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DNA from a 100-year-old holotype confirms the validity of a potentially extinct hummingbird species

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We used mtDNA sequence data to confirm that the controversial 100-year-old holotype of the Bogotá sunangel (*Heliangelus zusii*) represents a valid species. We demonstrate that *H. zusii* is genetically well differentiated from taxa previously hypothesized to have given rise to the specimen via hybridization. Phylogenetic analyses place *H. zusii* as sister to a clade of mid- to high-elevation Andean species currently placed in the genera *Taphrolesbia* and *Aglaiocercus*. *Heliangelus zusii*, presumed extinct, has never been observed in nature by biologists. We infer that the species occupied a restricted distribution between the upper tropical and temperate zones of the northern Andes and that it was most probably driven to extinction by deforestation that accompanied human population growth during the nineteenth and early twentieth centuries. We demonstrate the feasibility of obtaining DNA from nearly microscopic tissue samples from old hummingbird specimens and suggest that these methods could be used to resolve the taxonomy of dozens of avian taxa known only from type specimens.

Keywords: ancient DNA; Andes Mountains; hummingbird; hybrid; extinction

1. INTRODUCTION

Cataloguing the biota of biologically unexplored regions often follows closely on the heels of human population expansion and subsequent waves of anthropogenically caused extinctions (Fuller 2001). Many species that were rare when first encountered by biologists have not been observed since the type specimens were collected. Species known only from unique specimens are rightfully viewed with caution because they may represent hybrids or aberrant phenotypes. Living populations of these biological mysteries are occasionally 'rediscovered' in nature many decades or even centuries after their scientific descriptions, providing hope to conservationists and new data to systematists (Cohn-Haft 1993; Bauer *et al.* 2000; Buzzetti & Carlos 2005; Mauro 2005; Lane *et al.* 2006; Round *et al.* 2007). More frequently, the mysteries remain unsolved, hinting at an unknown history of hybridization or of possible extinction. For these cases, ancient DNA (aDNA) methods provide the means for resolving disputes regarding the validity of species known only from unique holotypes. We have used this approach to investigate the contentious case of a spectacular 100-year-old hummingbird specimen, the holotype and the only known specimen of the Bogotá sunangel *Heliangelus zusii*.

In 1947, Brother Nicéforo María presented a specimen he purchased in 1909 in Bogotá, Colombia, to Rodolphe Meyer de Schauensee at the Academy of Natural Sciences of Philadelphia (ANSP). Meyer de Schauensee was perplexed by the specimen (ANSP 159261) and solicited the opinions of many leading ornithologists of his day, including John Zimmer, James Peters and Alexander Wetmore. There was no consensus among the experts, and Meyer de Schauensee referred the specimen to *Neolesbia nehrkorni*, a taxon based on two specimens now thought to be hybrids (Hinkelmann *et al.* 1991). Graves (1993) conducted an exhaustive analysis of plumage and morphology, comparing the specimen to all hummingbird taxa housed in several of the largest museums. Following a well-established method of hybrid diagnosis (Graves 1990), he concluded that the specimen represented the only known example of a valid species, which he named *H. zusii*. This conclusion was controversial, in part, because the exact provenance of the specimen is unknown and because hummingbirds are known to hybridize extensively (McCarthy 2006). The taxon has not been accepted by many authorities (Schuchmann 1999).

2. MATERIAL AND METHODS

(a) *Laboratory procedures*

Skin cells were scraped from the feet of ANSP 159261 using a sterile scalpel blade at the National Museum of Natural History, Smithsonian Institution. Extraction and PCR setup (with negative controls and replications) were performed in the dedicated aDNA laboratory at the New York State Museum, where no previous work on hummingbirds had been performed. We employed a modified version of the silica-based DNA extraction method of Höss & Pääbo (1993). We designed six PCR primer pairs to target short segments of the mitochondrial *ND2* and *ND4* genes and their flanking tRNAs. Extraction protocol, primer sequences and PCR conditions are described in the electronic supplemental material. In a separate laboratory at University of California, Berkeley, we obtained sequences from frozen tissues of 95 hummingbird species and from five swifts (Apodidae), a treeswift (Hemiprocnidae) and an owlet-nightjar (Aegothelidae), which were used to root phylogenetic trees. Taxon sampling within the hummingbird family Trochilidae included species from all nine major trochilid clades and all 18 genera within the 'coquette' clade (Lophornithini) (McGuire *et al.* 2008), including an additional 16 species beyond those included in McGuire *et al.* (2007) because we expected *H. zusii* to be nested within that group. Methods and primers for amplification and sequencing from frozen samples are available in McGuire *et al.* (2007). Specimen details and GenBank (NCBI) accession numbers are in the electronic supplementary material, table S1.

