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RESEARCH ARTICLE

Description of New American Carduelis/Spinus Bird Species in La Paz (Bolivia): *C./S. lapazensis.*

Antonio Arnaiz-Villena[1,*], Valentín Ruiz-del-Valle[1], Fabio Suarez-Trujillo[1], Adrian Lopez-Nares[1], Alvaro Callado[1], Eduardo Gomez-Casado[2], Estefania Crespo-Yuste[1] and Cristina Campos[1]

[1]*Department of Immunology, School of Medicine, University Complutense,, Madrid, Spain*
[2]*Department of Biotechnology, INIA, Madrid, Spain*

Abstract:

Introduction:

South American siskins (*Genus Carduelis/Spinus*) are the outcome of regional evolutionary radiation from an extant (or other extinct) species: *C. notata*, a North America siskin, which thrives in Mexico subtropical areas and is parental of one of the three described North American siskin radiations.

Methods:

Speciation and/or subspeciation of this South American siskin radiation have probably occurred during Pleistocene Epoch. In the present paper, a new species/subspecies akin to *C./S. atrata* is described by genetic and phenotypic parameters: this new species/subspecies was previously considered a subspecies of *C./S. xanthogastra,* which thrives further North and is separated about 1,762 km, 1,094 miles, from this described subspecies, *Carduelis/ Spinus xanthogastra stejnegeri*.

Results:

Our genetic study using mt cyt b, phenotypic and behavior observations show that this putative *C./S. xanthogastra* subspecies is either a different species or a *C./S.atrata* subspecies; we have proposed a provisional name for this finch, *C./S. lapazensis,* instead *of C./S. x. stejnegeri.*

Conclusion:

Species definition is movable and controversial, and it is uncertain in South American siskins, which all show a close genetic and phenotypical relationship, which may be still immersed in speciation processes since Pleistocene Epoch.

Keywords: Songbirds, Finches, *Serinus*, *Carduelis*, *Spinus*, Carduelinae, Siskins, Bolivia, *Lapazensis*, La Paz, *Atrata*, *Xanthogastra*, *Stejnegeri*, Fringillinae, Fringilidae, *Notata*, Andes, Ecuador, Peru, Bird.

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1. INTRODUCTION

Genus *Carduelis* (family Fringillidae, subfamily Fringillinae, tribe Carduelini) includes goldfinches, siskins, redpolls, greenfinches, and crossbills, among others [1, 2]. It comprises over 30 species, and it is widespread all over the World except for Subsaharan Africa and Australia. It belongs to the Fringillidae family of birds, which also includes many sparrows, bramblings, and chaffinches [3]. Many of the species comprised within this genus and other genera have recently been classified by using Molecular Systematics and the mitochondrial cytochrome b (cyt b) gene [4, 5].

* Address correspondence to this author at the Department of Immunology, School of Medicine, University Complutense, Madrid, Spain;
Tel: +34 913941632; +34 606993161; E-mails: aarnaiz@med.ucm.es, arnaizville@hotmail.com and chopo.pntic.mec.es/biolmol

The estimated divergence time for most of the genus *Carduelis* species suggests that they appeared in a range of time between the Miocene and Pliocene; there is no evidence for a divergence time consistent with late Pleistocene origin for most radiation groups [6 - 10]. This radiation was intermingled in time with *Serinus* species radiation. However, it is possible that certain *Carduelis* birds, classically considered as subspecies, originated during Pleistocene glaciations *i. e.*: the divergence time calculated for *C. carduelis* subspecies (grey-headed Asian and black-headed European goldfinch) is less than 800,000 years [6 - 10].

Three different and apparently parallel North and South American siskin evolutionary radiations have occurred in the last five million years, all three are genetically related, but a common ancestor, if any, is undetermined [3, 5]. Regarding

South American radiation, it has to be noted that Black-headed siskin (*Carduelis notata*) at present thriving at the Mexican mountains gave rise to these group of siskins [3, 7]. This happened after 3 MYA when Panama Isthmus closed and mesothermal plants, appropriate for siskin feeding, passed to the Andean Spine [3, 10].

In the present paper, the aim is to study the phylogenetic relationship of certain South American radiation birds: *Carduelis xanthogastra* (distributed in Costa Rica, Central America to Mt. Andes including most of Ecuador Country), *Carduelis xanthogastra stejnegeri* (Northern Bolivia) and *Carduelis atrata* (Mt. Andes of Peru, Bolivia, North Argentina and North Chile) (Figs. **1**, **2**). *C. x. stejnegeri* has been considered a subspecies of *C. xanthogastra*. *C. x. stejnegeri* description [11, 12] pointed out the differences between *C. xanthogastra* and *C. x. stejnegeri*. However, it has been observed that *C. x. stejnegeri* habitat was geographically different and separated from *C. xanthogastra* Fig. (**2**). In contrast, the geographical habitat was adjacent and sometimes overlapping to that of *C. atrata*, but generally in warmer valleys. We observed during several years *C. x. stejnegeri* behavior and habitat and carried out a genetic study in order to establish a relationship among these three species Fig. (**1**).

