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Carduelini New Sistematics: Crimson-winged Finch (*Rhodopechys sanguineus*) is Included in "Arid-Zone" *Carduelini* Finches by Mitochondrial DNA Phylogeny

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Abstract: *Rhodopechys sangineus* phylogeny together with a group of *Carduelini* finches has been analyzed. Mitochondrial cyt b molecule has been used for species comparison and maximum likelihood and Bayesian methods have been employed in order to obtain a solid phylogeny. Compared *Carduelini* finches groups include: Greenfinches, "Arid-Zone" finches and Genera *Rhodopechys* and *Pyrrhula* species. Our results lead to conclude: 1) Genus *Rhodopechys* included species should need a new taxonomic classification; 2) Genus *Pyrrhula* shares a common ancestor with "Arid-Zone" finches group; the latter is phylogenetically a separate clade, including species from *Carpodacus, Rhodopechys* and *Leucosticte* Genera, and 3) *Pinicola enucleator* belongs to Genus *Pyrrhula* and seems to be ancestral. Results show that a sistematics revision of *Carduelini* tribe bird species is required.

Keywords: "Arid-Zone" finches, *Carduelini*, *Carpodacus*, Greenfinches, *Pinicola*, *Pyrrhula*, *Rhodopechys*.

INTRODUCTION

Songbirds evolution (*Aves*, *Passeriformes*, *Passeri*) have frequently been studied. Their phenotypic and molecular evolutions are not always concordant for all cases [1, 2]. Thus, the similarities in morphology and behavior may originate shared features among genetically unrelated species occurring in similar environments; conversely, different features may be present among genetic sister taxa thriving under different environments. This phenotypic plasticity has already been described for other bird species [3, 4]. Thus, some genetic and/or phenotypic traits may not correlate with the evolutionary histories of the birds. On the other hand, mitochondrial cytochrome b gene (cyt-b) sequencing has been widely used for molecular systematics studies. Cyt-b gen has been proved to be helpful for defining evolutionary relationships among distant or closely related taxa [5, 6].

Crimson-winged Finch has the widest distribution among that of *Rhodopechys* /"Arid-Zone" finches [7, 8]. It thrives throughout deserts/ semi-desert marginal areas in North Africa (Sahara), Arabian Peninsula and Central Asia deserts [7] Fig. (**1**).

Rhodopechys sanguineus thrives discontinuously across all desert strip margins and mountains that go from Africa to China. Its presence in Atlas Mountains may be due to winter migration, but it has been recorded in breeding season [7]. It seems that this species is mostly an altitudinal migrant although in China it moves from breeding area in East China range to North Central China in winter. It is found in Lebanon, Mount Hermon (Israel) and probably Syria; strikingly it is also found in Caucasus Mountains, North Ossetia and Kashmir-Indian area (Chitral and Ladakh) [7].

Birds included within the *Carduelini* tribe (Genera *Rhodopechys*, *Carpodacus* and *Leucosticte*) have been shown to belong to the same evolutionary radiation by using molecular phylogenetic analyses [8]. Our phylogenetic analyses based cytochrome b gene (cyt-b) nucleotide sequences show that some of these birds (*Rhodopechys mongolica*, *Rhodopechys githaginea* and *Carpodacus nipalensis*) do not go in dendrograms with their respective phenetically defined allies and/or classical Genus classification [7]. This newly defined group of birds lives in both hot and cold ***arid areas*** and are phenetically distinct, probably because of their adaptation to diverse extreme environments. Both maximum likelihood and Bayesian inference dendrograms support the existence of this new group which seems to have appeared about 14 million years ago [8].

In the present work, we study the phylogeny of Crimson-winged Finch (*Rhodopechys sanguineus*) in order to complete the split family and new classification of *Rhodopechys* and "Arid-Zone" finches included species [8, 9, 10]. Our suggested taxonomic revisions are detailed at the end of Discussion section.