(b) *Phylogenetic analyses*

Four of the six primer pairs produced PCR products from the mitochondrial *ND4, ND2, tRNa-His* and *tRNa-Ser* genes, totalling 356 base pairs (bp) of concatenated sequence. Phylogenies were reconstructed using maximum parsimony (MP), maximum likelihood (ML) and Bayesian approaches to analyse both a taxonomically broad sample of hummingbirds for only those characters for which we had data for *H. zusii* (95 hummingbird species plus outgroups, 356 bp), and a larger alignment of 4133 bp of the above

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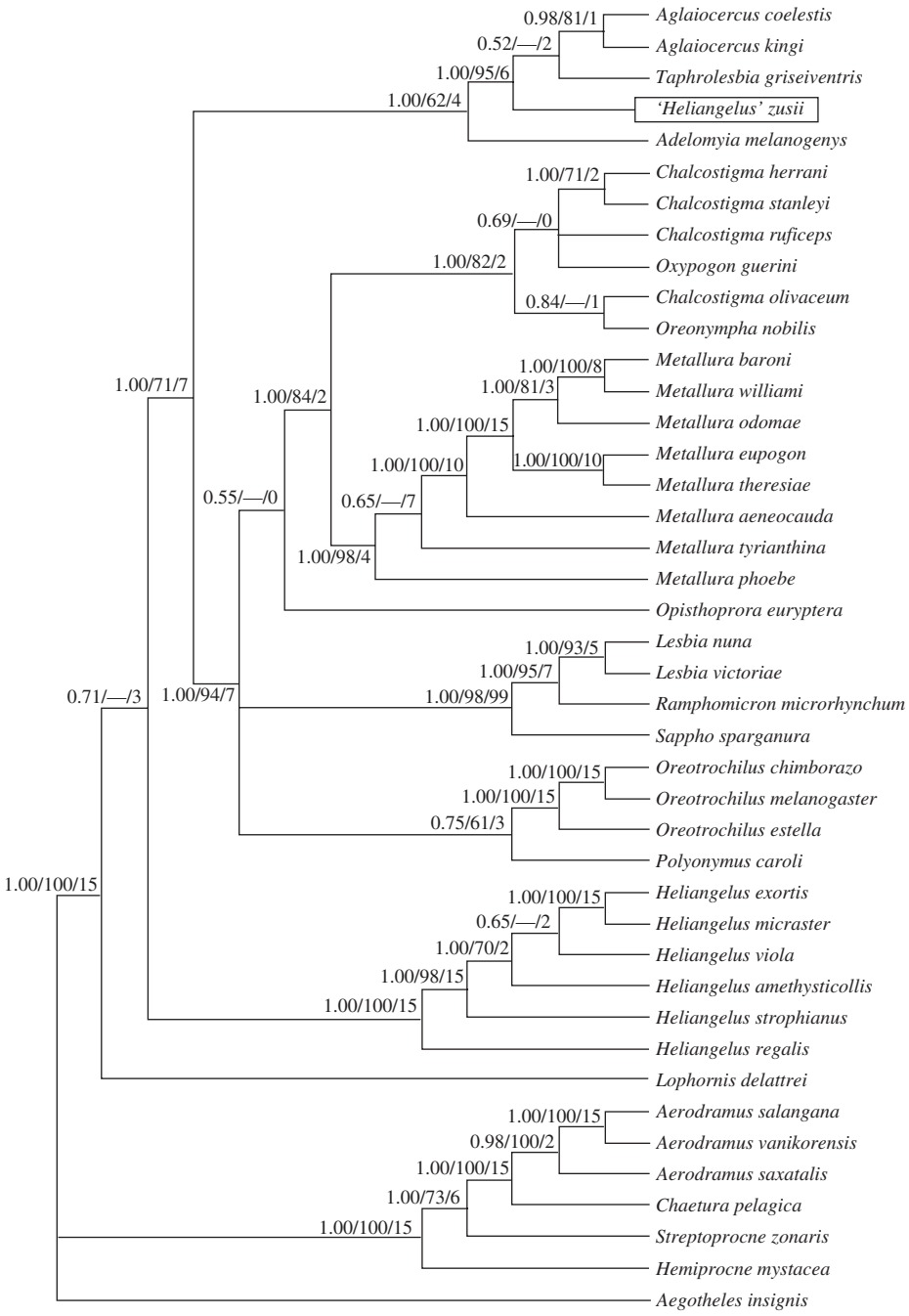

Figure 1. Consensus Bayesian phylogeny of select hummingbird species showing the relationship of *H. zusii* to other high-elevation coquettes. Numbers at nodes are Bayesian posterior probabilities/ML bootstrap support (1000 replicates)/MP Bremer support. Trees were rooted with an outgroup comprising swifts, a treeswift and an owlet-nightjar.

mitochondrial genes plus the nuclear β-fibrinogen intron 7 and adenylate kinase 1 intron 5 genes for 33 species of montane coquettes, one lowland coquette, *H. zusii* and outgroups. This combination of analyses allowed us to initially place *H. zusii* within the broad phylogenetic context of all hummingbird lineages, and then to bring as much data as possible to bear on the relationship of *H. zusii* to its closest relatives. We used the program Nona (Goloboff 1995) for MP and to compute Bremer support values (Bremer 1994). ML tree searches and bootstrapping (1000 replicates) were run in PhyML (Guindon & Gascuel 2003). Bayesian analysis using MrBayes (Huelsenbeck & Ronquist 2001) included two runs of 10 million generations, sampling every 10 000 generations and excluding the first one million generations as burn-in. Details of substitution model selection are given in the electronic supplementary material.