Fig. (1). Males of *Carduelis* species targeted for analysis. a) *C. x. stejnegeri* (Photographed by Fabian Beltrán at Botanic Gardens, La Paz, Bolivia) [11]. Adult male, female and juvenile characters and their distribution in [12] **b)** *C. xanthogastra* [11, 13]. Adult male, female and juvenile description, status, habitat, behaviour and their distribution in the study [12] (Photographed by A. Arnaiz-Villena. Bird from Valencia, Venezuela) **c)** *C. atrata* [11, 14]. Description adult male, female and juvenile characters and their distribution in the study [12] (Photographed by A. Arnaiz-Villena. Bird from La Paz, Bolivia).

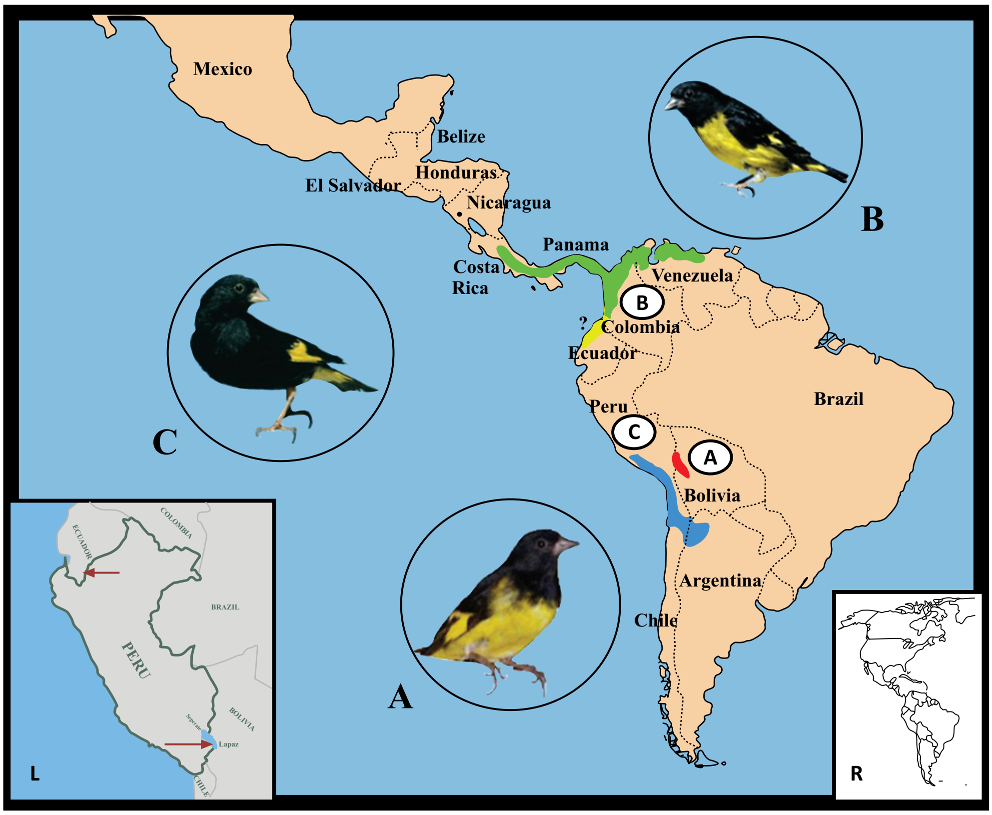

Fig. (2). Distribution map of studied species and species photographs [11, 12]. **A.***C. x. stejnegeri* (Distribution is shown in red colour), **B.***C. xanthogastra* (Distribution is shown in green colour) and **C.***C. atrata* (Distribution is shown in blue colour). **A** photography is taken by ornithologist Fernando Dolset. **B** photography is also shown in Fig. **1** (A. A-V). **C** Photograph is taken by A. Arnaiz-Villena in El Alto, La Paz, Bolivia. **Inset R**: Americas map. **Inset L**: Arrows mark distance of thriving margins of *C. xanthogastra* (North: southern Ecuador) and *C.xanthogastra stejnegeri* (South: northern Bolivia).A gap of 1,094 miles,1,765 km, separates both birds thriving ranges. It is not discarded that *C. x. stejnegeri* may thrive in southernmost Peru [15, 16].

Table 1. Origin and Cytochrome b GenBank accession numbers of the species used in this study.

Scientific Name	Common Name	GenBank	Origin
Carduelis xanthogastra	Yellow-bellied siskin	L76389	San Jose, Costa Rica
Carduelis xanthogastra stejnegeri	**Yellow-bellied Siskin (*stejnegeri*)**	**Mk 733355**	**La Paz, Bolivia**
Carduelis atrata	Black siskin	L76385	Sucre, Bolivia
Carduelis barbata	Black-chinned siskin	L77868	Magallanes, Chile
Carduelis crassirostris	Thick-billed siskin	L77869	Mendoza, Argentina
Carduelis cucullata	Red siskin	L76299	Venezuela
Carduelis magellanica	Hooded siskin	U79016	Misiones, Argentina
Carduelis notata	Black-headed siskin	U79019	Chiapas, Mexico
Carduelis olivacea	Olivaceus siskin	L77871	Lima, Perú
Carduelis pinus perplexus	Pine siskin	DQ246804	Quetzaltenango, Guatemala
Carduelis psaltria columbiana	Lesser goldfinch	U78324	Maracay, Venezuela
Carduelis spinescens	Andean siskin	U79017	Merida, Venezuela
Carduelis spinus	Eurasian siskin	L76391	Eurasia, N. África (N. America)
Carduelis tristis	American goldfinch	U79022	San Francisco (CA), USA
Carduelis yarrellii	Yellow-faced siskin	U83200	Recife, Brasil
Fringilla coelebs	Chaffinch	L76609	Madrid, Spain

2. MATERIALS AND METHODS

For the original description of the following species, (Appendix 1): *C. xanthogastra* (yellow-bellied siskin), *C. x. stejnegeri* (yellow-bellied siskin), *C. atrata* (black siskin).

