



An extinct hummingbird species that never was: a cautionary tale about sampling issues in molecular phylogenetics

JORGE L. PÉREZ-EMÁN^{1,7}, JHONIEL PERDIGÓN FERREIRA^{1,5}, NATALIA GUTIÉRREZ-PINTO^{2,6},
ANDRÉS M. CUERVO³, LAURA N. CÉSPEDES², CHRISTOPHER C. WITT⁴ & CARLOS DANIEL CADENA²

¹Instituto de Zoología y Ecología Tropical, Universidad Central de Venezuela, Caracas, Venezuela.

²Departamento de Ciencias Biológicas, Universidad de los Andes, Bogotá, Colombia.

³Instituto Alexander von Humboldt, Bogotá, Colombia.

⁴Museum of Southwestern Biology, University of New Mexico, Albuquerque, New Mexico, USA.

⁵Current address: Department of Evolutionary Biology and Environmental Studies, University of Zurich—Irchel.

⁶Current address: School of Biological Sciences, University of Nebraska—Lincoln.

⁷Corresponding author. E-mail: jorge.perez@ciens.ucv.ve

The selection of species and individuals for molecular analyses critically affects inferences in various fields of systematic biology including phylogenetics, phylogeography, and species delimitation. Especially in areas like the Neotropical region where molecular analyses have recovered substantial within-species divergence and unexpected affinities of populations (Turchetto-Zolet *et al.* 2013), biases resulting from incomplete taxonomic or geographic sampling may compromise the understanding of phylogenetic relationships (Avenidaño *et al.* 2017). Here we describe a case in which assessments of the validity of a potentially extinct species of Neotropical bird were likely compromised because within-species variation was not accounted for in phylogenetic analyses evaluating the alternative hypothesis that the only known specimen may represent a hybrid.

Hummingbirds (Trochilidae) are well known for their propensity to hybridize (Banks & Johnson 1961). Hybrid hummingbird specimens are common in natural history collections and have caused substantial taxonomic confusion because many were described as distinct species by museum-based ornithologists. Therefore, stringent protocols exist to diagnose hybrid hummingbirds and thus avoid treating hybrids as taxa (Graves 1990). A notable exception to multiple situations in which hummingbird specimens were described as distinct species but were later determined to be hybrids is that of a skin purchased in 1909 by Brother Nicéforo María in Bogotá, Colombia. Upon examining this specimen, which had puzzled ornithologists for decades and lacked precise locality data, Graves (1993) concluded it was not an aberrant individual of a known taxon and ruled out the possibility that it may represent a hybrid. Therefore, he designated it as the holotype of a new species, the Bogota Sunangel *Heliangelus zusii* Graves, 1993, which he noted may have gone extinct due to habitat destruction, representing a “relic of a lost world” (Graves 1993). Despite the careful consideration and rejection of alternative hypotheses for what this specimen might represent, its treatment as a species was received with skepticism by some researchers (Schuchmann 1999).

A study analyzing mitochondrial DNA (mtDNA) sequences of the holotype of *H. zusii* was considered to settle disagreements about its validity as a species (Kirchman *et al.* 2010). Phylogenetic analyses indicated that the specimen is not closely related to species of *Heliangelus*; rather, they suggested that it belongs in a clade with two species of *Agelaiocercus* from northern South America, Long-tailed Sylph *A. kingii* (Lesson) and Violet-tailed Sylph *A. coelestis* (Gould), and the Gray-bellied Comet, *Taphrolesbia griseiventris* (Taczanowski), a species in a monotypic genus endemic to north-central Peru (Kirchman *et al.* 2010). In addition, sequence divergence between *H. zusii* and specimens of *Agelaiocercus* and *Taphrolesbia* was considered substantial (>5% and 3% p-distance, respectively), consistent with status as a distinct species (Kirchman *et al.* 2010). Accordingly, *H. zusii* is presently considered a valid species (Remsen *et al.* 2017).

