



## The *Basilinna* genus (Aves: Trochilidae): an evaluation based on molecular evidence and implications for the genus *Hylocharis*

### El género *Basilinna* (Aves: Trochilidae): una evaluación basada en evidencia molecular e implicaciones para el género *Hylocharis*

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**Abstract.** Hummingbirds are one of the most diverse families of birds and the phylogenetic relationships within the group have recently begun to be studied with molecular data. Most of these studies have focused on the higher level classification within the family, and now it is necessary to study the relationships between and within genera using a similar approach. Here, we investigated the taxonomic status of the genus *Hylocharis*, a member of the Emeralds complex, whose relationships with other genera are unclear; we also investigated the existence of the *Basilinna* genus. We obtained sequences of mitochondrial (ND2: 537 bp) and nuclear genes (AK-5 intron: 535 bp, and c-mos: 572 bp) for 6 of the 8 currently recognized species and outgroups. Our analyses, using 3 different inference methods (Maximum Parsimony, Likelihood and Bayesian methods), corroborate the existence of the hummingbird genus *Basilinna* as composed of 2 species commonly assigned to the genus *Hylocharis*: *leucotis* and *xantusii*. Our study also supports that *Hylocharis* is a paraphyletic genus that includes species belonging to the genus *Amazilia*.

Key words: phylogenetic taxonomy, molecular phylogeny, *Basilinna leucotis*, *Basilinna xantusii*, hummingbirds.

**Resumen.** Los colibríes son una de las familias de aves más diversa y las relaciones filogenéticas dentro del grupo están empezando a entenderse mejor gracias a estudios con datos moleculares. La mayoría de esos estudios se ha enfocado a las relaciones filogenéticas de alto nivel dentro de la familia y ahora también es necesario estudiar las relaciones entre y dentro de los géneros con un enfoque semejante. En este estudio investigamos la situación taxonómica del género *Hylocharis*, miembro del complejo de las Esmeraldas, cuyas relaciones con otros géneros no están del todo claras; también investigamos la existencia del género *Basilinna*. Obtuvimos secuencias mitocondriales (ND2: 537 bp) y nucleares (intrón AK-5: 535 bp y c-mos: 572 bp) para 6 de las 8 especies actualmente reconocidas, así como para los grupos externos. Nuestros análisis, usando 3 métodos de inferencia distintos (máxima parsimonia, máxima verosimilitud e inferencia bayesiana), corroboran la existencia del género *Basilinna* conformado por 2 especies que actualmente se asignan al género *Hylocharis*: *leucotis* y *xantusii*. Nuestro estudio también sugiere que el género *Hylocharis* es parafilético e incluye especies asignadas al género *Amazilia*.

Palabras clave: taxonomía filogenética, filogenia molecular, *Basilinna leucotis*, *Basilinna xantusii*, colibríes.

## Introduction

Hummingbirds are a clearly defined clade of birds whose internal relationships have only recently begun to be understood through a series of molecular studies

(Bleiweiss et al., 1997; Bleiweiss, 1998a, b, c; Altshuler et al., 2004; McGuire et al., 2007). Most of these studies have focused on the higher level classification within the family, whereas studies focused on the relationships among and within genera are rather sparse. Inter and intra genera studies are important because phenotypic variation and distributional patterns suggest that the species limits are not always clear (Schuchmann, 1978, 1984, 1989, 1995,

1999; Schuchmann and Duffner, 1993; Schuchmann and Züchner, 1997). However, only a few genera have been studied in detail using molecular tools, such as *Metallura* (García-Moreno et al., 1999), *Lampornis* (García-Moreno et al., 2006), *Cynanthus* (García-Deras et al., 2008), *Coeligena* (Parra et al., 2009) and *Adelomyia* (Chaves and Smith, 2011).