2.1. Bird Samples, DNA Extraction and PCR Amplifications

Original species bird descriptions are detailed in **Appendix 1** after the Acknowledgements section.

Fifteen species of *Carduelis* (order Passeriformes) have been included in this study (Table **1**). They belong to the tribe Carduelini. One male and two females of *C. x. stejnegeri* [11, 12] were collected, sexed (Labs Edyma, Valdepeñas, Spain), DNA sequenced and photographed. Blood from living birds was drawn as described in our previous papers [6, 7] and preserved in EDTA at 4ºC until use; otherwise, DNA was taken from feathers. DNA extraction was performed using a commercial DNA purification kit (QuickGene DNA Whole Blood Kit S, FUJIFILM, Tokyo, Japan). Amplification and sequencing of mt cyt b gene 924 base pairs (bp) were performed as previously described [8, 17]. Internal primers used for sequencing were: H15149 3'-TGCAGCCCCTCA GAATGA TATTTGTCCTCA-5' and L15299 5'-GGATT CTTCGCCCT GCACTTCCTCC-3'. *C. x. stejnegeri* flocks were observed during 3 different years around La Paz City small valleys, ravines and La Paz Cactus Gardens.

One of our *C. x. stejnegeri* sample was a female whose photographs are shown in the discussion section. It cannot be confused with *C. atrata* females, which may thrive in the same area, but usually at higher altitudes (see below). *C. atrata* females show a full black back; the back is less bright than it is in males. Sometimes both sexes are difficult to distinguish [12]. In order to avoid confusion when authors refer to this work and before nomenclature committees decide a name for the species or subspecies described in this paper, we propose a provisional name: *Carduelis/Spinus lapazensis.*

2.2. Phylogenetic Analyses

DNA sequences were aligned by using the MEGA 5 computer program [18] for further phylogenetic calculations and calculating the cytochrome b DNA distances and cytochrome b protein distances. MEGA 5 has also been used to compare differences in the codons between *C. x. stejnegeri*, *C. atrata* and *C. xanthogastra*. The final length of the sequences used was 924 nucleotides. Chaffinch, *Fringilla coelebs* (family Fringillidae, subfamily Fringillinae), was chosen as an outgroup to root the phylogenetic dendrograms. Phylogenetic dendrograms were obtained using the Maximum Likelihood (ML) methodology [19] with PAUP* v. 4.0b10 program [20] and Bayesian Inference (BI) methodology using Mr. Bayes program [21, 22]. Model test v.3.7 [23] was used to find out a DNA substitution model that fits the data best. The best model was used prior to both ML and BI analyses. Linearized ML dendrograms were obtained with PAUP* v.4.0b10 [18] with the estimated branch length [24], which assumes that the rates among the evolutionary lineages may not be constant.

3. RESULTS

3.1. Phylogeny of *C. x. stejnegeri*

Fig. (**3**) shows a linearized Bayesian dendrogram (Fig. **3A**). Sample DNA sequence of *C. x. stejnegeri* (Fig. **3A**) is not related phylogenetically with *C. xanthogastra,* as it had been thought. However, *C. x. stejnegeri* is related to *C. atrata* with 92% of the bootstrap value. In addition, these results are supported by (Fig. **3B**), a Linearized Maximum Likelihood dendrogram (Fig. **3B**): the close relationship between *C. atrata* and *C. x. stejnegeri has also been observed.* In addition, *C. xanthogastra* appeared on Earth earlier than *C. atrata* and *C. x. stejnegeri*, as shown in Fig. (**3B**) and more amplified in Fig. (**3C**).

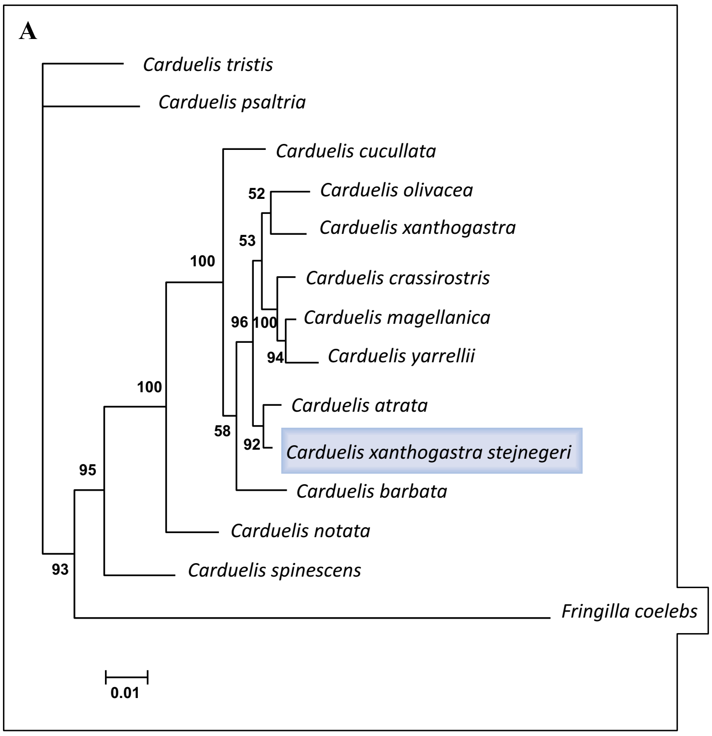

Fig. (3A). A Linearized Bayesian dendrogram based on mitochondrial cytochrome b (mt cyt b) DNA sequences.

