

***Estrildinae* Finches (*Aves*, *Passeriformes*) from Africa, South Asia and Australia: a Molecular Phylogeographic Study**

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Abstract: Estrildid finches are distributed throughout Africa, South Asia, Australia and neighbouring islands in the Indian and Pacific Oceans. Some specific phylogenetic and systematic debated questions have been clarified in the present study by mitochondrial cytochrome b DNA sequencing of 61 species of Estrildids and subsequent analyses of results by both Bayesian Inference and Maximum Likelihood methodologies. Our results support that Estrildids are a monophyletic group with polytomies that may have started evolving by Middle Miocene Epoch (about 16, 5 million years ago). This proposed timing is coincidental with the *Fringillinae* finches' radiation starting time and also with the biggest Himalayan and Tibetan Plateau uplift, triggered by the Indian tectonic plate strongest collision; this established present day southern Asia monsoon regime and other drastic climatic changes, like a dryer weather in Tibetan Plateau and China deserts. The Estrildid finches form a monophyletic group which includes several polytomies and comprises African, Asian and Australian birds. The most ancient evolutive group comprises African (African silverbill), Asian (Indian silverbill) and Australian (diamond firetail); this suggests that the whole Estrildids radiation might have originated around India. More Estrildid species will be studied in order to further establish this group phylogeography. In addition, monophyletic radiations include species from different Continents. Finally, *Ploceinae* Genus *Quelea* finches is a group separate and basal from Estrildini and Viduini species in our dendrograms.

Keywords: Estrildids, finches, firefinches, grassfinches, munias, mannikins, silverbills, waxbills, weavers, quelea, ploceinae.

INTRODUCTION

The Estrildids represent an old group of songbirds (order *Passeriformes*) distributed throughout Africa, South Asia and Australia, including the Indian and South Pacific Islands. They feed almost exclusively of seeds, have a small size and are very sociable and active, showing a great variety of colours and well-developed behavioural patterns [1]. They have been traditionally classified as the family *Estrildidae*, and three groups can be differentiated: the African waxbills (genera *Estrilda*, *Pytilia*, *Lagonosticta*, *Uraeginthus* and others), the Australian grassfinches (genera *Poephila*, *Neochmia* and others) [2,3] and the paleotropical mannikins (genera *Lonchura*, *Erythrura* and others). However, we have followed the classification proposed by Sibley [4], which is based on a kind of molecular data (DNA / DNA hybridation) and considers the Estrildids as a subfamily (*Estrildinae*) with two tribes (Estrildini and Viduini), close to the weavers (subfamily *Ploceinae*) and the sparrows (subfamily *Passerinae*), being all of them included within the family *Passeridae*. According to this classification, 29 genera and 158 species are included within the subfamily *Estrildinae*.

The origin and evolution of the Estrildids, and even their monophyly, is yet to be solved. The true relationship among the African, Asian Indian, and Austro-Pacific *Estrildinae* groups, the hypothetical original place of radiation (if only one existed), the inclusion of the whydahs within the Estrildids, and the relationships between the Estrildids and other groups, like the weavers and sparrows, is still debated. Also, the remarkable case of the Australian *Neochmia temporalis*, which shows a very similar phenotype to the African *Estrilda astrild rubriventris*, introduces the controversy on convergent evolution versus genetic relatedness [1]. On the other hand, the nomenclature may need be revised. Indeed, some species are identified with at least two different scientific names (*Chloebia* or *Erythrura gouldiae*, *Padda* or *Lonchura oryzivora*, *Emblema* or *Stagonopleura guttata*, *Aidemosyne* or *Neochmia modesta*, *Aegintha* or *Neochmia temporalis*, *Lonchura griseicapilla* or *Spermestes caniceps* or *Odontospiza caniceps*). Also, genera are named differently by different authors; i.e.: the African munias are designated either as *Lonchura* or *Spermestes*; and the Australian genus *Taeniopygia* is also known as *Poephila* [1,4,5]. Finally, several genera contain only one or two species (*Emblema*, *Mandingoa*, *Clytospiza*, *Amadina*, *Hypargos* or *Euschistospiza*) and the position of some of them within the Estrildids remains unclear.

Studies based on molecular data have addressed some of these problems. Phylogenetic analyses of some species of Estrildids based on the nuclear gene β -fibrinogen [6] and on the mitochondrial genes cytochrome b [6,7] and ND1, ND2,

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ND6 and other mitochondrial regions [8] have suggested the monophyly of the Estrildids [6,8], the separation between the African and Australian ones [6], and the genetic difference between the Estrildids and the sparrows (subfamily *Passerinae*) and weavers (subfamily *Ploceinae*) [7].