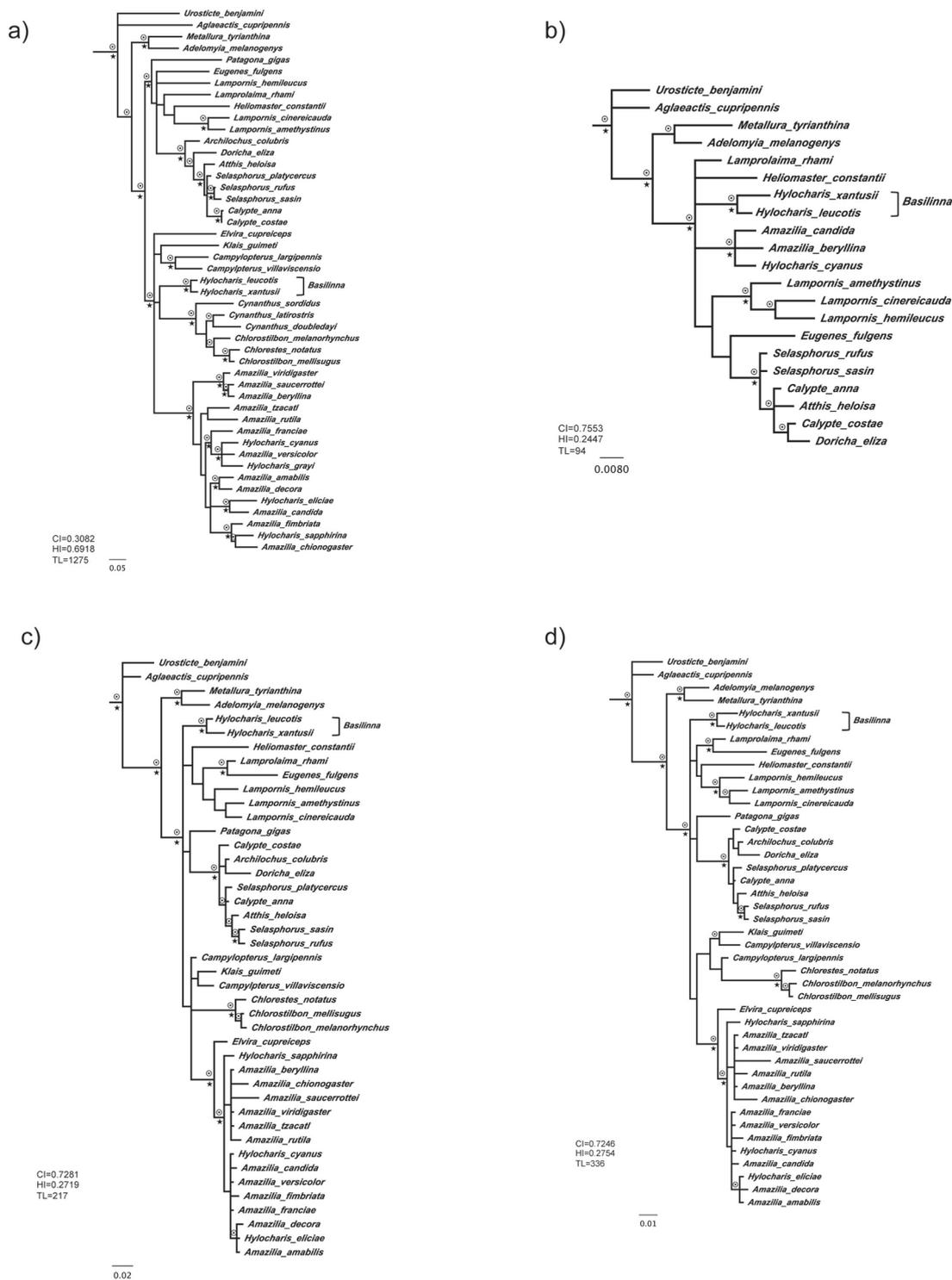
Both DNA-DNA hybridization (Bleiweiss et al., 1997) and DNA sequencing (Altschuler et al., 2004; McGuire et al., 2007) point out to the same main clades within the family. The most basal node is an unresolved polytomy between Hermits (e.g., *Eutoxeres*, *Glaucis*, *Threnetes* and *Phaethornis*), non-Hermits, and a small clade formed by *Topaza* and *Florisuga* (McGuire et al., 2007). Within the non-Hermits there are 6 large groups originally identified by Bleiweiss et al. (1997) and later corroborated by DNA sequencing studies (Altschuler et al., 2004), with the Mangoes (e.g., *Doryfera*, *Colibri*, *Anthracothonax*) being the most basal of them. Brilliants (e.g., *Heliodoxa*, *Boissonneaua*, *Coeligena*, *Aglaeactis*, *Eriocnemis*, *Haplophaedia*) are the sister clade to Coquettes (e.g., *Heliangelus*, *Sephanoides*, *Discosura*, *Lophornis*, *Aglaiocercus*, *Oreotrochilus*, *Lesbia*, *Chalcostigma*, *Metallura*), and together they form a clade sister to *Patagona*, Emeralds, Bees, and Mountain Gems. The relationships among these latter groups are unresolved. Mountain gems (e.g., *Lampornis*, *Heliomaster*) are the sister group to Bees (e.g., *Selasphorus*, *Calypte*, *Archilochus*, *Calliphlox*), and the clade formed by these 2 is part of an unresolved polytomy with *Patagona gigas* and the Emeralds (e.g., *Chlorostilbon*, *Campylopterus*, *Chalybura*, *Thalurania*, *Eupherusa*, *Elvira*, *Amazilia*, *Hylocharis*). Moreover, addition of species to the larger DNA-sequence phylogeny has resulted in local topological changes in this part of the tree (compare figure 1 from Altschuler et al., 2004 with figure 2 from McGuire et al., 2007), but Mountain Gems in particular remain poorly sampled in the overall hummingbird phylogeny.