MATERIALS AND METHODS

Bird Samples, DNA Extraction and PCR Amplifications

Sixty two species of songbirds (order *Passeriformes*, suborder *Passeri*) have been included for study (see Table **1**). They belong to the tribe *Carduelini*. *Rhodopechys sanguineus* was DNA-sequenced (four differents birds) and analyzed in the present work together with relevant *Carduelini* species. Other sequences used in this analysis were retrieved from the GenBank (see Table **1**) [8, 9, 11-13]. DNA extraction was done using a standard protocol [14]. Amplification and sequencing of cyt b gene 924 base pairs (bp) was performed as previously described [9].

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

Scientific Name	Common Name	GenBank	Origin
Carduelis ambigua	Black-headed greenfinch	U78322	Szechwan, China
Carduelis atriceps	Black-capped siskin	AF342863	Quetzaltenango, Guatemala
Carduelis carduelis caniceps	European goldfinch	L76388	Katmandu, Nepal
Carduelis carduelis parva	European goldfinch	L76387	Madrid, Spain
Carduelis chloris	Greenfinch	L76297	Madrid, Spain
Carduelis dominicensis	Antillean siskin	AF342864	Constanza, Dominican Rep.
Carduelis flammea	Common redpoll	L76386	Brussels, Belgium
Carduelis hornemanni	Artic redpoll	U83201	Antwerp, Belgium
Carduelis lawrencei	Lawrence´s goldfinch	L76392	San Diego (CA), USA
Carduelis notata	Black-headed siskin	U79019	Chiapas, Mexico
Carduelis pinus pinus	Pine siskin	U79020	Jackson (WY), USA
Carduelis pinus perplexus	Pine siskin	DQ246804	Quetzaltenango, Guatemala
Carduelis psaltria colombiana	Dark-backed goldfinch	U78324	Maracay, Venezuela
Carduelis psaltria hesperophilus	Dark-backed goldfinch	L76390	Sacramento (CA), USA
Carduelis sinica	Grey-capped greenfinch	L76592	Szechwan, China
Carduelis spinoides	Yellow-breasted greenfinch	U79018	Katmandu, Nepal
Carduelis spinus	Eurasian siskin	L76391	Madrid, Spain
Carduelis tristis	American goldfinch	U79022	San Francisco (CA), USA
Carpodacus erythrinus	Common rosefinch	AF342883	Islamabad, Pakistan
Carpodacus nipalensis	**Dark-breasted rosefinch**	**AF342866**	**Katmandu, Nepal**
Carpodacus roseus	Palla’s rosefinch	AF342867	Beijing, China
Carpodacus rubicilloides	Streaked rosefinch	AF342868	Katmandu, Nepal
Carpodacus thura	White-browed rosefinch	AF342869	Katmandu, Nepal
Carpodacus trifasciatus	Three-banded rosefinch	AF342870	Szechwan, China
Fringilla coelebs	Chaffinch	L76609	Madrid, Spain
Haematospiza sipahi	Scarlet finch	AF342875	Katmandu, Nepal
Leucosticte arctoa arctoa	**Asian rosy-finch**	**DQ257460**	**Ulaanbaatar, Mongolia**
Leucosticte arctoa tephrocotis	**American rosy-finch**	**AY156385**	**Pribilof Islands (AK), USA**
Loxia curvirostra curvirostra	Common crossbill	AF342876	Alcala de Henares, Spain
Loxia curvirostra japonica	Common crossbill	AF342877	Beijing, China
Loxia leucoptera	Two-barred crossbill	AF342878	Siberia, Russia
Pinicola enucleator	Pine grosbeak	AF342882	Novorsibirsk, Russia
Pyrrhula erythaca	Beavan´s bullfinch	AF342862	Beijing, China
Pyrrhula nipalensis	Brown bullfinch	AF342884	Katmandu, Nepal
Pyrrhula pyrrhula cineracea	Common Bullfinch	AF342886	Novasibirsk, Russia
Pyrrhula pyrrhula griseiventris	Common Bullfinch	AF342881	Beijing, China
Pyrrhula pyrrhula iberiae	Common Bullfinch	AF342885	Santander, Spain

(Table 1) contd….

