

Bayesian phylogeny of Fringillinae birds: status of the singular African oriole finch *Linurgus olivaceus* and evolution and heterogeneity of the genus *Carpodacus* *

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Abstract Oriole finch *Linurgus olivaceus* is a songbird thriving in African tropical forests. It has been classified as the only species within the genus *Linurgus*. The phylogenetic relationships of the oriole finch with at least one species of each extant Passerine families and subfamilies have been studied by mitochondrial DNA sequencing. *L. olivaceus* has also been genetically compared with most extant Canaries, Siskins and other members of the subfamily Fringillinae. In total, 83 Fringillinae species have been used for the present study, and a phylogenetic revision of this subfamily has been addressed. Phylogenetic trees have been constructed by using a Bayesian methodology. Our results show that *Linurgus olivaceus* belongs to the subfamily Fringillinae and clusters within the tribe Carduelini, grouping with canaries (genus *Serinus*), goldfinches and siskins (genus *Carduelis*) and crossbills (genus *Loxia*). The oriole finch appears to be a basal single species that has evolved together with (and separately from) other *Serinus* and *Carduelis* species. Extinct species lacking in the analysis may have existed that were genetically closer to *Linurgus olivaceus*. On the other hand, the hawfinch *Coccothraustes coccothraustes* is definitively included within Eurasian grosbeaks (genera *Eophona* and *Mycerobas*), and the American *Carpodacus* species seem to be an evolutionary radiation apart from the Asian ones [Acta Zoologica Sinica 53 (5): 826–834, 2007].

Key words *Carpodacus*, *Coccothraustes*, Hawfinch, House finch, *Linurgus olivaceus*, Oriole finch, Passeriformes, Songbirds

燕雀亚科鸟类的贝叶斯系统发生：非洲鹂雀的分类地位及朱雀属的进化与异质起源 *

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摘要 鹂雀 (*Linurgus olivaceus*) 是非洲热带森林中一种独特的鸣禽, 为鹂雀属 (*Linurgus*) 的唯一物种。在已有的研究中, 通过对鹂雀和雀形目现存鸟类中每个科及亚科至少一个物种的线粒体 DNA 序列测定, 分析了鹂雀与其它现存雀形目鸟类的系统发生关系; 在遗传上, 对鹂雀与金丝雀、金翅雀及燕雀亚科其它鸟类亦有比较研究。本研究共使用了燕雀亚科 83 种鸟类, 重点对该亚科的系统发生进行了修订。使用贝叶斯法构建了系统发生树, 结果表明: 鹂雀属于燕雀亚科, 系统发生树中聚在金翅雀族 (Carduelini), 与金丝雀属 (*Serinus*)、金翅雀属 (*Carduelis*) 及交嘴雀属 (*Loxia*) 的种类形成一组; 在系统发生中, 鹂雀可能是一个基部物种, 它同金丝雀属和金翅雀属鸟类一同进化并分歧出来。在本研究中未能涉及的一些已灭绝种类, 可能与鹂雀有着较近的遗传学关

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系。另一方面, 研究也表明锡嘴雀 (*Coccothraustes coccothraustes*) 肯定包括在欧亚蜡嘴雀 (蜡嘴雀属 *Eophona* 和拟蜡嘴雀属 *Mycerobas*) 中, 美洲的朱雀 (*Carpodacus*) 可能是从亚洲种类分歧出来并经过进化辐射形成 [动物学报 53 (5): 826–834, 2007]。

关键词 朱雀 锡嘴雀 蜡嘴雀 家朱雀 鹎雀 雀形目 鸣禽

The evolutionary histories of songbirds have been broadly studied. Sometimes, phenotypic, behavioural and molecular evolution are not concordant (Sibley and Ahlquist, 1990; Pasquet and Thibault, 1997). Thus, the ecological convergence of morphological and behavioural characters may lead to shared features among non-closely genetically related species occurring in similar or quasi-identical environments, and conversely, disparate features may occur among genetically sister taxa thriving under different environments. Evidence of this phenotypic plasticity has already been given for bird species (Marchetti, 1993; Kusmierski et al., 1997; McCracken et al., 1999; van Tuinen et al., 2001; Haavi et al., 2004). Therefore, some phenotypic traits may be not coincidental with the information about the evolutionary histories of birds under study.

Mitochondrial cytochrome *b* gene (mt *cyt-b*) sequencing has been widely used in molecular systematics. This gene has been proved to be helpful for defining evolutionary relationships among relatively distant or closely related birds, even at the subspecies level (Friesen et al., 1996; Questiau et al., 1998). Songbirds (order Passeriformes, suborder Passeri) comprise about 4 000 worldwide species. The Fringillidae family is included within songbirds. Many songbird species have been phylogenetically analysed previously in our and others' studies by using molecular markers (Pasquet and Thibault, 1997; Arnaiz-Villena et al., 1998, 1999, 2001; Sangster, 2000; Yuri and Mindell, 2002; Zamora et al., 2006a, b).