The genus *Hylocharis*, the focus of this study, is a member of the Emeralds complex. Its relationships to other genera within this clade are still unclear. McGuire et al. (2009) indicated that *Amazilia* does not represent a monophyletic genus, as several genera are nested within it (*i.e.* *Hylocharis*, *Lepidopyga*, *Chrysuronia* and *Damophila*; see also García-Moreno et al., 2006); these authors suggested that further morphological and genetic studies, with dense intra- and interspecific sampling, are necessary in order to clarify relationships within the Emeralds complex. The genus *Hylocharis*, as currently recognized by the American Ornithologists' Union (AOU, 1998; Remsen et al., 2013), includes 8 species (*H. grayi*,

*H. eliciae*, *H. leucotis*, *H. xantusii*, *H. sapphirina*, *H. cyanus*, *H. chrysura* and *H. humboldtii*) and has the widest distribution of any genus within the family, ranging from the south of the United States to the north of Argentina (Schuchmann, 1999). *H. leucotis* is found on pine and pine-oak forest and edges, in the highlands of Mexico and northern Central America, between 1 200 and 3 500 m, resulting roughly in 4 discontinuous blocks: the Sierra Madre Occidental and Oriental and the Neovolcanic Axis; the Sierra Madre del Sur; the highlands of Chiapas and Guatemala; and the mountains of western Honduras, including the northern parts of El Salvador and the northwestern highlands of Nicaragua (Arizmendi et al., 2010a). *H. xantusii* occurs in Baja California Sur (Mexico) along the Sierra de la Laguna and the Sierra de la Giganta, into the southernmost part of Baja California, and the islands of Cerralvo and San José in the Gulf of California (Arizmendi et al., 2010b).

Ridgway (1911) proposed that *H. leucotis* and *H. xantusii* belong to a different genus, *Basilinna*, created by Boie in 1831. According to Ridgway (1911), *Basilinna* is “similar to *Hylocharis*, but wing relatively longer (3 times as long as exposed culmen) and style of coloration very different, the side of head with a broad white postocular streak and a black (male) or dusky (female) auricular stripe” (p. 377); those 2 characteristics (wing and white postocular stripe) are not present in the other species of the genus *Hylocharis*. More recently, Howell and Webb (1995) and Schuchmann (1999) resurrected this proposal. In a molecular study focused in the genus *Lampornis*, García-Moreno et al. (2006) also found evidence suggesting the existence of a clade formed by *Basilinna leucotis* and *B. xantusii*. This work included an analysis of mitochondrial DNA sequences from a broad sample of hummingbird species (100 species from 62 genera) and found several things relevant to the present study: the 3 species of *Hylocharis* included in the sampling scheme nested unambiguously within the Emerald group, and not with *Lampornis* as was suggested by Schuchmann (1999). Within this Emerald clade, *Hylocharis* did not form a monophyletic group: whereas *H. leucotis* and *H. xantusii* formed a well supported clade sister to *Chlorostilbon*, the third *Hylocharis* species was deeply nested in another clade together with *Chrysuronia*, *Lepidopyga*, *Amazilia*, *Taphrospilus*, and *Elvira*.

Here we evaluate the taxonomic status of *H. leucotis* and *H. xantusii* using data from mitochondrial and nuclear genes—both coding and non-coding—looking for further evidence for the existence of the *Basilinna* genus, as well as of its relationships with other genera (*Lampornis* and *Hylocharis*).



**Figure 1.** Phylogenetic relationships among *Hylocharis* hummingbirds as inferred using Bayesian methods and the evolutionary models indicated in Results. a), Partial mitochondrial protein coding ND2 gene; b), partial nuclear protein coding c-mos gene; c), complete intron 5 of Adenylate Kinase (AK5); d), concatenate nuclear genes (c-mos and AK5). Branching patterns from maximum likelihood and maximum parsimony analyses agree with the trees depicted. A star below the branch depicts a posterior probability  $\geq 0.95$ ; a double circle above the branch refers to bootstrap values  $\geq 50\%$ .



**Figure 2.** Phylogenetic relationships among *Hylocharis* hummingbird species inferred using Bayesian methods on the concatenated sequences of the 3 amplified gene fragments. Branching patterns from maximum likelihood and maximum parsimony analyses agree with the tree depicted. A star below the branch depicts a posterior probability  $\geq 0.95$ ; a double circle above the branch refers to bootstrap values  $\geq 50\%$ .

