Hybrid speciation leads to novel male secondary sexual ornamentation of an Amazonian bird

Alfredo O. Barrera-Guzmán, Alexandre Aleixo, Matthew D. Shawkey, and Jason T. Weir

Hybrid speciation is rare in vertebrates, and reproductive isolation arising from hybridization is infrequently demonstrated. Here, we present evidence supporting a hybrid-speciation event involving the genetic admixture of the snow-capped (Lepidothrix nattereri) and opal-crowned (Lepidothrix iris) manakins of the Amazon basin, leading to the formation of the hybrid species, the golden-crowned manakin (Lepidothrix vilasboasi). We used a genome-wide SNP dataset together with analysis of admixture, population structure, and coalescent modeling to demonstrate that the golden-crowned manakin is genetically an admixture of these species and does not represent a hybrid zone but instead formed through ancient genetic admixture. We used spectrophotometry to quantify the coloration of the species-specific male crown patches. Crown patches are highly reflective white (snow-capped manakin) or iridescent whitish-blue to pink (opal-crowned manakin) in parental species but are a much less reflective yellow in the hybrid species. The brilliant coloration of the parental species results from nanostructural organization of the keratin matrix feather barbs of the crown. However, using electron microscopy, we demonstrate that the structural organization of this matrix is different in the two parental species and that the hybrid species is intermediate. The immediate nature of the crown barbs, resulting from past admixture appears to have rendered a duller structural coloration. To compensate for reduced brightness, selection apparently resulted in extensive thickening of the carotenoid-laden barb cortex, producing the yellow crown coloration. The evolution of this unique crown-color signal likely culminated in premating isolation of the hybrid species from both parental species.

Significance

Hybridization between species can produce reproductively isolated lineages by combining parental genotypes in novel ways. Here, we used thousands of genetic markers to demonstrate that the recently rediscovered golden-crowned manakin represents an avian hybrid species from the Amazon basin. This hybrid species has a unique golden-colored crown patch used for display, which differs from the brilliant white coloration of the parental species. We used microscopy to demonstrate that, despite its unique coloration, the crown has intermediate color-producing morphological features at the nanoscale. We propose that these intermediate features disrupted the high reflectivity of the parental species, resulting in a dull hybrid population. Selection then sequestered carotenoids to the crown to compensate for its low reflectivity.


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Data deposition: The sequences reported in this paper have been deposited in the National Center for Biotechnology Information GenBank database (accession nos. MG662253–MG662371) and the Sequence Read Archive (SRA) database (accession nos. SRX637033–SRX637054).

1To whom correspondence may be addressed. Email: abareneguzm@gmail.com or jason.wear@utoronto.ca.

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L. vilasboasi has been difficult because the species had not been observed since the 1950s until 2002, when it was rediscovered with a substantial but geographically restricted population (35). Preliminary phylogenetic analyses with mitochondrial DNA nest L. vilasboasi within adjacent populations of L. iris (36). The lack of distinctive mitochondrial DNA could be compatible either with very recent divergence of L. vilasboasi or with mitochondrial introgression between L. iris and L. vilasboasi. A third possibility is that L. vilasboasi represents a genetic admixture between L. nattereri and L. iris and therefore is of hybrid origin. Here we used a large genome-wide SNP dataset to assess whether L. vilasboasi represents its own independent evolutionary lineage, a hybrid zone between L. nattereri and L. iris, or a hybrid species. We show that L. vilasboasi represents a genetically admixed population from the two proposed parental species. We then used spectrometry and transmission electron microscopy to determine whether the structural elements that produce the distinctive crown coloration of L. vilasboasi have morphological components intermediate between the two parental species, which would suggest that reproductive isolation driven by crown color evolved as a consequence of the admixture event.