Scientific Name	Common Name	GenBank	Origin
Rhodopechys githaginea	**Trumpeter finch**	**AF342887**	**Gran Canaria Island, Spain**
Rhodopechys mongolica	**Mongolian finch**	**AF342888**	**Gilgit, Pakistan**
Rhodopechys obsoleta	Desert finch	AF342889	Kabul, Afghanistan
Rhodopechys sanguineus	**Crimson-winged Finch**	**KJ608059**	**Mount Hermon, Israel**
Serinus alario	Black-headed canary	L76276	Capetown, South Africa
Serinus albogularis	Whited-throated canary	L78705	Capetown, South Africa
Serinus atrogularis	Yellow-rumped seedeater	L76267	Capetown, South Africa
Serinus canaria	Island canary	L76266	Canary Islands, Spain
Serinus canicollis	Yellow-crowned canary	L78706	Capetown, South Africa
Serinus citrinella citrinella	Citril finch	L77872	Madrid, Spain
Serinus citrinella corsicanus	Citril finch	AY583725	Sardinia, Italy
Serinus citrinelloides	African citril	L77555	Nairobi, Kenya
Serinus citrinipectus	Lemon-breasted canary	L78707	Maputo, Mozambique
Serinus dorsostriatus	White-bellied canary	L76278	Dar es Salam, Tanzania
Serinus flaviventris	Yellow canary	L76280	Capetown, South Africa
Serinus gularis	Streaky-headed seedeater	L77556	Capetown, South Africa
Serinus leucopygius	White-rumped seedeater	L76264	Dakar, Senegal
Serinus mozambicus	Yellow-fronted canary	L76265	Dar es Salam, Tanzania
Serinus pusillus	Red-fronted serin	L77873	Sin Wiang, China
Serinus serinus	European serin	L76263	Madrid, Spain
Serinus striolatus	Streaky seedeater	L77557	Nairobi, Kenya
Serinus sulphuratus	Brimstone canary	L76294	Capetown, South Africa
Serinus syriacus	Syrian serin	AY570547	Mount Hermon, Israel
Serinus totta	Cape serin	AY570548	Cape Town, South Africa
Uragus sibiricus	Long-tailed rosefinch	AF365877	Beijing, China

Phylogenetic Analyses

Sequence was analyzed for stop codons with MEGA v. 3.1 as described [15]. Phylogenetic dendrograms were obtained using maximum likelihood (ML) [16] in PAUP* v. 4.0b10 [17] and Bayesian (BI) [18, 19]. Model test v. 3.7 [20] was used to find out a DNA substitution model that fits the data best. Best model was then used in both the ML and BI analyses. Linearized ML dendrograms were obtained with PAUP* v. 4.0b10 [17] with the estimated branch length according [21] which assumes that the rates among the evolutionary lineages may not be constant. Tree calculation strategy consisted of a heuristic search with NNI (Nearing-Neighbor Interchange) swapping algorithm. Robustness of nodes was assessed by 1000 bootstrap replicates in the ML analyses. The parameters rates defining the model of evolution were allowed to change in the BI analysis after each generation in order to increase the likelihood of resulting trees. Therefore, none of the parameters were a priori fixed. 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’. Split frequencies average standard deviation approached to zero being around 0.01 at the end of the analysis. Posterior probability values (ppv) indicate the robustness of the nodes in the BI. Phylogenetic analyses the lark-like bunting, and chaffinch, *Fringilla coelebs* (family *Fringillidae*, subfamily *Fringillinae*), was used as an outgroup (Table **1**).

RESULTS

Eurasian thriving range of *Rhodopechys* and *Leucosticte* relevant species are shown in Fig. (**1**).

Phylogenetic analyses using ML and BI methodology gave on a congruent topology (Figs. **2**, **3**). Several monophyletic clades were found, i.e.: crossbills (Genus *Loxia*; ML bootstrap=100 (Fig. **2**), BI ppv=1.00 (Fig. **3**)) and bullfinches