3. RESULTS AND DISCUSSION

Initial analysis of the complete taxon sample (356 bp) placed *H. zusii* (ANSP 159261) unambiguously within the high-elevation group of coquettes (McGuire *et al.* 2008) in a clade consisting of species placed in *Taphrolesbia* and *Aglaiocercus*, but did a poor job recovering well-established, higher-level hummingbird clades. Our MP, ML and Bayesian analyses of the more character-rich (4133 bp) alignment of 35 coquette species plus outgroups provided strong support for the hypothesis that *H. zusii* represents a distinct lineage allied with *Taphrolesbia* and *Aglaiocercus* (figure 1), with high Bremer support (6), ML bootstrap (95%) and Bayesian posterior probabilities (1.00) for the critical node linking *H. zusii* to its sister taxa. ML branch lengths (not shown) as well as pairwise genetic distances indicate that *H. zusii* is not a hybrid, but is a distinct taxon exhibiting

Table 1. Genetic distances between *H. zusii* and closely related coquette genera. (Distances are uncorrected per cent sequence divergences, including mean (number of species sampled) and range. —, sequence not available.)

	Heliangelus	*Aglaiocercus*	*Adelomyia*	*Taphrolesbia*
ND2 + *ND4* (245 bp)	13.86 (6) 12.25–17.42	5.51 (2) 5.31–5.71	9.80 (1)	3.28 (1)
tRNA-His + *tRNA-Ser* (112 bp)	4.24 (6) 2.81–4.74	3.84 (2) 3.84–3.84	1.92 (1)	—

species-level divergence from all sampled species (at least 3.3% at *ND2* + *ND4*, 245 bp) and is 12.3–17.4% divergent from six species of *Heliangelus* (table 1).

In rejecting the hybrid hypothesis for *H. zusii*, we feel certain that all potential parental species were sampled. Although we did not sample *Aglaiocercus berlepschi* (Venezuelan sylph), this taxon is morphologically similar to *Aglaiocercus kingi*, and the two are considered conspecific by many authorities. The three species of *Aglaiocercus* (sylphs) undoubtedly form a monophyletic group based on morphology. The 19 species of coquettes not sampled are in the genera *Lophornis*, *Discosura*, *Sephanoides*, *Oreotrochilus*, *Heliangelus*, *Chalcostigma*, *Metallura* and *Phlogophilus*; all these genera form well-supported clades that are genetically divergent from the clade containing *H. zusii*, and only *Chalcostigma* is not monophyletic (figure 1; McGuire *et al.* 2007). Robust support for the affinity of *H. zusii* with the *Taphrolesbia–Aglaiocercus* clade necessitates generic reassignment of *zusii*. We defer this action pending a general review of generic limits within the Trochilidae.

Our findings permit us to more accurately circumscribe the probable geographical range and habitat of *zusii*. Tens of thousands of hummingbird trade skins were exported from Bogotá for the millinery trade in the nineteenth century (Doughty 1975), and nearly all refer to species restricted to the northern Andean region of Colombia and adjacent Ecuador (Berlioz & Jouanin 1944). Graves (1993) speculated that *zusii* originated from the Eastern Cordillera of the Colombian Andes within a few hundred kilometres of Bogotá, or possibly in the Central Cordillera, in cloud forest between 1400 and 2200 m (altitude above sea level). The fact that only a single specimen is known despite the extensive collection of showy hummingbirds suggests that it had a relictual or restricted geographical distribution when collected. The three species of *Aglaiocercus* inhabit humid Andean forest (900–3000 m) from the Coastal Range of Venezuela south to northern Bolivia, whereas *Taphrolesbia griseiventris* occurs in a semi-arid region (2750–3170 m) of northern Peru, characterized by cacti and other xerophytic plants (Collar *et al.* 1992). Assuming that the ecology of *zusii* falls between these extremes, the search for *zusii* should be expanded to include semi-arid habitat in the Andes as high as 3200 m from northwestern Venezuela south of northern Peru. We presume that *zusii* is extinct, but hummingbird species continue to be discovered (Fitzpatrick *et al.* 1979; Graves 1980; Cortes-Diago *et al.* 2007), suggesting that *zusii* may persist in an unexplored region of the Andes, such as an outlying cordillera (e.g. *Heliangelus regalis*) or an isolated peak (e.g. *Ramphomicron dorsale*).

aDNA from unique types may help resolve long-standing taxonomic problems in ornithology and other disciplines. Our finding that mtDNA from ANSP 159261 is highly divergent from potential parental species rules out a hybrid origin and precludes sequencing nuclear DNA, which is much more difficult to obtain from century-old archival material (Kirchman 2009). The use of silica-based techniques, originally developed for extractions from organic remains of Pleistocene origin, should enable genetic characterization of other extremely small samples of archival material such as those we have obtained from the tiny feet of a hummingbird museum specimen.