## Materials and methods

**Taxon sampling.** Table 1 presents a full list of the taxa included in this study. We took advantage of many sequences already deposited in GeneBank and complemented these with our own generated DNA sequences. We obtained tissue samples from *Hylocharis leucotis* and *H. xantusii*, as well as selected species that could be related to these of the Emerald group (*Amazilia candida* and *A. beryllina*, *Cynanthus*, *Chlorostilbon*), Bee group (*Selasphorus sasin*, *S. rufus*, *Doricha eliza*, *Calypte costae*, *C. anna*, *Atthis heloisa* and *Archilochus colubris*) and Mountain Gem group (*Lamprolaima rhami*, *Lampornis hemileucurus*, *L. cinereicauda*, *L. amethystinus*, *Heliomaster constantii* and *Eugenes fulgens*). We also included several other species of the genus *Hylocharis*: *grayi*, *sapphirina*, *eliciae* and *cyaneus*, resulting in the broadest sampling of *Hylocharis*

species in a molecular study as far as we know (Table 1) – though unfortunately the analysis of the genus is incomplete, as we lack samples of *H. chrysura* and *H. humboldtii*. We sequenced several other species of hummingbirds in order to have a well represented outgroup that included species from clades basal to the Emeralds according to the most current understanding of the Trochilidae phylogeny (Altshuler et al., 2004; McGuire et al., 2007). We restricted the outgroup to 4 genera: *Adelomyia* and *Metallura* from the coquettes group, and *Aglaeactis* and *Urosticte* from the brilliants group. Coquettes and brilliants form the sister clade to a group that comprises *Patagona*, emeralds, mountain gems, and bees (see Fig. 3 in McGuire et al., 2007).

**DNA amplification and sequencing.** We extracted DNA from tissue samples using the Qiagen DNeasy extraction kit, following the manufacturer's protocols. We amplified 3 DNA fragments of similar size of different regions and characteristics: the full length of a non coding intron (intron 5 of Adenylate kinase, or AK5), and partial sequences of 2 protein coding genes, 1 nuclear (proto-oncogen *c-mos*, intronless codes for a kinase) and 1 mitochondrial (NADH dehydrogenase subunit 2, or ND2). The 3 amplified fragments have comparable lengths: for ND2 we amplified and sequenced a fragment 537 base pairs (bp) long using the primers L5215 and H5766 (Sorenson et al., 1999); the intron, AK5, is 580 bp and was amplified using different combinations of the primers reported by Shapiro and Dumbacher (2001); and the nuclear coding gene *c-mos* has a length of 572 bp, and was amplified following the conditions reported by Cooper and Penny (1997). Nuclear fragments were only amplified and sequenced for a subset of samples that included *H. leucotis* and *H. xantusii* and related genera. Amplified products were cleaned by gel filtration using Sephadex G50 columns (*Sigma*), and sequenced using dye-labelled terminators (BigDye chemistry, *Applied Biosystems*). Sequencing reaction products were cleaned by gel filtration in the same way as PCR products, and resolved with an ABI 377 automated sequencer. All sequences generated for this study have been deposited in Genbank under accession numbers from KM389474 to KM389529 (Table 1). Sequences were aligned and proofread using SeAl v. 2.0a11 (Rambaut, 2003) and ClustalX (Thompson et al., 1997). We corroborated the origin of all our sequences by combining at least 2 of the following methods: amplifying overlapping gene segments, amplifying or sequencing 1 region with different primer sets, sequencing both DNA strands for all amplified fragments, or using multiple individuals of a single species. We found no evidence of numt contamination of our mtDNA sequences (Bensasson et al., 2001; Sorenson and Quinn, 1998; Zhang and Hewitt,

Autor, revisar palabras marcadas en rojo, significa que si van acentuadas en español, deberán acentuarse aquí; sólo países no se acentúan en inglés.