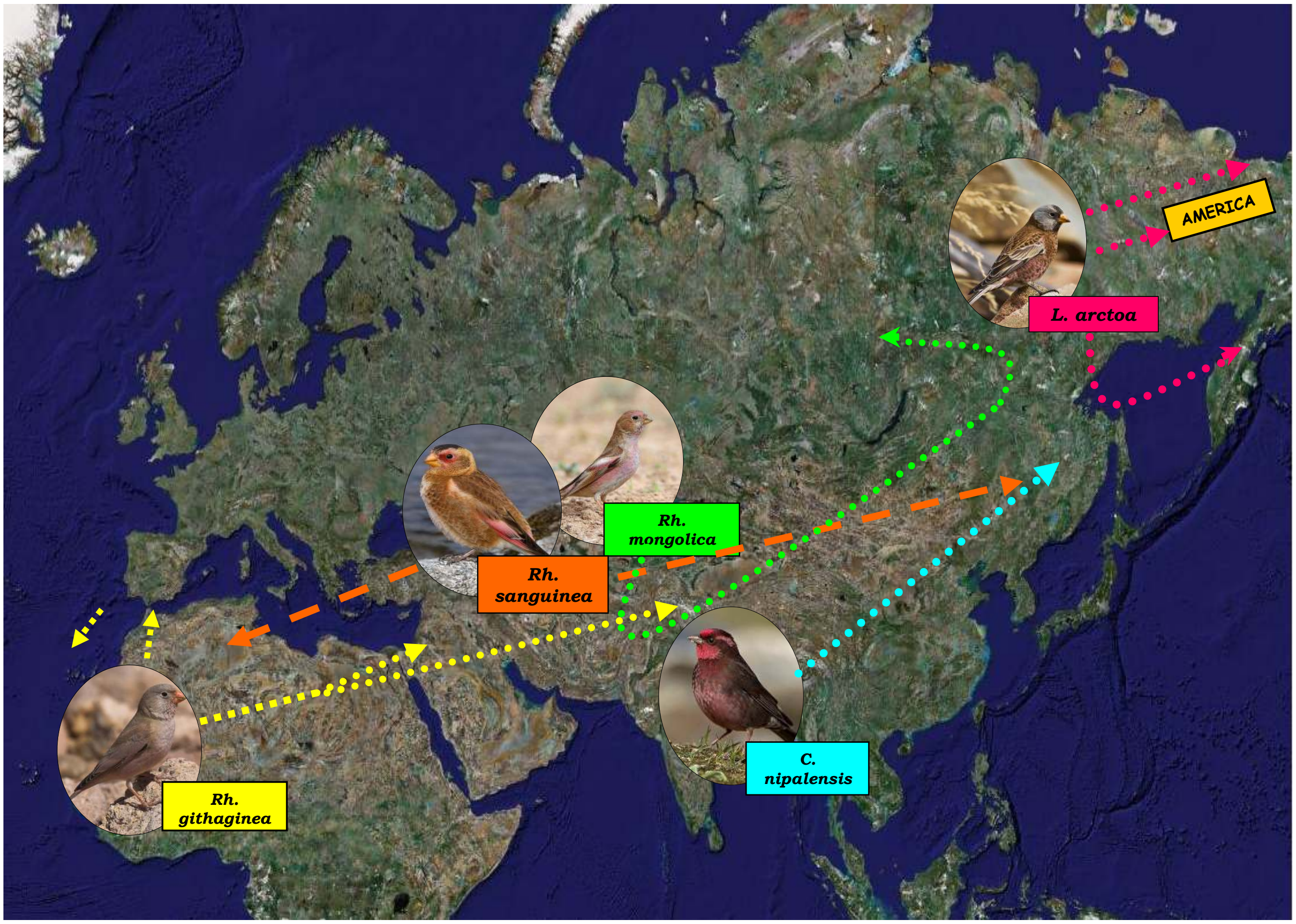

Fig. (1). Geographic distribution and photographs of present paper studied species. ***Leucosticte arctoa*** (red) thrives from Central Asia (Altai) to eastern Siberia, Japan, Alaska to western Canada and western USA; ***Rhodopechys sanguineus*** (orange) thrives in Morocco, central Turkey and Middle East to northwest China mountains; ***Rhodopechys githaginea*** (yellow) thrives *in* Canary Islans North África, Middle East to Central Asia and northern mountain; ***Rhodopechys mongolica*** (green) is distributed in Eastern Turkey to Central Asia, Kashmir, western and northern China; ***Carpodacus nipalensis*** (blue) inhabit in Himalayas from northern India to southern Tibet and western China [7].