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Supplementary material

Fig. S1. Holotype of "*Heliangelus*" *zusii* (ANSP 159261)

Fig. S2. "*Heliangelus*" *zusii* and its close relatives (upper left, *Aglaiocercus kingi*; upper middle, *Aglaiocercus coelestis;* upper right, *Heliangelus zusii*; and lower right, *Taphrolesbia griseiventris*). Painting by Jon Fjeldså ©, University of Copenhagen (see next page).

On-line supplementary material

(http://rsbl.royalsocietypublishing.org/content/6/1/112/suppl/DC1)

Ancient DNA Extraction, Amplification, and Sequencing: Contamination controls carried out in the ancient DNA lab include negative extraction controls (containing no tissue), glove changes between handling each sample, ultraviolet irradiation of all plastics and buffers, exclusive use of aerosol-barrier pipette tips, and daily sanitation of all equipment and surfaces with 10% bleach solution. The skin sample from ANSP 159261was incubated for 48 h with agitation at 55°C in 600 uL of an extraction buffer consisting of 7.5 M guanidinium thiocyanate, 0.1 M Tris-HCl (pH 6.4), 0.02 M EDTA, and 1.3% Triton X-100. After centrifugation, 500uL of supernatant was removed to a second tube containing 500uL of binding buffer (7.5 mol/L guanidinium thiocyanate, and 0.3M sodium acetate [pH 5.2]) and 40uL of saturated silica suspension (SiO2 in water). DNA was bound to the silica for 3 hr at 27°C, pelleted by centrifugation, washed once with binding buffer and twice with 52% ethanol supplemented with 0.125M sodium chloride, 0.01M Tris [pH 8.0] and 0.5M EDTA. Bound DNA was eluted by incubating the pellet twice in 75uL volumes of TE buffer for 30 minutes at 55°C.

PCR conditions and primer sequences: Amplifications were performed in 50uL reactions containing 5.0 uL of DNA extract, 0.5uM of each primer, 0.4mM dNTPs , 2.5mM $MgCl_2$, 0.5mM bovine serum albumen, and 1 unit of AmpliTaq Gold DNA polymerase. Primer pairs that successfully amplified DNA from ANSP159261 were F1 (GCAACATTCCTAACAGCCTCAT) R1 (GATGTTCTCGTGAGGTTGAGTTT), F2 (AAACATTAGCCTGTGATCCTAAAAA) R2 (TCCTAAGACCAGTGGATTAACTG), F4 (TCCAAGAACTCTCCAAACAAGA) R4

(GGGAGGGTGATTGTTGAGTAGT), and F5 (TCCYTRCTGCTAACCRCAGCAA) R5 (ATGTGAGGAAGAGAATAGTGAT). PCR products were cut from agarose gels, purified using a Qiagen MinElute gel extraction kit, and were sequenced on an Applied Biosystems 3730XL DNA Sequencer.

We used MODELTEST (Posada, D., & Crandall, K. A. 1998. *Bioinformatics* **14**, 817–818. doi:10.1093/bioinformatics/14.9.817) to select appropriate models for seven data partitions used in subsequent ML and Bayesian analyses. The partitions and models are as follows:

Data Partition	Number of substitution types	Among-site rate variation
tRNAs	2	Proportion of invariant sites
ND2 1st codon position	6	Proportion of invariant sites + Gamma
ND2 2nd codon position	6	Proportion of invariant sites + Gamma
ND2 3rd codon position	6	Proportion of invariant sites + Gamma
AK1	6	Gamma distribution
bfib	6	Gamma distribution
ND4 1st codon position	6	Proportion of invariant sites + Gamma
ND4 2nd codon position	6	Proportion of invariant sites + Gamma
ND4 3rd codon position	6	Proportion of invariant sites + Gamma