**Table 1.** List of species, voucher IDs, localities, and GenBank accession numbers

Main Clades	Species	ND2 (537 bp)	c-mos (572 bp)	AK5 (535 bp)	Catalogue No.	Locality
Outgroup (Brilliantis)	<i>Aglaeactis cupipennis</i>	KM389474	KM389514	AY830535	- LSUMZ B6304 - LSU B32662 - ZMUC P73	- Ecuador, Pichincha Province - Peru, Cajamarca Department - Peru, Ancash Department - Peru, Junin Department - Peru, Pasco Department, Playa Pampa - Peru, Loja Department - Peru, Azuay Department - Ecuador, Imbabura Department - Peru, Apurimac Department - Ecuador, Pichincha Province - Costa Rica, San José Province - Mexico, Sinaloa, El Palmito - Mexico, Oaxaca, Yucunino - Mexico, Oaxaca, El Naranjo - Mexico, Hidalgo, Tlanchinol - Costa Rica, Cartago
		KM389475	KM389515	KM389493	- ZMUC NK4	- Costa Rica, Heredia
Coquettes	<i>Adelomyia melanogenys</i>	AY830457	KM389516	KM389494	- LSUMZ B8025 - ZMUC P14 - ZMUC P43	- Mexico, Oaxaca, Martín Caballero - USA, Louisiana, East Baton Rouge Parish - Mexico, Guerrero
		KM389476	KM389517	KM389495	- ZMUC P280 - ZMUC P272	- Mexico, Hidalgo, La Cabaña - Mexico, Oaxaca, Teotitlán
<i>Patagona</i>	<i>Patagona gigas</i>	AY830510	×	AY830585	- LSUMZ B6303	- Mexico, Oaxaca, Yucunino - Mexico, Oaxaca, El Naranjo - Mexico, Hidalgo, Tlanchinol - Costa Rica, Cartago
Mt. Gems	<i>Eugenius fulgens</i>	AY830481	DQ223957	KM389496	- LSUMZ B9964 - MZFC SIN115	- Mexico, Oaxaca, Yucunino - Mexico, Oaxaca, El Naranjo - Mexico, Hidalgo, Tlanchinol - Costa Rica, Cartago
		KM389477	KM389518	KM389497	- MZFC OMVP750 - MZFC CONACYT 1282	- Mexico, Oaxaca, Yucunino - Mexico, Oaxaca, El Naranjo - Mexico, Hidalgo, Tlanchinol - Costa Rica, Cartago
Bees	<i>Heliomaster constantii</i>	KM389478	DQ223947	KM389498	- LSUMZ 394256	- Mexico, Oaxaca, Yucunino - Mexico, Oaxaca, El Naranjo - Mexico, Hidalgo, Tlanchinol - Costa Rica, Cartago
		KM389479	DQ223951	KM389499	- LSUMZ B19791	- Mexico, Oaxaca, Yucunino - Mexico, Oaxaca, El Naranjo - Mexico, Hidalgo, Tlanchinol - Costa Rica, Cartago
Bees	<i>Lampornis amethystinus</i>	KM389480	DQ223952	KM389500	- LSUMZ B16006	- Mexico, Oaxaca, Martín Caballero - USA, Louisiana, East Baton Rouge Parish - Mexico, Guerrero
		KM389481	DQ223955	KM389501	- MZFC OMVP1037	- Mexico, Oaxaca, Martín Caballero - USA, Louisiana, East Baton Rouge Parish - Mexico, Guerrero
Bees	<i>Lampornis cinereicauda hemileucus</i>	AY830465	×	KM389502	- LSUMZ B5270 - MZFC CONACYT 971	- Mexico, Oaxaca, Martín Caballero - USA, Louisiana, East Baton Rouge Parish - Mexico, Guerrero
		KM389482	KM389519	KM389503	- MZFC BCTDA052 - MZFC CONACYT 775	- Mexico, Oaxaca, Martín Caballero - USA, Louisiana, East Baton Rouge Parish - Mexico, Guerrero
Bees	<i>Archilochus colubris</i>	KM389481	DQ223955	KM389501	- MZFC OMVP1037	- Mexico, Oaxaca, Martín Caballero - USA, Louisiana, East Baton Rouge Parish - Mexico, Guerrero
		AY830465	×	KM389502	- LSUMZ B5270 - MZFC CONACYT 971	- Mexico, Oaxaca, Martín Caballero - USA, Louisiana, East Baton Rouge Parish - Mexico, Guerrero
Bees	<i>Atthis heloisa</i>	KM389482	KM389519	KM389503	- MZFC BCTDA052 - MZFC CONACYT 775	- Mexico, Oaxaca, Martín Caballero - USA, Louisiana, East Baton Rouge Parish - Mexico, Guerrero
		KM389481	DQ223955	KM389501	- MZFC OMVP1037	- Mexico, Oaxaca, Martín Caballero - USA, Louisiana, East Baton Rouge Parish - Mexico, Guerrero