In 2011, observations of a striking hummingbird in the Reserva Rogitama, Eastern Cordillera of the Andes in Boyacá, Colombia, led ornithologists and birders to suspect this might be the rediscovery of *H. zusii*. However,

after careful examination of the single individual that was captured and released, it was concluded that the Rogitama hummingbird was not *H. zusii*; rather, various phenotypic characters suggested it was a hybrid, with *A. kingii* and Tyrian Metaltail, *Metallura tyrianthina* (Loddiges), hypothesized to be its parents (Stiles & Cortés-Herrera 2015). Thus, *H. zusii* remains a lost taxon with no records other than the type, and as such is considered critically endangered if not extinct (Parra 2016).

We recently generated mtDNA sequence data from a feather sample of the Rogitama hummingbird to compare it with data from other taxa. Upon initial analyses, we found that the sequence we obtained was remarkably similar to the published sequence of *H. zusii* (Kirchman *et al.* 2010) in the few nucleotide positions in which they overlapped. Because phenotypic traits indicate that the Rogitama hummingbird is not *H. zusii*, we began to entertain a new hypothesis, namely that both of these individuals are hybrids, with their similar mtDNA indicating a shared maternal species. Our ongoing work on the phylogeography of *A. kingii* allowed us to compare sequences of this species from various geographical regions with those of the Rogitama hummingbird, the holotype of *H. zusii*, and other hummingbird taxa to evaluate this hypothesis.

Detailed analyses of the phylogeny and phylogeography of *Agelaiocercus* are not the goal of this study and will be published elsewhere. Here we report on sequences of the ND2 mitochondrial gene for 33 individuals of *A. kingii* and three *A. coelestis*, including samples previously analysed by Kirchman *et al.* (2010). Sampling covered most of the geographical range of *A. kingii*, but was especially thorough in montane Colombia, considering the geographic origin of the Rogitama hummingbird and, hypothetically, of *H. zusii* (Figure 1a, Table 1). We isolated whole genomic DNA using the DNeasy Tissue Kit (Qiagen, Valencia, California) following manufacturer's instructions or a phenol-chloroform method. Amplification of subunit 2 of the protein-coding gene NADH (ND2, 1041 bp) was done using the polymerase chain reaction (PCR) in a 2720 thermocycler (Applied Biosystems) and primers L5219 and H6313 (Sorenson *et al.* 1999). PCR conditions included an initial denaturation at 95°C for 8 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 50°C for 30 s, and an extension phase of 72°C for 60 s. These cycles were ended with a final extension phase of 72°C for 10 m. The PCR reactions contained 1–2 mL of DNA template, 0.125 U of *Taq* polymerase (PROMEGA), 14.375 mL H₂O, 1.5 mL MgCl₂, 5 mL buffer solution, 1.25 mL of each primer, and 0.5 mL dNTPs, in a total volume of 25 mL. These PCR products were purified in a 1% low-melting point agarose gels and cycle-sequenced using 1 mL DNA template. Because molecular work with the Rogitama hummingbird was conducted in a separate lab at Universidad de los Andes and the data for *H. zusii* were obtained from GenBank, contamination of samples is not a plausible explanation for our results. We combined our data with published sequences of *H. zusii* (GenBank Accession GU166851), *T. griseiventris* (GU166856), *Adelomyia melanogenys* (Fraser) (JF894047), and *Chalcostigma herrani* (Delattre & Bourcier) (EU042536), with the latter two species designated as outgroups (Kirchman *et al.* 2010; McGuire *et al.* 2014). New sequences were deposited in GenBank (accession numbers MF442357–MF442391). Because we could not obtain complete ND2 sequences for the Rogitama hummingbird (we obtained 543 base pairs) and the available ND2 sequence of *H. zusii* is also incomplete, overlap in data between these two specimens was restricted to 71 base pairs. Overlap with sequences of other specimens, however, was more extensive.

We estimated ND2 gene trees using maximum-likelihood and Bayesian inference methods. Maximum-likelihood reconstructions were conducted in RAxML-HPC version 8 (Stamatakis 2014) and nodal support was calculated using 1000 pseudoreplicates. The Bayesian analysis was run in MrBayes v.3.2.6 (Ronquist *et al.* 2012) and consisted of four independent runs with four MCMC chains run for 10 million generations sampling every 1000 generations, discarding the first 25% as burn-in. We implemented a general time-reversible model of nucleotide substitution with gamma-distributed rate heterogeneity (GTR+G) in both analyses, as selected using the Akaike Information Criterion.