taxon	Museum catalog number	ND2 Genbank No.	ND4 and tRNAs Genbank No.	Bfib7 Genbank No.	AK1 Genbank No.	Tribe	locality
Adelomyia melanogenys	LSUMZ B-8025	AY830457	EU042274	AY830608	AY830533	Lophornithini	Peru, Pasco Department, Playa Pampa, 8 km NW Cushi.
Aegotheles insignis	KU 5081	EU042514	EU042194	EU042350	EU042432	z-Outgroup	Papua New Guinea, Simbu Province, 06 deg 41'40'S, 145 deg 06'24'E.
Aerodramus salangana	Unknown	AY294488	-	AF182648		z-Outgroup	Unknown.
Aeronautes saxatalis	GM42600	GU166847	-	GU166838	-	z-Outgroup	USA.
Aeronautes vanikorensis	USNM B-04039	EU042510	EU042190	EU042347	-	z-Outgroup	Papua New Guinea, New Ireland Island, New Ireland Province, Weitin River Valley.
Aglaeactis cupripennis	LSUMZ B-6304	AY830459	EU042276	AY830610	AY830535	Coeligenini	Ecuador, Pichincha Province, Yanacocha N slope of Cerro Pichincha.
Aglaiocercus coelestis	LSUMZ B-7813	AY830460	EU042277	AY830611	AY830536	Lophornithini	Ecuador, El Oro Province, 9.5 km road to Pinus, 900m.
Aglaiocercus kingi	LSUMZ B-8017	AY830461	EU042278	AY830612	AY830537	Lophornithini	Peru, Pasco Department, Playa Pampa, 8 km NW Cushi.
Amazilia chionogaster	LSUMZ B-17165	AY830462	EU042279	AY830613	AY830538	Trochilini	Argentina.
Amazilia franciae	LSUMZ B-12063	EU042521	EU042201	EU042357	EU042439	Trochilini	Ecuador, Pichincha Province, Mindo.
Amazilia saucerrottei	FMNH 393025	EU042523	EU042203	EU042359	EU042441	Trochilini	Costa Rica, Guanacaste Province, Santa Cruz, 17 km SSW, Cerro Vista al Mar.
Amazilia tzacatl	LSUMZ B-16538	EU042524	EU042204	EU042360	EU042442	Trochilini	Panama, Panama Province, Old Gamboa Road-2 ponds, 5km NW of Paraiso; 9 deg 03' 30'' N, 79 deg 39' 00'' W.
Amazilia versicolor	FMNH 395409	EU042525	EU042205	EU042361	EU042443	Trochilini	Brazil, Sao Paulo State, Boracei.
Androdon aequatorialis	LSUMZ B-1402	AY830463	EU042280	AY830614	AY830539	Polytmini	Panama, Darien Province, Ca 9 km NW Cana on slopes Cerro Pirre.
Anthracothorax nigricollis	LSUMZ B-9552	EU042527	EU042207	EU042363	EU042445	Polytmini	Bolivia, Pando Department, Nicolas Suarez; 12 km by road S of Cobija, 8 km W on road to Mucden.
Aphantochroa cirrochloris	FMNH 399167	EU042528	EU042208	EU042364	EU042446	Trochilini	Brazil, Pernambuco State, Taquaritinga.
Archilochus colubris	LSUMZ B-5270	AY830465	EU042282	AY830616	AY830541	Mellisugini	USA, Louisiana, East Baton Rouge Parish, Baton Rouge, 15533 Geraldine St.
Boissonneaua matthewsii	LSUMZ B-8130	AY830466	EU042283	AY830617	AY830542	Coeligenini	Peru, Pasco Department, Playa Pampa, ca 8 km NW Cushi on trail to Chaglla.
Calypte anna	LSUMZ B-24864	EU042532	EU042212	EU042368	EU042450	Mellisugini	USA, California, San Bernardino County, E. Mojave Desert, SW base Clark Mtn., Pachalka Spring.
Campylopterus largipennis	LSUMZ B-4474	AY830467	EU042285	AY830618	AY830543	Trochilini	Peru, Loreto Department, Lower Rio Napo region, E bank Rio Yanayacu, ca 90 km N Iquitos.
Chaetura pelagica	LSUMZ B-3346	AY830455	GU166857	AY830606	AY830531	z-Outgroup	USA, Louisiana, Cameron Parish, East Jetty Woods, 2 mi. S Cameron.
Chalcostigma herrani	LSUMZ B-6277	EU042536	EU042216	EU042372	EU042454	Lophornithini	Ecuador, Pichincha Province, Mt. Pichincha.
Chalcostigma olivaceum	LSUMZ B-3924	GU166848	GU166858	GU166839	GU166828	Lophornithini	Peru, Lima Department.
Chalcostigma ruficeps	LSUMZ B-1293	AY830469	EU042288	AY830620	AY830545	Lophornithini	Bolivia, La Paz Department, Ca 1km S Chuspipata.
Chalcostigma stanleyi	MSB NK-159810	GU167255	GU166924	GU167144	-	Lophornithini	Peru, Cuzco Department, 12 km N Urubamba.
Chalybura buffonii	LSUMZ B-26911	EU042537	EU042217	EU042373	EU042455	Trochilini	Panama, Panama Province, Old Gamboa Road, 5 km NW Paraiso, 9 deg 03' 30" N, 79 deg 39' 03" W.