Table 1. Continue

Main Clades	Species	ND2 (537 bp)	c-mos (572 bp)	AK5 (535 bp)	Catalogue No.	Locality
	<i>Calypte anna</i>	KM389483	KM389520	KM389504	- MZFC CONACYT 615	- Mexico, Baja California, Rancho Monte Alto
	<i>Calypte costae</i>	KM389484	KM389521	KM389505	- MZFC SIN025	- Mexico, Sinaloa, El BateI
	<i>Doricha eliza</i>	KM389485	KM389522	KM389506	- MZFC B0589	- Mexico, Yucatán, Dzilam de Bravo
	<i>Selasphorus platycercus</i>	AY830522	×	AY830597	- LSUMZ B23428	- USA, Texas, Jeff Davis County
	<i>Selasphorus rufus</i>	KM389486	KM389523	KM389507	- MZFC BMM475	- Mexico, Sinaloa, El BateI
	<i>Selasphorus sasin</i>	KM389487	KM389524	KM389508	- MZFC CONACYT 268	- Mexico, Sonora, Tiburón Island
Emeralds	<i>Amazilia amabilis</i>	EU042518	×	EU042436	- LSUMZ B28483	- Panama, Colón Province
	<i>Amazilia beryllina</i>	KM389488	KM389525	KM389509	- MZFC BMM480	- Mexico, Sinaloa, El BateI
	<i>Amazilia candida</i>	KM389489	KM389526	KM389510	- MZFC OMVP504	- Mexico, Oaxaca, Chalchijapa
	<i>Amazilia chionogaster</i>	AY830462	×	AY830538	- LSUMZ B17165	- Argentina
	<i>Amazilia decora</i>	EU042519	×	EU042437	- LSUMZ B100024	- Costa Rica, Puntarenas Province
	<i>Amazilia fimbriata</i>	EU042520	×	EU042438	- LSUMZ B5956	- Ecuador, Morona- Santiago Province
	<i>Amazilia franciae</i>	EU042521	×	EU042439	- LSUMZ B12063	- Ecuador, Pichincha Province
	<i>Amazilia rutila</i>	EU042522	×	EU042440	- UWBM 56002	- Nicaragua, Puerto Cabezas
	<i>Amazilia saucerrottei</i>	EU042523	×	EU042441	- FMNH 393025	- Costa Rica, Guanacaste Province
	<i>Amazilia tzacatl</i>	EU042524	×	EU042442	- LSUMZ B16538	- Panama, Panamá Province
	<i>Amazilia versicolor</i>	EU042525	×	EU042443	- FMNH 395409	- Brazil, Sao Paulo State, Boracei
	<i>Amazilia viridigaster</i>	EU042526	×	EU042444	- LSUMZ B7490	- Venezuela, Amazonas State, Cerro de la Neblina
	<i>Campylopterus largipennis</i>	AY830467	×	AY830543	- LSUMZ B4474	- Peru, Loreto Department
	<i>Campylopterus villaviscensio</i>	AY830468	×	AY830544	- LSUMZ B5588	- Peru, San Martín Department

Table 1. Continue

Main Clades	Species	ND2 (537 bp)	c-mos (572 bp)	AK5 (535 bp)	Catalogue No.	Locality
CONACYT861	<i>Chlorostilbon melanorhynchus</i>	AY830470	×	AY830546	- LSUMZ B6327	- Ecuador, Pichincha Province
	<i>Chlorostilbon mellisugus</i>	AY830471	×	AY830547	- LSUMZ B9450	- Bolivia, Pando Department
	<i>Chlorostilbon notatus</i>	EU042539	×	EU042457	- FMNH 392810	- Brazil, Alagoas State, Piranhas
	<i>Cyananthus doubledayi</i>	EU418751	×	×	- MZFC CONACYT 991	- Mexico, Guerrero, San Luis Acatlán
	<i>Cyananthus latirostris</i>	EU418758	×	×	- MZFC	
	- Mexico, Colima, Tepames					
	<i>Cyananthus sordidus</i>	EU418755	×	×	- MZFC OMVP747	- Mexico, Oaxaca, Cerro Piedra Larga
	<i>Elvira cupreiceps</i>	AY830481	×	AY830553	- LSUMZ B16066	- Costa Rica, Heredia Province
	<i>Hylocharis cyanus</i>	KM389490	KM389527	KM389511	- ZMUC P1419	- Bolivia, Santa Cruz Department
	<i>Hylocharis eliciae</i>	EU042562	×	EU042478	- LSUMZ B16074	- Costa Rica, Puntarenas Province
<i>Hylocharis grayi</i>		EU042563	×	×	- ANSP 5064	- Ecuador, Imbabura
	<i>Basilinna leucotis</i>	KM389491	KM389528	KM389512	- MZFC OMVP413	- Mexico, Oaxaca, Cerro Piedra Larga
<i>Hylocharis sapphirina</i>		EU042564	×	EU042479	- LSUMZ B12912	- Bolivia, Santa Cruz Department
	<i>Basilinna xantusii</i>	KM389492	KM389529	KM389513	- MZFC CONACYT730	- Mexico, Baja California Sur, San Dionisio
<i>Klais guimeti</i>	AY830495	×	AY830570	- LSUMZ B6168	- Ecuador, Morona-Santiago Province	
TOTAL		47	21	43		

ANSP= Academy of Natural Sciences.

FMNH= Field Museum of Natural History.

LSUMZ= Louisiana State University, Museum of Natural Sciences.

MZFC= Museo de Zoología, Facultad de Ciencias.

UWBM= University of Washington Burke Museum.

ZMUC= Zoological Museum University of Copenhagen.

a \*= after the accession number, indicates those sequences generated for this study.