(Genus *Pyrrhula*; ML bootstrap=83 (Fig. **2**), BI ppv=1.00 (Fig. **3**)). Rosefinches (Genus *Carpodacus*) form a polyphyletic clade, as it was already shown [11]. Genera *Carduelis* and *Serinus* species, however, are clustered in several paraphyletic groups as already described [13]. Also, some monotypic Genera group with another different Genus: *Pinicola enucleator* (pine grosbeak) forms a clade with Genus *Pyrrhula* (ML bootstrap=81 (Fig. **2**), BI ppv=0.91 (Fig. **3**)), *Uragus sibiricus* (long-tailed rosefinch) goes with *Carpodacus rubicilloides* (ML bootstrap= 30 (Fig. **2**), BI ppv=0.92 (Fig. **3**)) and *Haematospiza sipahi* (scarlet finch) is clustered together with *Carpodacus erythrinus* (ML bootstrap=49 (Fig. **2**), BI ppv=0.97 (Fig. **3**)); this is expected from our previous work [11]. *R. obsoleta* is grouped with Greenfinches (*Carduelis*), splitting Genus *Rhodopechys*, as expected [9]. Citril finch (*Carduelis citrinella*) groups with the European goldfinch (*Carduelis carduelis*), which is congruent with previous reports [9, 22]. A phylogenetic group consistently contained two species of 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 has a bootstrap value of 60 in the ML dendrogram (Fig. **2**) 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. We expected that *Rhodopehcys sanguineus*, *Leucosticte brandti*, and *Leucosticte nemoricola* may probably belong to this group [7]. Samples and further analyses are necessary to assess this hypothesis.

However, it is clear from present paper results that *Rhodopechys sanguineus* is in fact included in within "Arid-Zone" finches group (Fig. **2**, **3**), probably being one of the most ancient extant bird of these species. Considering the linearized maximum likelihood genetic timing and the corresponding cladogenesis divergence among the taxa analyzed (see Fig. **2** footnote), Genera *Serinus* and *Carduelis* diverged more recently and probably in a simultaneous fashion. Rosefinches seem to have diverged earlier; and the bullfinches (*Pyrrhula*) as well as the "Arid-Zone" finches diverged even earlier at the base of the tree (Fig. **2**).

Bayesian phylogenetic trees are fully concordant with our maximum likelihood results (Figs. **2**, **3**; not shown).

Fig. (2). *Carduelinae* species dendrogram shows that Genus *Rhodopechys* (including *Rhodopechys sanguineus*) may be included into "Arid-Zone" finches Genus or Super Genus together with *Leucosticte* and *Carpodacus nipalensis* finches [8]. Greenfinches include *Rhodopechys obsoleta* (Desert Finch), which is the Greenfinches ancestor in dendrogram [9]. Newly studied in this paper *Rhodopechys sanguineus* is ancestral in *Rhodopechys* Genus. Genus *Pyrrhula* seems to share a common ancestor with "Arid-Zone" finches clade. 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, [21] 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 [21] 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, 12, 27, 28]. "Arid-Zone" finches are highlighted in red.