Chlorostilbon mellisugus	LSUMZ B-9450	AY830471	EU042290	AY830622	AY830547	Trochilini	Bolivia, Pando Department, Nicolv°s Suarez; 12 km by road S of Cobija, 8 km W on road to Mucden.
Chrysolampis mosquitus	LSUMZ B-35902	EU042540	EU042220	EU042376	EU042458	Polytmini	Trinidad and Tobago, Caroni County, Arena Forest, ca. 5 km S San Rafael.
Chrysuronia oenone	LSUMZ B-5381	AY830472	EU042291	AY830623	AY830548	Trochilini	Peru, San Martin Department, 20 km by road NE Tarapoto on road to Yurimaguas.
Coeligena coeligena	LSUMZ B-8015	EU042541	EU042221	EU042377	EU042459	Coeligenini	Peru, Pasco Department, Playa Pampa, 8 km NW Cushi on trail to Chaglla.
Colibri coruscans	LSUMZ B-6310	AY830476	EU042295	AY830627	GU166829	Polytmini	Ecuador, Pichincha Province, Yanacocha, N slope of Cerro Pichincha.
Discosura conversii	LSUMZ B-16064	AY830519	EU042296	AY830670	AY830594	Lophornithini	Costa Rica, Heredia Province, Finca La Fortuna; 4 km SE Virgendel Socorro.
Discosura popelairii	LSUMZ B-5950	EU042546	EU042226	EU042382	EU042464	Lophornithini	Ecuador, Morona-Santiago Province, near Tayuza.
Doryfera ludovicae	LSUMZ B-1802	AY830477	EU042297	AY830628	AY830552	Polytmini	Peru, Pasco Department, Santa Cruz; about 9 km SSE Oxapampa.
Ensifera ensifera	LSUMZ B-8224	AY830479	EU042299	AY830630	AY830554	Coeligenini	Peru, Pasco Department, Millpo, E Tambo de Vacas on Pozuzo- Chaglla trail.
Eriocnemis vestita	LSUMZ B-324	EU042551	EU042231	EU042387	GU166830	Coeligenini	Peru, Cajamarca Department, Cerro Chinguela, 5 km NE Sapalache.
Eugenes fulgens	LSUMZ B-9964	AY830481	EU042301	AY830632	AY830556	Lampornithini	Costa Rica, San Jose Province, La Georgina, km 95 Pan-Am Hwy.
Eulampis holosericeus	USNM B02121	AY830530	EU042302	AY830681	AY830605	Polytmini	St. Vincent and the Grenadines, St. George Parish, Indian Bay, 2.5 mi SE Kingstown.
Eupherusa nigriventris	LSUMZ B-16055	EU042553	EU042233	EU042389	EU042469	Trochilini	Costa Rica, Heredia Province, Finca La Fortuna; 4 km SE Virgendel Socorro.
Eutoxeres condamini	LSUMZ B-8094	AY830484	EU042305	AY830635	AY830559	Phaethornithinae	Peru, Pasco Department, Playa Pampa, ca 8 km NW Cushi on trail to Chaglla.
Florisuga mellivora	LSUMZ B-9646	AY830485	EU042306	AY830636	AY830560	Topazini	Bolivia, Pando Department, Nicolas Suarez; 12 km by road S of Cobija, 8 km W on road to Mucden.
Glaucis hirsutus	LSUMZ B-28468	AY830486	EU042307	AY830637	AY830561	Phaethornithinae	Panama, Colon Province, Achiote Road at Rio Providencia.
Haplophaedia aureliae	LSUMZ B-32971	AY830487	EU042308	AY830638	AY830562	Coeligenini	Peru, Cajamarca Department, Ca 3 km NNE San Jose de Lourdes; 5 deg 23.0' S 78 deg 46.3' W.
Heliangelus amethysticollis	LSUMZ B-6246	AY830489	EU042310	AY830640	AY830564	Lophornithini	Ecuador, Morona-Santiago Province, W slope Cordillera del Cutucu, S trail from Logrono to Yaupi.
Heliangelus exortis	Unvouchered	GU166849	GU166859	GU166840	GU166831	Lophornithini	Colombia.
Heliangelus micraster	LSUMZ B-31990	EU042556	EU042236	EU042392	EU042472	Lophornithini	Peru, Cajamarca Department, Quebrada Lanchal, ca. 8 Km ESE Sallique 5 deg 41.2'S, 79 deg 15.0'W.
Heliangelus regalis	LSUMZ B-5532	GU166850	GU166860	GU166841	GU166832	Lophornithini	Peru, San Martin Department.
Heliangelus strophianus	LSUMZ B-6296	GU167231	GU166900	GU167121	GU167177	Lophornithini	Ecuador, Pichincha Province.
Heliangelus viola	LSUMZ B-32370	GU167232	GU166901	GU167122	GU167178	Lophornithini	Peru, Cajamarca Department.
Heliangelus zusii	ANSP 159261	GU166851	GU166861	-	-	Lophornithini	"Bogota"
