



# The phylogenetic relationships of Przevalski's Finch *Urocynchramus pylzowi*, the most ancient Tibetan endemic passerine known to date

MARTIN PÄCKERT,<sup>1\*</sup> JOCHEN MARTENS,<sup>2</sup> YUE-HUA SUN<sup>3</sup> & PATRICK STRUTZENBERGER<sup>1</sup>

<sup>1</sup>Senckenberg Natural History Collections, Museum of Zoology, Königsbrücker Landstraße 159, D-01109, Dresden, Germany

<sup>2</sup>Institut für Zoologie, Johannes Gutenberg-Universität, 55099, Mainz, Germany

<sup>3</sup>Key Laboratory of Animal Ecology and Conservation, Institute of Zoology, Chinese Academy of Sciences, 100101, Beijing, China

Competing systematic hypotheses have placed the Tibetan endemic Przevalski's Finch *Urocynchramus pylzowi* either with the Old World buntings (Emberizidae) or with the cardueline finches (Fringillidae, Carduelinae). Recent studies based on limited genetic evidence instead suggest an isolated position within Passeroidea and advocate a separate family, Urocynchramidae, as had been suggested much earlier on the grounds of morphology. We provide a time-calibrated multi-locus phylogeny for Passeroidea including Przevalski's Finch based on three mitochondrial markers and three nuclear introns that placed *U. pylzowi* in a clade together with Estrildidae, Viduidae and Ploceidae. A sister group relationship of *U. pylzowi* and weavers (Ploceidae) was concordant among three multilocus reconstructions but received only poor support. Divergence time estimates inferred from a fossil/biogeographical molecular dating approach suggested a late Oligocene split of *U. pylzowi* from its closest relatives at roughly 25 million years ago, making this the oldest known Tibetan endemic passerine. In addition to the molecular data, behavioural peculiarities and egg coloration further strengthen an isolated placement of *U. pylzowi*.

**Keywords:** nine-primaried oscines, Passeroidea, Qinghai-Tibet Plateau, time-calibrated phylogeny.

Przevalski's Finch *Urocynchramus pylzowi* Przevalski 1876 (Fig. 1) is a Chinese endemic passerine with a small distribution at the eastern margin of the Qinghai-Tibet Plateau. It was first described from the Tatung River in the Nan Shan of north-eastern Qinghai and from there its range extends to south-western Gansu, eastern Xizang and western Sichuan (Collar & Newton 2010). It occupies semi-open and open bush and scrub vegetation between 3050 m and the upper limit of shrub and bush vegetation below the nival ecotone. The species has a strong preference for dwarf willow species, such as *Salix alfredii*, and dwarf rhododendrons *Rhododendron* spp. as well as for stands of *Potentilla tenuifolia* and *Caragana jubata* (Schäfer 1938, Schäfer & Meyer de Schauensee 1938,

Gebauer *et al.* 2006). Apart from the nominate form, one subspecies, *U. p. coloratus* Tugarinov and Stegmann (1929), has been described from the headwaters of the Blue River, but most authorities do not recognize this taxon (Vaurie 1956). *Urocynchramus pylzowi* is one of the Asian high-elevation bird species whose biology and ecology is very little known. Its general behaviour, nest and eggs have been insufficiently described, and its peculiar acoustic display was described only recently (Gebauer *et al.* 2006).

Since its initial scientific description, the phylogenetic relationships and systematic placement of *Urocynchramus* have been controversial. The scientific name of its monotypic genus is a compound word that refers to its phenotypic similarities to the long-tailed rosefinch *Uragus sibiricus* on the one hand (cf. Badyaev 1997) and similarities in beak morphology with the emberizid subgenus

\*Corresponding author.

Email: martin.paeckert@senckenberg.de



**Figure 1.** Przevalski's Finch *Urocynchramus pylzowi*: (a) adult male, Qinghai, photo: Paul Jones, Ottawa, Canada; (b) adult female from near Lake Qinghai (Koko Nor), photo: Axel Gebauer, <http://www.gebauer-wildphoto.com>; (c) adult female from near Koko Nor, note the bunting-like beak, photo: J. Martens.

*Cynchramus* on the other (Fig. 1). The various vernacular names given to *Urocynchramus* are symptomatic of the controversy regarding its systematic affiliations: some authorities refer to it as Pink-tailed Bunting and include it in the Emberizidae (Inskipp *et al.* 1996) or in the subfamily Emberizinae of the Fringillidae (Monroe & Sibley 1993), whereas others include it as the Pink-tailed

Rosefinch in the Fringillidae, with close affiliations to Carduelinae (Vaurie 1956, 1959, 1972, King 1997, Clement 1999, for a criticism of that classification see Parkes 2001, Collar & Newton 2010).

Although researchers of the early 20th century had already noticed significant peculiarities of *Urocynchramus* beyond superficial characters such as the bunting-like beak, rosefinch-like reddish coloration and *Uragus*-like long tail, its affiliation to finches (Fringillidae) and/or buntings (Emberizidae) was common until the first molecular genetic analysis was published (Groth 2000), which did not support such affiliations. However, even in the light of Groth (2000), some authors still suggest an affiliation with finches and buntings. For example, Collar and Newton (2010) in the *Handbook of the Birds of the World* placed *Urocynchramus* between *Uragus sibiricus* (which was recently shown to be a true *Carpodacus* rosefinch; Zuccon *et al.* 2012, Tietze *et al.* 2013) and *Carpodacus*, although they highlighted recent molecular findings.