Linurgus olivaceus is the only species within genus *Linurgus*. It thrives in tropical forests of central Africa and its name makes reference to the resemblance with the African white-headed oriole (*Oriolus larvatus*, Family Corvidae), which occurs in overlapping areas (Clement et al., 1993). Non-molecular (phenotypic and behavioural) characters, however, have related oriole finch to the Canaries species *Serinus totta* (cape serin) and the black-headed canary *Serinus alario* (Badyaev, 1996, 1997). *L. olivaceus* is included within the Subfamily Fringillinae (order Passeriformes, family Fringillidae) as a single species genus, according to Sibley's birds classification (Sibley and Ahlquist, 1990); however, inclusion in Carduelini tribe (with canaries and goldfinches) was not proposed by Sibley (Sibley and Ahlquist, 1990).

In the present work, we aim to study for the first time the taxonomic status and evolutionary history of the oriole finch by using mt *cyt b* DNA sequences. DNA from

species belonging to every extant Passerine families and subfamilies have been compared together with *L. olivaceus*, particularly those from the three tribes comprising Subfamily Fringillinae (Drepanidini, Carduelini and Fringillini). The phylogenetic analysis shows that the oriole finch belongs to tribe Carduelini, grouping with canaries (genus *Serinus*), goldfinches-siskins (genus *Carduelis*) and crossbills (genus *Loxia*). It is important that Grosbeaks are included in the analysis since the relative thick and big oriole finch's beak resembles that of the Grosbeaks.

We also make a revision of the hawfinch *Coccothraustes coccothraustes* phylogeny and analyse the phylogenetic status of the North American *Carpodacus* species compared to the Asian ones. In a previous work (Arnaiz-Villena et al., 2001), hawfinch classification was uncertain because the available DNA sequence was too short. A longer sequence has been analysed in the present study, definitively placing the Hawfinch within Eurasian grosbeaks. The American house finch *Carpodacus mexicanus* appeared to be separated from the Asian *Carpodacus* species (Arnaiz-Villena et al., 2001); in this paper we have included a new American species, Cassin's finch *Carpodacus cassinii*, which further supports the separation between Asian and American *Carpodacus* species radiation.

1 Materials and methods

1.1 Bird samples and DNA sequences

Oriole finch *L. olivaceus* was sampled in a Spanish former colony (Equatorial Guinea, Africa) at Bioko Island (former Fernando Poo Island). The place coordinates are 3.383° N, 8.767° E, at the Gulf of Guinea. The bird was captured close to the capital, Malabo, where the standard species type was described as *Coccothraustes olivaceus* by Frazer in 1842 (Clement et al., 1993). The hawfinch *C. coccothraustes* sample was taken at a Madrid city garden (Spain) during the autumn southern emigration. This bird thrives in mixed woods, bushes, parks and gardens of Europe and Asia, in palearctic lowlands and mountains up to 3 000 m, including Spain, North Africa and Japan. The Cassin's finch *C. cassinii* was sampled in Mount Charleston (Nevada, USA). Its habitat is restricted to mountain pine forest of western North America. Blood extraction, amplification and sequencing of mt *cyt b* gene (924 base pairs, from 100 to 1 024) were carried out as previously described (Arnaiz-Villena et al., 1992; Arnaiz-Villena et al., 1998). The new DNA sequences obtained were submitted

to GenBank and given the accession numbers DQ257461 (*L. olivaceus*), EF371060 (*C. coccothraustes* and EF371059 (*C. cassini*). *L. olivaceus* and *C. dominicensis* photographs were taken out by Antonio Arnaiz-Villena (see Fig.1).

1.2 Species used for phylogenetic calculations

Linurgus olivaceus was first compared with mt cyt *b* DNA sequences from many other songbirds (order Passeriformes, suborder Passeri) to confirm its current taxonomic status at the family (or subfamily) level (Sibley and Ahlquist, 1990; Clement et al., 1993). Families, subfamilies and species of analysed birds, and the corresponding GenBank accession numbers are detailed in Table 1. Chicken (Galliformes: Phasianidae: *Gallus gallus*, X52392) was set as outgroup. These sequences were taken from the National Center of Biotechnology Information (<http://www.ncbi.nlm.nih.gov>) and from our own results (Arnaiz-Villena et al., 1998, 1999; Allende et al., 2001; Zamora et al., 2006a, 2006b; Arnaiz-Villena et al., 2007). In a second approach, the oriole finch was compared with species belonging to the three tribes (Carduelini, Drepanidini and Fringillini) from Subfamily Fringillinae to better define its position at the species level. *C. coccothraustes* and *C. cassini* were included in this analysis. Lark-like bunting *Emberiza impetuani* (Family Fringillidae, Subfamily Emberizinae) was used as outgroup. The species used in this second analysis are indicated in Table 2, and these results are shown in Fig.1.

