

Mitochondrial DNA Phylogenetic Definition of a Group of 'Arid-Zone' Carduelini Finches

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Abstract: Birds included within the Carduelini tribe (genera *Rhodopechys*, *Carpodacus* and *Leucosticte*) apparently belong to the same radiation according to molecular phylogenetic analyses. Our phylogenetic analyses based on nucleotide sequences of the *cytochrome b* gene (cyt-b) indicate that some of these birds (*Rhodopechys mongolica*, *R. githaginea* and *Carpodacus nipalensis*) do not cluster together with their respective phenetically defined allies. This new group of birds thrives in both hot and cold arid zones and are phenetically distinct, probably because of their adaptation to different extreme environments. Both maximum likelihood and Bayesian inference methods support the existence of this new evolutionary basal group among finches which might have originated about 14 million years ago.

Key Words: Carduelini, *Carpodacus*, finch, *Fringillinae*, *Leucosticte*, phylogeny, *Rhodopechys*, rosefinches.

INTRODUCTION

The evolutionary histories of songbirds (class *Aves*, order *Passeriformes*, suborder *Passeri*) have been broadly studied. Their phenotypic, behavioural and molecular evolution are not always concordant [1, 2]. Thus, the parallelism in morphology and behavioural characters may lead to shared features among non-closely genetically related species occurring in similar or quasi-identical environments, and conversely, distinct features may occur among genetic sister taxa thriving under different environments. Evidence of this phenotypic plasticity has already been documented for other bird species [3-7]. Therefore, some genetic and/or phenetic traits apparently may not correlate with the evolutionary histories of the birds under study.

Mitochondrial *cytochrome b* gene (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 taxa, even at the subspecies level [8-10].

Here, we study some passerine species that belong to the genera *Rhodopechys*, *Carpodacus* and *Leucosticte* which do not cluster together with their corresponding genera according to molecular phylogenetic studies [1, 11, 12]. These songbirds belong to the tribe Carduelini (family *Fringillidae*, subfamily *Fringillinae*) and thrive in extreme environments, either in deserts (*Rhodopechys*) or high altitude habitats (*Carpodacus* and *Leucosticte*). Birds from the genus *Rhodopechys* inhabit deserts or very arid areas of Africa and Central Asia and have pale or sandy coloured wings and bills [13]. Four species are included within this genus: *R. githaginea* (trumpeter finch), *R. mongolica* (Mongolian finch), *R. obsoleta* (desert finch) and *R. sanguineus* (crimson-winged finch). The desert finch has recently been reported not to be genetically close to *Rhodopechys* species, but to be an ancestor of greenfinches from the genus *Carduelis* [12]. The rosefinches (genus *Carpodacus*) are high-altitude birds that have probably originated in the Himalayas [13]. Only one species (out of 21), the common rosefinch (*Carpodacus erythrurus*), has a Palearctic distribution and breeds at low altitudes. All the species of the genus *Carpodacus* are distributed in Asia (including Middle East), except *C. mexicanus* (house finch), *C. cassinii* (Cassin's finch) and *C. purpureus* (purple finch) which thrive in North America. The dark-breasted rosefinch (*Carpodacus nipalensis*) does not phylogenetically cluster with either American or Asian *Carpodacus* [11, 14]. The genus *Leucosticte* comprises three species: *L. nemoricola* (plain mountain finch), *L. brandti* (black-headed mountain finch) and *L. arctoa* (rosy finch). They are dark-plumaged sparrow-like birds that thrive in high altitude habitats of Central Asia and, in the case of *Leucosticte arctoa*, also in western North America. Some authors separate the rosy finch into two (or even three) different species (or subspecies) depending on the distribution range [13]. Here, we consider one species with two subspecies: the Asian rosy finch (*L. arctoa arctoa*) and the American rosy finch (*L. a. tephrocotis*).

In the present work we analyze the genetic relatedness among tribe Carduelini species using mt cyt-b DNA sequences previously obtained by us [11, 12, 15-17], including the only *Leucosticte* cyt-b DNA sequence available so far (*L. arctoa tephrocotis*, AY156385) and a new one obtained by us for this study (*L. arctoa arctoa*, DQ257460). Our work has also been enhanced because we previously had preliminary results suggesting that a small group of 'arid-zone' finches (species from genera *Carpodacus*, *Rhodopechys* and *Leucosticte*) might be defined.