Results

Genetic Patterns. Genetic analyses from genome-wide SNP and mtDNA data suggest that L. vilasboasi is genetically admixed between L. nattereri and L. iris. First, for genome-wide SNPs, we find that L. vilasboasi individuals are genetically more similar to one or the other putative parental species than the parental species are to each other based on coancestry (P < 0.00001, two-tailed t test) (Fig. 2A and SI Appendix, Table S1) and fixation index (FST) (P = 0.0000, based on 1,000 bootstrap datasets) (SI Appendix, Table S1), which is consistent either with a hybrid origin or with L. vilasboasi representing a distinct lineage that has had substantial gene flow from the other species. FST values were significantly greater than 0 but were low (0.14 to 0.2) compared with other Amazonian species pairs (37) suggesting recent divergence. Second, a principal coordinate analysis (PCoA) indicates an intermediate position of L. vilasboasi along the first principal coordinate (PCo1), as is consistent with genetic admixture (Fig. 2B). While the position of L. vilasboasi along PCo1 is similar to that of several L. iris x L. nattereri hybrids from a recently discovered contact zone (37), L. vilasboasi individuals are differentiated in PCo2 from both the parental species and their hybrids, as expected if L. vilasboasi has had sufficient time for sorting of some ancestral alleles or evolved distinct alleles following the initial admixture event. PCoA results were mirrored by Bayesian analyses of population structure and admixture (Fig. 2C). The best-fit models supported either two or three distinct populations (with only a modest increase in likelihood under the three-population model) (SI Appendix, section 3 and Fig. S2) differing in whether L. vilasboasi was treated as a distinct population recognized L. vilasboasi as distinct, albeit with substantial admixture with L. iris. In contrast, in the two-population model, L. vilasboasi was not a distinct population but instead derived 15–20% of its genome from L. nattereri and the remainder from L. iris. These results, for both the structure plots and PCoA, are consistent with a hybrid origin of L. vilasboasi resulting in a population that has become weakly differentiated genetically since its initial origin but could also be consistent with L. vilasboasi representing its own, recently differentiated independent lineage. Third, the phylogenetic network connecting the three species shows extensive reticulation, demonstrating that the three species likely do not share a bifurcating, tree-like history (Fig. 2D), although this analysis does not discriminate between reticulation derived from admixture versus ancestral polymorphism. Finally, our mtDNA haplotype network (Fig. 2E) indicates a lack of differentiation of L. vilasboasi haplotypes from adjacent L. iris haplotypes as previously reported (36). Our sole sequenced sample of L. iris iris found east of the Xingu River was highly differentiated in mtDNA from L. iris eucephala found west of the Xingu. In contrast, the two subspecies of L. iris were not differentiated in the genome-wide SNP data (Fig. 2B). This lack of nuclear differentiation might suggest that males freely cross the Xingu River, preventing differentiation in nuclear markers, while females are more dispersal limited, with the Xingu forming a barrier to gene flow and promoting mtDNA differentiation. If correct, then L. vilasboasi may have inherited the mtDNA of...
L. *iris eucephala* as a consequence of a past hybridization event. Alternatively, the mtDNA of *L. iris eucephala* might have originated in *L. vilasboasi* following its formation either as a hybrid species or as a nonhybrid lineage and then more recently introgressed across species boundaries into adjacent populations of *L. iris* west of the Xingu River. Both scenarios for the mtDNA data are consistent with hybridization playing a role in the formation of this species complex. We point out that results from each of these genetic analyses are consistent with an initial hybrid origin for *L. vilasboasi*, but some of these results could also be consistent with a nonhybrid origin. We next used coalescent modeling to test these alternatives.

**Coalescent Test for Hybrid Origins.** Coalescent modeling of genome-wide, noncoding nuclear markers not closely linked to coding regions (and thus presumably neutral) clearly favored a hybrid speciation model leading to the formation of *L. vilasboasi* over models in which *L. vilasboasi* is sister to either *L. nattereri* or *L. iris* or is basal to these (Table 1). The maximum likelihood parameter estimates of the hybrid speciation model suggested that 62% (95% CI: 54–72%) of the genome of *L. vilasboasi* was derived from *L. iris* and 38% (28–46%) from *L. nattereri* (SI Appendix, Fig. S4), which is slightly different from the admixture proportions reported from our analyses of population structure (Fig. 2B). Assuming the neutral mutation rate calculated from pedigree analyses in *Ficedula* flycatchers (38) and a generation length of 2 y, *L. nattereri* and

![Genetic analyses of Lepidothrix in southern Amazonia.](image)

**Table 1. Support for coalescent models in which *L. vilasboasi* (V) arises with (A1) and without (T1, T2, T3) genetic admixture from *L. nattereri* (N) and *L. iris* (I)**

<table>
<thead>
<tr>
<th>Model</th>
<th>T1</th>
<th>T2</th>
<th>T3</th>
<th>A1</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. samples</td>
<td>6</td>
<td>6</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>ΔAIC</td>
<td>33.3</td>
<td>124.4</td>
<td>130.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Akaike weights</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>1.00</td>
</tr>
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</table>