Hartert (1910), alluding to the systematic position of nine-primaried and 10-primaried songbirds, found a relatively long 10th primary in *U. pylzowi* and suggested strong similarities of that species with the weavers (Ploceidae). Based on results from his morphological analyses, von Domaniewski (1918) was the first to suggest family rank to a monotypic Urocynchramidae. This was later followed by Wolters (1975–1982), who did not add any further arguments. The isolated systematic position of *Urocynchramus* was subsequently corroborated by molecular evidence. A comparison of a cytochrome-*b* (*cytb*) sequence acquired from a specimen collected by Ernst Schäfer in 1934 with those of other passerines allowed Groth (2000) to reject any phylogenetic relationship of *Urocynchramus* to either Fringillidae or Emberizidae. He placed it as a basal sister of a large passeroid clade including all so-called 'nine-primaried' families plus Estrildidae and Ploceidae. These results were later confirmed by Gebauer *et al.* (2006), who also added bioacoustic and ecological arguments against a closer relationship of *Urocynchramus* to either buntings or finches. In the same year, Yang *et al.* (2006) provided a two-marker phylogeny (*cytb* and a further nuclear marker *c-myc*) that likewise rejected a close relationship between *U. pylzowi* and rosefinches (*Carpodacus* including *Uragus sibiricus*), instead suggesting an isolated position of Urocynchramidae basal to the nine-primaried

oscines. All subsequent phylogenetic supertree and supermatrix reconstructions that included *Urocynchramus* (Jönsson & Fjeldså 2006, Jetz *et al.* 2012) referred to Groth's (2000) phylogenetic hypothesis but without using his original sequence data. To date, a single complete cytochrome-oxidase I (COI) sequence of *U. pylzowi* (1249 bp length) is available at GenBank submitted by team members of Yang *et al.* (2006), who apparently had not used this sequence for their study. Some taxonomic authorities have recently relied on the genetic evidence from Groth (2000) to confirm the family status of Urocynchramidae (Clements *et al.* 2014, Dickinson & Christidis 2014). However, to date, a phylogenetic hypothesis for the placement of *Urocynchramus* in relation to other Passeroidea based on firm multi-locus evidence is still missing. Here we aim to provide a more robust hypothesis of the phylogenetic relationships of *U. pylzowi* based on a multi-locus sequence dataset and to investigate the temporal dimension when ancestors of this enigmatic Tibetan endemic split from its closest relatives using time-calibrated phylogenetic trees.

## METHODS

We obtained samples of *U. pylzowi* from the lower slopes of northern Nan Shan Mts close to the southern shore of Lake Qinghai (Koko Nor), 3 km southeast of Heimahe at 3300 m, sampling one adult female (voucher catalogue number: MTD-C64769) and one juvenile (voucher catalogue number: MTD-C64770). We amplified and sequenced four mitochondrial regions (*cytb*, NADH dehydrogenase subunit 2 (ND2), 16S rRNA, COI) and three nuclear markers (myoglobin intron 2, fibrinogen intron 7, ornithine-decarboxylase intron 7) from DNA extracts obtained from muscle tissue. For phylogenetic analyses we assembled a multi-locus sequence dataset of 100 taxa. We filled gaps in published sequence datasets of buntings and rosefinches from previous studies (Tietze *et al.* 2013, Päckert *et al.* 2015b) and added newly generated sequence data for members of other passeroid families such as Prunellidae. For details on DNA extraction, PCR and sequencing protocols see the studies cited above. For sources of sequence data see Table S1.

Sequences were aligned with MEGA 6 (Tamura *et al.* 2011). We reconstructed a multi-locus

phylogeny based on six markers (COI sequences were not used for multi-locus analyses due to data deficiency for many taxa) with BEAST v.1.8.1 (Drummond & Rambaut 2007). We ran BEAST for 50 million generations (trees sampled every 5000 generations) under the uncorrelated lognormal clock model for all loci with the 'auto-optimize' option activated and a birth-death process prior (with incomplete sampling assumed) applied to the tree. The best partitioning scheme was determined with PARTITIONFINDER 1.1.1 (Lanfear *et al.* 2012) using the corrected Akaike information criterion (AICc), the 'beast' model set and the greedy search algorithm. The best scheme was determined to be a 10-partition scheme with *cytb* and ND2 partitioned by codon position and all other markers set as a separate partition. According to these results we applied (i) the GTR+I+ $\Gamma$  model to the partitions *cytb* 1st codon position, ND2 1st and 2nd codon position and 16S rRNA; (ii) the GTR+ $\Gamma$  model to the partitions *cytb* 3rd codon position, ND2 3rd codon position, *myo*, *fib7* and *ODC*; (iii) the HKY+I+ $\Gamma$  model to *cytb* 2nd codon position.

For inference of divergence time estimates, we calibrated our phylogeny using four fossil and two biogeographical calibration points (three of them have been previously applied by Kennedy *et al.* 2012). We used the following fossil ages as node constraints: (i) a fossil bunting *Ammodramus hatcheri* from the Clarendonian–Hemphillian (Steadman 1981, node: all Passerellidae), (ii) a fossil *Passerina* sp. from the Hemphillian (Steadman 1981, Steadman & McKittrick 1982, Kennedy *et al.* 2012, node: *Cyanocompsa* and *Passerina*), (iii) a fossil tentatively identified as Parulidae from the early Miocene (Kennedy *et al.* 2012, Mayr 2013, node: Parulidae, Icteridae, Emberizidae and Passerellidae), and (iv) the earliest fossil representing the crown group Passeriformes for time to most recent common ancestor (tmrca) of the root separating *Acanthisitta* from other passerines (Mayr 2013, the normal prior interval was defined using the separation of New Zealand from Australia as a soft minimum; see Kennedy *et al.* 2012). We furthermore applied the following three palaeogeographical node constraints: (v) the palaeovolcanic age of São Miguel, Azores, for the crown group of *Pyrrhula* bullfinches (Johnson *et al.* 1998, Töpfer *et al.* 2011, node: *Pyrrhula murina* and *P. pyrrhula*), and (vi) the formation of the Hawaiian Kauai/Nilhau complex for Hawaiian honeycreepers (Lerner *et al.* 2011). We slightly modified the dating approach

of Kennedy *et al.* (2012) by applying a lognormal tmrca prior distribution to four calibration points (instead of a 'hard maximum') according to the standards for fossil calibrations outlined in Benton *et al.* (2009). For two calibration nodes a normal prior distribution was applied due to apparent incompatibilities between lognormal tmrca prior intervals that arose in first test runs with BEAST (palaeovolcanic age of São Miguel, *Pyrrhula*; separation of New Zealand from Australia, *Acanthisitta*). For details on constraint nodes, calibration points and tmrca prior settings, see Table 1 and Fig. S1. For comparison, we performed a second run with BEAST and applied an empirical substitution rate of 0.0105 substitutions per site per lineage per million years (Weir & Schluter 2008) to the *cytb* partition and left the rates of all other loci to be estimated relative to the *cytb* rate. The log files were examined with

