (*Lanius excubitor*), no. 671. *In* A. Poole and F. Gill [EDS.], The birds of North America. The Birds of North America, Inc., Philadelphia.

CLEMENTS, J. F., T. S. SCHULENBERG, M. J. ILIFF, B. L. SULLIVAN, AND C. L. WOOD [ONLINE]. 2009. The Clements checklist of birds of the world, version 6.4. <http://www.birds.cornell.edu/clementschecklist/Clements%206.4.xls/view> (12 October 2010).

DEGEUS, D. W., AND L. B. BEST. 1991. Brown-headed Cowbirds parasitize Loggerhead Shrikes: first records for family Laniidae. Wilson Bulletin 103:504–506.

DRUMMOND A. J., AND A. RAMBAUT. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evolutionary Biology 7:214.

FLEISCHER, R. C., J. J. KIRCHMAN, J. P. DUMBACHER, L. BEVIER, C. DOVE, N. C. ROTZEL, S. V. EDWARDS, M. LAMMERTINK, K. MIGLIA, AND W. S. MOORE. 2006. Mid-Pleistocene divergence of Cuban and North American Ivory-billed Woodpeckers. Biology Letters 2:466–469.

GONZALEZ, J., M. WINK, E. GARCIA-DEL-REY, AND G. D. CASTRO. 2008. Evidence from DNA nucleotide sequences and ISSR profiles indicates paraphyly in subspecies of the Southern Grey Shrike (*Lanius meridionalis*). Journal of Ornithology 149:495–506.

HELM-BYCHOWSKI, K., AND J. CRACRAFT. 1993. Recovering phylogenetic signal from DNA sequences: relationships within the corvine assemblage (class Aves) as inferred from complete sequences of the mitochondrial DNA cytochrome-*b* gene. Molecular Biology and Evolution 10:1196–1214.

KLASSERT, T. E., M. ÁNGELES HERNÁNDEZ, F. CAMPOS, O. INFANTE, T. ALMEIDA, N. M. SUÁREZ, J. PESTANO, AND M. HERNÁNDEZ. 2008. Mitochondrial DNA points to *Lanius meridionalis* as a polyphyletic species. Molecular Phylogenetics and Evolution 47:1227–1231.

KOCHER, T. D., W. K. THOMAS, A. MEYER, S. V. EDWARDS, S. PÄÄBO, F. X. VILLABLANCA, AND A. C. WILSON. 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. Proceedings of the National Academy of Sciences U.S.A. 86:6196–6200.

LAHTI, D. C. 2006. Persistence of egg recognition in the absence of cuckoo brood parasitism: pattern and mechanism. Evolution 60:157–168.

LEFRANC, N. 1997. Shrikes: a guide to the shrikes of the world. Pica Press, Sussex, England.

LOVÁSZI, P., AND C. MOSKÁT. 2004. Break-down of arms race between the Red-backeD Shrike (*Lanius collurio*) and Common Cuckoo (*Cuculus canorus*). Behaviour 141:245–262.

LOVETTE, I. J., BERMINGHAM, E., SEUTIN, G., AND RICKLEFS, R. E. 1998. Evolutionary differentiation in three endemic West Indian warblers. Auk 115:890–903.

LOWTHER, P. E. [ONLINE]. 2010. Host list of avian brood parasites—2—Cuculiformes; Cuculidae. http://fm1.fieldmuseum.org/aa/Files/lowther/OWcList.pdf (12 October 2010).

MAYR, E. 1946. The history of North American bird fauna. Wilson Bulletin 58:3–41.

MUNDY, N., AND A. J. HELBIG. 2004. Origin and evolution of tandem repeats in the mitochondrial DNA control region of shrikes (*Lanius* spp.). Journal of Molecular Evolution 59:250–257.

NAKAMURA, H., S. KUBOTA, AND R. SUZUKI. 1998. Coevolution between the Common Cuckoo and its major hosts in Japan, p. 94–112. *In* S. I. Rothstein and S. K. Robinson [EDS.], Parasitic birds and their hosts: studies in coevolution. Oxford University Press, New York.

PANOV, E. N. 1995. Superspecies of shrikes in the former USSR, p. 26–33. *In* R. Yosef and F. E. Lohrer [EDS.], Shrikes (Laniidae) of the world: biology and conservation. Proceedings of the Western Foundation of Vertebrate Zoology 6.