1996), and we obtained congruent sequences of our nuclear genes that aligned well with sequences of other avian species –particularly with hummingbirds when available. **Data analyses.** We conducted phylogenetic analysis using maximum parsimony (MP) and maximum likelihood (ML), with combined nuclear sequences (c-mos+AK5), separate genes (c-mos, AK5, ND2) and the 3 concatenate genes (c-mos+AK5+ND2). There is disagreement on whether the best approach for phylogenetic analyses is the combination of all existing information (total evidence) or the congruence between independent sets. We used both approaches by performing analyses of our individual gene fragments (independent sets) as well as a combination of all sequences. Moreover, because the taxon sampling varied somewhat between the different gene fragments, it was important to ensure through the analyses of each individual gene fragment that this taxon sampling did not bias our main conclusions. MP and ML analysis were performed using PAUP\* (Swofford, 2002) unless otherwise stated. MP analyses used a heuristic search using a TBR branch-swapping option and with all positions equally weighted, support for each node was obtained by 1 000 bootstrap replicates (Felsenstein, 1985). We used jModeltest v. 0.1.1 (Posada, 2008) to evaluate the model parameters for the ML searches. The best fitting models for our combined sequences and for each gene were: c-mos+AK5, HKY+I+G; c-mos, TrN+I+G; AK5, HKY+G, and ND2, GTR+I+G. The ML analyses were performed using heuristic searches and nodal support was estimated via 1000 bootstrap replicates, with the gaps defined as missing data.

We performed Bayesian Inference (BI) on the combined nuclear sequences (AK5 and c-mos), the separate genes, and the 3 concatenate gene fragments. When using more than 1 fragment at a time we defined partitions corresponding to each fragment, and allowed for different evolutionary rates in each partition. We used the model of evolution that best explained our data as estimated with jModeltest (see above). BI analyses were conducted using MrBayes 3.0 (Huelsenbeck and Ronquist, 2002). Each analysis consisted of 4 Markov chains, random starting trees, and uniform prior distribution of parameters. The chains were run for 10 million generations, sampling trees every 250th generation. The asymptote was determined visually, the first 1 000 trees were discarded as burn-in, and the remaining trees from the plateau phase were then used to estimate Bayesian posterior probabilities. We considered that clades were strongly supported if they were present in at least 95% of the sample trees (Huelsenbeck and Ronquist, 2002; Wilcox et al., 2002).

All trees obtained were rooted with the same species as outgroup (*Adelomyia* and *Metallura* from the coquettes

group, and *Aglaeactis* and *Urosticte* from the brilliants group) as suggested by previous results (Bleiweiss, 1998a; Altshuler et al., 2004; García-Moreno et al., 2006; McGuire et al., 2007).

## Results

Analysis of mitochondrial sequences using 3 different search strategies, maximum parsimony, maximum likelihood and bayesian inference, in all cases identified a well supported ( $\geq 95\%$ ) monophyletic clade integrated by *H. leucotis* and *H. xantusii* (Fig. 1a) distinct from other *Hylocharis* species which appear several nodes away, always nested within *Amazilia*. The clade formed by *Hylocharis leucotis* and *H. xantusii* is more closely related to the *Cyananthus* and *Chlorostilbon* clade, which in turn is the sister group of a large clade containing the rest of the emeralds and including the other *Hylocharis* species (Fig. 1a).

Analyses of both separated and concatenated sequences of the nuclear fragments also retrieved the *H. leucotis* - *H. xantusii* clade (Figs. 1b-d). The nuclear sequences do not resolve completely the relationships within the emeralds clade, nor the relationships between the bees and mountains gems groups. Nevertheless, the nuclear sequences also suggest that the *H. leucotis*-*H. xantusii* pair is not closely related to other *Hylocharis* species, as these always appeared nested within a clade that included the *Amazilia* species included in this study.

Besides the *Basilinna* clade, we recovered a well resolved relationship between *Hylocharis* species and different *Amazilia* species (Figs. 1a-d) (García-Moreno et al., 2006; McGuire et al., 2007).

The position of the *H. leucotis*-*H. xantusii* clade within the phylogeny is still unresolved. The pair came out as sister to a clade containing *Cyananthus*, *Chlorostilbon* and *Chlorestes* species in our analyses with ND2 sequences, but without sufficient support ( $< 95\%$ ). In the analyses with the AK5 intron the *H. leucotis*-*H. xantusii* clade is part of a polytomy between several well supported clades, whereas *Chlorostilbon* and *Chlorestes* are nested with some support (though not unambiguously) within a large complex that includes *Campylopterus*-*Klais* clade and a larger *Elvira*-*Hylocharis*-*Amazilia*. No sequences of *Chlorostilbon* or *Campylopterus* were available for an analysis with c-mos.

## Discussion

Our results strongly suggest that the genus *Hylocharis* as currently understood is a paraphyletic group, as already suggested in the broad study of McGuire et al. (2007). We

found this result using 3 genetic markers with different characteristics (an intron, AK5; a proto-oncogen, c-mos; and a mitochondrial protein coding gene, ND2), 3 different inference methods (maximum parsimony, maximum likelihood, and bayesian inference), and 6 of the 8 species that the AOU recognizes in the genus (Remsen et al 2013).