TRACER v1.4.8 (Drummond & Rambaut 2007) to ensure adequate effective sample sizes (ESSs). We considered ESSs satisfactory for each target distribution if they were at least 100 (preferably above 200; Drummond & Bouckaert 2015). Trees were summarized with TREEANNOTATOR v1.4.8 (posterior probability limit = 0.5) using a burn-in value of 3000 (trees) and the median height annotated to each node.

To validate the phylogeny obtained from BEAST we also calculated a maximum likelihood (ML) tree with RAxML v7.2.6 (Stamatakis 2006; using the GUI PYTHON application v0.93 by Silvestro & Michalak 2012) and another Bayesian tree with MRBAYES v3.1.2 (Ronquist & Huelsenbeck 2003). Partitioning of the dataset was identical to that used for runs in BEAST. For MRBAYES, the MCMC chains ran for 10 million generations and trees were sampled every 100th generation. We used

**Table 1.** Calibration points used for molecular dating and tmrca prior settings in BEAST. Five lognormal priors (including zero offset, minimum node age (= fossil age), log mean and standard deviation (sd)), and two normal priors; tmrca priors for fossil calibration points 1–4 were set to a lognormal distribution roughly covering the interval of the stratigraphic layer with the fossil age as zero offset (see Benton *et al.* 2009). The tmrca prior for the Drepanidinae clade was set according to the respective node age estimated by Lerner *et al.* (2011) (zero offset) and with a lognormal distribution covering the period of the biogeographical formation of the oldest Hawaiian islands (compare Lerner *et al.* 2011). Node 5 of the Azorean endemic *Pyrrhula murina* and its sister species *Pyrrhula pyrrhula* was constrained using the volcanic age of São Miguel.

Calibration taxon	Node dated	Calibration source	Date used (Ma)	Zero offset	log mean	sd	Reference(s)
Parulidae	1. Parulidae + Icteridae + Emberizidae + Passerellidae	Fossil: tentative identification as Parulidae, early Miocene	18–23	18	1.0	0.1	Kennedy <i>et al.</i> (2012), Mayr (2013)
<i>Passerina cyanea</i>	2. <i>Cyanocompsa cyanooides</i>	Fossil: <i>Passerina</i> , Hemphillian	4.5–10	4.5	0.5	0.75	Kennedy <i>et al.</i> (2012), Steadman and McKittrick (1982)
<i>Ammodramus humeralis</i>	3. Passerellidae	Fossil: <i>Ammodramus hatcheri</i> , Clarendonian–Hemphillian	4.3–10	4.3	0.5	0.75	Steadman (1981)
Drepanidinae	4. Drepanidinae	Biogeographical: formation of Kauai/Nilhau complex	4.9–5.7	4.9	0.01	0.5	Lerner <i>et al.</i> (2011)
			Date used (Ma)	Normal mean		sd	
<i>Pyrrhula murina</i>	5. <i>P. murina</i> / <i>P. pyrrhula</i>	Biogeographical: age of São Miguel	0–0.88	0.88		0.35	Töpfer <i>et al.</i> (2011)
<i>Acanthisitta chloris</i>	6. All passerines	Biogeographical: separation of New Zealand from Australia Fossil: earliest crown group passerines from Australia	23–85	54		15.8	Kennedy <i>et al.</i> (2012) Mayr (2013)

TRACER v1.4.8 to check ESS values for convergence of model parameters for the combined output files. The first 30 000 samples were discarded as burn-in and the model parameters and the posterior probabilities were estimated from the remaining samples. The remaining trees were summarized in a 50% majority rule consensus tree. ML bootstrap support was obtained with RAXML using 100 replicates under the GTR+ $\Gamma$ +I model (thorough bootstrap option).

For ESSs for the data likelihood, the mean in the BEAST run was 1395 (range 329–5291; Table S2) indicating satisfactory convergence. In comparison, the mean ESS from the run with MRBAYES was 8913 (range 107–45 770) indicating satisfactory convergence for all parameters except mean relative rates for each of the 10 partitions ( $m\{1\}$  –  $m\{10\}$ ; Table S3). Failure of Bayesian analysis to converge, particularly for large datasets, has been previously reported, e.g. for the deep phylogeny of birds by Hackett *et al.* 2008 (their fig. S3), and might be due to inaccurate branch-length estimates several magnitudes longer compared with those resulting from likelihood analysis (Brown *et al.* 2009). On account of this criticism and given that crucial parameters such as prior and likelihood reached satisfactory ESS in our analysis, we considered the non-convergence of the relative rate parameters and a possible effect on branch lengths of minor importance in the MRBAYES tree, as that lacked a time calibration. Nevertheless, the ML reconstruction using RAXML might be the more reliable and more robust phylogenetic hypothesis to be compared with the time-calibrated BEAST tree.

We also reconstructed a single-locus phylogeny for an enlarged ND2 sequence dataset of *U. pylzowi* plus Ploceidae, Viduidae and Estrildidae (data from GenBank; most sequences from Sorenson & Payne 2001, Sorenson *et al.* 2004, Prager *et al.* 2008). Phylogenetic reconstructions with BEAST were performed according to the settings described above including partitioning by codon position of the ND2 dataset.