We found that sequences of the holotype of *H. zusii* and the Rogitama hummingbird are nested within *Agelaiocercus*, where they belong in a clade formed by individuals of *A. kingii* from the three cordilleras of the Colombian Andes (Figure 1b). Sequences of both “problem” birds are most similar to sequences of individuals from the Eastern Cordillera, the general provenance of the type locality of *A. kingii* (Boissonneau 1840). In fact, the ND2 sequence of *H. zusii* (181 bp) is identical to a sequence of *A. kingii* from Huila (IAvH BT1210) and differs in only one nucleotide from sequences of *A. kingii* from Santander and Norte de Santander. Likewise, the ND2 sequence of the Rogitama hummingbird is identical to sequences from Huila, Santander and Norte de Santander (Colombia), and from eastern Ecuador and Peru. Indeed, the sequences of *H. zusii* and of the Rogitama

hummingbird are identical along the 71 nucleotide sites in which they overlap. Because of the short sequences of both *H. zusii* and the Rogitama hummingbird, we also analyzed the data considering only the 181-bp ND2 fragment from *H. zusii*, and results were the same (genetic distances among sequences based on this short fragment are correlated to distances based on the complete gene; Figure 2). A striking additional result is the considerable population structure exhibited by *A. kingii*, with distinct mtDNA lineages occupying different geographical regions (Figure 1b). Major clades of *A. kingii* formed a polytomy with sequences of *A. coelestis* and *T. griseiventris*.