ΔAIC, change in Akaike information criterion.
L. iris are estimated to have split 242 kya, with the hybrid-speciation event leading to L. vilasboasi occurring 158 kya. We also tested more complex models in which gene flow occurred among all three species. While a hybrid speciation model with gene flow received substantial support over a model without gene flow (SI Appendix, Table S3), the resulting parameter estimates relating to admixture proportions had broad CIs (SI Appendix, Fig. S4), suggesting that the signal in the data may not be sufficient to obtain precise estimates for this parameter from models of this complexity. Moreover, various issues related to genome reduction datasets are known to influence parameter estimates in coalescent models (39, 40), and so we treat all parameter estimates with caution. Nevertheless, the better fit of a hybrid speciation model with gene flow (SI Appendix, Table S3) suggests that gene flow among these species occurred following the hybrid-speciation event.

Crown Feather Coloration. Feather coloration and the brilliance of the ornamental male crown patches differed in the three species (Figs. 3 and 4). The dramatic change in reflectance (with colors ranging from white to blue to pink) (Fig. 1C) with probe angle (UV/blue at 45° and red at 90°) in L. iris is indicative of strong iridescence, likely produced by the linearly arranged ordered layers of air and keratin in the spongy matrix (Fig. 3) (41–43). L. iris barbs also possessed a thin cortex (Fig. 4C) with some melanosomes present, while barbules were filled with melanosomes (SI Appendix, Fig. S5A). Melanosomes are thought to play little role in the crown coloration of L. iris (41). In contrast, L. nattereri and L. vilasboasi did not exhibit strong iridescence, with the former lacking or having few ordered layers in the spongy matrix and the latter having an intermediate number of layers (Figs. 3 and 4). L. nattereri possessed a brilliant white color with flatter reflectance spectra caused by incoherent scattering from a disordered matrix in the absence of melanosomes (Fig. 3 and SI Appendix, Fig. S5B). The melanin pigments in melanosomes can absorb incoherently scattered light at certain wavelengths (42–45). The absence of melanin allows a brilliant white coloration, as observed in albino individuals of normally blue- or black-colored avian species (44). Thus, both L. nattereri and L. iris possess brilliant crown coloration that is strongly influenced by the morphology of the spongy matrix, but the structural coloration of each species is obtained through different arrangements of the components of this matrix. In contrast, the sharp drop in reflectance below 500 nm in L. vilasboasi at both angles is characteristic of yellow pigmentation caused by carotenoids (46–48) that disappears after performing carotenoid extraction (Fig. 3). The unusually thick cortex of L. vilasboasi (Fig. 4C) contains structures that resemble carotenoid pigments reported for other bird species (46), and we did not observe these structures in L. iris or L. nattereri (SI Appendix, Fig. S5). Melanosomes were also present in the thinnest parts of the barb cortex and throughout the barbules (SI Appendix, Fig. S5C). The nanostructure of L. vilasboasi is mostly quasi-ordered, as in L. nattereri, and has an intermediate number of ordered layers of the keratin matrix (Fig. 4A). The observed yellow coloration is likely produced by a combination of light scattering by the keratin matrix and filtering by carotenoids as in yellow budgerigars (48). The average diameter of the air pockets in the keratin matrix of L. vilasboasi is also intermediate between the other two species (Fig. 4A and B). L. vilasboasi exhibited much lower reflectance values (44% at peak) than L. nattereri (109%) or L. iris (380%), resulting in a much duller look both before and after carotenoid extraction. Crown feathers of the L. iris × L. nattereri F1-like hybrid have some iridescence, as in L. iris, but their reflectance is lower than in both parental species and is slightly higher than in L. vilasboasi.

![Fig. 3](image-url)  
(Left) Percentage of reflectance (90° and 45° to feather). Percentage of reflectance is show before (red trace) and after (blue trace) carotenoid extraction in L. vilasboasi. Reflectance curves and their SEs are taken from five measures of two stacked feathers. (Center) Transmission electron micrographs (TEMs) of the barb cortex and keratin spongy layer of male crown patch. (Scale bars: 1 μm.) (Right) Crown patch photographs for three species of Lepidothrix and an F1-like L. nattereri × L. iris hybrid.