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MATERIALS AND METHODOLOGY

Bird Samples, DNA Extraction and PCR Amplifications

Sixty one species of songbirds (order *Passeriformes*, suborder *Passeri*) have been included in this study (see Table 1). They belong to the tribe Carduelini (family *Fringillidae*, subfamily *Fringillinae*). The species highlighted in grey were sequenced and analyzed in this study together with relevant species of the tribe Carduelini. Other sequences included in this analysis were retrieved from the GenBank (see Table 1) [11, 12, 15-17].

DNA was obtained from blood taken by cutting one claw after anesthetizing with a lidocaine ointment. Blood was collected in EDTA and frozen until use. DNA extraction was performed using a standard phenol-chloroform protocol [18]. Amplification and sequencing of 924 base pairs (bp) of the *cyt-b* gene was carried out as previously described by Zamora *et al.* [12]. Nuclear mitochondrial insertions (“numts”) were not detected, since their evolutionary rate is different and they usually have stop codons. Our phylogeny methodologies would have detected these different evolutionary rates by leaving “numts” as outliers.

Phylogenetic Analyses

The sequences were aligned and checked for stop codons with MEGA v. 3.1 [19]. The phylogenetic dendrograms were reconstructed using maximum likelihood (ML) [20] in PAUP* v. 4.0b10 [21] and Bayesian inference (BI) [22] in MrBayes v. 3.1.2 [23]. Modeltest v. 3.7 [24] was employed to find the model of DNA substitution that fits the data best. The best model was then used in both the ML and BI analyses. The linearized ML dendrograms were calculated with PAUP* v. 4.0b10 [21] with the branch lengths estimated according to the model proposed by Thorne *et al.* [25] which assumes that the rates among the evolutionary lineages may not be constant. The tree reconstruction strategy consisted of a heuristic search with NNI (Neighbour-Neighbour Interchange) swapping algorithm. Robustness of nodes was assessed by 1000 bootstrap replicates in the ML analyses. The rates of the parameters defining the model of evolution were allowed to change in the BI analysis after each generation in order to increase the likelihood of the resulting trees. Thus, none of the parameters were fixed *a priori*. In BI analyses, two independent runs (with one cold and three heated chains each) were performed along with 5 million generations. Trees were sampled every 100 generations and the first 12,500 samples were discarded as ‘burn-in’. The average standard deviation of split frequencies approached to zero being around 0.01 at the end of the analysis. The posterior probability values (ppv) indicate the robustness of the nodes in the BI. In our phylogenetic analyses the lark-like bunting, *Emberiza impetuani* (family *Fringillidae*, subfamily *Emberizinae*, tribe *Emberizini*), was used as an outgroup (see Table 1).

RESULTS

The phylogenetic analyses using ML and BI methods converged on a congruent topology (Figs. 1, 2). In general, we found several monophyletic clades, namely, crossbills (genus *Loxia*; ML bootstrap=100, BI ppv=1.00) and bullfinches (genus *Pyrrhula*; ML bootstrap=83, BI ppv=1.00).

Rosefinches (genus *Carpodacus*) form a polyphyletic clade, as it was already shown by Arnaiz-Villena *et al.* [11]. The species of the genera *Carduelis* and *Serinus*, however, are clustered in several paraphyletic groups as described in a previous study [17]. On the other hand, some monotypic genera group with another different genus: *Pinicola enucleator* (pine grosbeak) forms a clade with genus *Pyrrhula* (ML bootstrap=81, BI ppv=0.91), *Uragus sibiricus* (long-tailed rosefinch) goes with *Carpodacus rubicilloides* (ML bootstrap=30, BI ppv=0.92) and *Haematospiza sipahi* (scarlet finch) is clustered together with *Carpodacus erythrurus* (ML bootstrap=49, BI ppv=0.97); this is concordant with previous results [11]. Also, *Rhodopechys obsoleta* is grouped with the greenfinches (*Carduelis*), splitting genus *Rhodopechys*, as expected [12]. Citril finch (*Serinus citrinella*) groups with the European goldfinch (*Carduelis carduelis*), which is congruent with previous findings [17, 26]. Finally, a phylogenetic group consistently contained two species of the genus *Rhodopechys* (*R. mongolica* and *R. githaginea*), two subspecies of *Leucosticte arctoa* (*L. a. arcota* and *L. a. tephrocotis*) and the dark-breasted rosefinch (*Carpodacus nipalensis*). This group is supported by a bootstrap value of 60 in the ML tree (Fig. 1) and a posterior probability value of 1.00 in the Bayesian tree (Fig. 2). This new group might represent a Carduelini group of ‘arid-zone’ birds. Note that *Rhodopechys sanguineus*, *Leucosticte brandti*, and *Leucosticte nemoricola* may probably belong to this group. Samples and further analyzes are necessary to assess this hypothesis.