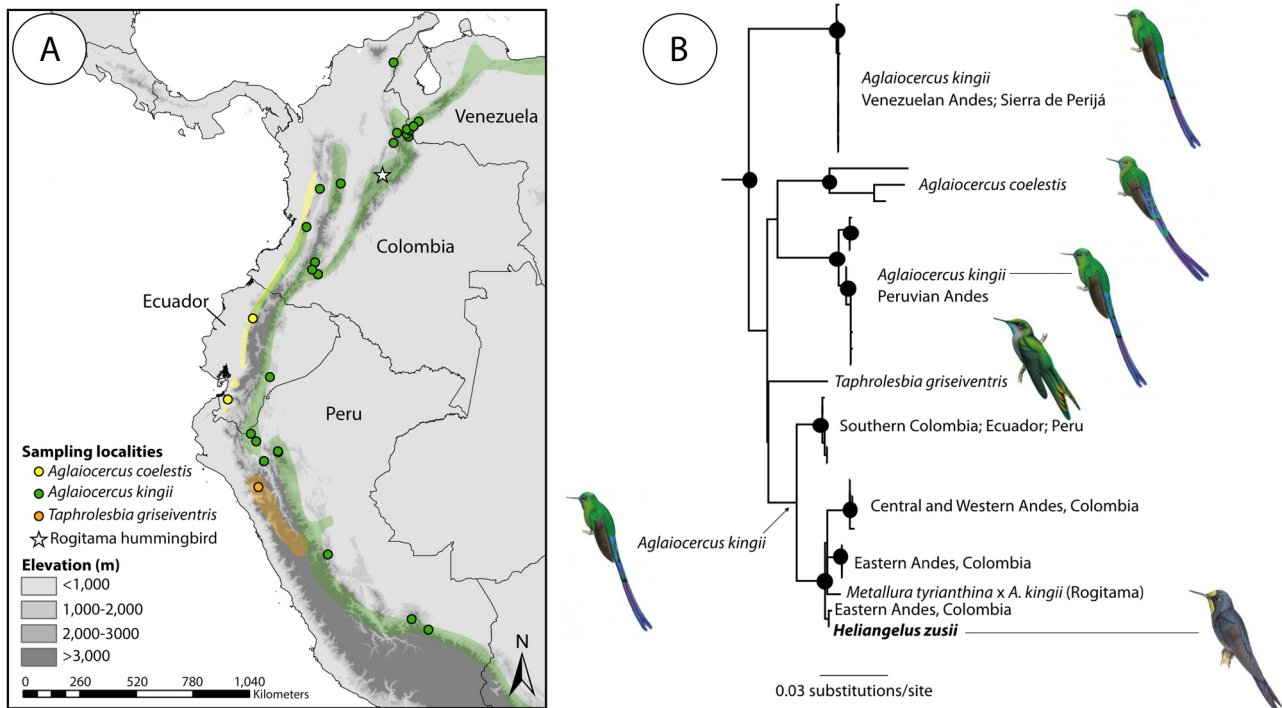


FIGURE 1. (A) Distributions of *Aglaiocercus kingii*, *A. coelestis*, and *Taphrolesbia griseiventris* (polygons), and geographic provenance of specimens of these species and of the Rogitama hybrid hummingbird included in molecular analyses (dots and star). (B) Phylogenetic relationships among species and populations of *Aglaiocercus*, *Taphrolesbia*, the Rogitama hummingbird, and *Heliangelus zusii* based on sequences of the ND2 gene. Black dots indicate strongly supported nodes (0.95 Bayesian posterior probability, > 80% maximum-likelihood bootstrap). The Rogitama hummingbird and *H. zusii* have haplotypes closely allied to those of *A. kingii* from the Eastern Cordillera of Colombia, indicating they are hybrids sharing *A. kingii* as female parent. Sister taxon, *Adelomyia melanogenys*, and outgroup *Chalcostigma herrani*, are not shown. Illustrations courtesy of Lynx Edicions (del Hoyo *et al.* 2018).

A potential explanation for our results is that *H. zusii* is a valid species closely related to *A. kingii*, with which it still shares mtDNA sequences owing to recent speciation and incomplete lineage sorting. We believe a recent speciation scenario is unlikely, however, considering that recently diverged species of hummingbirds differ mainly in plumage and not in shape or structure (e.g. *Coeligena* and *Lampornis*; García-Moreno *et al.* 2006; Parra 2010), and *H. zusii* differs substantially from *A. kingii* in bill shape and tail structure (Graves 1993). Alternatively, we suggest that the simplest interpretation of our results is that the holotype of *H. zusii* is probably not a representative of a valid species. Rather, based on the identity of its mtDNA with that of *A. kingii* and its morphological distinctiveness, it is most likely a hybrid. Because mtDNA is maternally inherited, the only specimen referable to *H. zusii* ever collected probably resulted from a cross between a female *A. kingii* and another species of hummingbird. Furthermore, given phylogeographic structure observed in *A. kingii*, our data suggest strongly that this hybridization event likely took place in the Eastern Cordillera of the Colombian Andes. Thus, in addition to resolving the status of an enigmatic specimen, our analysis partly clarifies its geographic origin, considering that “Bogota” trade skins may be from multiple areas (Graves 1993). Our results also confirm the hypothesis that the female parent of the Rogitama hummingbird was *A. kingii* (Stiles & Cortés-Herrera 2015). The phenotypic differences between *H. zusii* and the Rogitama hummingbird (Stiles & Cortés-Herrera 2015) suggest that although they share *A. kingii* as female parent, they were sired by males of different species.

TABLE 1. Tissue samples sequenced in this study for the mitochondrial ND2 gene. Collection acronyms: Colección Ornitológica Phelps (COP); Instituto Alexander von Humboldt (IAvH); Instituto de Ciencias Naturales Colombia (ICN); Louisiana State University Museum of Natural Science (LSUMZ); Museum of Southwestern Biology University of New Mexico (MSB); Museo de Zoología of the Universidad Católica del Ecuador (QCAZ).