1.3 Statistical analyses

924 base pairs (bp) DNA sequences of mt cyt *b* gene from the bird species analysed were aligned and checked for stop codons with MEGA3.1 computer program (Kumar et al., 2004). Phylogenetic trees were calculated using a Bayesian analysis (Huelsenbeck and Ronquist, 2001) with MrBayes 3.1.2 software (Ronquist and Huelsenbeck, 2003). The model of evolution chosen was the one that most likely fit for the data set, according to Modeltest 3.1 program (<http://darwin.uvigo.es>) (Posada and Crandall, 1998); this model was GTR + I + G, that is, a general time reversible model which considers up to six different nucleotide substitution types, the proportion invariable sites and a gamma distribution of rates at variable sites, with the associated shape parameter 'alpha'. Two independent runs, with one cold and three heated chains each, were performed along 5 000 000 generations, sampling every 100 generations. The 'burn-in' was determined to be 25% (1 250 000 generations), so the first 12 500 samples were discarded. After that, both runs had converged to a stationary distribution and the average standard deviation of split frequencies approached to zero, being less than 0.01 at the end of the analysis.

2 Results

The Bayesian analysis place *Linurgus olivaceus* within subfamily Fringillinae (Family Fringillidae) when compared with members from most of the families and subfamilies of songbirds (Suborder Passeri). All compared Passeri species are detailed in Table 1 and the dendrograms of results are not shown. *L. olivaceus* appears as a basal species of genera *Carduelis* and *Serinus* polytomic radiations.

Calculations carried out with species from tribes Carduelini, Drepanidini and Fringillini (Subfamily Fringillinae) (see Table 2) show that tribes Drepanidini and Fringillini form two distinct well-defined monophyletic groups, strongly supported in the Bayesian analysis with posterior probability values of 1.00 for both groups (Fig.1). However, tribe Carduelini comprises a number of paraphyletic groups. The oriole finch is included into these Carduelini groups, and clusters by itself among all analysed Canaries (genus *Serinus*), Goldfinches-Siskins (genus *Carduelis*) and crossbills (genus *Loxia*), having a high posterior probability value of 0.99 in the Bayesian tree (Fig.1, see node 'A'). This later 'A' Carduelini group shows a paraphyletic distribution of smaller consistent and independent subgroups of *Carduelis* and *Serinus* species, like the Asian-African canaries, North and South American siskins, African canaries, redpolls and greenfinches (including the desert finch *Rhodopechys obsoleta*). *Linurgus olivaceus* appears as a basal and separated species in the African Canaries subgroup.

Other well-defined Carduelini groups are the bullfinches (genus *Pyrrhula*), that include the pine grosbeak *Pinicola enucleator*, and the rosefinches, grouping Asian *Carpodacus* species with the long-tailed rosefinch *Uragus sibiricus* and the scarlett finch *Haematospiza sipahi*. The American *Carpodacus cassini* (Cassin's finch) and house finch *Carpodacus mexicanus* cluster together and separated from the rest of Carduelini finches, including the Asian *Carpodacus* species. Finally, the hawfinch *Coccothraustes coccothraustes* clusters with grosbeaks (genera *Eophona* and *Mycerobas*), basal to genus *Eophona*.

3 Discussion

3.1 Oriole finch taxonomic status

The Bayesian analysis places *Linurgus olivaceus* within Subfamily Fringillinae (Family Fringillidae) when compared with members from most of the families and subfamilies of songbirds specified in Table 1, what is concordant with Sibley's birds classification (Sibley and Ahlquist, 1990). Once oriole finch was shown to be placed within Fringillinae birds, similar phylogenetic calculations were carried out to compare the oriole finch with species from this Fringillinae subfamily tribes

Table 1 Species representing most of the families and subfamilies of songbirds (Order Passeriformes, Suborder Passeri), used to determine the phylogenetic status of the Oriole Finch among them