In the present work we have studied mitochondrial cytochrome b (mt cyt-b) DNA sequences from 61 species of Estrildids (57 from our own stock and 4 from the GenBank, see Table 1), belonging to 21 different genera, for analyzing the genetic relationships existing among them in order to determine the taxonomic position of these widespread genera and species of finches and to correlate the phylogenetic distribution obtained with the biogeographical location of the species analysed. Our aims were also to compare the relative times of divergence among the Estrildid evolutive groups, and to identify the place (or places) where the radiation of this heterogeneous and diverse group of birds might have started.

1. MATERIALS AND METHODS

1.1. Bird Samples, DNA Extraction, Amplification and Sequencing

Sixty one species of Estrildids (order *Passeriformes*, family *Passeridae*, subfamily *Estrildinae*, tribes Estrildini and Viduini) have been analysed for the first time by mitochondrial cytochrome b DNA sequencing. A list of the species studied is given in Table 1, together with the origin of the samples and the GenBank accession numbers corresponding to the cyt-b DNA sequences used. Blood samples (2-3 drops) were obtained from wild birds in their natural thriving areas, by cutting one claw (after anesthetising with a lidocaine ointment), and frozen in an EDTA solution until use. DNA extraction was performed using a standard Phenol-Chloroform methodology [9] or a commercial DNA purification kit (*QuickGene DNA Whole Blood Kit S*, FUJIFILM, Tokyo, Japan). 924 base pairs of the mt cyt-b gene were specifically amplified by polymerase chain reaction (PCR) with the primers L14841 and H15767 [10] in a *Mastercycler ep gradient S* thermal cycler (EPPENDORF, Hamburg, Germany). The final reaction volume was 20 µl, containing 200-400 ng of whole DNA and the rest of PCR reagents according to the requirements of the polymerase used (*Taq DNA polymerase, recombinant*, INVITROGEN, Carlsbad, CA, USA). The PCR conditions consisted of one denaturation cycle (95°C, 5 minutes), followed by 45 cycles of denaturation (96°C, 30 seconds), primer annealing (50°C, 35 seconds) and polymerase extension (72°C, 70 seconds) steps, and a final extension cycle (72°C, 20 min.). Amplification products were separated by electrophoresis in 2% agarose and the fragments purified with a commercial kit (*Mini Elute Gel Extraction Kit*, QUIAGEN, Hilden, Germany). The sequencing process was carried out in an Applied Biosystems 970 Automated Sequencer using the primers L14841, L15299 and H15149 [10]. At least, two independent amplifications (and further sequencing) were performed for each sample to confirm the sequences obtained.

1.2. Statistical Analyses and Time Calculation Hypothesis

DNA sequences were aligned and checked for stop codons with MEGA 3.1 computer program [11] for further

phylogenetic calculations. The final length of the sequences used was 924 nucleotides. The Blackcap, *Sylvia atricapilla* (family *Sylviidae*, subfamily *Sylvinae*, tribe *Sylvini*; GenBank accession number: DQ270389), was chosen as outgroup to root the phylogenetic dendrograms, which were constructed using Bayesian Inference (BI) [12] and Maximum Likelihood (ML) [13] methodologies. The BI dendrogram was calculated with MrBayes 3.1.2 computer program [14]. The model of nucleotide substitution used was GTR+I+G, that is, a general time reversible model (which considers six different nucleotide substitution types), implemented with the proportion of invariable sites and the gamma distribution of rates at variable sites. The default settings were left as priors for the analysis. Two independent runs (with one cold and three heated chains each) were performed and the analysis was stopped after 4 million generations, being the standard deviation of split frequencies around 0.01. The trees were sampled every 100 generations along the Markov Chain-Monte Carlo process, and the first 10,000 samples (25%) were discarded as burn-in. The posterior probability values indicate the robustness of the nodes (see Fig. 1). To perform the ML analysis, the program Modeltest 3.7 [15], together with PAUP4.0b10 [16], was used to choose the model of nucleotide substitution that best fits the data; it resulted to be a GTR+I+G model. The likelihood scores estimated for that model were used as prior settings for the ML analysis (see Fig. 2 footnote) in PAUP 4.0b10 computer program [16]. The ML tree was constructed using a heuristic search with a NNI (Neighbour-Neighbour Interchange) swapping algorithm, and linearized estimating the branch lengths according to the model proposed by Thorne [17], which allows the rates not to be constant among the evolutionary lineages. The robustness of the nodes was obtained after 1,000 bootstrap replications. In order to assign approximate divergence times to the nodes, a smaller ML linearized tree (not shown) was constructed including sixteen representative Estrildids from both of the subfamily *Estrildinae* tribes (Estrildini and Viduini) together with greenfinches (*Carduelis chloris*, *C. sinica*, *C. ambigua*, *C. spinoides*) and the Chaffinch (*Fringilla coelebs*). The outgroup *Sylvia atricapilla* was also included.