Taxon	Catalog number	Locality
<i>Aglaiocercus kingii</i>	COP (JM1113)	Venezuela, Táchira, P. N. El Tamá
<i>Aglaiocercus kingii</i>	COP (YPL205)	Venezuela, Táchira, P. N. El Tamá
<i>Aglaiocercus kingii</i>	COP (YPL226)	Venezuela, Táchira, P. N. El Tamá
<i>Aglaiocercus kingii</i>	COP (YPL233)	Venezuela, Táchira, P. N. El Tamá
<i>Aglaiocercus kingii</i>	COP (JM1053)	Venezuela, Táchira, P. N. Chorro El Indio
<i>Aglaiocercus kingii</i>	COP (YPL121)	Venezuela, Táchira, P. N. Páramos El Batallón y La Negra
<i>Aglaiocercus kingii</i>	ICN 37200 (JPL255)	Colombia, Cesar, Manaure
<i>Aglaiocercus kingii</i>	IAvH (SS1172)	Colombia, Norte de Santander, Orocué
<i>Aglaiocercus kingii</i>	IAvH (SS1252)	Colombia, Norte de Santander, Orocué
<i>Aglaiocercus kingii</i>	IAvH (AMC1018)	Colombia, Norte de Santander, Asiria
<i>Aglaiocercus kingii</i>	IAvH BT1780	Colombia, Norte de Santander, Cucutilla
<i>Aglaiocercus kingii</i>	IAvH BT1831	Colombia, Norte de Santander, Cucutilla
<i>Aglaiocercus kingii</i>	IAvH BT5331	Colombia, Santander, Piedecuesta
<i>Aglaiocercus kingii</i>	IAvH BT1210	Colombia, Huila, Acevedo, camino al pesebre
<i>Aglaiocercus kingii</i>	IAvH BT2330	Colombia, Huila, P. N. Puracé
<i>Aglaiocercus kingii</i>	IAvH BT7358	Colombia, Huila, San Agustín, Reserva Natural Los Yalcones
<i>Aglaiocercus kingii</i>	IAvH BT2435	Colombia, Valle, La Cumbre, cuenca alta del río Bitaco
<i>Aglaiocercus kingii</i>	IAvH BT2450	Colombia, Valle, La Cumbre, cuenca alta del río Bitaco
<i>Aglaiocercus kingii</i>	IAvH BT4070	Colombia, Risaralda, Pueblo Rico, P. N. Tatamá
<i>Aglaiocercus kingii</i>	IAvH BT4673	Colombia, Caldas, La Miel
<i>Aglaiocercus kingii</i>	LSUMZ B6216	Ecuador, Morona Santiago, W slope cordillera del Cutucú
<i>Aglaiocercus kingii</i>	LSUMZ B34830	Perú, Cajamarca, Cordillera del Condor, Picorana
<i>Aglaiocercus kingii</i>	LSUMZ 33579	Perú, Cajamarca, Nuevo Perú
<i>Aglaiocercus kingii</i>	MSB:Bird:32143	Perú, Amazonas, 4.5 km N Tullanya
<i>Aglaiocercus kingii</i>	MSB:Bird:32885	Perú, Amazonas, 4.5 km N Tullanya
<i>Aglaiocercus kingii</i>	MSB:Bird:32887	Perú, Amazonas, 4.5 km N Tullanya
<i>Aglaiocercus kingii</i>	LSUMZ 44328	Perú, San Martín, ca 22 km ENE Florida
<i>Aglaiocercus kingii</i>	LSUMZ B44400	Perú, San Martín, ca 22 km ENE Florida
<i>Aglaiocercus kingii</i>	LSUMZ B44447	Perú, San Martín, ca 22 km ENE Florida
<i>Aglaiocercus kingii</i>	LSUMZ B8017	Perú, Pasco, Playa Pampa, 8 km NW Cushi
<i>Aglaiocercus kingii</i>	MSB:Bird:27271	Perú, Cusco, San Pedro
<i>Aglaiocercus kingii</i>	MSB:Bird:33213	Perú, Cusco, Abra Bella Vista
<i>Aglaiocercus kingii</i>	MSB:Bird:33250	Perú, Cusco, Abra Bella Vista
<i>Aglaiocercus coelestis</i>	QCAZ (CARS159)	Ecuador, Pichincha, Estación Científica Río Guajalito
<i>Aglaiocercus coelestis</i>	QCAZ (CARS168)	Ecuador, Pichincha, Estación Científica Río Guajalito
<i>Aglaiocercus coelestis</i>	LSUMZ B7813	Ecuador, El Oro, 9.5 km road to Pinus