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that produce the strong iridescence found in L. iris and the brilliant white found in L. nattereri, as suggested by the much lower reflectance values in the hybrid species. Extraction of the carotenoids of these feathers resulted in a dull grayish-white appearance in L. vilasboasi (Fig. 3 and SI Appendix, Fig. S6), which probably is close to the original appearance of the crown following the initial hybridization event, as also suggested by the pattern of crown reflectance recorded for L. iris × L. nattereri hybrids (see below). To compensate, we propose that sexual selection, probably mediated by female choice, resulted in extensive thickening of the carotenoid-laden barb cortex, with a duller look of the crown as the outcome (SI Appendix, Fig. S7). The evolution of this unique yellow crown color was thus not a direct consequence of hybridization but instead was selected to compensate for the significant loss of reflectance following hybridization. It is plausible that the yellow crown could have evolved in a relatively short period, given that sexual selection can accelerate the evolution of male plumage characters related to premating isolation (52). Preference for the novel yellow crown phenotype in hybrid females (not tested here) also could have evolved in parallel after the initial hybridization event. Experimental interspecific crosses in fishes (53) and insects (54–56) have demonstrated that early generation hybrid females often show mating preferences for novel hybrid phenotypes, and such mating preferences are believed to promote hybrid speciation (53, 54, 57). Furthermore, it is documented in other manakin species that female choice can promote the establishment of certain hybrid phenotypes in some populations (58), while in Darwin’s finches the preference for hybrid phenotypes has been demonstrated to occur as late as the early generations following hybridization (59). As crown patches are displayed prominently during courtship of lekking males (30, 34), it is likely that this character plays a strong role in premating reproductive isolation between the Lepidodrornis species. In contrast, song, which is used by the males to attract females to leks, is not known to differ among these species (60), and we have successfully used the songs of L. iris to attract L. nattereri females, showing that songs are not causing strong premating reproductive isolation.

One way to support the hypothesis for the dull origin of L. vilasboasi would be to observe hybrids between L. iris and L. nattereri. L. iris and L. nattereri currently form a narrow hybrid zone in the southern part of their distributions (37). The sole adult male F1-like hybrid that we collected had structural features of the barb keratin matrix in its crown that were intermediate between those in the parental species and closely matched L. vilasboasi (Fig. 3 and 4). Like L. vilasboasi, this hybrid individual had greatly reduced reflectance of the crown, although the color remained whitish and not yellow as in L. vilasboasi. The droller crown in the hybrid supports our hypothesis that L. vilasboasi began as a dull-crowned hybrid swarm before sequestering carotenoids in the crown feathers. The even duller look of L. vilasboasi compared with the early generation L. iris × L. nattereri hybrid might have derived from increased recombination of the parental genomes in later-generation hybrids that resulted in still further disruption of the structural elements fine-tuned to generate high reflectivity.
Despite species-specific ornamentation, reproductive isolation between *L. nattereri*, *L. iris*, and *L. vilasboasi* appears to be incomplete, as is often true of other morphologically distinctive manakins and for birds in general. First, coalescent model testing with and without gene flow favored a model with ongoing migration among each of the three species following the initial admixture event leading to *L. vilasboasi*. However, the maximum likelihood estimates of migration rates in SNPs far from coding regions (and which are presumably neutral) are slightly less than a single individual per generation (SI Appendix, Fig. S4). This level of gene flow is unlikely to result in homogenization of the gene pools of these species. Second, populations of *L. nattereri* west of the Tapajós and Jurumã Rivers possess a considerable genome proportion of *L. iris* and/or *L. vilasboasi* origin, as shown by structure and PCO plots (Fig. 2A and B). The introgression appears to be one-sided, with *L. nattereri* genes not occurring in *L. iris* along the east bank of the Tapajós River. A likely explanation is that the Tapajós River, which forms a barrier between these and many other pairs of species (37, 51), may have originally flowed further west. A change in its course brought it east to its current position, transferring a small population of *L. iris* and possibly also *L. vilasboasi* to the west bank of the Tapajós, where they became genetically subsumed into adjacent populations of *L. nattereri*. The proportion of *L. iris* or *L. vilasboasi* genes in the *L. nattereri* populations decreased from north to south, consistent with this interpretation (in Fig. 2B these individuals are arranged from left to right corresponding to a south-to-north gradient). Changes in the course of the Tapajós River have also been proposed as an explanation for complex phylogeographical patterns of other bird species in the region (61–65).