Considering the maximum likelihood genetic divergences and the corresponding cladogenesis timing among the taxa analysed (see Fig. 1 footnote), the genera *Carduelis* and *Serinus* diverged recently and simultaneously. Moreover, rosefinches diverged earlier; and the bullfinches (*Pyrrhula*) as well as the ‘arid-zone’ finches diverged even earlier at the base of the tree (Fig. 1).

DISCUSSION

Phylogenetic Relationships

Our aim in the present work was to find the phylogenetic position of some Carduelini species that have been left out from ‘their own’ predicted genera in previous analyses, e.g. *Rhodopechys mogolica*, *Rhodopechys githaginea* and *Carpodacus nipalensis* [11, 12], and to test whether they group together with other ‘extreme environment’ Carduelini finches like *Leucosticte* species. The new ‘arid-zone’ group was supported by both ML and BI analyses (Figs. 1, 2) (the same results were found with neighbor-joining and maximum parsimony analyses, data not shown). Our results, particularly the Bayesian analysis (Fig. 2), confirm previous phylogenetic finding on the tribe Carduelini [11, 15-17]. These phylogenetic analyses had not been done under Bayesian premises until now.

Biogeographical and Evolutionary Pattern Hypotheses

The ML tree was linearized in order to obtain a rough estimate of phylogenetic divergences and the age of different lineages among Carduelini. A molecular clock model was applied to the *cytochrome b* gene using two different estimations of divergence time: one based on galliform birds [11, 15, 16] and another based on Hawaiian honeycreepers [12,

Table 1. Origin of Samples and *Cytochrome b* GenBank Accession Numbers of the Species Analyzed in this Study

Scientific Name	Common Name	GenBank	Origin
<i>Carduelis ambigua</i>	Black-headed greenfinch	U78322	Szechwan, China
<i>Carduelis atriceps</i>	Black-capped siskin	AF342863	Quetzaltenango, Guatemala
<i>Carduelis carduelis caniceps</i>	European goldfinch	L76388	Katmandu, Nepal
<i>Carduelis carduelis parva</i>	European goldfinch	L76387	Madrid, Spain
<i>Carduelis chloris</i>	Greenfinch	L76297	Madrid, Spain
<i>Carduelis dominicensis</i>	Antillean siskin	AF342864	Constanza, Dominican Rep.
<i>Carduelis flammea</i>	Common redpoll	L76386	Brussels, Belgium
<i>Carduelis hornemanni</i>	Artic redpoll	U83201	Antwerp, Belgium
<i>Carduelis lawrencei</i>	Lawrence’s goldfinch	L76392	San Diego (CA), USA
<i>Carduelis notate</i>	Black-headed siskin	U79019	Chiapas, Mexico
<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, Venezuela
<i>Carduelis psaltria hesperophila</i>	Dark-backed goldfinch	L76390	Sacramento (CA), USA
<i>Carduelis sinica</i>	Grey-capped greenfinch	L76592	Szechwan, China
<i>Carduelis spinoides</i>	Yellow-breasted greenfinch	U79018	Katmandu, Nepal
<i>Carduelis spinus</i>	Eurasian siskin	L76391	Madrid, Spain
<i>Carduelis tristis</i>	American goldfinch	U79022	San Francisco (CA), USA
<i>Carpodacus erythrinus</i>	Common rosefinch	AF342883	Islamabad, Pakistan
<i>Carpodacus nipalensis</i>	Dark-breasted rosefinch	AF342866	Katmandu, Nepal
<i>Carpodacus roseus</i>	Palla’s rosefinch	AF342867	Beijing, China
<i>Carpodacus rubicilloides</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>Emberiza impetuani</i> (OUTGROUP)	Lark-like bunting	L77902	Bostwana
<i>Haematospiza sipahi</i>	Scarlet finch	AF342875	Katmandu, Nepal
<i>Leucosticte arctoa arctoa</i>	Asian rosy-finch	DQ257460	Ulaanbaatar, Mongolia
<i>Leucosticte arctoa tephrocotis</i>	American rosy-finch	AY156385	Pribilof Islands (AK), USA
<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</i>	Two-barred crossbill	AF342878	Siberia, Russia
<i>Pinicola enucleator</i>	Pine grosbeak	AF342882	Novorsibirsk, Russia
<i>Pyrrhula erythaca</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 grisseiventris</i>	Comon bullfinch	AF342881	Beijing, China
<i>Pyrrhula pyrrhula iberiae</i>	Comon bullfinch	AF342885	Santander, Spain
<i>Rhodopechys githaginea</i>	Trumpeter finch	AF342887	Gran Canaria Island, Spain
<i>Rhodopechys mongolica</i>	Mongolian finch	AF342888	Gilgit, Pakistan