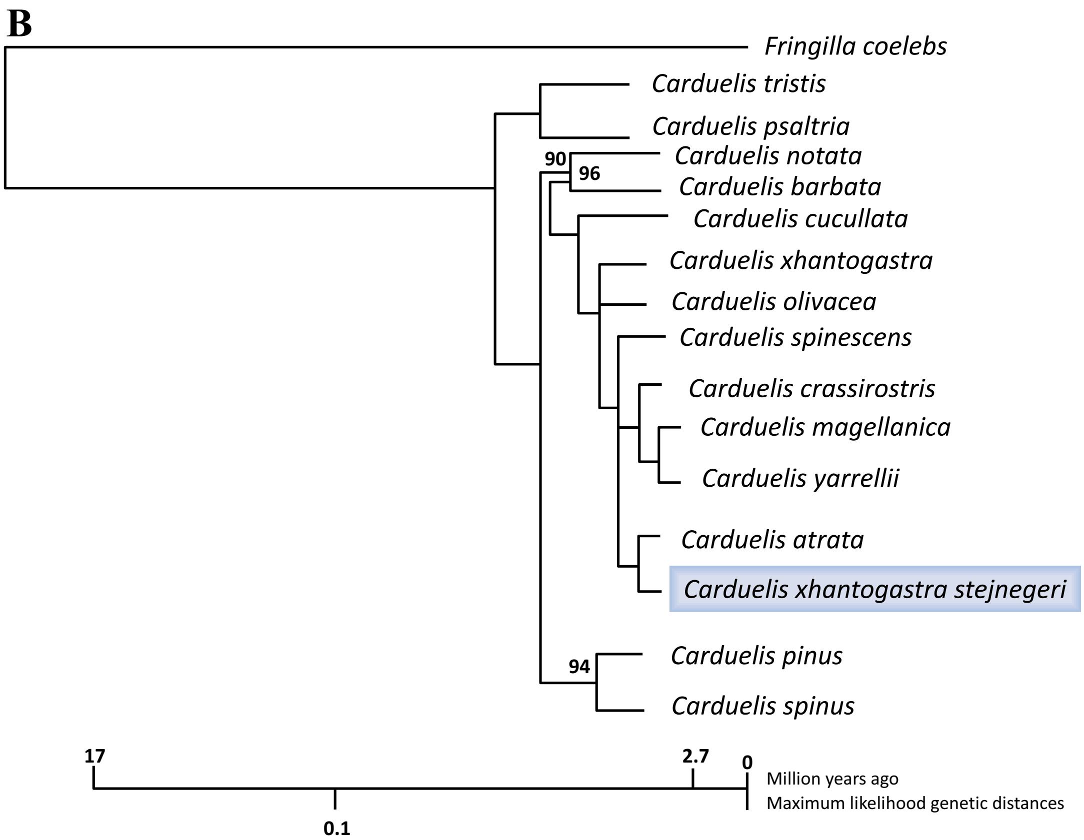

Fig. (3B). Linearized Maximum Likelihood dendrogram based on mt cyt b DNA sequences. *Fringilla coelebs* was chosen as an outgroup for both trees [3, 7, 8]. Only significant bootstrap values are depicted, which are concordant with previous studies [3, 8].