A third indicator of incomplete reproductive isolation comes from a parapatric contact zone between *L. nattereri* and *L. iris* in the headwater region between the Teles Pires and Xingu Rivers (37). We found both species syntropically at a few localities in this contact zone (Fig. L4). Genetic analyses identified only two early generation hybrids (F1, F2, or backcrosses of these with parentals) among our contact zone sample (SI Appendix, Fig. S3), although a small amount of introgression is seen in a substantial number of individuals of either species from the contact zone (Fig. 2A and B). The small number of early generation hybrids suggests substantial reproductive isolation between *L. iris* and *L. nattereri*, which may include premating isolation (resulting in the formation of the hybrid swarm). However, tests are needed to assess levels of postzygotic isolation. The formation of these species, including the hybrid-speciation event leading to *L. vilasboasi*, are likely to represent cases in which moderate pre- and postmating isolating barriers are in place but fail to prevent small amounts of hybridization between species.

The combined effect of river barriers and wet forest retraction during past climatic oscillations may have provided periods of allopatry (33, 37, 67, 68) facilitating the origin of *L. vilasboasi* following hybridization of the parental species. *L. vilasboasi* is located in a very restricted geographical region west of the Jamanxim River and east of the Cachimbo range and Tapajós River. These geographic features may form partial barriers to gene flow in the north (although at one locality *L. vilasboasi* and *L. iris* occur sympotopically along the east bank of the Jamanxim River in our samples, and contact between *L. vilasboasi* and *L. nattereri* around the north end of the Cachimbo range is likely, given continuous habitat and a lack of river barriers) (SI Appendix, Fig. S6), but there is currently no obvious barrier to the south where all three species are likely to come into contact (SI Appendix, Fig. S6). We suspect that during a past humid period *L. nattereri* and *L. iris* came into geographic contact and formed a hybrid zone in the region now occupied by *L. vilasboasi*. With the onset of the next glacial period, the southern edge of humid forest in Amazonia retracted to the north (37), isolating the hybrid zone populations in this region from the broader ranges of *L. nattereri* and *L. iris* and forcing them to collapse into a hybrid swarm. The disruption of the brilliant structural crown colors of the parental species in the hybrid swarm precipitated the sequestration of carotenoids in crown feathers, leading to the formation of the unique ornamentation of *L. vilasboasi*. During interglacials, humid forest expanded to the south, bringing *L. vilasboasi* back into contact with its parental species, as is likely at present (SI Appendix, Fig. S6). Although our estimates of the timing of these events vary depending on whether we allow postspeciation gene flow between species (i.e., migration essentially doubles the estimated dates), model estimates nevertheless place the separation of *L. nattereri* and *L. iris* within the past 600,000 y, coinciding with the Mid- to Late-Pleistocene severe climatic cycles that most likely caused oscillation of the southern extent of humid forest in Amazonia (69). It thus seems likely that, following its initial separation, *L. vilasboasi* has come back into contact with the other parental species during one or two interglacial periods when humid forests expanded maximally to the south and has maintained its morphological distinction and species status despite this contact.

We have presented substantial evidence that *L. vilasboasi* is of hybrid origin. Whether *L. vilasboasi* also represents a hybrid species depends largely on how hybrid species are defined. A recent review (10) narrowly defined hybrid species as having had a hybrid origin, exhibiting reproductive isolation from parental species, and in which reproductive isolation originated as a direct consequence of hybridization. Here we demonstrate a hybrid origin and argue that the unique yellow crown likely results in substantial (but incomplete) premating reproductive isolation. However, we favor a scenario in which the yellow crown coloration originated as an indirect, rather than a direct, consequence of the hybrid event. We argue that the initial admixture event may have triggered a chain of events that ultimately and indirectly resulted in reproductive isolation, as proposed here, and that such generations are valid hybrid-speciation events. We do caution, however, that a key aspect of our scenario remains speculative. We have demonstrated that both *L. vilasboasi* and an early generation hybrid between the parental species have a duller crown color associated with an intermediate nanostructural organization of the keratin matrix of crown barbs. We argue that selection favored sequestration of carotenoids (which are present across the yellow body plumage of these birds) in crown feathers as a way to render dull-crowned males more attractive to females. However, an alternative is that the yellow crown coloration evolved for reasons unrelated to hybridization. Unfortunately, we are not aware of any way to test these alternatives, short of trying to replicate this chain of events through impractical laboratory-based formation of a hybrid swarm. Nevertheless, it seems highly unlikely that *L. vilasboasi* would have evolved a dull yellow crown from the brilliant white or opalescent crown of the parental species without the intermediate nanostructural arrangement of the barbs in the hybrid reducing both iridescence and the incoherently scattered white, as suggested by early generation hybrids between *L. iris* and *L. nattereri*.