It was estimated in a previous work that the chaffinch separated from the greenfinches about 16,5 million years ago [18]. Initially, a mixed external fossil and molecular record divergence time (pheasant/chicken) was used to calibrate our dendrograms. A continuum of small songbirds speciation was found during the Miocene, Pliocene and Pleistocene Epochs [18]. Also, last glaciations may have separated western European siskins (*C. spinus*) from the Far East subspecies by an ice-induced vicariance effect [19]. A linearized Maximum Likelihood (ML) tree was constructed to infer the hypothetical evolutive radiation times of appearance on Earth [20].

2. RESULTS

DNA sequences of mt cyt-b gene from 61 different species of Estrildids have been obtained by us (except for 4 of them) and compared by using phylogenetic methodologies to analyse the genetic relationships existing among them and to study the biogeographic distribution pattern (see Table 1). BI (Fig. 1) and linearized ML (Fig. 2) dendrograms were constructed, both showing a very similar topology with several highly supported phylogenetic clusters.

Table 1. Species Studied in the Present Work. The Distribution Range is Indicated. The GenBank Accession Numbers Correspond to the Mitochondrial Cytochrome b DNA Sequences Used in the Phylogenetic Analyses

Scientific Name	Common Name	GenBank	Distribution Range
<i>Amadina erythrocephala</i>	Red-headed Finch	EU423967	Africa
<i>Amadina fasciata</i>	Cut-throat Finch	EU423946	Africa
<i>Amandava amandava</i>	Red Avadavat	EU423958	Indochina, Indonesia
<i>Amandava subflava</i>	Zebra Waxbill	EU423929	Africa
<i>Cryptospiza reichenovii</i> ¹	Red-faced Crimson-wing	AY228056	Africa
<i>Emblema pictum</i>	Painted Fairtail	EU423956	Australia
<i>Erythrura gouldiae</i>	Gouldian Finch	DQ270375	Australia
<i>Erythrura prasina</i>	Pin-tailed Parrotfinch	EU423928	Malaysia, Sumatra, Borneo
<i>Erythrura trichroa</i>	Blue-faced Parrotfinch	EU423932	New Guinea, Pacific Islands
<i>Erythrura tricolor</i>	Tricolored Parrotfinch	EU423973	Lesser Sunda Islands ²
<i>Estrilda astrild rubriventris</i>	Common Waxbill	EU423942	Africa
<i>Estrilda caeruleus</i>	Lavender Waxbill	EU423930	Africa
<i>Estrilda erythronotos</i>	Black-cheeked Waxbill	EU423943	Africa
<i>Estrilda melanosis</i>	Swee Waxbill	EU423940	Africa
<i>Estrilda melpoda</i>	Orange-cheeked Waxbill	EU423931	Africa
<i>Estrilda nonnula</i>	Black-crowned Waxbill	EU423955	Africa
<i>Estrilda rhodopyga</i>	Crimson-rumped Waxbill	EU423964	Africa
<i>Estrilda troglodytes</i>	Black-rumped Waxbill	EU423937	Africa
<i>Euschistospiza dybowskii</i>	Dybowski's Twinspot	EU423952	Africa
<i>Hypargos niveoguttatus</i>	Peters's Twinspot	EU423935	Africa
<i>Lagonosticta senegala</i>	Red-billed Firefinch	EU423959	Africa
<i>Lonchura cantans cantans</i>	African Silverbill	EU423950	Africa
<i>Lonchura castaneothorax</i>	Chestnut-breasted Munia	EU423947	Australia, New Guinea
<i>Lonchura kelaarti</i>	Black-throated Munia	EU423972	India, Sri Lanka
<i>Lonchura leucogastroides</i>	Javan Munia	EU423974	Sumatra, Java, Bali, Borneo
<i>Lonchura maja</i>	White-headed Munia	EU423951	Malaysia, Sumatra, Java, Bali
<i>Lonchura malabarica</i>	White-throated Sylverbill	EU423948	India
<i>Lonchura malacca malacca</i>	Indian Black-headed Munia	EU423961	India, Sri Lanka
<i>Lonchura malacca sinensis</i>	Indian Black-headed Munia	DQ270353	Thailand, Malaysia
<i>Lonchura oryzivora</i>	Java Sparrow	DQ270364	Java
<i>Lonchura pallida</i> ¹	Pale-headed Munia	AY495406	Lesser Sunda Islands ²
<i>Lonchura punctulata punctulata</i>	Scaly-breasted Munia	EU423954	India
<i>Lonchura quinticolor</i>	Five-colored Munia	EU423965	Lesser Sunda Islands ²
<i>Lonchura striata</i>	White-rumped Munia	DQ270352	Indochina, Sumatra
<i>Mandingoa nitidula schlegeli</i>	Green-backed Twinspot	EU423949	Africa
<i>Neochmia modesta</i>	Plum-headed Finch	AY495401	Australia
<i>Neochmia ruficauda clarescens</i>	Star Finch	EU423977	Australia
<i>Neochmia temporalis</i>	Red-browed Firetail	EU423970	Australia
<i>Nesocharis capistrata</i>	Grey-headed Oliveback	EU423953	Africa