(Table 1) contd....

Scientific Name	Common Name	GenBank	Origin
<i>Rhodopechys obsoleta</i>	Desert finch	AF342889	Kabul, Afghanistan
<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</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</i>	Yellow canary	L76280	Capetown, South Africa
<i>Serinus gularis</i>	Streaky-headed seedeater	L77556	Capetown, South Africa
<i>Serinus leucopygius</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 totta</i>	Cape serin	AY570548	Cape Town, South Africa
<i>Uragus sibiricus</i>	Long-tailed rosefinch	AF365877	Beijing, China

The 'Arid-Zone' Finches are in Bold

17]. Both estimations of divergence timing did not exhibit significant differences, but they are only approximations.

The Miocene Epoch is characterized by an initial cold peak (Mi-1) followed by a general Earth warming; the tundra was replaced by conifer woods in the corresponding areas between 17 and 14.5 million years ago (MYA) [27] when the temperatures were 6°C higher than at present times [27]. However, after 14.5 MYA the temperatures started to drop and finally the Antarctica and Greenland became glaciated around 6 MYA. Simultaneously with this general cooling, vast arid regions appeared in Asia and Africa [27]. In addition, the Tibet plateau underwent its most important uplift during this cold Miocene period [27, 28]. The heavy rain regime in the high Tibet-Himalayan peaks gave rise to the birth of deep and plentiful rivers (Ganges, Bramaputra, Yangtze and Mekong) which carry 25% of the total suspension materials that reach the oceans on Earth.

The ancestors of the new group of 'arid-zone' Carduelini might have appeared 13.5 MYA. This is roughly coincidental with the appearance of vast arid areas in Africa and Asia. This radiation might have started around this time in Asia (or less likely in Africa) from where some of the lineages later might have undergone dispersal during warmer conditions.

This may be the case of *Leucosticte arctoa* now thriving both in Asia and America.

The 'arid-zone' finches' plumage colours are not homogeneous and some *Leucosticte* and *Carpodacus* finches bear more melanine pigment. This may suggest a more recent and disparate change in the 'arid-zone' finches' environments. More humid habitats (i.e., those of *Leucosticte* and *Carpodacus nipalensis* in comparison with *Rhodopechys* habitats) favour melanine dyed feathers, because it protects plumage from bacterial degradation by humidity [29]. Carotenoids, the other major pigments responsible for plumage colouration [30], are scarce in dry areas, but *Carpodacus nipalensis* also feeds on berries at high altitudes [13] and this may cause the intense male purple face colouring at breeding. Also, *C. nipalensis* has the darkest plumage colours among rosefinches [13], resembling *Leucosticte* finches in this respect.

In summary, according to our phylogenetic analyses based on mitochondrial cytochrome b DNA sequences, the genera *Rhodopechys* and *Carpodacus* should be revised because this 'arid-zone' separate evolutionary group comprises species belonging to both of the genera. Further analyses using nuclear markers could be required to complete the