Two of the species commonly assigned to the genus, *H. leucotis* and *H. xantusii*, form a strongly supported monophyletic group that is separated from other *Hylocharis* species by several genera (Figs. 1, 2); this clade is equivalent to the *Basilinna* genus proposed by Boie (1831) and Ridgway (1911). The other 4 *Hylocharis* species included in the study (*H. grayi*, *H. sapphirina*, *H. eliciae*, and *H. cyanus*) do not form a monophyletic group either (Figs. 1a-d), but instead appear in different places within the Emeralds clade, intermixed within the genus *Amazilia*, which is also shown to be paraphyletic (Fig. 1). Although we were unable to determine the precise location of the *H. leucotis* - *H. xantusii* clade within the phylogeny, our results suggest a relationship with the Emeralds.

Based on the results presented here and in García-Moreno et al. (2006), we suggest that the genus *Basilinna* be brought back into use. The use of the genus *Basilinna* has been supported recently by some authors (Howell and Webb, 1995; Schuchmann, 1999), but had never been properly defined from a phylogenetic perspective. In a study focused on the Mountain Gems of the genus *Lampornis* that included nuclear and mitochondrial DNA sequences, García-Moreno et al. (2006 –their Fig. 2) already found *Basilinna* as a well supported clade that fell within the Emerald group close to *Chlorostilbon*, while the other *Hylocharis* species included in that study, *H. cyanus*, also appeared deep within the Emerald clade and separated from *H. leucotis* and *H. xantusii* by several genera, a result that we are confirming in this more focused study.

Our results do not support Schuchmann's (1999) proposal, based in the shared presence of a broad white postocular stripe, that *Basilinna* is closely related to *Lampornis*. This character, however, can also be seen in *Adelomyia melanogenys*, a genus from the Coquettes clade and clearly different from *Lampornis* and *Basilinna* (McGuire et al., 2007). None of our results suggest a particularly close relationship between *Lampornis* and *Basilinna*. Analyses based on individual fragments did not have the power to resolve the deeper relationships between the different clades. Nevertheless, whereas the *H. leucotis* - *H. xantusii* group was unresolved with respect to other clades, in most analyses *Lampornis* came out close to *Heliomaster*, *Eugenes*, and *Lamprolaima*. We never recovered a topography implying a sister relationship between *Lampornis* and *H. leucotis* - *H. xantusii*. It is worth mentioning that although we were unable to amplify

the same set of species for each gene fragment, this does not seem to affect the main conclusions of this work, namely the paraphyly of *Amazilia* and *Hylocharis*, the existence of a *Basilinna* clade, and the lack of a close relationship between *Basilinna* and *Lampornis* (Figs. 1, 2). Our total-evidence analysis, using the concatenated sequences of the 3 amplified DNA fragments, suggests a closer relationship of *H. leucotis* - *H. xantusii* with the Emeralds than with *Lampornis*. Ridgway (1911) provides a good description of the morphology and color patterns of the genus *Basilinna* and its 2 species, emphasizing differences with *Hylocharis*. In particular, the broad white postocular stripe present in *H. leucotis* and *H. xantusii*, but not in other members of *Hylocharis*, was one of the main characters leading Boie (1831) and Ridgway (1911) to propose the existence of *Basilinna*.

As for the other *Hylocharis* species, they appear in different places within the Emerald clade. Our hypothesis using all the available sequence information results in only 2 species forming part of the same clade, *H. grayi* and *H. cyanus*, which also includes *Amazilia versicolor*; whereas *H. sapphirina* and *H. eliciae* form 2 separate clades with other *Amazilia* species, namely and *Amazilia chionogaster* and *A. candida* respectively (Fig. 2). Although comparisons are not straightforward because the taxon sampling differs between the studies, in their more comprehensive phylogenetic study of hummingbirds McGuire et al. (2007) also found its 4 *Hylocharis* species – the same ones included in this study – interspersed within a clade rich in *Amazilia* species, with only 2 *Hylocharis* forming part of the same clade (*H. eliciae* and *H. cyanus*). Our results, together with those of other authors, indicate that a thorough revision of the genera *Amazilia* and *Hylocharis*, including other related genera (e.g., *Chrysuronia*, *Lepidopyga*), is necessary.

In conclusion, our results support the existence of a clearly defined clade formed by the 2 species of hummingbirds currently known as *H. leucotis* and *H. xantusii*, which other authors in the past have recognized as a genus on its own. We therefore propose the recognition of the genus *Basilinna* (Boie, 1831) encompassing 2 species: *B. leucotis*, distributed along the highlands of Mexico and Central America down to Nicaragua, and *B. xantussi*, restricted to the Baja California Peninsula in northwest Mexico. The results presented here also suggest that *Hylocharis* and *Amazilia* are currently paraphyletic groups in need of a thorough revision (McGuire et al., 2007).

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