Family	Subfamily	Species	GenBank accession numbers
Climacteridae	—	<i>Cormobates placens</i>	AY064278
Menuridae	—	<i>Menura novaehollandiae</i>	AY064276
Ptilonorhynchidae	—	<i>Chlamydera lauterbachii</i>	U76506
Turnagridae	—	<i>Turnagra capensis</i>	U51734
Maluridae	—	<i>Stipiturus mallee</i>	AY488404
Pardalotidae	Pardalotinae	<i>Pardalotus striatus</i>	AY488398
	Dasyornithinae	<i>Dasyornis broadbenti</i>	AY488394
	Acanthizinae	<i>Sericornis perspicillatus</i>	AY488400
Petroicidae	—	<i>Tregellasia leucops</i>	AY443259
Orthonychidae	—	<i>Orthonyx temminckii</i>	AY064275
Laniidae	—	<i>Lanius bucephalus</i>	AB159157
Vireonidae	—	<i>Vireo latimeri</i>	AF383108
Corvidae	Corcoracinae	<i>Corcorax melanorhamphos</i>	AY064274
	Pachycephalinae	<i>Pachycephala pectoralis</i>	AY228089
	Corvinae	<i>Oriolus xanthornus</i>	AF094615
	Dicrurinae	<i>Dicrurus paradiseus</i>	AF096473
	Malaconotinae	<i>Malaconotus sulfureopectus</i>	AF096456
Callaeatidae	—	<i>Callaeas cinerea</i>	AF433209
Picathartidae	—	<i>Chaetops frenatus</i>	AY228052
Bombycillidae	—	<i>Ptilogonys cinereus</i>	AY443258
Cinclidae	—	<i>Cinclus mexicanus</i>	AY329454
Muscicapidae	Turdinae	<i>Turdus merula</i>	AY286396
	Muscicapinae	<i>Ficedula parva</i>	AY329460
Stumidae	—	<i>Sturnus vulgaris</i>	AF285790
Sittidae	Sittinae	<i>Sitta pygmaea</i>	AY352538
Certhiidae	Certhiinae	<i>Certhia familiaris</i>	AY352523
	Troglodytinae	<i>Troglodytes aedon</i>	AY352547
	Polioptilinae	<i>Polioptila caerulea</i>	AY352535
Paridae	Remizinae	<i>Remiz pendulinus</i>	AY228081
	Parinae	<i>Parus major</i>	AF551784
Aegithalidae	—	<i>Aegithalos caudatus</i>	AB159172
Hirundinidae	Hirundininae	<i>Hirundo rustica</i>	AY509626
Regulidae	—	<i>Regulus calendula</i>	AY329472
Pycnonotidae	—	<i>Hypsipetes amaurotis</i>	AB159164
Cisticolidae	—	<i>Prinia bairdii</i>	AY352536
Zosteropidae	—	<i>Zosterops japonicus</i>	AB159168
Sylviidae	Acrocephalinae	<i>Urosphena squameiceps</i>	AB159179
	Megalurinae	<i>Megalurus pryori</i>	AJ004323
	Garrulicinae	<i>Garrulax chinensis</i>	AY333176
	Sylviinae	<i>Sylvia melanothorax</i>	AJ534546
Alaudidae	—	<i>Alauda arvensis</i>	AY228047
Nectariniidae	Nectariniinae	<i>Nectarinia sovimanga</i>	AY235553
Passeridae	Passerinae	<i>Passer domesticus</i>	AF094639
	Motacillinae	<i>Motacilla alba</i>	AF445529
	Prunellinae	<i>Prunella atrogularis</i>	AY329471
	Ploceinae	<i>Quelea cardinalis</i>	AF255709
	Estrildinae	<i>Lonchura cucullata</i>	L76611
Fringillidae	Peucedraminae	<i>Peucedramus taeniatus</i>	AF290139
	Fringillinae	<i>Serinus serinus</i>	L76263
	Emberizinae	<i>Emberiza elegans</i>	L78806

Table 2 List of species used in the phylogenetic calculations. Origin of samples and GenBank accession numbers to *cyt b* DNA sequences are indicated