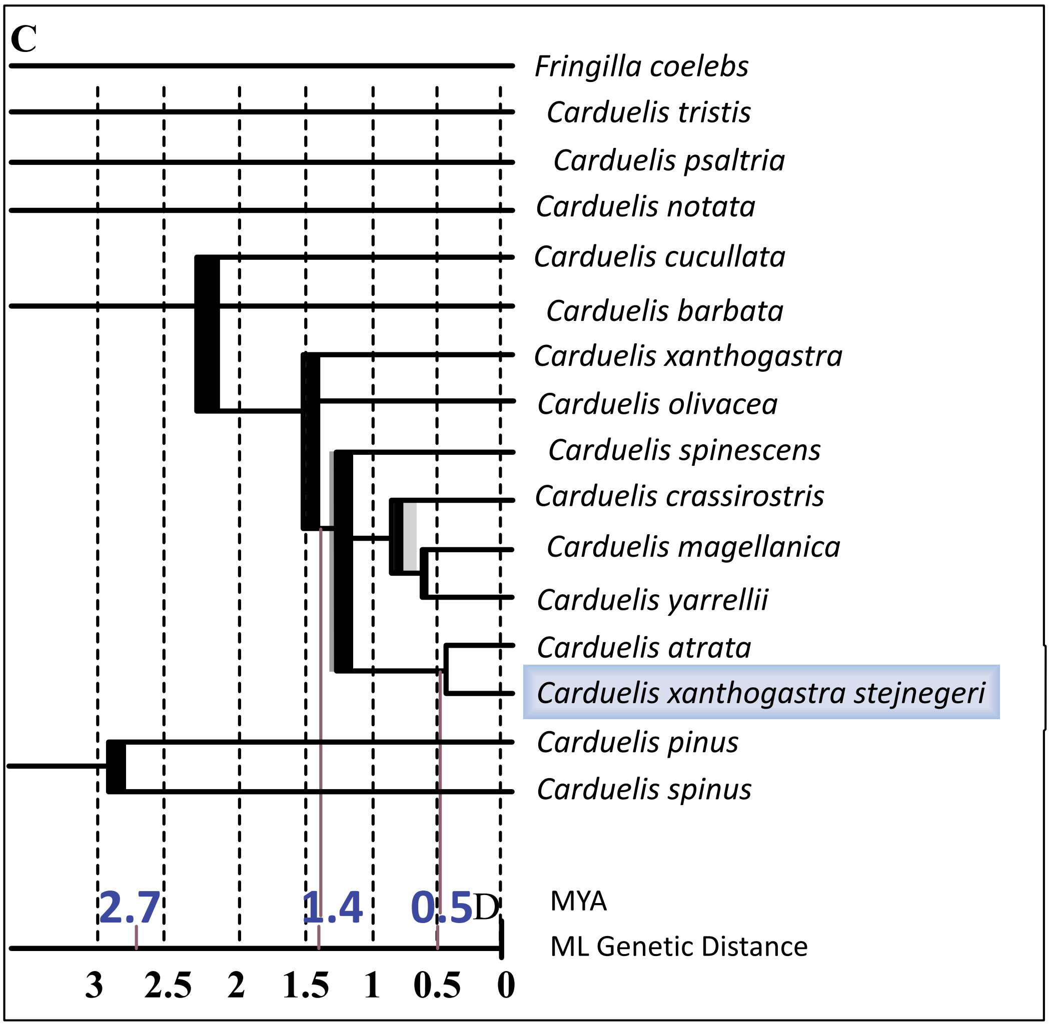

Fig. (3C). Amplified part of 4B dendrogram showing also that speciation occurred for *C. atrata/ C. x. stejnegeri* about 0.5 MYA (million years ago) and for *C. xanthogastra* about 1.4 MYA.

Similar to the results obtained in dendrograms from Fig. (**3**) with mt cytochrome b DNA genetic distances, (Table **2**) shows a close relationship between *C. x. stejnegeri* and *C. atrata* (0.004) than that between *C. x. stejnegeri* and *C. xanthogastra* (0.012). Although at the DNA level, there were differences between these three taxa, not at the protein level, because these DNA differences were synonymous mutations.

Table 2. Cytochrome b DNA genetic distances between the species used in the analysis. DNA genetic distances between *C. x. stejnegeri, C. xanthogastra* and *C. atrata* are marked by grey shadow.

DNA Genetic Distances	*C. xanthogastra stejnegeri*	*C. atrata*	*C. cucullata*	*C. olivacea*	*C. crassirostris*	*C. barbata*	*C. notata*	*C. magellanica*	*C. yarrellii*	*C. tristis*	*C. psaltria*	*C. spinus*	*C. xanthogastra*
C. xanthogastra stejnegeri	-	-	-	-	-	-	-	-	-	-	-	-	-
C. atrata	0.004	-	-	-	-	-	-	-	-	-	-	-	-
C. magellanica	0.008	0.053	-	-	-	-	-	-	-	-	-	-	-
C. yarrellii	0.009	0.061	0.024	-	-	-	-	-	-	-	-	-	-
C. crassirostris	0.009	0.061	0.015	0.015	-	-	-	-	-	-	-	-	-
C. olivacea	0.011	0.055	0.023	0.023	0.021	-	-	-	-	-	-	-	-

(Table 2) cont.....

DNA Genetic Distances	*C. xanthogastra stejnegeri*	*C. atrata*	*C. cucullata*	*C. olivacea*	*C. crassirostris*	*C. barbata*	*C. notata*	*C. magellanica*	*C. yarrellii*	*C. tristis*	*C. psaltria*	*C. spinus*	*C. xanthogastra*
C. xanthogastra	0.012	0.055	0.034	0.037	0.035	0.036	-	-	-	-	-	-	-
C. barbata	0.014	0.061	0.019	0.014	0.005	0.02	0.031	-	-	-	-	-	-
C. cucullata	0.015	0.06	0.022	0.018	0.011	0.021	0.035	0.008	-	-	-	-	-
C. notata	0.03	0.059	0.015	0.013	0.009	0.019	0.032	0.01	0.013	-	-	-	-
C. spinus	0.045	0.043	0.062	0.066	0.063	0.062	0.056	0.063	0.062	0.066	-	-	-
C. tristis	0.054	0.047	0.051	0.054	0.052	0.051	0.042	0.51	0.052	0.05	0.048	-	-
C. psaltria	0.061	0.059	0.019	0.016	0.014	0.022	0.029	0.013	0.019	0.014	0.063	0.046	-
Fringilla coelebes	0.146	0.143	0.149	0.15	0.147	0.156	0.146	0.144	0.14	0.146	0.152	0.141	0.0143

Table 3. Cytochrome b DNA differences between *C. x. stejnegeri* and *C. atrata*.

DNA differences between *C. x. stejnegeri* and *C. atrata*					
Codon Number	Location of Difference within the Codon	*C. x. stejnegeri*	*C. atrata*	Type of Difference	Protein
4	First base	TTA	CTA	Transition	-
71	Third base	ATC	ATT	Transition	Synonymous
80	Third base	GAA	GAG	Transition	-
260	Third base	CTA	CTG	Transition	-

Table 4. Cytochrome b DNA differences between *C. x. stejnegeri* and *C. xanthogastra*.