The possibility that *H. zusii* was a hybrid (i.e. between species of *Aglaiocercus* and *Heliangelus*) was raised by both Peters and Zimmer in response to questions by Meyer de Schauensee about the identity of this specimen (Graves 1993). This hypothesis was rejected by Graves (1993) because he was unable to find a combination of two parental species that would produce such a distinctive specimen given the morphological intermediacy expected

for hybrid hummingbirds and the lack of characters present in potential parents. For example, Graves (1993) reasoned that one should expect traces of an awl-shaped bill shape in hybrids involving members of *Agelaiocercus*, yet the *Agelaiocercus* x *Metallura* hybrid from Rogitama lacks such a bill shape (G. Stiles pers. comm.). Moreover, the observation of phenotypic characters not present in either parental species as shown by recent studies of vocalizations (Stiles & Cortés-Herrera 2015) and plumage (Clark *et al.* 2013) complicates hybrid diagnosis in hummingbirds, even when rigorous protocols (e.g., Graves 1990, 1993) are employed. Given current information, we cannot reject the possibility that a species in the genus *Heliangelus* may be the other parent of *H. zusii*, due to morphological similarities between the specimen and members of this genus. Analyses of nuclear genes from the holotype of *H. zusii* would be necessary to establish with certainty its other parental species but even in the absence of this information and given that the sequence of *H. zusii* is identical to sequences of *A. kingii* from the Eastern Cordillera of Colombia, the burden of proof has shifted towards those claiming that this taxon is a valid species.

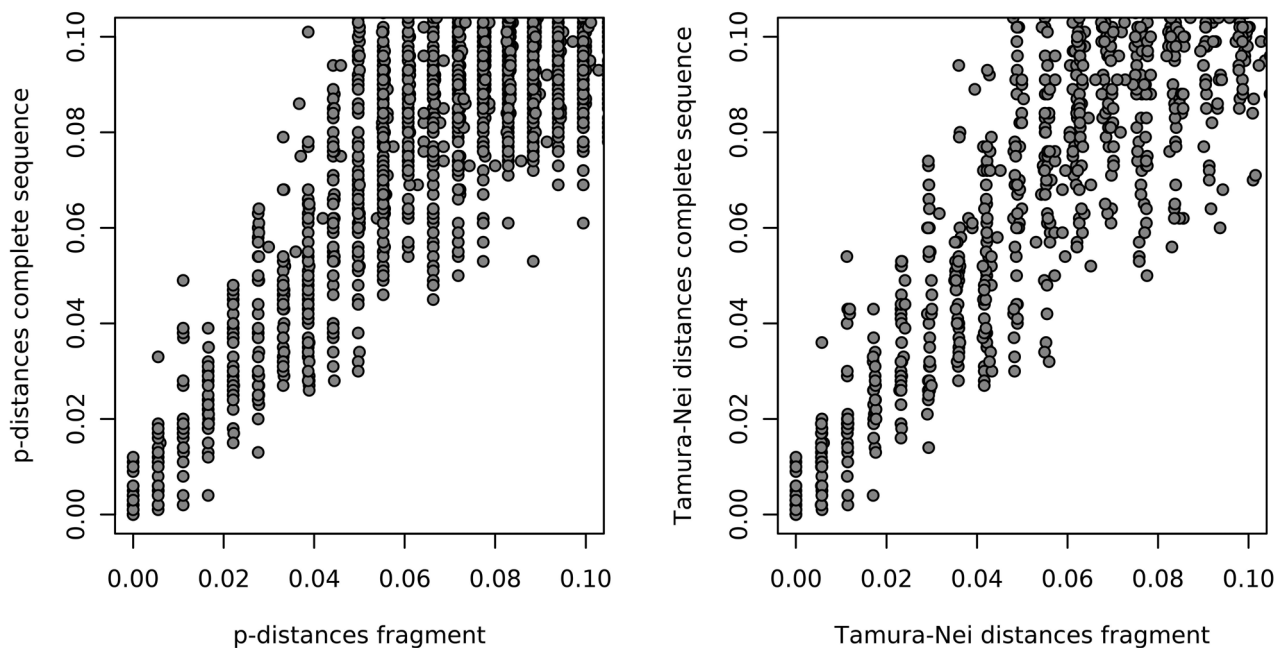


FIGURE 2. Correlation between genetic divergence (p and Tamura-Nei distances) based on the small fragment available for *Heliangelus zusii* (fragment) with divergence in complete sequences (ND2 gene) across 315 hummingbird taxa (McGuire *et al.* 2014 dataset). Genetic distances were calculated in MEGA6 (Tamura *et al.* 2013) and all positions with missing data were not considered when calculating pairwise genetic distances. The plot is cropped at 10% p-distance, thus providing a better view of the data spread in the area of low divergence relevant to the *H. zusii* issue. The correlation between the small fragment available for *H. zusii* and all the ND2 data is strong, and it is especially tight near the origin: 0% divergence in that small fragment essentially means no (or up to approximately 1%) divergence in the rest of the sequence. This suggests that our observation of identical sequences in *H. zusii* and *A. kingii* is not an artifact resulting from the small amount of available sequence data.