Heliodoxa leadbeateri	LSUMZ B-6036	AY830492	EU042314	AY830643	AY830567	Coeligenini	Ecuador, Morona-Santiago Province, West slope of Cordillera del Cutucu on trail from Lagrono to Yaupi.
Heliodoxa schreibersii	LSUMZ B-5440	EU042559	EU042239	EU042395	EU042475	Coeligenini	Peru, San Martin Department, 20 km by road NE Tarapoto on road to Yurimaguas.
Heliomaster longirostris	LSUMZ B-18268	AY830493	EU042315	AY830644	AY830568	Lampornithini	Bolivia, Santa Cruz Department, Velasco; Parque Nacional Noel Kempff Mercado 86 km ESE Florida.
Heliothryx barroti	LSUMZ B-12039	AY830494	EU042316	AY830645	AY830569	Polytmini	Ecuador, Esmeraldas Province, El Placer, ca 670m, 0 deg 52'N, 78 deg 33'W.
Hemiprocne mystacea	UWBM 68087	EU042517	EU042197	EU042353	EU042435	z-Outgroup	Solomon Islands, Western Province, New Georgia Group, New Georgia, Arara, Eagon Resource Development Company, 2.5 km NE.
Klais guimeti	LSUMZ B-6168	AY830495	EU042317	AY830646	AY830570	Trochilini	Ecuador, Morona-Santiago Province, W slope Cordillera del Cutucu, Yapitya, on Logrono-Yaupi trail.
Lafresnaya lafresnayi	LSUMZ B-32771	AY830496	EU042318	AY830647	AY830571	Coeligenini	Peru, Cajamarca Department, Quebrada Las Palmas, ca 13 km WSW Chontali, 5 deg 40.0' S 79 deg 12.2' W.
Lampornis calolaemus	LSUMZ B-28169	EU042565	EU042245	EU042400	EU042480	Lampornithini	Panama, Chiriqui Province, Dist. Gualaca, Cordillera Central, 4.3 km by road S Lago Fortuna dam.
Lesbia nuna	LSUMZ B-3615	AY830498	EU042320	AY830649	AY830573	Lophornithini	Peru, Huanuco Department, Quebrada Shugush, 30 km on Huanuco-La Union road.
Lesbia victoriae	ZMC 113657	AY830499	GU166862	AY830650	AY830574	Lophornithini	Ecuador, Pichincha Province, S slope Pasochoa.
Lophornis chalybea	LSUMZ B-9399	GU167239	GU166908	GU167129	GU167185	Lophornithini	Bolivia, Pando Department.
Lophornis delattrei	LSUMZ B-22644	AY830500	EU042321	AY830651	AY830575	Lophornithini	Bolivia, La Paz Department, Prov. B. Saavedra, 83 km by road E Charazani, Cerro Asunta Pata.
Lophornis pavoninus	KU 4063, voucher KUMNH 92432	EU042568	EU042248	EU042403	EU042483	Lophornithini	Guyana, Mt. Roraima (05 deg 15' N, 60 deg 44' W).
Metallura aeneocauda	LSUMZ B-1236	AY830501	EU042322	AY830652	AY830576	Lophornithini	Bolivia, La Paz Department, ca 1km S Chuspipata.
Metallura baroni	ZMC 113658	GU167240	GU166909	GU167130	GU167186	Lophornithini	Ecuador.
Metallura eupogon	LSUMZ B-8293	GU166852	GU166863	GU166842	GU166833	Lophornithini	Peru, Pasco Department.
Metallura odomae	LSUMZ B-353	GU167241	GU166910	GU167131	GU167187	Lophornithini	Peru, Cajamarca Department, Cerro Chinguela.
Metallura phoebe	LSUMZ B-103876	EU042569	EU042249	EU042404	EU042484	Lophornithini	Peru, Arequipa Department, Ca 12 Road km E Chiguata.
Metallura theresiae	LSUMZ B-3534	GU166853	GU166864	GU166843	GU166834	Lophornithini	Peru, Huanuco Department.
Metallura tyrianthina	LSUMZ B-6272	AY830502	EU042323	AY830653	AY830577	Lophornithini	Ecuador, Pichincha Province, Mt. Pichincha.
Metallura williami	LSUMZ B-5939	EU042570	EU042250	EU042405	EU042485	Lophornithini	Ecuador, Morona-Santiago Province.
Microchera albocoronata	LSUMZ B-35764	EU042571	EU042251	EU042406	EU042486	Trochilini	Costa Rica, Cartago Province, 11 km SW Pejibaye, 9 degrees 47'N, 83 degrees 45'W.
Myrtis fanny	LSUMZ B-9292	AY830503	GU166865	AY830654	AY830578	Mellisugini	Peru, Huanuco Department, Nvevas Flores (Cullquish) on Rio Maranon.
Ocreatus underwoodii	LSUMZ B-22594	AY830504	EU042324	AY830655	AY830579	Coeligenini	Bolivia, La Paz Department, Prov. B. Saavedra, 88 km by road E Charazani, Cerro Asunta Pata.
Opisthoprora euryptera	ZMC 115166	EU042572	EU042252	EU042407	EU042487	Lophornithini	Ecuador, Napo Province, Oyacachi, 3050 m.