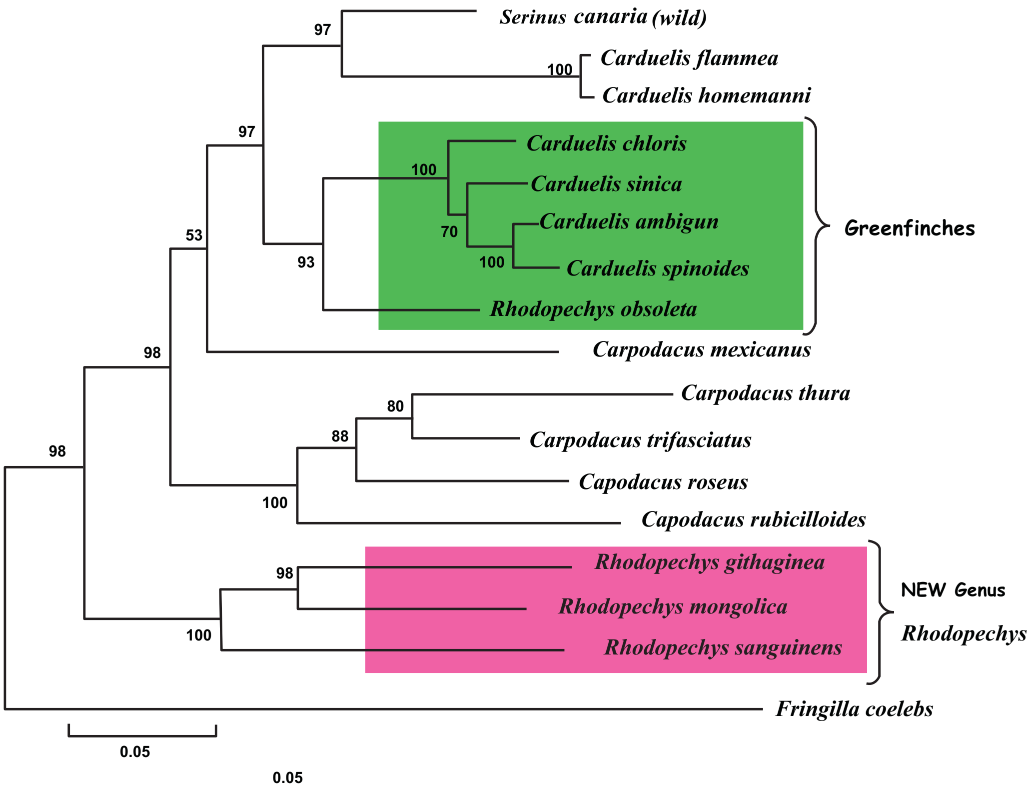

Fig. (3). Greenfinches Genus includes *Rhodopechys obsoleta*. *Rhodopechys* Genus has "missed" Desert Finch, which is now a Greenfinch. *Rhodopechys sanguineus* is the phylogenetic ancestor of Genus *Rhodopechys.* Linearized Bayesian Dendrogram based on cytochrome b DNA sequences [8, 9].

DISCUSSION

Some topics and questions arise from our results.

Miocene Climate Change

The Miocene Epoch had a initial cold phase (Mi-1) followed by a general Earth warming; tundra was substituted by conifer woods in the corresponding areas between 17 and 14.5 million years ago (MYA) [23]; temperatures by then were 6ºC higher than at present [23]. However, after about 10 MYA temperatures dropped and finally Antarctica and Greenland became glaciated about 6 MYA. Together with this general cooling, vast arid regions were established in Asia and Africa [23]. In addition, Tibet plateau underwent its higher uplift during this cold Miocene period [23, 24]. Then a heavy rain regime in the high Tibetan-Himalayan Mountains occurred that gave birth to the deep and big rivers (Ganges, Bramaputra, Yangtze and Mekong), which carry 25% of the total suspension materials that reach Oceans on Earth. Ancestors of "Arid-Zone" *Carduelini* finches might have appeared 13.5 MYA together with Genus *Pyrrhula* (Bullfinches) witch seems a sister Genera which includes *Pinicola enucleator*. Bullfinches would have occupied a more humid and full of vegetation habitat than "Arid-Zone" finches (Table **1**) [7].

Rhodopechys Sanguineus and Genus *Rhodopechys* Split

Rhodopechys sanguineus has been included within "Arid-Zone" finches together with Genus *Leucosticte* and *Carpodacus nipalensis*. They all share one phenotypic characteristic: a different degree of reddish-pink plumage. Also, all of them thrive in extreme climates over the World; habitats are correspondingly desert margins or mountains with scarce vegetation. *Rhodopechys sanguineus* seems to be ancestral to *Rhodopechys githaginea* (Trumpeter Finch) and *Rhodopechys mongolica* (Mongolian Finch); It appeared on Earth about 10 million years ago according to our linearized ML and Bayesian dendrograms results (Figs. **2** and **3**).

In addition, this *Rhodopechys* group is separated from *Rhodopechys obsoleta* (Desert Finch), which is a pale colour Greenfinch and their extant ancestor (Figs. **2**, **3**) [8, 10].

Tree placement and origin timing suggest that *Rhodopechys sanguineus* is an ancestral species to *Rhodopechys mongolica* and *Rhodopechys githaginea* (Figs. **2**, **3**).