Scientific name	Common name	GenBank	Sample region
<i>Linurgus olivaceus</i>	Oriole finch	DQ257461	Bioko, Equatorial Guinea
Subfamily Emberizinae, Tribe Emberizini			
<i>Emberiza impetuani</i>	Lark-like bunting	L77902	Bostwana
Subfamily Emberizinae, Tribe Cardinalini			
<i>Pinicola enucleator</i>	Pine grosbeak	AF342882	Novorsibirsk, Russia
Subfamily Fringillinae, Tribe Carduelini			
<i>Carduelis ambigua</i>	Black-headed greenfinch	U78322	Szechwan, China
<i>Carduelis atrata</i>	Black siskin	L76385	Sucre, Bolivia
<i>Carduelis atriceps</i>	Black-capped siskin	AF342863	Quetzaltenango, Guatemala
<i>Carduelis barbata</i>	Black-chinned siskin	L77868	Magallanes, Chile
<i>Carduelis cannabina</i>	Linnet	L76298	Madrid, Spain
<i>Carduelis carduelis caniceps</i>	European goldfinch	L76388	Katmandu, Nepal
<i>Carduelis carduelis parva</i>	European goldfinch	L76387	Madrid, Spain
<i>Carduelis chloris aurantiventris</i>	Greenfinch	L76297	Madrid, Spain
<i>Carduelis crassirostris</i>	Thick-billed siskin	L77869	Mendoza, Argentina
<i>Carduelis cucullata</i>	Red siskin	L762299	Venezuela
<i>Carduelis dominicensis</i>	Antillean siskin	AF342864	Constanza, Dominican Rep.
<i>Carduelis flammea</i>	Common redpoll	L76386	Brussels, Belgium
<i>Carduelis flavirostris</i>	Twite	U83199	Antwerp, Belgium
<i>Carduelis hornemanni</i>	Arctic redpoll	U83201	Antwerp, Belgium
<i>Carduelis lawrencei</i>	Lawrence's goldfinch	L76392	San Diego (CA), USA
<i>Carduelis magellanica</i>	Hooded siskin	U79016	Misiones, Argentina
<i>Carduelis notata</i>	Black-headed siskin	U79019	Chiapas, Mexico
<i>Carduelis olivacea</i>	Olivaceous siskin	L77871	Lima, Perú
<i>Carduelis pinus pinus</i>	Pine siskin	U79020	Jackson (WY), USA
<i>Carduelis pinus perplexus</i>	Pine siskin	AF901951	Quetzaltenango, Guatemala
<i>Carduelis psaltria colombiana</i>	Dark-backed goldfinch	U78324	Maracay, Vanezuela
<i>Carduelis psaltria hesperofila</i>	Dark-backed goldfinch	L76390	Sacramento (CA), USA
<i>Carduelis sinica</i>	Grey-capped greenfinch	L76592	Szechwan, China
<i>Carduelis spinescens</i>	Andean siskin	U79017	Merida, Venezuela
<i>Carduelis spinoides</i>	Yellow-breasted greenfinch	U79018	Katmandu, Nepal
<i>Carduelis spinus</i>	Eurasian siskin	L76391	Madrid, Spain
<i>Carduelis tristis salicamans</i>	American goldfinch	U79022	San Francisco (CA), USA
<i>Carduelis xanthogastra</i>	Yellow-bellied siskin	L76389	San Jose, Costa Rica
<i>Carduelis yarrellii</i>	Yellow-faced siskin	U83200	Recife, Brasil
<i>Carpodacus cassinii</i>	Cassin's finch	EF371059	Mt. Charleston (NV), USA
<i>Carpodacus erythrurus roseatus</i>	Common rosefinch	AF342883	Islamabad, Pakistan
<i>Carpodacus mexicanus frontalis</i>	House finch	AF342865	Los Angeles (CA), USA
<i>Carpodacus roseus</i>	Palla's rosefinch	AF342867	Beijing, China
<i>Carpodacus rubicilloides lucifer</i>	Streaked rosefinch	AF342868	Katmandu, Nepal
<i>Carpodacus thura</i>	White-browed rosefinch	AF342869	Katmandu, Nepal
<i>Carpodacus trifasciatus</i>	Three-banded rosefinch	AF342870	Szechwan, China
<i>Coccothraustes coccothraustes</i>	Hawfinch	EF371060	Madrid, Spain
<i>Eophona migratoria</i>	Yellow-billed grosbeak	AF342871	Beijing, China
<i>Eophona personata</i>	Japanese grosbeak	AF342872	Beijing, China
<i>Haematospiza sipahi</i>	Scarlet finch	AF342875	Katmandu, Nepal