PEER, B. D., AND E. K. BOLLINGER. 1997. Explanations for the infrequent cowbird parasitism on Common Grackles. Condor 99:151–161.

PEER, B. D., AND S. I. ROTHSTEIN. 2010. Phenotypic plasticity in Common Grackles (*Quiscalus quiscula*) in response to repeated brood parasitism. Auk 127:293–299.

PEER, B. D., S. I. ROTHSTEIN, K. S. DELANEY, AND R. C. FLEISCHER. 2007. Defence behaviour against brood parasitism is deeply rooted in mainland and island scrub-jays. Animal Behaviour 73:55–63.

PEER, B. D., S. I. ROTHSTEIN, M. J. KUEHN, AND R. C. FLEISCHER. 2005. Host defenses against cowbird (*Molothrus* spp.) parasitism: implications for cowbird management. Ornithological Monographs 57:84–97.

PEER, B. D., AND S. G. SEALY. 2000. Conspecific brood parasitism and egg rejection in Great-tailed Grackles. Journal of Avian Biology 31:271–277.

PEER, B. D., AND S. G. SEALY. 2004a. Correlates of egg rejection in hosts of the Brown-headed Cowbird. Condor 106:580–599.

PEER, B. D., AND S. G. SEALY. 2004b. Fate of grackle (*Quiscalus* spp.) defenses in the absence of brood parasitism: implications for long-term parasite–host coevolution. Auk 121:1172–1186.

ROTHSTEIN, S. I. 1990. A model system for coevolution: avian brood parasitism. Annual Review of Ecology and Systematics 21:481–508.

ROTHSTEIN, S. I. 2001. Relic behaviours, coevolution and the retention versus loss of hosts defences after episodes of avian brood parasitism. Animal Behaviour 61:95–107.

ROTHSTEIN, S. I., AND B. D. PEER. 2005. Conservation solutions for threatened and endangered cowbird (*Molothrus* spp.) hosts: separating fact from fiction. Ornithological Monographs 57:98–114.

SCHNEIDER, S., D. ROESSLI, AND L. EXCOFFIER. 2000. ARLEQUIN version 2.000: a software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Geneva, Switzerland.

SLIKAS, B., JONES, I. B., DERRICKSON, S. R. AND FLEISCHER, R. C. 2000. Phylogenetic relationships of Micronesian white-eyes based on mitochondrial sequence data. Auk 117: 355–365.

SORENSON, M. D., AND R. C. FLEISCHER. 1996. Multiple independent transpositions of mitochondrial DNA control region sequences to the nucleus. Proceedings of the National Academy of Sciences, U.S.A. 93:15239–15243.

STAMATAKIS, A., P. HOOVER, AND J. ROUGEMONT. 2008. A rapid bootstrap algorithm for the RAxML web-servers. Systematic Biology 75:758–771.

SWOFFORD, D. L. 2003. PAUP*. Phylogenetic analysis using parsimony (*and other methods), version 4. Sinauer Associates, Sunderland, MA.

WEIR, J. T., AND D. SCHLUTER. 2008. Calibrating the avian molecular clock. Molecular Ecology 17:2321–2328.

YAMAGISHI, S., I. NISHIUMI, AND C. SHIMODA. 1992. Extrapair fertilization in monogamous Bull-headed Shrikes revealed by DNA fingerprinting. Auk 109:711–721.

YOSEF, R. 1996. Loggerhead Shrike (*Lanius ludovicianus*), no. 231. *In* A. Poole and F. Gill [EDS.] The birds of North America. Academy of Natural Sciences, Philadelphia.

APPENDIX 1. Collection localities and specimen data for tissue samples of *Lanius* used in this study.