In sum, our analyses are not consistent with the idea that *H. zusii* is a valid taxon (Kirchman *et al.* 2010), a conclusion evident only because of our increased geographic sampling. Specifically, because the earlier study sampled a single individual per species of *Agelaiocercus* (see also McGuire *et al.* 2014), the authors were unable to detect that *H. zusii* has *Agelaiocercus* mtDNA and to uncover the complex pattern of genealogical relationships among populations of *A. kingii*, other *Agelaiocercus*, and *Taphrolesbia*. Given our findings and those of others (e.g. Zucker *et al.* 2016; Avendaño *et al.* 2017), we stress that addressing questions about the phylogeny and phylogeography of Neotropical birds—even in cases involving the affinities of a single specimen—requires comprehensive sampling across taxonomy and geography.

Acknowledgements

We thank Roberto Chavarro for permission to obtain samples of the Rogitama hummingbird and to F. Gary Stiles

for providing them. We thank Colección Ornitológica Phelps (COP), Instituto Alexander von Humboldt (IAvH), Instituto de Ciencias Naturales Colombia (ICN), Louisiana State University Museum of Natural Science (LSUMZ), Museum of Southwestern Biology University of New Mexico (MSB), and Museo de Zoología of the Universidad Católica del Ecuador (QCAZ) for tissue samples. Funding was provided by the Consejo de Desarrollo Científico y Humanístico de la Universidad Central de Venezuela (JLP-E) and University of New Mexico (CCW). Research was conducted under authorization from the Oficina de Diversidad Biológica del Ministerio del Ambiente (Venezuela) and Ministerio de Ambiente, Vivienda y Desarrollo Territorial (Colombia). We thank Elisa Bonaccorso for sequences of Ecuadorian material and J. Miranda and M. Castro for help with laboratory work. Thanks to George Sangster, Gary Stiles and two anonymous reviewers who made valuable comments, Gary R. Graves for providing a constructive critique of the manuscript, and P. Rasmussen for editorial comments on an earlier version of this manuscript.