Continued Table 2

Scientific name	Common name	GenBank	Sample region
<i>Loxia curvirostra curvirostra</i>	Common crossbill	AF342876	Alcala de Henares, Spain
<i>Loxia curvirostra japonica</i>	Common crossbill	AF342877	Beijing, China
<i>Loxia leucoptera bifasciata</i>	Two-barred crossbill	AF342878	Siberia, Rusia
<i>Mycerobas affinis</i>	Collared grosbeak	AF342879	Katmandu, Nepal
<i>Mycerobas carnipes</i>	White-winged grosbeak	AF342880	Katmandu, Nepal
<i>Pyrrhula erythaca wilderi</i>	Beavan's bullfinch	AF342862	Beijing, China
<i>Pyrrhula nipalensis</i>	Brown bullfinch	AF342884	Katmandu, Nepal
<i>Pyrrhula pyrrhula cineracea</i>	Comon bullfinch	AF342886	Novasibirsk, Russia
<i>Pyrrhula pyrrhula griseiventris</i>	Comon bullfinch	AF342881	Beijing, China
<i>Pyrrhula pyrrhula iberiae</i>	Comon bullfinch	AF342885	Santander, Spain
<i>Rhodopechys obsoleta</i>	Desert finch	AF342889	Kabul, Afganistan
<i>Serinus alario</i>	Black-headed canary	L76276	Capetown, South Africa
<i>Serinus albogularis</i>	Whited-throated canary	L78705	Capetown, South Africa
<i>Serinus atrogularis</i>	Yellow-rumped seedeater	L76267	Capetown, South Africa
<i>Serinus canaria</i>	Island canary	L76266	Canary Islands, Spain
<i>Serinus canicollis canicollis</i>	Yellow-crowned canary	L78706	Capetown, South Africa
<i>Serinus citrinella citrinella</i>	Citril finch	L77872	Madrid, Spain
<i>Serinus citrinella corsicanus</i>	Citril finch	AY583725	Sardinia, Italy
<i>Serinus citrinelloides</i>	African citril	L77555	Nairobi, Kenya
<i>Serinus citrinipectus</i>	Lemon-breasted canary	L78707	Maputo, Mozambique
<i>Serinus dorsostriatus</i>	White-bellied canary	L76278	Dar es Salam, Tanzania
<i>Serinus flaviventris quintoni</i>	Yellow canary	L76280	Capetown, South Africa
<i>Serinus gularis endemion</i>	Streaky-headed seedeater	L77556	Capetown, South Africa
<i>Serinus leucopygius riggenbachi</i>	White-rumped seedeater	L76264	Dakar, Senegal
<i>Serinus mozambicus</i>	Yellow-fronted canary	L76265	Dar es Salam, Tanzania
<i>Serinus pusillus</i>	Red-fronted serin	L77873	Sin Wiang, China
<i>Serinus serinus</i>	European serin	L76263	Madrid, Spain
<i>Serinus striolatus</i>	Streaky seedeater	L77557	Nairobi, Kenya
<i>Serinus sulphuratus</i>	Brimstone canary	L76294	Capetown, South Africa
<i>Serinus syriacus</i>	Syrian serin	AY570547	Mount Hermon, Israel
<i>Serinus tibetanus</i>	Tibetan siskin	L76279	Szechwan, China
<i>Serinus totta</i>	Cape serin	AY570548	Cape Town, South Africa
<i>Uragus sibiricus lepidus</i>	Long-tailed rosefinch	AF365877	Beijing, China
Subfamily Fringillinae, Tribe Drepanidini			
<i>Hemignathus munroi</i>	Akiapolaau	AF015760	Hawaii Islands, USA
<i>Hemignathus virens</i>	Hawaii amakihi	AF015755	Hawaii Islands, USA
<i>Oreomystis bairdi</i>	Kauai creeper	AF015763	Hawaii Islands, USA
<i>Oreomystis mana</i>	Hawaii creeper	AF015758	Hawaii Islands, USA
<i>Paroreomyza montana</i>	Maui creeper	AF015759	Hawaii Islands, USA
<i>Pseudonestor xanthophrys</i>	Maui parrotbill	AF015762	Hawaii Islands, USA
Subfamily Fringillinae, Tribe Fringillini			
<i>Fringilla coelebs</i>	Chaffinch	L76609	Madrid, Spain
<i>Fringilla montifringilla</i>	Brambling	L77903	Denmark
<i>Fringilla teydea</i>	Teydefinch	AF002894	Canary Islands, Spain

All the sequences have been obtained by us (Arnaiz-Villena et al., 1998; Arnaiz-Villena et al., 1999; Arnaiz-Villena et al., 2001; Zamora et al., 2006b), except *Fringilla teydea* and the Hawaiian honeycreepers species (tribe Drepanidini), taken from the GenBank (<http://www.ncbi.nlm.nih.gov>). Three new sequences are included for the present work (in grey).