DNA differences between *C. x. stejnegeri* and *C. xanthogastra*					
Codon Number	Location of Difference within the Codon	*C. x. stejnegeri*	*C. xanthogastra*	Type of Difference	Protein
168	First base	TTA	CTA	Transition	-
30	Third base	GCT	GCC	Transition	-
71	Third base	ATC	ATT	Transition	-
99	Third base	GGT	GGC	Transition	-
123	Third base	ACA	ACG	Transition	-
186	Third base	ATT	ATC	Transition	Synonymous
199	Third base	GGC	GGA	Transversion	-
238	Third base	CCT	CCC	Transition	-
271	Third base	CTT	CTC	Transition	-
278	Third base	TCC	TCT	Transition	-
306	Third base	GTG	GTA	Transition	-

In order to further study this close relationship between *C. x. stejnegeri* and *C. atrata*, cytochrome b DNA sequences of the concerned three taxa were compared codon by codon according to their position number and the mutation order inside codon. Two comparisons were made: *C. x. stejnegeri vs C. atrata* (Table **3**) and *C. x. stejnegeri vs C. xanthogastra* (Table **4**).

C. x. stejnegeri and *C. atrata* comparison show four mutations, one with a mutation in the first base of codon number 4, and the other three located at the third position of codon 71, 80 and 260 (Table **3**). All of these mutations were synonymous, they do not imply change at the protein level, and they were transitions. More abundant mutations have been found between *C. x. stejnegeri* and *C. xanthogastra* DNA sequences (Table **4**). One of them was found in the first base of the codon (168 codon), and the rest were found in the third position of codons 30, 71, 99, 123, 186, 199, 238, 271, 278 and 306. All of these mutations were synonymous: they do not imply change at protein level; however, one of them was a transversion as compared to (Table **3**) (C. *x. stejnegeri vs C. atrata*) where of them all were transitions.

4. DISCUSSION

South American siskins present close molecular genetics and phenotype relationships, as observed in Fig. (**3**). In fact, Fig. (**3B**) shows a very rapid regional radiation of most South American siskins except for *Carduelis barbata*, which seems the closest extant relative to parental *Carduelis barbata*. This is striking since present-day habitats of both birds are the most distant ones, (Mexico for *C. notata* and southern Chile for *C. barbata)* [12]. It was established that South American siskin radiation occurred between 5-3 MYA [3, 7, 10] and speciation of most South American siskins occurred rapidly and in more recent time (Pleistocene glaciations), (Figs. **3B**, **3C**) and [3, 7, 10].

4.1. Singular Peru Coast Geography and Climate

Most Peru coast except its northernmost part is dry and desert; some parts where any river cut it and ravines appear, more humid weather may occur. In addition, most coastal Peru is high and is a part of Mt. Andes. The cold Humboldt ocean stream makes the coastal climate unexpectedly cold according to its latitude. It should be tropical-like according to its equatorial proximity, and similar to that of Ecuador Country. Peru extreme coast climate and also Mt Andes may have stopped *C. xanthogastra* to thrive in Peru while it does in southernmost Ecuador. In addition, it has been shown that South American siskin/golfinch speciation has particularly been favoured in Mt. Andes [25]. Most of *C.xanthogastra* thriving range has a tropical and or less extreme weather and altitude (Fig. **2**) [11, 12, 14, 26]: its range includes, the Cordillera Central to Cordillera Talamanca, Costa Rica, to western Chiriquí, western Panama, Andes of Colombia (except Nariño), Perija Mountains, Colombia/Venezuela, also coastal mountains (west to Yaracuy) and Andes, south of Merida, Venezuela; also irregularly recorded in El Oro and Pichincha, western Ecuador; extreme southeast Peru (Puno) to central Bolivia (La Paz and Santa Cruz); scarce in Ecuador. All thriving range with tropical and/or mild weather except for southern Peru and adjacent Bolivia highlands,where C/S x.stegnegeri inhabits. All these factors may have stop *C. xanthogastra* to thrive throughout Peru (Fig. **2**).

4.2. *Carduelis xanthogastra vs Carduelis x. stejnegeri*

Carduelis xanthogastra and its still considered subspecies of *C. x. stejnegeri,* as described in the study [11 - 13]. The resemblance between these two “subspecies” was quite strong, phenotypes were similar in males. However, nominal *C. xanthogastra* female is different from the male [1, 11 - 13]. No uniform black is seen and the general colour is dull olive-green above [12]. Nominal *C. xanthogastra* habitat was described from Costa Rica to Ecuador, including areas of Colombia and Venezuela (Fig. **2**). In contrast, *C. x. stejnegeri* male was described as being similar to nominal, but the female colour was also similar to the male. According to Mr. Buckley, no dull olive-green like in nominal was observed, but black and yellow similar to males were obvious. In Dr. Sclater's collection, a Bolivian specimen collected by Mr. D. Forbes had dark olive-green above with some yellow spots in the crown: [11]. In either case, the plumage of hen is very different from nominal *C. xanthogastra* hen, (Figs. **5**, **6**) [11, 12].