In conclusion, we provide evidence of a hybrid origin for a morphologically distinctive Neotropical bird species. Our results highlight the importance of hybridization both as a source of genetic variation for the speciation process and as a source of evolutionary novelty. This study adds to the growing number of putative cases of homoploid hybrid speciation in terrestrial
vertebrates, particularly birds. In contrast to other reported avian examples, such as the Italian sparrow (24–27, 29), the Audubon's warbler (23) and the Hawaiian duck (28), the yellow-crown phenotype of *L. vilasboasi* is not intermediate between the parents. This yellow crown coloration could represent a novel transgressive phenotype that arose purely through the recombination of parental alleles (1, 7, 49, 50, 70, 71). Instead, our analyses of crown feather coloration and nanostructure suggest that the intermediate and mosaic nature of the nanostructural elements of the hybrids resulted in a dull white-gray crown ornamentation with subsequent sexual selection resulting in the sequestration of carotenoids leading to the novel yellow coloration (*SI Appendix, Fig. S7*). Although our current understanding of the levels of both pre- and postzygotic isolation is incomplete, the tight coupling of admixture proportions and low levels of heterozygosity suggest our sample of *L. vilasboasi* does not contain any early generation hybrids with parental species despite the sympatric presence of *L. vilasboasi* and *L. iris* at one of our sampling localities and the likely presence of parapatric contact zones with the parental species in the western and southern portions of its range (*SI Appendix, Fig. S6*). These results are consistent with substantial reproductive isolation, as also suggested by the fairly low migration rates estimated between *L. vilasboasi* and *L. iris/nattereri*. Nevertheless, our modeling results rule out introgression interspecies migration at neutral loci. The unique crown ornamentations of *L. vilasboasi*, *L. iris*, and *L. nattereri* appear to have evolved despite ongoing episodes of gene flow. Future work should (i) investigate the extent to which female *L. vilasboasi* prefer the yellow crown coloration over the crown color of the parental species; (ii) sample further putative parapatric contact zones to assess levels of hybridization; and (iii) evaluate whether genetic incompatibilities exist and are the result of mosaic inheritance of preexisting genetic variation found in the parental species, as suggested for other hybrid species.

Methods

Sampling Design. We collected specimen-voucherd genetic samples (deposited at the Museu Paraense Emílio Goeldi) during four field trips to Pará and Mato Grosso states of Brazil during 2012 (see further details in ref. 37, 2014, and 2015 and obtained additional samples from museum collections for a total of 144 individuals with tissue samples [21 *L. vilasboasi*, 47 *L. nattereri*, 21 L. Iris euechepha, 1 L. iris iris, and 54 individuals from the Xingu/Teles Pires headwaters contact zone between L. nattereri and L. Iris euechepha (*Fig. 1A* and Dataset S1)]. Approval was obtained from the Brazilian Government (collecting permits 4253-1, 40173-1, and 6381-1).

DNA Sequencing. A 966-bp fragment of the mtDNA gene cytochrome *b* was sequenced for 109 individuals using standard protocols (*SI Appendix, section 2*).


Genetic Analyses. Dataset 1 was used for coancestry estimates, and Dataset 2 was used to characterize genetic structure, population differentiation, and admixture patterns using PCoA, STRUCTURE plots (27), phylogenetic networks, and pairwise *F*st. A haplotype network was generated for the mtDNA dataset. For details, see *SI Appendix, section 2*.

Coalescent Modeling. We used coalescent modeling in a composite likelihood framework to compare the fit of six models for the origin of *L. vilasboasi* (see models in Table 1) using Dataset 3. These models differ in whether *L. vilasboasi* originated with or without genetic admixture between *L. iris* and *L. nattereri* and whether that admixture occurred as a point event in time, through ongoing gene flow, or a combination of the two. For details, see *SI Appendix, section 3*.

Spectrophotometry and Microscopy. Reflectance of male crown feathers for each species was obtained with a spectrometer held at an angle of 90° and 45°. For *L. vilasboasi*, this was done both before and after carotenoid extraction. Transmission electron microscopy was used in one feather per species and one from the early generation hybrid individual to quantify the number of ordered layers perpendicular to the barb surface and the spacing of air cavities in the keratin matrix of the barbs, barb cortex thickness, and to corroborate the presence of melanosomes. For details, see *SI Appendix, sections 4 and 5*.

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