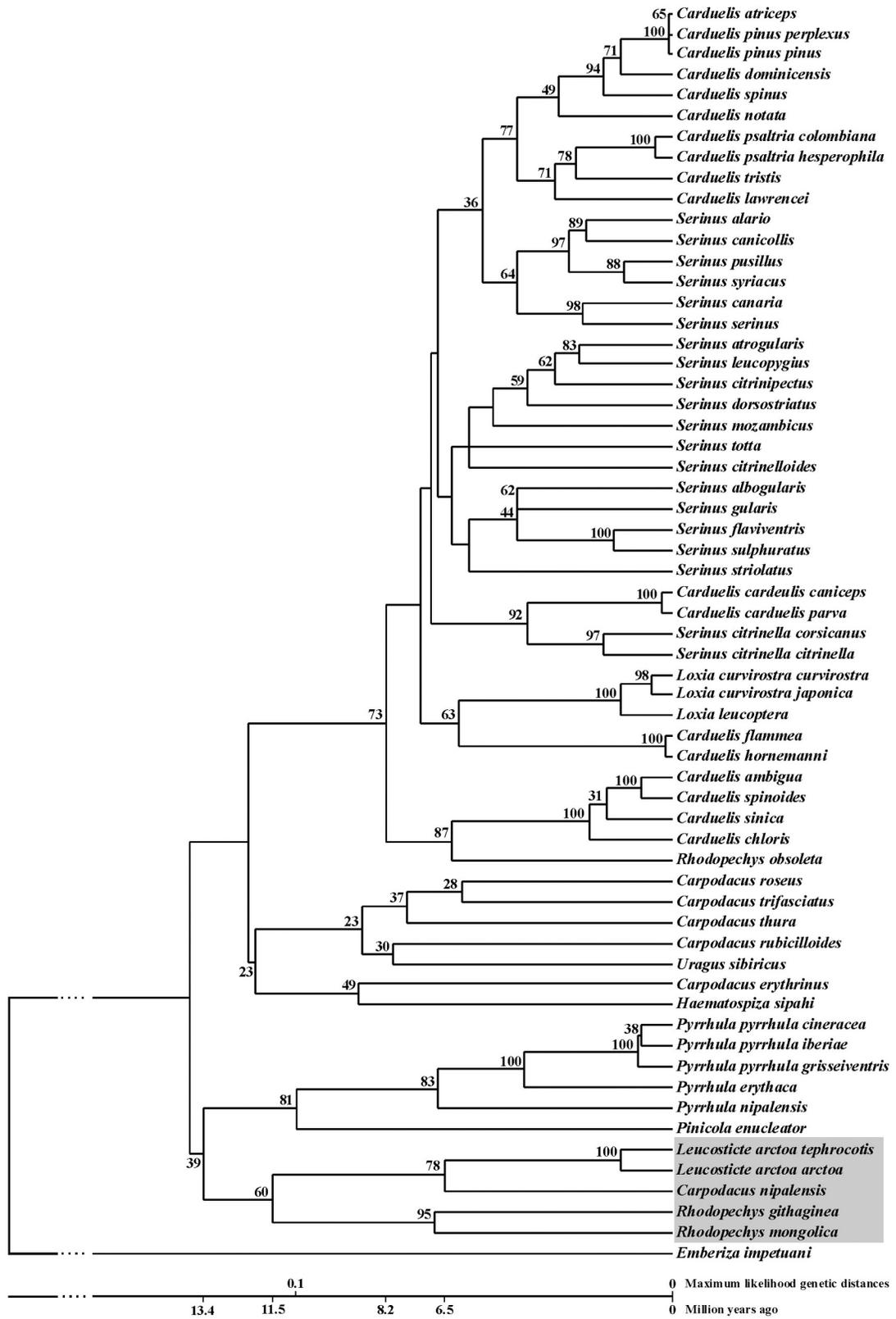


Fig. (1). Maximum likelihood (ML) linearized tree based on 924 bp mitochondrial *cytochrome b* DNA sequences ($\ln L = 8612.08$). The general time reversible model $GTR + I = 0.5788 + G = 1.2061$ was used as *a priori* settings in the analysis (base frequencies: A=0.29280, C=0.41180, G=0.13990, T=0.15550; rates of nucleotide substitution: AC=0.848200, AG=6.167300, AT=2.208400, CG=0.175600, CT=21.150900, GT=1.0000). Thorne's model [25] was used to estimate branch lengths. Bootstrap values are indicated for each node. The time scale indicates approximate the divergence times according to previous results [11, 15, 16, 31]. 'Arid-zone' finches are highlighted in grey.