References

- Avendaño, J.E., Arbeláez-Cortés, E. & Cadena, C.D. (2017) On the importance of geographic and taxonomic sampling in phylogeography: A reevaluation of diversification and species limits in a Neotropical thrush (Aves, Turdidae). *Molecular Phylogenetics & Evolution*, 111, 87–97.
<https://doi.org/10.1016/j.ympev.2017.03.020>
- Banks, R.C. & Johnson, N.K. (1961) A review of North American hybrid hummingbirds. *Condor*, 63, 3–28.
<https://doi.org/10.2307/1365419>
- Boissonneau, A. (1840) Oiseaux nouveaux ou peu connus de Santa-Fé de Bogota. *Revue Zoologique*, 3, 2–8.
- Clark, C.J., Feo, T.J. & van Dongen, W. (2013) Sounds and courtship displays of the Peruvian Sheartail, Chilean Woodstar, Oasis Hummingbird, and a hybrid male Peruvian Sheartail × Chilean Woodstar. *Condor*, 115, 560–577.
<https://doi.org/10.1525/cond.2013.120047>
- del Hoyo, J., Elliott, A., Sargatal, J., Christie, D.A. & de Juana, E. (Eds.) (2018) *Handbook of the Birds of the World Alive*. Lynx Edicions, Barcelona. Available from: <http://www.hbw.com/> (accessed 14 June 2017)
- García-Moreno, J., Cortés, N., García-Deras, G.M. & Hernández-Baños, B.E. (2006) Local origin and diversification among *Lampornis* hummingbirds: a Mesoamerican taxon. *Molecular Phylogenetics & Evolution*, 38, 488–498.
<https://doi.org/10.1016/j.ympev.2005.08.015>
- Graves, G.R. (1990) Systematics of the “green-throated sunangels” (Aves: Trochilidae): valid taxa or hybrids? *Proceedings of the Biological Society of Washington*, 103, 6–25.
- Graves, G.R. (1993) Relic of a lost world: a new species of sunangel (Trochilidae: *Heliangelus*) from “Bogotá”. *Auk*, 110, 1–8.
- Kirchman, J.J., Witt, C.C., McGuire, J.A. & Graves, G.R. (2010) DNA from a 100-year-old holotype confirms the validity of a potentially extinct hummingbird species. *Biology Letters*, 6, 112–115.
<https://doi.org/10.1098/rsbl.2009.0545>
- McGuire, J.A., Witt, C.C., Rensen Jr., J.V., Cori, A., Rabosky, D.L., Altshuler, D.L. & Dudley, R. (2014) Molecular phylogenetics and the diversification of hummingbirds. *Current Biology*, 24, 910–916.
<https://doi.org/10.1016/j.cub.2014.03.016>
- Parra, J.L. (2010) Color evolution in the hummingbird genus *Coeligena*. *Evolution*, 64, 324–335.
<https://doi.org/10.1111/j.1558-5646.2009.00827.x>
- Parra, J.L. (2016) *Heliangelus zusii*. In: Renjifo, L.M., Amaya-Villareal, A.M., Burbano-Girón, J. & Velásquez-Tibatá, J. (Eds.), *Libro Rojo de Aves de Colombia. Vol. II*. Universidad Javeriana e Instituto Alexander von Humboldt, Bogotá, Colombia, pp. 192.
- Remsen, J.V. Jr., Areta, J.I., Cadena, C.D., Claramunt, S., Jaramillo, A., Pacheco, J.F., Pérez-Emán, J., Robbins, M.B., Stiles, F.G., Stotz, D.F. & Zimmer, K.J. (2017) *A Classification of the Bird Species of South America*, American Ornithologists' Union. Available from: <http://www.museum.lsu.edu/~Remsen/SACCBaseline.htm> (accessed 13 July 2017)
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M.A. & Huelsenbeck, J.P. (2012) MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Systematic Biology*, 61, 539–542.
<https://doi.org/10.1093/sysbio/sys029>
- Schuchmann, K.-L. (1999) Hummingbirds. In: del Hoyo, J., Elliot, A. & Christie, D. (Eds.), *Handbook of the Birds of the World. Vol. 5. Barn-owls to Hummingbirds*. Lynx Edicions, Barcelona, pp. 468–680.
- Sorenson, M.D., Ast, J.C., Dimcheff, D.E., Yuri, T. & Mindell, D.P. (1999) Primers for a PCR-based approach to mitochondrial genome sequencing in birds and other vertebrates. *Molecular Phylogenetics & Evolution*, 12, 105–114.
<https://doi.org/10.1006/mpev.1998.0602>
- Stamatakis, A. (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30, 1312–1313.
<https://doi.org/10.1093/bioinformatics/btu033>

- Stiles, F.G. & Cortés-Herrera, J.O. (2015) Diagnosis and observations of a hybrid hummingbird (*Metallura tyrianthina* x *Agelaiocercus kingi*) in the Eastern Andes of Colombia. *Revista de la Academia Colombiana de Ciencias Exactas, Físicas y Naturales*, 39, 481–490.
<https://doi.org/10.18257/raccefyn.260>
- Tamura, K., Stecher, G., Peterson, D., Filipiński, A. & Kumar, S. (2013) MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Molecular Biology & Evolution*, 30, 2725–2729.
<https://doi.org/10.1093/molbev/mst197>
- Turchetto-Zolet, A.C., Pinheiro, F., Salgueiro, F. & Palma-Silva, C. (2013) Phylogeographical patterns shed light on evolutionary process in South America. *Molecular Ecology*, 22, 1193–1213.
<https://doi.org/10.1111/mec.12164>
- Zucker, M.R., Harvey, M.G., Oswald, J.A., Cuervo, A., Derryberry, E. & Brumfield, R.T. (2016) The Mouse-colored Tyrannulet (*Phaeomyias murina*) is a species complex that includes the Cocos Flycatcher (*Nesotriccus ridgwayi*), an island form that underwent a population bottleneck. *Molecular Phylogenetics & Evolution*, 101, 294–302.
<https://doi.org/10.1016/j.ympev.2016.04.031>