## RESULTS

The simplified multi-locus tree of our Passeroidea dataset is shown in Fig. 2 (for a tree showing all species, see Fig. S1). In our time-calibrated phylogeny, *U. pylzowi* was not included in the fully supported clade of ‘nine-primaried’ oscines

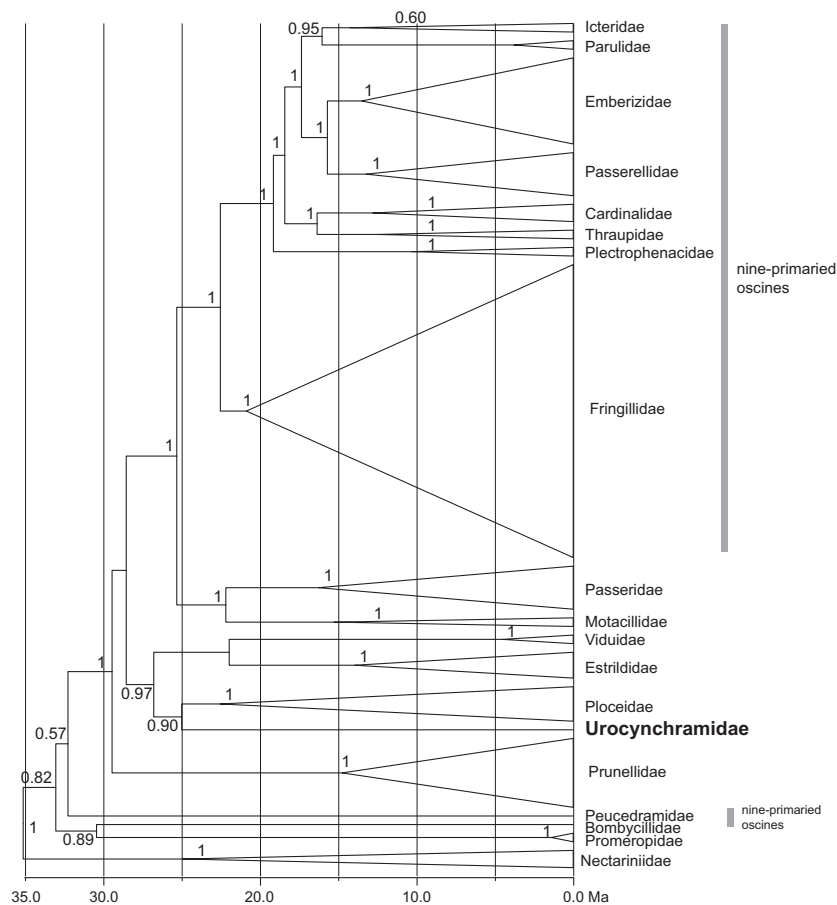
(although note that Peucedramidae was also excluded from the nine-primaried oscine clade) but was instead nested in a clade with Estrildidae, Viduidae and Ploceidae (Fig. 2). This entire clade received strong support in the dated BEAST phylogeny (Fig. 2), but only poor support from likelihood bootstrap and Bayesian posterior probabilities in the RAXML and MRBAYES trees (Fig. 3). The topology among *U. pylzowi* and these three families was identical in all multi-locus reconstructions with BEAST, MRBAYES and RAXML. However, the sister group relationship between *Urocynchramus* and Ploceidae received poor support in all analyses (Figs 2 and 3). The position of *Urocynchramus* as sister to Ploceidae could be driven by the signal of the mitochondrial markers, because this position was corroborated by the RAXML mtDNA tree; however, it received only poor support from likelihood bootstrap analyses (Fig. S2a). In contrast, for the nuclear and mtDNA tree inferred using MRBAYES, the Estrildidae/Viduidae clade, *Urocynchramus* and Ploceidae were successively basal to the crown clade of Motacillidae/Passeridae and the nine-primaried oscines (Fig. S2b). In the single-locus tree based on an enlarged ND2 sequence set, Estrildidae, Viduidae and Ploceidae appeared as three clearly separated lineages (however, only the first two received strong support) and *Urocynchramus* was nested within Ploceidae and formed a poorly supported clade with *Bubalornis*, *Dinemellia* and *Amblyospiza* (Fig. 4).

The mean divergence time to the tmrca of Ploceidae and Urocynchramidae inferred from the fossil calibration was 25 Ma (22.5–27.5 Ma); however, the tmrca estimate inferred from the *cytb* rate was considerably older (35 Ma (29.7–40.9 Ma)).

We compared our COI barcode sequences (688 bp, including that of a second juvenile from the same locality; cat. no. MTD-C64771) with the only GenBank sequence available for *U. pylzowi* (EU847720). The COI sequences differed by four substitutions, and the GenBank sequence showed three additional insertions at positions 403, 416 and 441 (all adenosine) that caused a reading-frame shift along that part of the COI gene.

## DISCUSSION

All our multi-locus reconstructions placed *U. pylzowi* sister to Ploceidae, in a larger clade that also