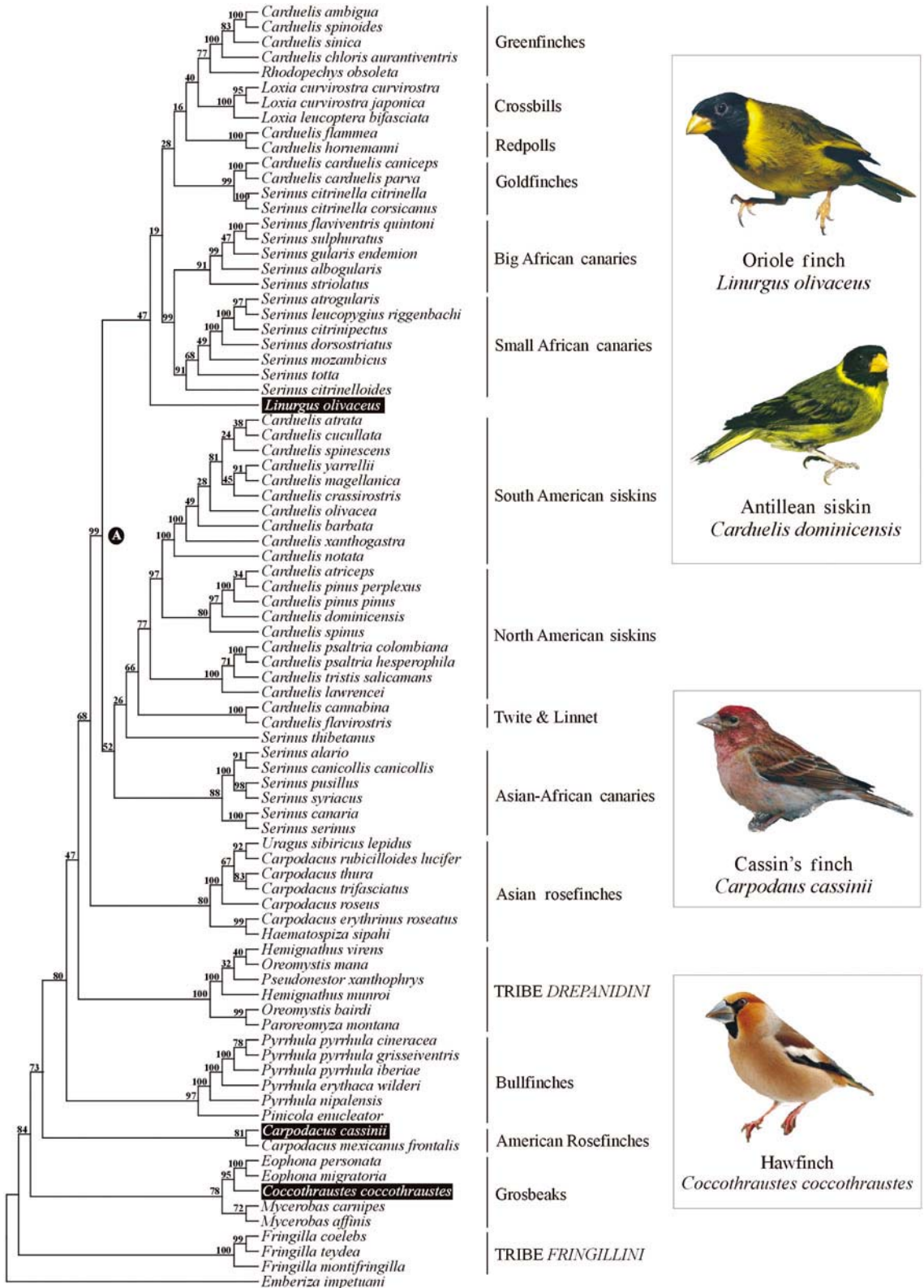


Fig. 1 Bayesian phylogenetic tree based on mitochondrial cytochrome *b* DNA sequences

Species belonging to the tribes Carduelini, Drepanidini and Fringillini from Subfamily Fringillinae are compared with the oriole finch *Linurgus olivaceus*. Hawfinch *Coccothraustes coccothraustes* and Cassin's finch *Carpodacus cassinii* are included in the tree (see also Table 2). Lark-like Bunting *Emberiza impetuumi* (Subfamily Emberizinae, tribe Emberizini) was set as outgroup. The evolutionary model chosen for the Bayesian analysis was GTR + I + G, which considers six different ratio of changes between nucleotides, the proportion of invariable sites and a gamma distribution of rates at variable sites. 5 000 000 generations were run, sampling every 100 generations and discarding the first 12 500 samples. The posterior probability values ($\times 100$) of the nodes are indicated. The three new species studied are highlighted in black.

(Carduelini, Drepanidini and Fringillini, see Table 2). Both tribes Drepanidini and Fringillini form distinct well-defined monophyletic groups, according to the Bayesian analysis (Fig.1), while tribe Carduelini seems to comprise a number of paraphyletic groups. The oriole finch was found to belong to tribe Carduelini, and it is integrated within a strongly supported group that clusters all the species from genera *Serinus*, *Carduelis* and *Loxia* (and also the desert finch *Rhodopechys obsoleta*, that groups with greenfinches (Zamora et al., 2006a) (Fig.1). *L. olivaceus* appears in a single branch as a basal species close to African Canaries (genus *Serinus*). No sister taxa have been found, most likely due to the lack of extinct species in the analyses (Arnaiz-Villena et al., 1999; Zamora et al., 2006b).

3.2 Oriole finch speciation timing

According to previous results (Arnaiz-Villena et al., 1998, 1999; Zamora et al., 2006b), the speciation of Canaries and Goldfinches started in the Miocene Epoch, about 9 – 10 MYA. The oriole finch is basal to many *Carduelis* and *Serinus* species (Fig.1); thus, *Linurgus*' ancestor may or may not be the ancestor of some extant canaries and goldfinches-siskins, but it is certain that it belongs to the same polyphyletic radiation, which started in the late Miocene Epoch (23 – 25 MYA).