Table 1. Contd....

Scientific Name	Common Name	GenBank	Distribution Range
<i>Ortygospiza atricollis atricollis</i>	African Quailfinch	EU423969	Africa
<i>Poephila acuticauda</i>	Long-tailed Finch	EU423978	Australia
<i>Poephila cincta cincta</i>	Black-throated Finch	EU423939	Australia
<i>Poephila personata personata</i>	Masked Finch	EU423941	Australia
<i>Pyrenestes sanguineus</i> ¹	Black-bellied Seedcracker	AY495395	Africa
<i>Pytilia hypogrammica</i>	Red-faced Pytilia	EU423927	Africa
<i>Pytilia melba melba</i>	Green-winged Pytilia	EU423963	Africa
<i>Pytilia phoenicoptera</i>	Red-winged Pytilia	EU423957	Africa
<i>Spermestes caniceps</i>	Grey-headed Silverbill	EU423934	Africa
<i>Spermestes bicolor bicolor</i>	Black-and-white Munia	EU423926	Africa
<i>Spermestes bicolor nigriceps</i>	Black-and-white Munia	EU423938	Africa
<i>Spermestes cucullatus</i>	Bronze Munia	AF255705	Africa
<i>Spermestes fringilloides</i>	Magpie Munia	EU423925	Africa
<i>Stagonopleura guttata</i>	Diamond Firetail	EU423976	Australia
<i>Taeniopygia bichenovii</i>	Double-barred Finch	EU423945	Australia
<i>Taeniopygia guttata</i>	Zebra Finch	DQ270390	Australia
<i>Uraeginthus bengalus</i>	Red-cheeked Cordonbleu	DQ270400	Africa
<i>Uraeginthus cyanocephalus</i>	Blue-capped Cordonbleu	EU423933	Africa
<i>Uraeginthus iantinogaster</i>	Purple Grenadier	EU423962	Africa
<i>Vidua chalybeata</i>	Village Indigobird	DQ270404	Africa
<i>Vidua macroura</i>	Pin-tailed Whydah	DQ270405	Africa
<i>Vidua paradisaea</i> ¹	Eastern Paradise-Whydah	U18865	Africa
<i>Quelea cardinalis</i> ³	Cardinal Quelea	AF255709	Africa
<i>Quelea quelea</i> ³	Red-billed Quelea	EU423982	Africa

¹ Mt cyt-b DNA sequences from these species were not obtained by us, but taken from the GenBank.

² Excluding Bali Island.

³ These two species belong to subfamily *Ploceinae* and were used in preliminary analyses to exclude the possibility of a genetic relationship between Estrildids and weavers, as found in (Allende *et al.*, 2001). They cluster in a separate branch basal to Estrildini and Viduini species (not shown).

The two tribes of Estrildids (Estrildini and Viduini) are clearly separated; the posterior probability values (ppv) in the BI tree (1.00 for both nodes, see Fig. 1, nodes K, L) as well as the bootstrap values (bv) in the ML tree (75 for the node grouping Estrildini species and 100 for Viduini species, see Fig. 2; nodes K, L, not shown) support this genetically distinct evolutionary groups.