Fig. (4). A*C. x. stejnegeri* (male) photographed by Fernando Dolset. **B***C. atrata* (male) photographed by A. Arnaiz-Villena (Specimen from El Alto, La Paz, Bolivia)

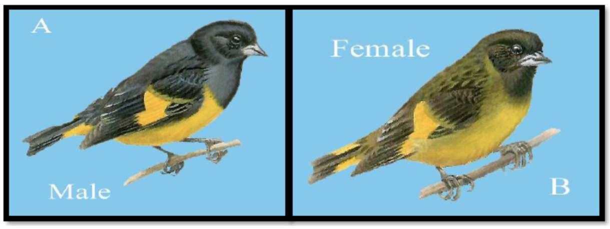

Fig. (5). A Scheme of male *C. x. stejnegeri* from [15]. **B** Scheme of female *C. x. stejnegeri* taken from [16].

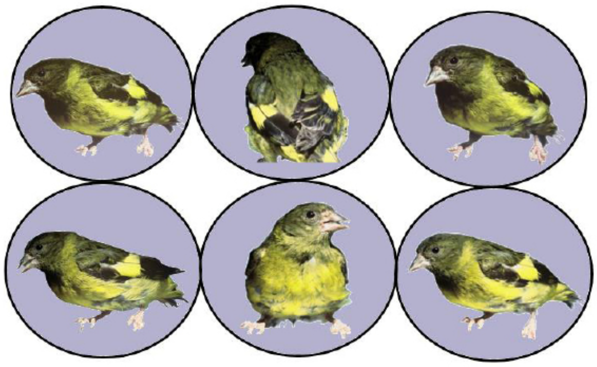

Fig. (6). ***Carduelis xanthogastra stejnegeri*****.** Sexed female. It cannot be confused with *C. atrata* females which may thrive in the same area (breeding season), usually at a higher altitude, see also Fig. (**2**, **5**). *C. atrata* females show a full black back; the back is less bright than in the male. Sometimes, both sexes are difficult to distinguish [12]. *C. xanthogastra stejnegeri,* female (La Paz, Bolivia) photographed by A. Arnaiz-Villena.

Our female specimen is shown in Figs. (**5** and **6**). *Carduelis x. stejnegeri* habitat was described to be in Bolivia (Sorata and Mapiri towns) [11]. Our genetic data show that *C. x. stejnegeri* is close to *C. atrata*, which is full black in both sexes except a yellow wing stripe and yellow lower abdomen and thighs [16] (Figs. **4**-**6**). *C. atrata* habitat was described in South Peru, Bolivia and North Argentina, down to Mendoza City [11, 12, 14]. In Fig. (**3B**), a linearized ML tree, nominal *C. xanthogastra* species appeared on Earth about 1.4 MYA in South America (Fig. **3C**), however ,both sister species *C. x. stejnegeri* and *C. atrata* appeared on Earth later, around 500,000 years ago, being one of them ancestral or ancestor has disappeared now. This is not distinguished by our present genetic study.

4.3. *Carduelis atrata vs Carduelis x. stejnegeri*

Carduelis atrata was described by Lafresnaye and D’Orbigny, 1837 [14]. This has been maintained as a monotypic species without subspecies, and it does not present sexual dimorphism. (Material and Methods Section). Both sexes are black and adult male shows brighter black colours, as shown in Figs. (**1**, **2**, **4**) [12, 14].

Carduelis. x. stejnegeri male is still regarded as a *Carduelis xanthogastra* subspecies in modern books but slightly larger [12]. It was originally described as “rather larger” [11] and was considered a separate species because of

C. x. stejnegeri shows less male/female dimorphism than expected like it occurs in *Carduelis atrata* where both sexes are black, with a yellow wings strip and yellow on belly and tail spots [11]. *C. x. stejnegeri* female was not "dull olive-green above" but similar to male or, according to others, a bit duller [11, 12]. Observations during 3 different years confirmed no sex marked differences (Figs. **5**, **6**). Habitat was described in Bolivia (Sorata, Nairapi) [11]; this is coincidental with our own observations. This species was observed in breeding raining season (December-February) around La Paz. It was not observed in Santa Cruz lower lands or Peru highlands, but only in warmer valleys about 2,500 – 3,500 meters (8,202 – 11,482 feet) altitude. This somewhat contradicts information detailed in the study [11], at least in the breeding season. Seasonal movements are not discarded in the non-breeding season.

Thus, *C. atrata* and *C. xanthogastra stejnegeri* are birds bigger than *C. xanthogastra*; sexes show little colour differences in contrast to *C. xanthogastra*, as shown in Figs. (**1**,**5** and **6**), and they have overlapping habitats, while *C. xanthogastra* habitats are further North-separated by Peru and 1,761.7 Km, 1,094.6 miles distance (Fig. **2**).

CONCLUSION

[1] *C. xanthogastra stegnejeri* is a subspecies or a close species of *C. atrata.* (Figs. **4A**, **4B**, **5A**, **5B**, **6**). Genetics analyses also confirm it, as shown in Figs. (**3**, **4**) and (Tables **2**, **3**, **4**).

[2] Linearized ML mt cytochrome phylogenetics show that both *C. x. stejnegeri* and *C. atrata* appeared on Earth much later than *C. xanthogastra.*

[3] Habitat of *C. atrata* and *C. x. stejnegeri* overlaps, although more restricted for the latter, which thrives at lower and warmer Bolivia highlands valleys in the breeding season.