3.3 Oriole finch relatedness

Phenotypic and DNA sequence-based classifications are not always concordant. In fact, the phylogenetic calculations do not show a close genetic relatedness between the Oriole Finch and morphologically similar species, like the Antillean siskin *Carduelis dominicensis*, which is very similar in plumage and size, but genetically unrelated (Arnaiz-Villena et al., 2007 and Fig.1). On the other hand, the Bayesian phylogenetic analysis shows that phenotypically distinct birds are indeed genetically close (Fig.1). Some examples were previously reported: 1) the Citril finch *Serinus citrinella* clusters genetically with the goldfinch *Carduelis carduelis* (Arnaiz-Villena et al., 1999; Zamora et al., 2006b); 2) the scarlet finch *Haematospiza sipahi* and the long-tailed rosefinch *Uragus sibiricus* genetically group with rosefinches from genus *Carpodacus* (Arnaiz-Villena et al., 2001) in spite of a disparate morphology; 3) pine grosbeak *Pinicola enucleator* seems to be genetically a quite apparently different bullfinch (genus *Pyrrhula*) (Arnaiz-Villena et al., 2001); and 4) the pale desert finch *Rhodopechys obsoleta* appears to be genetically the ancestor of fully coloured greenfinches (genus *Carduelis*) (Zamora et al., 2006a).

Convergent evolution towards black head and dark green colours are clearly found in both genetically unrelated oriole finch and antillean siskin. This is more probably due to the fact that these two birds live in humid forests and a dark colour (conferred by changes in carotenoid and melanine metabolism) (Gill, 1995),

protects feathers from microbial infections in humid habitats (Burt and Ichida, 2004), as seen for greenfinches (Zamora et al., 2006a).

3.4 Hawfinch *Coccothraustes coccothraustes*

In our previous work (Arnaiz-Villena et al., 2001) only a 307 bp cytochrome *b* DNA sequence was available from this species. This did not resolve the phylogenetic tree topology and the Hawfinch appeared to be separated from other Eurasian grosbeaks. Thus, a 924 bp sequence from mt cyt *b* was obtained for the present work and it was definitively shown that the hawfinch goes together with other Asian grosbeaks, basal to *Eophona* species. These results are supported by this work Bayesian methodology (Fig.1).

3.5 American *Carpodacus*: house finch and Cassin's finch

Our previous work showed a great heterogeneity within genus *Carpodacus*, showing polytomies and, more important, the North American house finch *C. mexicanus* was separated from the Asian species (Arnaiz-Villena et al., 2001). This separation is now confirmed with the analysis of a new American *Carpodacus*: *C. cassinii* (Cassin's finch). The Bayesian analysis (Fig.1) seems to establish that American *Carpodacus* belong to a radiation different to the Asian one. In the future, perhaps the addition of putative missing species in the analysis may join both Asian and American *Carpodacus* groups. In the present analysis they are separate phylogenetic groups. House finch thrives in western Canada, United States and Mexico, and eastern United States, while more purple coloured Cassin's finch lives in general in higher altitudes of western Canada, United States and Mexico. However, thriving range of both species frequently overlap in the western North American part. North American *Carpodacus* probably represent a radiation different from the Asian ones (Arnaiz-Villena et al., 2001). American *Carpodacus* ancestor probably appeared around 10 million years ago (MYA) (Arnaiz-Villena et al., 2001) in the second part of the Miocene Epoch when temperatures had already lowered and North America become isolated from Siberia by ice (Uriarte-Cantolla, 2003).

3.6 A brief comment on Fringillinae subfamily phylogeny

Conjoint phylogenetic analyses of *Carduelis* and *Serinus* genera show that they are the closest genera among Fringillinae. However, several polytomies were shown in both genera (Fig.1). This suggests an independent evolution of different small groups of Fringillinae birds, which started about 10 – 9 MYA in the Miocene Epoch and do not show a clear common ancestor (Zamora et al., 2006b). In the present paper, a more extensive and powerful Bayesian analysis further support these findings and helps to define that it is likely that several evolutionary radiations occurred separately within

Subfamily Fringillinae (Fig. 1).

The time of Fringillinae finches' appearance on Earth is consistent with 10 – 9 MYA in all analyses with Maximum Likelihood (ML) or Neighbor-Joining (NJ) linearized trees (Zamora et al., 2006b). Although some Paleoenvironment changes influencing the Fringillinae evolution have been put forward, like the Mediterranean Messinian salinity crisis (Zamora et al., 2006a) and the general Earth climate cooling after 14 MYA, which induced aridity in many Asian and African areas (Uriarte-Cantolla, 2003), direct clear environmental causes driving to the Fringillinae radiation have not yet been established. Finally, cladistic (Maximum Parsimony 'MP'), distance-based (Unweighted Pair Group Method with Arithmetic mean 'UPGMA' and Neighbor-Joining 'NJ') and Maximum Likelihood 'ML' analyses with *Carduelis*, *Serinus* and other relevant genera always gave consistent phylogenetic groups (Arnaiz-Villena et al., 1998; Arnaiz-Villena et al., 1999; Arnaiz-Villena et al., 2001; Zamora et al., 2006b). These groups are now confirmed in the Bayesian analysis (Fig. 1), which include *Linurgus olivaceus* as a separate and basal lineage within *Carduelis* and *Serinus* species subgroup, and confirms with a new American *Carpodacus* species (*C. cassini*) that American and Asian *Carpodacus* seem to be far separate radiations with the present day available data.

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