Different types of clusters may be observed among the Estrildini species. Some nodes group together all the species belonging to the same genus according to both trees (Figs. 1, 2), like *Amadina* (node B: ppv=1.00), *Pytilia* (node D: ppv=1.00), *Uraeginthus* (node E: ppv=0.91), *Amandava* (node F: ppv=1.00) and *Spermestes* (node I: ppv=1.00), and *Erythura* (node C: ppv=0.99). The genus *Estrilda* cluster includes *Nesocharis capistrata*, which is basal to *Estrilda* species (BI, node A: ppv=0.88). *E. caerulescens* and *E. erythronotos* seem to be somewhat separated from them (ppv=1.00, i.e.: a different evolutive radiation), and *E. melanotis* groups outside the other *Estrilda* species (Fig. 1). *Lon-*

chura species are joined under a strong supported node (Figs. BI, node H: ppv=1.00), with the exception of *L. malabarica* and *L. cantans* which form, together with *Stagonopleura guttata*, a very consistent phylogenetic group (node G: ppv=1.00) separated from the others (Fig. 1). Finally, the species belonging to the genera *Poephila*, *Taeniopygia*, *Emblema* and *Neochmia* are clustered together (see Fig. 1, node J), but supported by weak posterior probability values. The remaining species do not show a clear phylogenetic placement (see Fig. 1).

Regarding the distribution range, there is no genetic separation between the African and Australian Estrildids, being all of them intermixed in various phylogenetic groups (see Fig. 1, and also the Discussion section).

Fig. (3) shows present day Estrildids range. Four questions are addressed in this Figure:

1) A hypothetical original place of Estrildids may be India (see Fig. 1, node G).