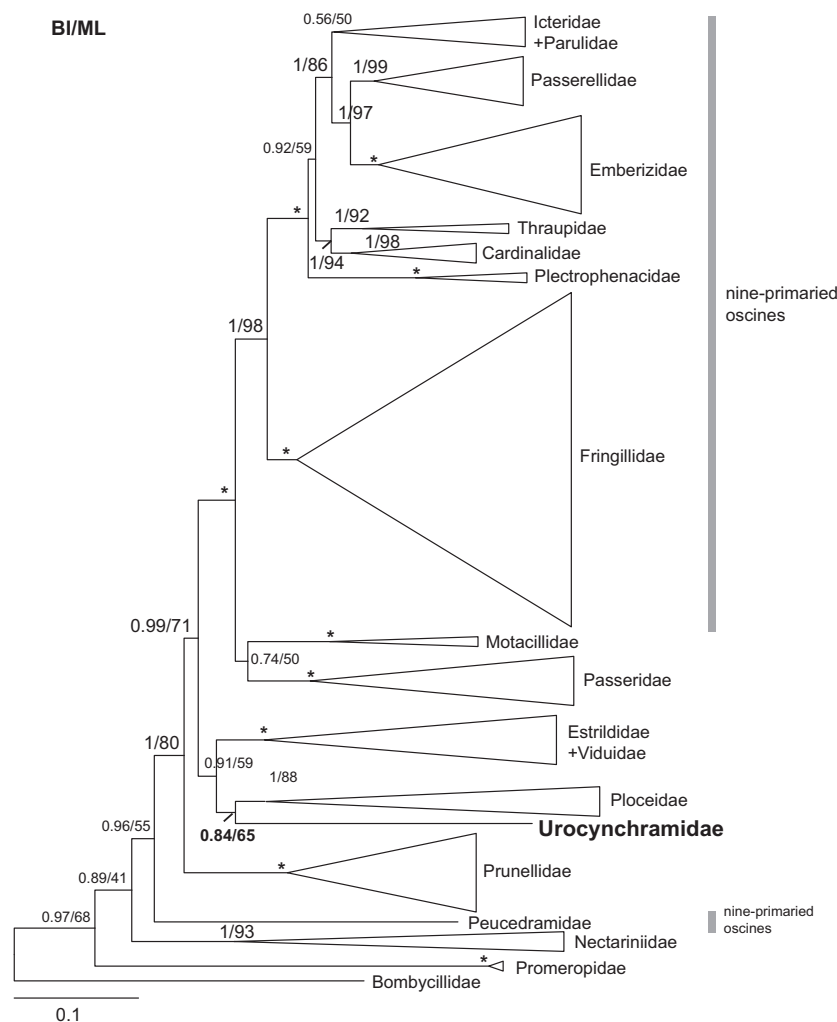


**Figure 2.** Multi-locus Bayesian phylogeny of Passeroidea (BEAST analysis) based on six markers (cytb: 1041 bp; ND2: 1041 bp; 16S rRNA: 856 bp; myoglobin: 746 bp; ODC: 543 bp; fib7: 705 bp). The tree was rooted with *Acanthisitta chloris* (pruned from the tree). Support from posterior probabilities is indicated at nodes.

included Estrildidae and Viduidae. That position is discordant with previous phylogenetic hypotheses, which placed *U. pylzowi* as a basal lineage of Passeroidea without any close affinities to at least one passeroid family (Groth 2000, Yang *et al.* 2006, Jönsson & Fjeldså 2006 (Passeroidea, clade 4)). Although node support was moderate to low for the clade uniting Urocynchramidae, Ploceidae, Estrildidae and Viduidae, there is further evidence of a closer relationship among *U. pylzowi* and weavers, waxbills and whydahs. Groth (2000) emphasized that in his *cytb* sequence dataset the lowest transversion distance between *U. pylzowi* and other species was with the estrildid genus *Parmoptila*; however, he refrained from suggesting a closer relationship between these taxa because they did not appear as sister groups from his phylogeny. Like Groth (2000), Yang *et al.* (2006) pointed out the isolated position of

*U. pylzowi* in their Bayesian and maximum parsimony consensus trees but also stressed that in their likelihood phylogeny *U. pylzowi* groups with three species of Ploceidae and Estrildidae (*Ploceus*, *Cryptospiza* and *Lonchura*), although with poor support. In contrast to our findings regarding the placement of *U. pylzowi*, the monophyly of the 'nine-primaried' oscines with the exception of Peucedramidae is in accordance with previous studies (Klicka *et al.* 2000, Yuri & Mindell 2002, Davis & Page 2014).

Strikingly, one of the major arguments against the inclusion of *U. pylzowi* in either the Fringillidae or the Emberizidae has been its well-developed 10th primary (von Domaniewski 1918, see also Payne: in Peters 1968). However, in passerines the number of primaries and the size of the 10th primary are known to carry only very limited phylogenetic information (Hall 2005). Hartert