Genus *Pyrrhula* and "Arid-Zone" Finches

Ancestors of the novel group of "Arid Zone" *Carduelini* finches might have appeared 13.5 MYA together with Genus *Pyrrhula* (Bullfinches) (Fig. **2**) which seems a sister Genus. Bullfinches would have occupied a more humid and full of vegetation habitat than most "Arid-Zone" finches (Table **1**) [7]; see below, more coloured *Leucosticte* finches and *C. nipalensis*. Also, all *Pyrrhula* species show a Paleartic distribution, including their extant ancestor *Pinicola enucleator* (Pine Grosbeak), which should be included within Genus *Pyrrhula* [11].

Pine Grosbeak (*P. enucleator*) seems to be the one *Pyrrhula* species found in North America in addition to show a Holoartic distribution it seems to be Genus *Pyrrhula* ancestor [11] (Figs. **2**, **3**) [7]. Coloured Bullfinch species are concordant with its wood bud-containing inhabitat.

This phylogeography information is roughly coincidental with the appearance of wide arid areas in Africa and Asia. "Arid-Zone" finches evolutionary radiation might have started around this time most likely in Asia (or in Africa) from where some of the lineages might have undergone dispersal during warmer conditions. The most ancient extant ancestor is *Rhodopechys sanguineus* (Fig. **2**, **3**). The "Arid-Zone" finches feather colours are not homogeneous and some *Leucosticte* and *Carpodacus* finches included in the group tend to bear more melanine pigment. This may suggest a more recent and separate change in the "Arid-Zone" finches environments. Habitats which are more humid (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 [25]. Carotenoids, the other major pigments responsible for plumage colouration [26], are not available for bird feeding in dry areas, but *Carpodacus nipalensis* also feeds on berries at high altitudes [7] and this may enhance its intense male purple face colouring at breeding. Also, *C. nipalensis* has the darkest plumage colours among rosefinches [7], 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" finches seems to be a separate evolutionary group that comprises species belonging to both of the previously admited Genera.

Also, Genus *Pyrrhula* should be regarded as sharing its extinct (or non-analyzed) ancestor with "Arid-Zone" finches (see Fig. **2**). Further analyses using other genetic markers could be required to complete these phylogenetic findings, and a taxonomical revision of these Genera (*Rhodopechys*, *Pyrrhula* and "Arid-Zone" finches) and *Carduelini* tribe is needed since only monophyletic clades should be used in a phylogenetical systematics [1, 11].

Thus, our suggested conclusions of present and previous studies [8, 9, 11] are:

1. The polytomies newly found in our *Carduelini* finches phylogenetic dendrograms (Figs. **2**, **3**) indicate that a systematics revision of these groups is required [8, 9, 11].
2. *Rhodopechys obsoleta* Desert Finch, is a Greenfinch ancestor of the other extant species [9]. This *Rhodopechys obsoleta* is a pale colour Greenfinch.
3. "Arid-Zone" finches included (*Leucosticte arctoa tephrocotis, Leucosticte arctoa arctoa, Carpodacus nipalensis, Rhodopechys githaginea, Rhodopechys mongolica* and *Rhodopechys sanguineus*) [8, 10, 29, 30].
4. *Carpodacus erythrinus* and *Haematospiza sipahi* form a single phylogenetic clade [8, 9, 11, 29, 30]
5. Grosbeaks: *Mycerobas* and *Eophona* Genera should also be reclassified [11, 29, 30].
6. Genus *Loxia* (Crossbills) are both placed as monophyletic and close to *Carduelis flammea* and *hornemanni* [11, 12, 29, 30]
7. *Pinicola enucleator* should be classified together with Bullfinches (*Pyrrhula* species). *Pinicola* seems the most extant ancient species within this monophyletic group (Fig. **2**) [11, 29, 30].

ABBREVIATIONS

BI = Bayesian inference
bp = Base pairs
Cyt-b = Cytochrome b
DMSO = Dimethyl Sulfoxide
ML = Maximum likelihood
MYA = Million years ago
NNI = Nearing-Neighbour Interchange
ppv = Posterior probability values

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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PATIENT'S CONSENT

Declared none.

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