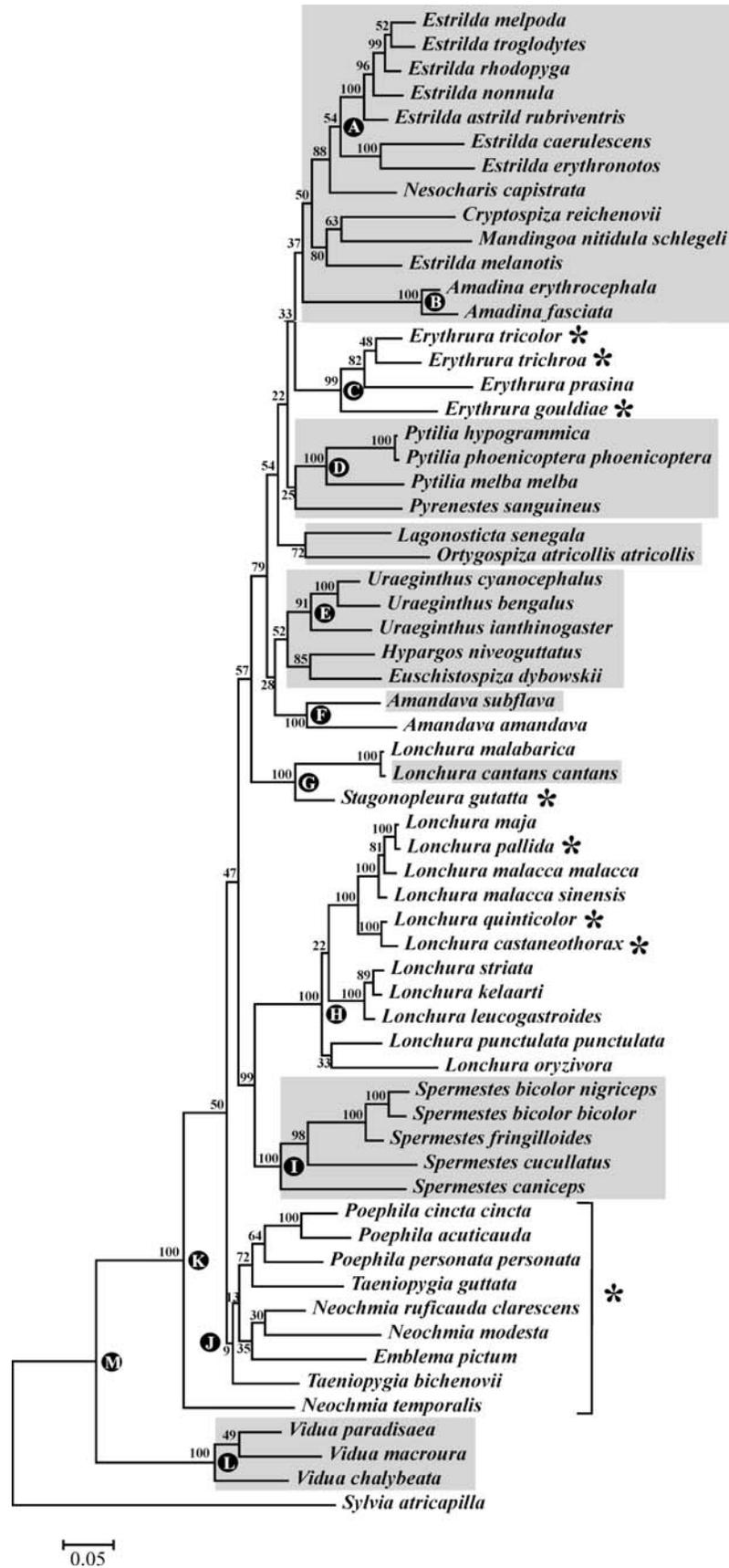


Fig. (1). Bayesian phylogram constructed assuming a GTR+I+G model of evolution. The posterior probability values (x100) are indicated for each node. The African species are shaded. Species thriving in the East side of Wallace's line (Australia and/ or neighbouring islands) are indicated by an asterisk (*). The rest of the species thrive in the West side of Wallace's line (South Asia and/ or Indian Ocean islands).

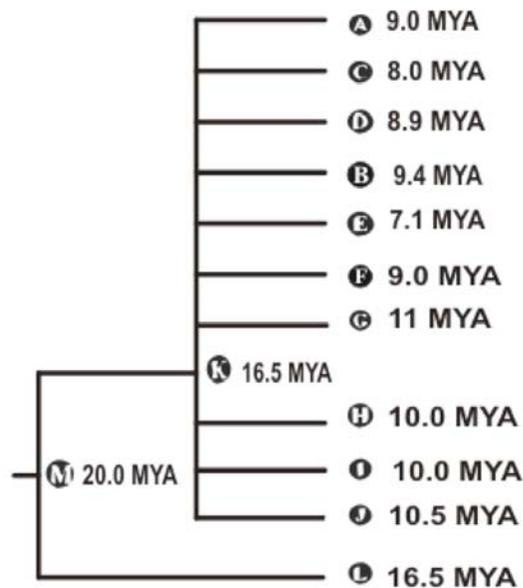


Fig. (2). Hypothesis. Scheme of linearized maximum likelihood phylogenetic tree, indicating approximate maximum coalescence times of nodes (MYA = million years ago). Nodes represented by letters are the same as in Fig. (1). Times of appearance on Earth hypotheses are indicated for each node (or evolutionary group of species) calculated as detailed in section 'Material and Methods' and taking genetic (and time) distances from the starting branch point before bifurcation. The original tree was constructed assuming GTR+I+G model of evolution (chosen by Modeltest), with branch lengths estimated according to Thorne's model.

- 2) African waxbills (Fig. 1; node A) are not genetically related to Australian Red-browed Firetail (Fig. 1; bottom). Their phenotype similarities are probably due to convergent evolution.
- 3) Silverbills do not cluster together (see Fig. 3 footnote).
- 4) Wallace's line is drawn between the Indonesian Islands of Bali and Lombok (both of them known as part of the Lesser Sunda Islands), and also between Borneo and Sulawesi (former Celebes) Islands to mark the boundary between the Oriental and Australian regions. The Wallace's line corresponds almost exactly with the outer limit of the continental shelf of South Asia. This was exposed by lowered sea level during Pleistocene glaciations [21]. Although many species of birds, mammals and plants are mostly thriving either at one or the other side of the line because of the relative dispersal barrier before Asian shelf during Pleistocene glaciations, no particular speciation of dispersal event is noticed around Pleistocene, 2 MYA and after (Fig. 2). This indicate that Estrildids speciation and radiation had occurred before Pleistocene, starting after 16,5 MYA (Fig. 2).

3. DISCUSSION

The results obtained in this work show that both tribes of Estrildids (Estrildini and Viduini) are clearly separated suggesting the monophyly of the Estrildids [6-8]. The lack of correlation between genetics and geographic distribution is also observed (Figs. 1, 2), contrary to others suggestion [6].

However, the Estrildid group of birds is composed itself of different evolutive radiations that need specific comments.