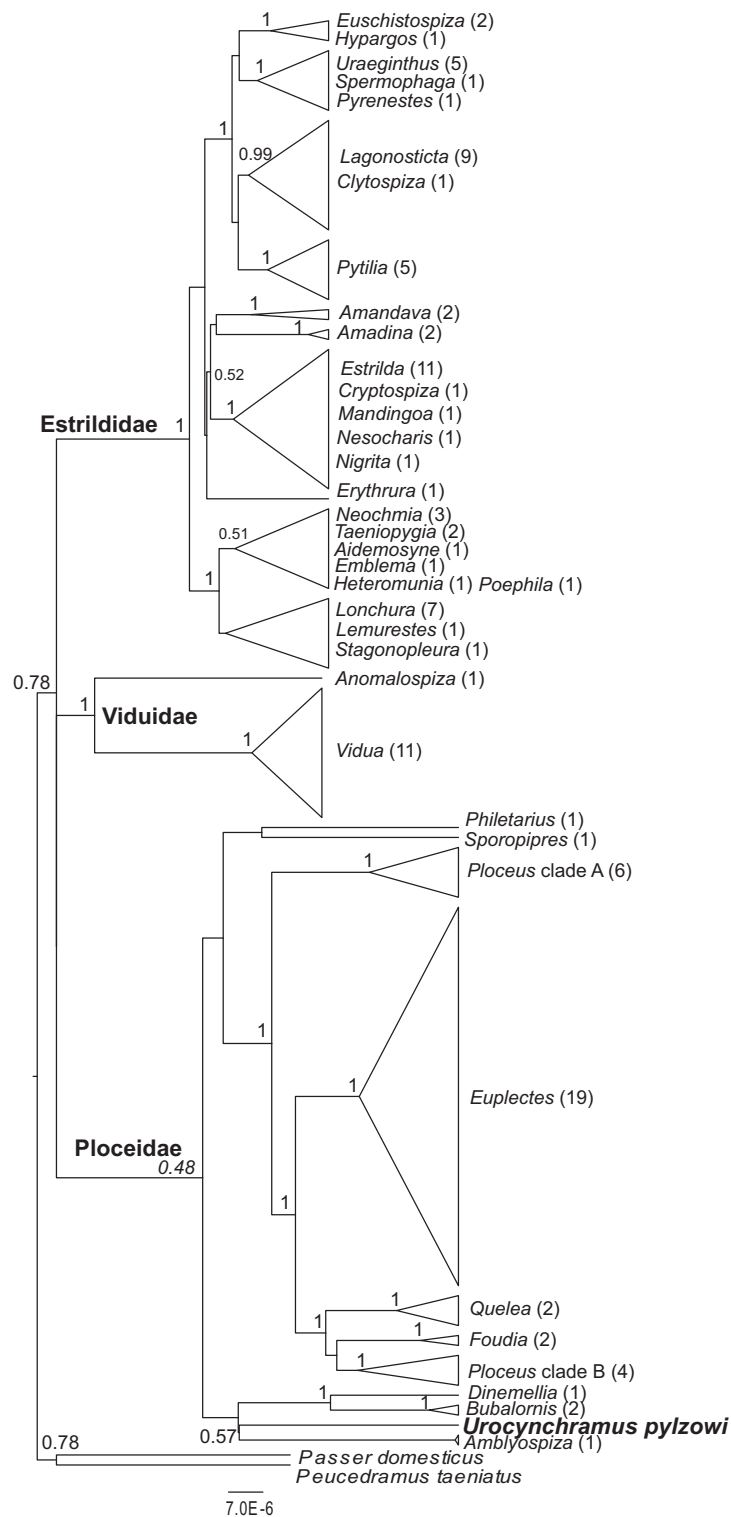


**Figure 3.** Multi-locus maximum likelihood phylogeny of Passeroidea based on six markers (cytb: 1041 bp; ND2: 1041 bp; 16S rRNA: 856 bp; myoglobin: 746 bp; ODC: 543 bp; fib7: 705 bp) inferred using RAxML. The tree was rooted with *Acanthisitta chloris* (pruned from the tree). Node support from ML bootstrap and Bayesian (BI) posterior probabilities is indicated, \*Full nodal support (ML = 100, BI = 1.0).

(1910) described the 10th primary of *U. pylzowi* as more than half the length of the ninth primary (he gives about 22 mm for its length) and he explicitly stated that the wing of *U. pylzowi* cannot be regarded as nine-primaries but rather resembles that of weavers, Ploceidae. Furthermore, Vaurie (1956: p. 35) describes the 10th primary of *U. pylzowi* as being somewhat similar in its development, although still longer, than that of the typical weavers of the genus *Ploceus*; similarly Stone (1933) also notes a similarity of the 10th primary to Ploceidae. In addition to the 10th primary, skull anatomy, e.g. the absence of an ossified inter-orbital septum, provides further arguments against a

closer relationship of *U. pylzowi* with either Emberizidae or Fringillidae (Zusi 1978).

In addition to morphology, its acoustic display behaviour strongly distinguishes *U. pylzowi* from finches and buntings. Its pipit-like display flight is most impressive and has been described in detail by Gebauer *et al.* (2006): the male starts suddenly from a bush perch, mostly when approached by a conspecific, and silently ascends at a steep angle with rapid and shallow wing beats. After reaching a height of 10–20 m, he starts singing and descends gliding with wings raised in a V-shape, the tail spread and slightly fanned upwards. The gliding back to the perch



**Figure 4.** Phylogenetic relationships of Estrildidae, Viduidae, Ploceidae and Urocynchramidae using 1041 bp from ND2. Bayesian inference of phylogeny was estimated using BEAST, partitioning by codon position. Numbers of species per genus are given in parentheses. Support from Bayesian posterior probabilities is indicated at nodes.

takes about 8–10 s, during which the male sings continuously. During the descent the long, partly reddish spread tail is a strong visual signal that can be seen well for a long distance. This display flight is unique and not shared by any finch, bunting or weaver species. A second distinct song type of *U. pylzowi* is regularly given from a perch. It differs in length and syntax from the flight song (Gebauer *et al.* 2006). The song is similar in auditory impression, and based on sonagram, to that of *Uragus sibiricus* as stated by Gebauer *et al.* (2006). Notes and frequency range are similar in both species, but statistically different in other characters. For example, verse length is shorter in *U. sibiricus* and that species does not use display flights. Song similarity to the Reed Bunting *Emberiza schoeniclus* has been suggested by Przewalski (1876), but Gebauer *et al.* (2006) suggest this may refer to certain call types, as the song of the Reed Bunting is much shorter and much more stereotyped than that of *U. pylzowi* and the syntax of both differs considerably.

The eggs of *U. pylzowi* are also peculiar and indicate an isolated position unrelated to finches and buntings. They are glossy, heavily and densely speckled on a dark olive background providing little contrast of the speckles and yielding an overall dark appearance (Schönwetter 1988, Gebauer *et al.* 2006, Dixon *et al.* 2013). In this aspect they resemble eggs of Common Nightingale *Luscinia megarhynchos* and Bluethroat *Luscinia svecica* and do not at all resemble the eggs of finches or buntings (Dixon *et al.* 2013).

Our divergence time estimates are in good concordance with the chronology of passerine evolution by Claramunt and Cracraft (2015), who suggest a late Eocene split between the sister clades Passeroidea and Bombycilloidea. Furthermore, our within-Passeroidea chronology is congruent with that proposed by Kennedy *et al.* (2012) and Barker *et al.* (2013), who both gave a tmrca of roughly 20 Ma for the basal node of their passeroid clade (comprising Fringillidae and the crown group of nine-primaried oscines). Deviations among more recent tmrca estimates within the nine-primaried passerine clade between the latter study and our results (younger tmrca of the sister clade of Fringillidae in Barker *et al.* 2013) are certainly due to the different taxon-samplings and different time-calibration strategies. Likewise, our fossil-calibration yielded a younger tmrca estimate for the Urocynchramidae/Ploceidae split

compared with the tmrca inferred from a fixed *cytb* substitution rate. Regardless of the deviations, our divergence time estimates suggest a long separation of *U. pylzowi* from its closest relatives that started possibly as early as the late Oligocene. Long-term separation and successive adaptations to extreme alpine environments might have masked its true phylogenetic relationships, as has been seen in other Tibetan endemics such as the Tibetan Ground Tit *Pseudopodoces humilis* (James *et al.* 2003, Qu *et al.* 2013). It remains open to question whether these Tibetan endemics have never diversified or whether they represent relic lineages of formerly more diverse clades (Favre *et al.* 2014, Päckert *et al.* 2015a). Nevertheless, according to our phylogenetic results *U. pylzowi* can be considered the oldest Tibetan endemic passerine bird species known to date. Placing *U. pylzowi* in a monotypic family still seems to be well justified in light of this deep genetic divergence.