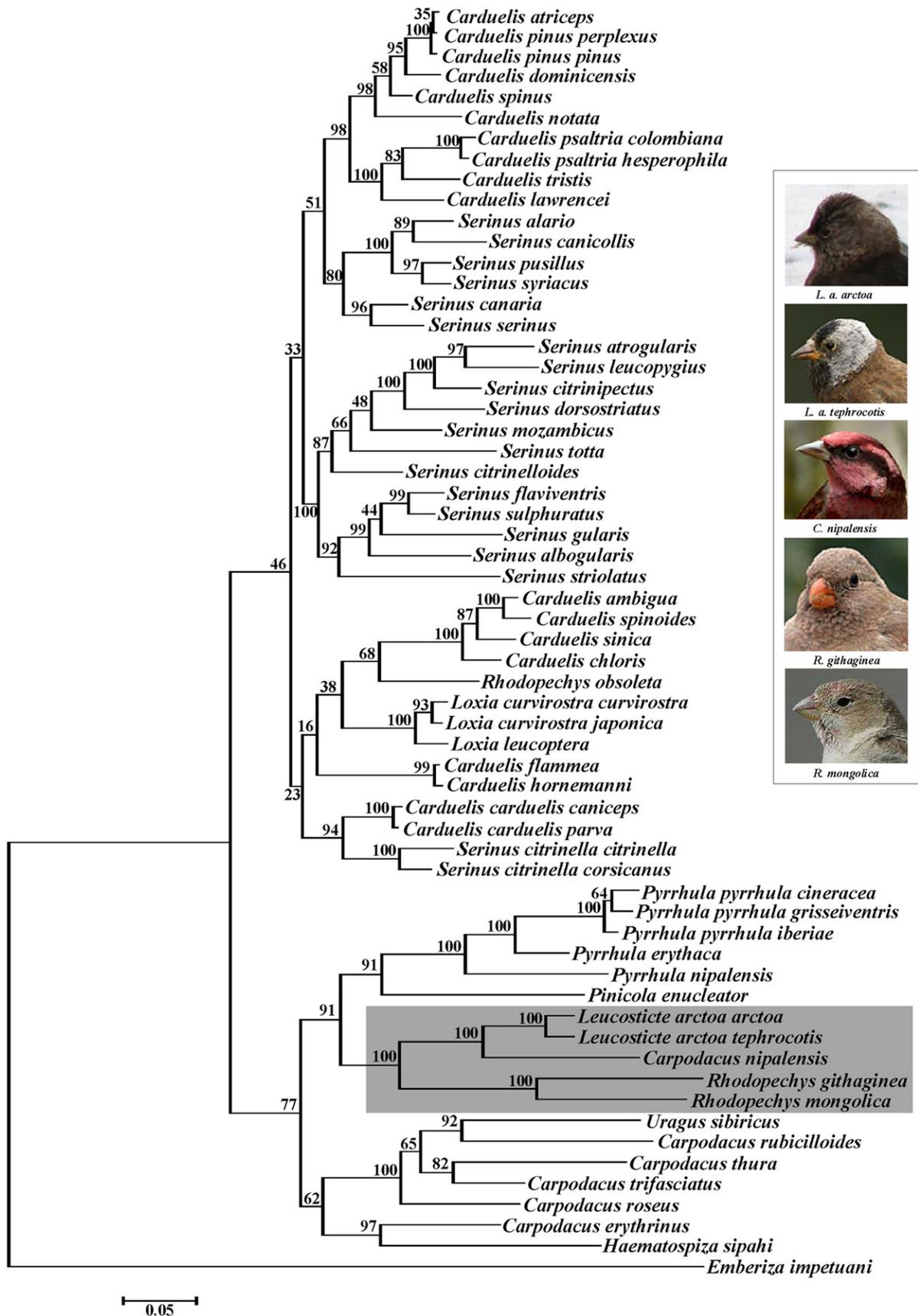


Fig. (2). Bayesian phylogenetic tree based on 924 bp of the mitochondrial *cytochrome b* DNA sequences. The model of evolution used was GTR + I + G. 5,000,000 generations were run, the sampling of the trees every 100 generations and the first 12,500 samples were discarded as 'burn-in'. The posterior probability values (x100) are indicated for each node. 'Arid-zone' finches are highlighted in grey along with their corresponding photographs.

phylogenetic findings presented in this work, and a taxonomical revision of these genera is needed since only monophyletic clades should be used in a biological systematics, not only with the species studied here [1, 11].

CONCLUSIONS

A long lasting microgeographical study of *Fringillinae* birds by using mitochondrial DNA phylogenies has allowed defining a group of monophyletic *Carpodacus/Rhodopechys/Leucosticte* *Carduelini* birds, all of which thrive in arid areas. This 'arid-zone' group is clearly separated from other *Carduelini* radiations according to maximum likelihood and Bayesian methodologies.

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ABBREVIATIONS

- BI = Bayesian inference
- bp = Base pairs
- cyt-b = Cytochrome b
- ML = Maximum likelihood
- MYA = Million years ago
- ppv = Posterior probability value

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