3.1. Tempo of Evolution Hypothesis

Once it is established the monophyly of the Estrildids' clade (Figs. 1, 2), it is feasible to address an approximation of the time of appearance of extant Estrildids. Fig. (2) shows a ML linearized tree which is calibrated for times by taking into account a mixed chicken / pheasant molecular and geological divergence and then a further calculation for *Fringilla coelebs* and *Sylvia atricapilla* [18]. The Estrildids radiation would have started about 16,5 MYA, according to node M (Fig. 2). At this time, the subfamily *Fringillinae* also started to radiate [22,23]. Different clear polytomies are pointed out in some nodes of the trees by letters in black circles (Figs. 1, 2). The oldest dichotomy seems to be the branch leading to node K, which is strongly supported (see Figs. 1, 2), and the time of divergence is around the Middle Miocene Epoch, about 12 MYA. This particular node comprises birds from Africa (genera *Estrilda*, *Pytilia*, *Amadina*, *Uraeginthus*, *Spermestes*), South Asia (genera *Lonchura*, *Amandava*) and Australia (genera *Erythrura*, *Neochmia*, *Poephila*) (see Figs. 1, 2, 3). This suggests that this node possibly represents the time of appearance of the original extinct ancestor of Estrildids (excluding whydahs).

The Indian plate crashed against the Asian plate 50 MYA, but the 'strongest push' occurred during the Miocene Epoch [21,24]. As a consequence, the Himalayan peaks established, the Tibetan plateau reached its nowadays altitude and dramatic changes happened in the Indian peninsula and Himalayan mountains (monsoon rains), and in the Tibetan plateau (colder and drier). The biggest rivers in India, Indochina and China established (starting at Tibetan Plateau) and their drastic climate change may have pushed the Estrildids' ancestor to radiate and give rise to node K (Figs. 1, 2) [21]. This fact would suggest an Indian origin for the extant Estrildids, which would have colonized Africa, Australia, South Asia and the Indian and South Pacific Islands (Fig. 3), because branch leading to node G is the oldest one found (Figs. 2, 3).

As previously stated, it is drawn from our own data on ML linearized tree (Fig. 2) and Miocene Epoch paleoenvironmental changes that Estrildid finches radiation might have originated in India (Fig. 3), according to the oldest branch of the ML linearized tree leading to node G.

3.2. Specific Phylogenetic Problems Addressed

- a) The Grey-headed Oliveback (*Nesocharis capistrata*), and presumably the two other *Nesocharis* species, *N. shelleyi* and *N. ansorgei* (not tested), groups with *Estrilda* species in a basal branch (Figs. 1, 2; node A), as previously shown with other DNA markers [8], and seems to be older than the *Estrilda* species (Fig. 2). Although more taxa are required, it is likely that genus *Estrilda* and genus *Nesocharis* belong to the same radiation and could be considered as members of the same genus, despite the remarkably different phenotype.
- b) The Gouldian Finch (*Chloebia gouldiae* / *Erythrura gouldiae*) is definitively included within the bright coloured birds of genus *Erythrura*, according to the BI den-