This study received substantial funding from Deutsche Forschungsgemeinschaft (DFG), PA 1818/3-1. J.M. received several grants from Deutsche Ornithologen-Gesellschaft (DO-G), Gesellschaft für Tropenornithologie (GTO) and from Feldbausch-Stiftung und Wagner-Stiftung both at Fachbereich Biologie, Johannes Gutenberg-Universität Mainz, Germany. Y-H.S. received research grants from the National Natural Science Foundation of China, project No. 31272286. A. Gebauer and M. Kaiser actively participated in the relevant expedition to Qinghai. J.M. had fruitful discussions with H. Pieper on the taxonomic placement of *Urocynchramus*. Paul Jones and Axel Gebauer kindly allowed publication of their *Urocynchramus* images. We are most grateful to the following colleagues and institutions who provided sample loans from their collections: U. Johannson, Swedish Museum of Natural History, Stockholm, Sweden; Jon Fjeldså, Zoological Museum at the Natural History Museum of Denmark, Copenhagen; Sharon Birks, Burke Museum of Natural History of Culture, Seattle, USA; National Museum of Natural Sciences, Taipei, Taiwan. We thank all friends, institutions and colleagues. Finally, we would also like to express our gratitude to the editors and three anonymous referees for their valuable comments and recommendations that substantially helped to improve the manuscript.

## REFERENCES

- Badyaev, A.V. 1997. Avian life history variation along altitudinal gradients: an example with cardueline finches. *Oecologia* **111**: 365–374.
- Barker, F.K., Burns, K.J., Klicka, J., Lanyon, S.M. & Lovette, I. 2013. Going to extremes: contrasting rates of