Oreonympha nobilis	FMNH 324113	AY830505	EU042325	AY830656	AY830580	Lophornithini	Peru, Cuzco Department, Huacarpay, 3300m.
Oreotrochilus chimborazo	ZMC 114549	AY830506	EU042326	AY830657	AY830581	Lophornithini	Ecuador, El Oro Province, Chimborazo.
Oreotrochilus estella	LSUMZ B-103835	AY830507	EU042327	AY830658	AY830582	Lophornithini	Peru, Ayacucho Department, Pampa Galeras, 25km WNW on Puquio.
Oreotrochilus melanogaster	LSUMZ B-2077	GU166854	GU166866	GU166844	GU166835	Lophornithini	Peru, Lima Department.
Oxypogon guerinii	ZMC 115370	EU042573	-	EU042408	EU042488	Lophornithini	Venezuela, Merida State, Mifafi Valle, El Domo, 4000m.
Patagona gigas	LSUMZ B-6303	AY830510	EU042330	AY830661	AY830585	Patagonini	Ecuador, Pichincha Province, Yanacocha N slope of Cerro Pichincha.
Phaethornis bourcieri	LSUMZ B-11017	EU042577	EU042256	EU042412	EU042492	Phaethornithinae	Peru, Ucayali Department, SE slope Cerro Tahuayo, ca ENE Pucallpa.
Phaethornis guy	LSUMZ B-35966	AY830511	EU042331	AY830662	AY830586	Phaethornithinae	Trinidad and Tobago, Caroni County, Arena Forest, ca. 5 km S San Rafael.
Phaethornis ruber	LSUMZ B-9481	AY830515	EU042335	AY830666	AY830590	Phaethornithinae	Bolivia, Pando Department, Nicolas Suarez; 12 km by road S of Cobija, 8 km W on road to Mucden.
Phlogophilus hemileucurus	LSUMZ B-5999	AY830517	EU042336	AY830668	AY830592	Lophornithini	Ecuador, Morona-Santiago Province, W slope of Cordillera del Cutucu on trail from Lagrono to Yaupi.
Polyonymus caroli	LSUMZ B-15503	GU166855	GU166867	GU166845	GU166836	Lophornithini	Peru.
Polytmus guainumbi	LSUMZ B-6691	EU042585	EU042264	EU042420	EU042500	Polytmini	Bolivia, Santa Cruz Department, 1km N 2km E YPFB Refinery.
Pterophanes cyanopterus	LSUMZ B-1296	AY830520	EU042337	AY830671	AY830595	Coeligenini	Bolivia, La Paz Department, ca 1km S Chuspipata.
Ramphomicron microrhynchum	LSUMZ B-8376	EU042587	EU042266	EU042422	EU042502	Lophornithini	Peru, Pasco Department, Millpo, E Tambo de Vacas Pozuzo-Chaglla Trail.
Rhodopis vesper	LSUMZ B-14277	EU042588	EU042267	EU042423	EU042503	Mellisugini	Peru.
Sappho sparganura	LSUMZ B-1195	GU167249	GU166918	GU167138	GU167194	Lophornithini	Bolivia, La Paz Department.
Schistes geoffroyi	LSUMZ B-6300	AY830521	EU042338	AY830672	AY830596	Polytmini	Ecuador, Pichincha Province, W slope of W Andes near Mindo.
Selasphorus platycercus	LSUMZ B-23428	AY830522	EU042339	AY830673	AY830597	Mellisugini	USA, Texas, Jeff Davis County, Davis Mountains State Park.
Sephanoides fernandensis	Michael S. Roy 620	EU042591	EU042270	EU042426	EU042506	Lophornithini	Chile, Juan Fernandez Islands, Isla Robinson Crusoe.
Streptoprocne zonaris	USNM B-13064	EU042511	EU042191	EU042348	EU042430	z-Outgroup	Guyana, Wiwitau Mountain, East Rupununi Savannah.
Taphrolesbia griseoventris	LSUMZ B-10388	GU166856	GU166868	GU166846	GU166837	Lophornithini	Peru, Cajamarca Department.
Thalurania colombica	LSUMZ B-11793	AY830524	EU042342	AY830675	AY830599	Trochilini	Ecuador, Esmeraldas Province, El Placer, ca 670m.
Threnetes ruckeri	LSUMZ B-28170	AY830527	EU042344	AY830678	AY830602	Phaethornithinae	Panama, Chiriqui Province, Distrito Gualaca, Cordillera Central, 4.3 km by road S Lago Fortuna dam.
Topaza pella	LSUMZ B-35362	AY830528	EU042345	AY830679	AY830603	Topazini	Brazil, Para State, E bank of Rio Teles Pires, 4 km. Upriver from mouth of Rio Sao Benedito.
Urochroa bougueri	ZMC 114654	EU042594	EU042273	EU042429	EU042509	Coeligenini	Ecuador, Napo Province, Hda San Isidro, 2100 m.
Urosticte benjamini	LSUMZ B-5546	AY830529	EU042346	AY830680	AY830604	Coeligenini	Peru, San Martin Department, 15 km by trail NE Jirillo on trail to Balsapuerto.