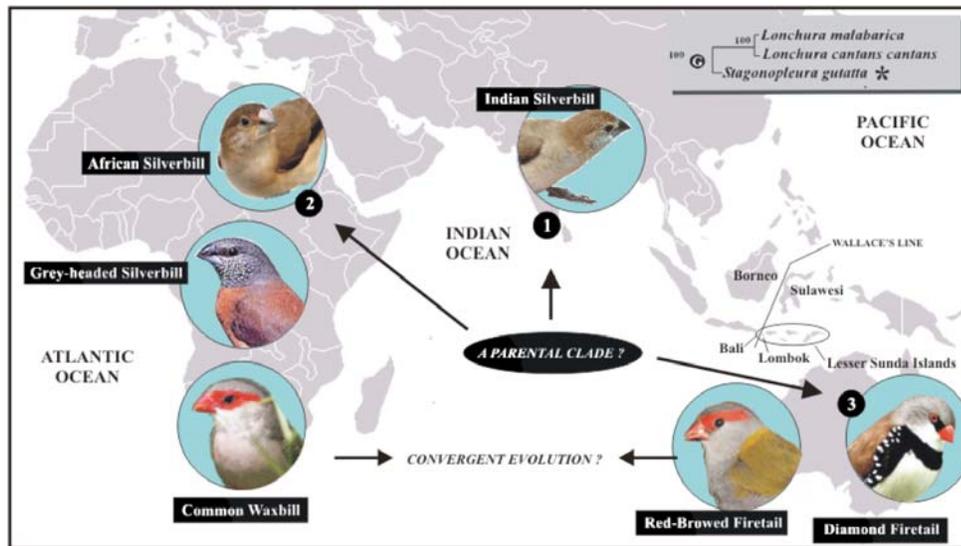


Fig. 3 Estrildids distribution range. Africa, South Asia (including Arabian Peninsula) and Indonesia, Australia and Pacific Islands. A possible original clade in India is represented. ① ② ③ See text and Fig. 1; basal node G. Australian Diamond Firetail (*Stagonopleura gutatta*) African Silverbill (*Lonchura cantans cantans*) and Indian Silverbill (*Lonchura malabarica*) also thrive in the tip of Arabian Peninsula. Also, African waxbills (genus *Estrilda*) do not have genetic connection with Australian Red-browed Firetail (*Neochmia temporalis*) (see Fig. 1; dendrogram, bottom). Grey-headed Silverbill (*Spermestes caniceps* or *Lonchura griseicapilla*) (See Fig. 1, node I) do not cluster with other silverbills (*L. malabarica*, *L. cantans*). Inset shows node G evolutionary group from the ML linearized tree (and bootstrap values, see Fig. 1 and 2). It may represent Estrildids' parental clade because it seems the oldest one and is distributed through Africa, India and Australia. Time is calculated from the origin of branch, before bifurcation where extant species may have existed.

dendrogram (Fig. 1; node C: $ppv=0.99$), as suggested by some authors [4,5] and as previously reported [6,8].

- c) The silverbills from genus *Lonchura* have been suggested to cluster together and separated from other *Lonchura* species [1]. This fact is partially confirmed by our results, showing the oldest Estrildinae evolutive radiation group that consistently clusters the African Silverbill (*Lonchura cantans*) with the Indian Silverbill (*Lonchura malabarica*), and also with the phenetically distinct Diamond Firetail (*Stagonopleura guttata*) from Australia (see Figs. 1, 2; nodes G, H). This group is not related to the rest of 'non-silverbill' *Lonchura* species. Another silverbill, however (the Grey-headed Silverbill, *Spermestes caniceps*), is placed within the *Spermestes* cluster (Figs. 1, 2, node I).
- d) The Java Sparrow (*Padda oryzivora* / *Lonchura oryzivora*) might be considered a *Lonchura* species according to both the BI and ML trees (Figs. 1, 2; node H), as previously suggested [4,5], although its position inside that phylogenetic group is not well defined because of low posterior probability values. This finding is also concordant with previous suggestions [6,8].
- e) The African munias form a distinct phylogenetic cluster with respect to the Asian and Australian munias (Figs. 1, 2; nodes H, I), corroborating the distinction proposed by some authors [4], who designate the former as belonging to the genus *Spermestes* and keep the latter as members of the genus *Lonchura*.
- f) The Plum-headed Finch (*Aidemosyne modesta* / *Neochmia modesta*) clusters with other Australian finches, close to *Neochmia* and *Poephila* species, as previously

seen [6,8]; however, this joining is slightly supported in the trees (Figs. 1, 2; node J) and both names may be accepted for this species [1,4,5].

- g) Clement, in the Introduction of his 1993 edition [1], pointed out that the Australian Red-browed Firetail (*Aegintha temporalis* / *Neochmia temporalis*) was very similar to some African waxbills from genus *Estrilda* (see Fig. 3). We have seen that it appears as a basal species of all Estrildini birds in the BI dendrogram (Fig. 1), but it is integrated among the Australian group in the ML tree with a weak support (Fig. 2; node J). In any case, it is not genetically related to the African waxbills and the red eye brow may be due to convergent evolution driven by unknown evolutive forces. The Australian Red-browed Firetail is probably an ancestral Australian Estrildid (Fig. 2).
- h) There is a group in the BI tree (Fig. 1; close to node A) clustering *Mandingoa nitidula*, *Cryptospiza reichenovii* and *Estrilda melanotis* with a low support ($ppv=0.80$). This group is not observed in the ML tree, where *Estrilda melanotis* is placed outside (Fig. 2). *Estrilda melanotis* was included, together with *Estrilda quartinia*, in genus *Coccyptigia* [8], and separated from other *Estrilda* species. Thus, *Mandingoa nitidula* and *Estrilda melanotis* may be joined to genus *Cryptospiza*.

Other Estrildid species, like *Pyrenestes sanguineus*, *Lagonostica senegala*, *Ortospiza atricollis*, *Hypargos niveoguttatus*, *Euschistospiza dybowskii* and *Emblema pictum* show a similar grouping, as previously suggested [6,8], but with low bootstrap (not shown) and posterior probability values in the corresponding trees (Figs. 1, 2), what may be probably due to a lack of species. Lack of extant species and

missing of extinct ones lead us to continue studying Estrildinae phylogeny in order to complete these clades' evolution and origins.

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