[4] *C. x. stejnegeri* sexes are more monotypic, similar to those of *Carduelis notata*, which is the extant parental species of the South American radiation.

[5] We propose for naming this species or *C. atrata* subspecies the provisional name of *C. lapazensis* since it has been first observed and characterized in phenotype and genetics in samples at La Paz (Bolivia) surroundings. Moreover, ongoing phenotypic studies are being performed.

[6] It is not genetically possible to establish a clear distinction between *C. atrata* / *C. x. stejgeneri*, as it occurs with other South American Siskin species, probably, because of close relatedness and recent speciation or phenotypic change [2, 3].

APPENDIX 1

ORIGINAL SPECIES BIRDS DESCRIPTIONS

C. xanthogastra description, may be consulted in Sharpe 1888 [11], Du Bus 1855 [13] and Clement *et al.* 1993 [12].

Description was like this: "Adult male: general colour above uniform black including the whole of the wing-coverts, bastard-wing, and primary-coverts: quills black, yellow at the base, with black shafts, on the secondaries, the innermost of which are entirely black; upper tail-coverts and center tail-feathers black, the remainder yellow for the basal half; head all round, sides of neck, and entire throat black; remainder of under surface yellow, greener on the sides of body and flanks; thighs black; under tail-coverts yellow; under wing-coverts and axillaries yellow, the former with blackish, yellow towards the base of the inner web. Total length 4.5 inches, Culmen 0.4, wing 2.6, tail 1.5, tarsus 0.6.

Adult female: different from the male. General colour above dull olive-green; lesser wing-coverts like the back; median and greater coverts dusky blackish, tipped with pale olive-green, whitish at the ends; bastard-wing, primary-coverts, and quills dusky blackish, fringed with pale olive-green, whitish at the ends of the secondaries; upper tail-coverts like the back; tail-feathers dusky blackish, edged with olive-green; crown of head like the back; sides of face olive-greenish, a little yellower on the fore part of the cheeks; throat and under tail-coverts; thighs ashy; under wing-coverts and axillaries ashy, fringed with olive-yellow; quills below dusky; ashy along the inner web. Total length 4 inches, culmen 0.4, wing 2.35, tail 1.45, tarsus 0.45."

C. x. stejnegeri was described (first in 1855 by Du Bus), may be consulted in Sharpe 1888 [11] and Clement *et al.* 1993 [12].

Description was like this: "Adult male: similar to *C. xanthogastra*, but rather larger, and distinguished by its yellow things, larger yellow wing-patch (the greater coverts being tipped with yellow), and whitish edgings to 4 inches, culmen 0.45, wing 2.7, tail 1.55, tarsus 0.55.

Adult female: According to Mr. Bukley is like the male, but with colours not so bright. Total length 4 inches, culmen 0.4, wing 2.5, tail 1.55, tarsus 0.5.

If the similarity in the colour of the sexes is really correctly determined, it is another proof of the difference between *C. x. stejnegeri* and *C.xanthogastra,* with which it has always been united.

In Dr. Sclater's collection is a Bolivian specimen collected by Mr. D. Forbes and marked a female (apparently by Dr. Sclater himself), which is dark olive-green above with some yellow spots on the crown; the head and sides of face are dingy greenish, blacker on the lores and region of the eye; wing-coverts greenish or edged with the latter colour; otherwise the wing marked as in the male but more dingy black, the greater coverts being tipped with yellow; under surface of body yellow, the throat being dull greenish. This seems to me more likely to be the plumage of the adult female, and I think Mr. Bukley's identification must be wrong. In either case the plumage of the hen bird is very different from that of the female of *C. xanthogastra***.**"

C. atrata was described in Lafresnaye and d'Orbigny 1837 [14], Sharpe 1888 [11] and Clement *et al.* 1993 [12].

Description was like this: "Adult male: general colour above sooty black. Wing-coverts black. The greater coverts tipped with yellow. Bastard-wing and primary-covers black. Quills yellow at base, black at the ends, the inner secondary entirely black. Upper tail-coverts and center tail-feathers black, the rest yellow at base, black at the ends. Head black all rounds as well as the entire under surface, excepting the abdomen and under tail-coverts, which are yellow. Thi~~n~~gs pale yellow. Under wing-coverts and axillaries yellow, the lower primary-coverts black: quills below blackish, yellow at base. Total length 4.8 inches, culmen 0.4, wing 3.15, tail 1.9, tarsus 0.6.

Adult female: differs from the male only in being more brownish black. The median as well as the greater coverts tipped with pale yellow, and the quills edged with yellowish white near the end of the outer web. The yellow on the under surface is rather paler and extends up to the fore neck, but is mottled with brown on the breast. Total length 4.8 inches, culmen 0.4, wing 3.2, tail 2, tarsus 0.6."

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethics Committée of University Complutense, Spain on March 13th 2008. Ethical approval number is 2008-AAV-UC.003.

HUMAN AND ANIMAL RIGHTS

No humans were used in this study. All animal procedures were performed in accordance with the International and Madrid Regional Laws. Permission for publication is issued for Researches attached to University Complutense, Madrid, Spain.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Material is deposited in Immunology Dept, University Complutense, Madrid, Spain [https://www.ucm.es/micro biologia-1/antonio-arnaiz-villena]. International data is also deposited in GeneBank [https://www.ncbi.nlm.nih.gov/nucleo tide/].

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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