Taxon[a]	Locality	Museum catalog number[b]	GenBank number[c]
L. ludovicianus mexicanus			
	California		AY030105
L. ludovicianus			
	U.S.A.		X74259
	Dakota Co., Minnesota	UMBM X7756	
	Minnesota	UMBM X7758	
	St. Croix, Co., Wisconsin	UMBM X7780	
	San Patricio Co., Texas	UMBM JK96002, UMBM JK96023	
	Clay Co., Minnesota	UMBM KSW705	
	Platte Co., Missouri	KU 1692	
	Comanche Co., Kansas	KU 1804	
	Brewster Co., Texas	UMMMZ 234774	
	Arizona	AMNH GFB1160, AMNH GFB1170, AMNH GFB1250	
	New Mexico	AMNH GFB1162, AMNH GFB1233	
L. ludovicianus gambeli			
	San Diego Co., California	FMNH 342075	
L. ludovicianus ludovicianus			
	Highlands Co., Florida	FMNH 364687, FMNH 387729, FMNH 393577	
L. excubitor borealis			
	Itasca City, Minnesota	FMNH 385515	
	Lake Co., Minnesota	FMNH 385516	
	Sherburne Co., Minnesota	FMNH 385517	
	Price Co., Wisconsin	FMNH 428951	
L. excubitor			
	Cook Co., Minnesota	UMBM JDW0035	
	Clearwater Co., Minnesota	UMBM JK95028	
	Mille Lacs Co., Minnesota	UMBM X7458	
	Minnesota	UMBM X7530	
	Kittitas Co., Washington	UWBM 58346	
	Grant Co., Washington	UWBM 60536, UWBM 62203	
	Minnesota		AY030106
L. excubitor bianchii			
	Sakhalin Province, Russia	UWBM 47014	
L. excubitor			
	Yamal–Nenets Autonomous District, Russia	UWBM 59641	
	Moscow Province, Russia	UWBM 57238	
	Mongolia	AMNH LMC95-23, AMNH LMC95-24, AMNH LMC95-44, AMNH LMC97-87	
L. excubitor excubitor			
	Poland		EF635016, EF635014, AM494430, EF635015
	Poland		AM494428
	Hungary		AM494429
L. meridionalis pallidirostris			
	Kazahkstan		AM494431
L. meridionalis koenigi			
	Canary Islands		EF635013, EF635027, EF635026, AM494419, EF635028, AM494416, AM494418, AM494415, AM494417, AM494424, AM494423, AM494421, AM494420, EF635030, AM494422, EF635029
L. meridionalis algeriensis			
	Tunisia		EF637082
	Algeria		AM494425

(*continued*)

APPENDIX 1. Continued.

Taxon[a]	Locality	Museum catalog number[b]	GenBank number[c]
L. meridionalis aucheri			
	Israel		AM494426, AM494427
L. meridionalis meridionalis			
	Spain	EF635024	EF635024, EF635023, EF635025, AM494435, AM494436, AM494938, AM494437, AM494433, AM494432, AM494434, AM494439, AM494444, AM494443, AM494442, AM494440, AM494441
L. sphenocercus sphenocercus			
	Eastern Russia		AM494445
	Primorskiy Kray, Russia	UWBM 75445	
L. schach			
	China		EF621581, EF621576, EF621584, EF621585, EF621593, EF621592, EF621591
	Hong Kong	Lsc[d]	
L. cristatus			
	China		EF621580, EF621579, EF621578
L. tephronotus			
	China		EF621574, EF621573
L. bucephalus			
	China		EF621577, EF621583
L. bucephalus bucephalus			
	Japan		AB159154, AB159155
	Korea		AB159157, AB159156
L. isabellinus			
	China		EF621586, EF621587, EF621588
L. isabellinus phoenicuroides			
	Kazakhstan		EF635031, EF635017
L. collurio			
	China		EF621572, EF621589, EF621590
	Italy	Lcc[d]	
L. collurio collurio			
	Turkey		EF635033
	Germany		EF635032
L. tigrinus			
	China		EF621582
L. minor			
	China		EF621575
	Greece		EF635019, EF635020
L. senator			
	Greece		EF635036
	France		EF635034
	Spain		AM494446
	Israel		EF635035
L. collaris			
	South Africa		EF635018
L. nubicus			
	Israel		EF635021
Corvus brachyrhynchos			
			AY030112

[a]Identifications to subspecies (or not) are as given to us by the sources, the reason for inconsistency.
[b]AMNH, American Museum of Natural History; UMBM, University of Minnesota Bell Museum of Natural History; UWBM, University of Washington Burke Museum; UMMZ, University of Michigan Museum of Zoology; FMNH, Field Museum of Natural History; KU, University of Kansas Natural History Museum.
[c]Sequences have been submitted to GenBank.
[d]Samples from Mundy and Helbig (2004).