- diversification in a recent radiation of New World passerine birds. *Syst. Biol.* **62**: 298–320.
- Benton, M.J., Donoghue, P.C. & Asher, R.J. 2009. Calibrating and constraining molecular clocks. In Hedges, S.B. & Kumar, S. (eds) *The Timetree of Life*: 35–86. Oxford: Oxford University Press.
- Brown, J.M., Hedtke, S.M., Lemmon, A.R. & Lemmon, E.M. 2009. When trees grow too long: investigating the causes of highly inaccurate Bayesian branch-length estimates. *Syst. Biol.* **59**: 145–161.
- Claramunt, S. & Cracraft, J. 2015. A new time tree reveals Earth history's imprint on the evolution of modern birds. *Evol. Ecol.* **1**: e1501005.
- Clement, P. 1999. *Finches and Sparrows*. Princeton: Princeton University Press.
- Clements, J.F., Schulenberg, T.S., Iliff, M.J., Roberson, D., Fredericks, T.A., Sullivan, B.L. & Wood, C.L. 2014. The eBird/Clements checklist of birds of the world: Version 6.9. Downloaded from <http://www.birds.cornell.edu/clements-checklist/download/> (accessed 14 February 2015).
- Collar, N.J. & Newton, I. 2010. Family Fringillidae (finches). In del Hoyo, J., Elliott, A. & Christie, D. (eds) *Handbook of the Birds of the World*, Vol. 15: 440–617. Barcelona: Lynx Edicions.
- Davis, K.E. & Page, D.R.M. 2014. Reweaving the tapestry: a supertree of birds. *PLoS Curr.* **6**: ecurrents.tol.c1af68dda7c999ed9f1e4b2d2df7a08e.
- Dickinson, E.C. & Christidis, L. 2014. *The Howard and Moore Complete Checklist of the Birds of the World*. 4th edn, Vol. 2. Eastbourne: Aves Press.
- Dixon, A., Ming, M., Ashford, C. & Stafford, P. 2013. Notes on the breeding biology of Pink-tailed Bunting *Urocynchramus pylzowi*. *BirdingASIA* **20**: 98–99.
- von Domaniewski, J. 1918. Die Stellung des *Urocynchramus pylzowi* Przew. in der Systematik. *J. Ornithol.* **66**: 421–424.
- Drummond, A.J. & Bouckaert, R.R. 2015. *Bayesian evolutionary analysis with BEAST*. Cambridge: Cambridge University Press.
- Drummond, A.J. & Rambaut, A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evol. Biol.* **7**: 214.
- Favre, A., Päckert, M., Pauls, S., Jähmig, S., Uhl, D., Michalak, I. & Muellner-Riehl, A. 2014. The role of the uplift of the Qinghai-Tibetan Plateau for the evolution of Tibetan biotas. *Biol. Rev.* **90**: 236–253.
- Gebauer, A., Kaiser, M. & Wassmann, C. 2006. Remarks on biology, vocalisations and systematics of *Urocynchramus pylzowi* Przewalski (Aves, Passeriformes). *Zootaxa* **1325**: 75–98.
- Groth, J.G. 2000. Molecular evidence for the systematic position of *Urocynchramus pylzowi*. *Auk* **117**: 787–791.
- Hackett, S.J., Kimball, R.T., Reddy, S., Bowie, R.C., Braun, E.L., Braun, M.J., Chojnowski, J.L., Cox, W.A., Han, K.L., Harshman, J., Huddleston, C.J., Marks, B.D., Miglia, K.J., Moore, W.S., Sheldon, F.H., Steadman, D.W., Witt, C.C. & Yuri, T. 2008. A phylogenomic study of birds reveals their evolutionary history. *Science* **320**: 1763–1768.
- Hall, K.S.S. 2005. Do nine-primaried passerines have nine or ten primaries? The evolution of a concept. *J. Ornithol.* **146**: 121–126.
- Hartert, E. 1910. *Die Vögel der paläarktischen Fauna*, 1st edn. Berlin: Friedländer.
- Inskipp, T., Lindsey, N. & Duckworth, W. 1996. *An annotated checklist of the birds of the Oriental region*. Sandy: Oriental Bird Club.
- James, H.F., Ericson, P.G.P., Slikas, B., Lei, F., Gill, F.B. & Olson, S.L. 2003. *Pseudopodoces humilis*, a misclassified terrestrial tit (Paridae) of the Tibetan plateau: evolutionary consequences of shifting adaptive zones. *Ibis* **145**: 185–202.
- Jetz, W., Thomas, G.H., Joy, J.B., Hartmann, K. & Mooers, A.O. 2012. The global diversity of birds in space and time. *Nature* **491**: 444–448.
- Johnson, C.L., Wjibrans, J.R., Constable, C.G., Gee, J., Staudigel, H., Tauxe, L., Forjaz, V.H. & Salgueiro, M. 1998.  $^{40}\text{Ar}/^{39}\text{Ar}$  ages and paleomagnetism of São Miguel lavas, Azores. *Earth Planet. Sci. Lett.* **160**: 637–649.
- Jönsson, K.A. & Fjeldså, J. 2006. A phylogenetic supertree of oscine passerine birds (Aves: Passeri). *Zool. Script.* **35**: 149–186.
- Kennedy, J.D., Weir, J.T., Hooper, D.M., Tietze, D.T., Martens, J. & Price, T. 2012. Ecological limits on diversification of the Himalayan core Corvoidea. *Evolution* **66**: 2599–2613.
- King, B.F. 1997. *Checklist of the Birds of Eurasia*. Vista: Ibis Publishing Company.
- Klicka, J., Johnson, K.P. & Lanyon, S.M. 2000. New World nine-primaried oscine relationships: constructing a molecular framework. *Auk* **117**: 321–336.
- Lanfear, R., Calcott, B., Ho, S.Y.W. & Guindon, S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol. Biol. Evol.* **29**: 1695–1701.
- Lerner, H.R.L., Meyer, M., James, H.F., Hofreiter, M. & Fleischer, R.C. 2011. Multilocus resolution of phylogeny and timescale in the extant adaptive radiation of Hawaiian honeycreepers. *Curr. Biol.* **21**: 1–7.
- Mayr, G. 2013. The age of the crown group of passerine birds and its evolutionary significance – molecular calibrations versus the fossil record. *Syst. Biodiv.* **11**: 7–13.
- Monroe, B.L. Jr & Sibley, C.G. 1993. *A World Checklist of Birds*. New Haven: Yale University Press.
- Päckert, M., Martens, J., Sun, Y.-H. & Tietze, D.T. 2015a. Evolutionary history of passerine birds (Aves: Passeriformes) from the Qinghai-Tibetan plateau – from a pre-Quaternary perspective to an integrative biodiversity assessment. *J. Ornithol.* **156**(Suppl.): 355–365.
- Päckert, M., Sun, Y.-H., Strutzenberger, P., Valchuk, O., Tietze, D.T. & Martens, J. 2015b. Phylogenetic relationships of endemic bunting species (Aves, Passeriformes, Emberizidae, *Emberiza koslowi*) from the eastern Qinghai-Tibetan Plateau. *Vert. Zool.* **65**: 135–150.
- Parkes, K.C. 2001. Finches and Sparrows. *Wilson Bull.* **113**: 120–121.
- Peters, J.L. 1968. *Check-list of Birds of the World*, 14th edn. Cambridge, MA: Museum of Comparative Biology.
- Prager, M., Johansson, E.I. & Andersson, S. 2008. A molecular phylogeny of the African widowbirds and bishops, *Euplectes* spp. (Aves: Passeridae: Ploceinae). *Mol. Phylogenet. Evol.* **46**: 290–302.
- Przewalski, N.M. 1876. Mongolija i strana Tangutov: Trechletnee putešestvie v vostochnoj nagornoj Azii N. Príeval'skago, Podpolkovnika Generalnago Staba/Nikolaj Michajlovic Przeval'skij T. 2. Russkago geograficheskago Obščestva U. S. Balašev Sanktpeterburg.

- Qu, Y., Zhao, H., Han, N., Zhou, G., Song, G., Gao, B., Tian, S., Zhang, J., Zhang, R., Meng, X., Zhang, Y., Zhang, Y., Zhu, X., Wang, W., Lambert, D., Ericson, P.G.P., Subramanian, S., Yeung, C., Zhu, H., Jiang, Z., Li, R. & Lei, F. 2013. Ground Tit genome reveals avian adaptation to living at high altitudes in the Tibetan plateau. *Nat. Commun.* **4**: 2071.
- Ronquist, F. & Huelsenbeck, J.P. 2003. MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**: 1572–1574.
- Schäfer, E. 1938. Ornithologische Ergebnisse zweier Forschungsreisen nach Tibet. *J. Ornithol.* **86**(Suppl.): 1–349.
- Schäfer, E. & Meyer de Schauensee, R. 1938. Zoological results of the second Dolan expedition to western China and eastern Tibet, 1934–1936, Part II, Birds. *Proc. Acad. Natl Sci. Philadelphia* **90**: 185–260.
- Schönwetter, M. 1988. Weitere Nachträge zu Band 2 “Handbuch der Oologie”. In Meise, W. (ed.) *Handbuch der Oologie*, Lieferung 44. Berlin: Akademie-Verlag.
- Silvestro, D. & Michalak, I. 2012. raxmlGUI: a graphical front-end for RAxML. *Org. Divers. Evol.* **12**: 335–337.
- Sorenson, M.D. & Payne, R.D. 2001. A single ancient origin of brood parasitism in African finches: implications for host-parasite coevolution. *Evolution* **55**: 2550–2567.
- Sorenson, M.D., Balakrishnan, C.N. & Payne, R.B. 2004. Clade-limited colonization in brood parasitic finches (*Vidua* spp.). *Syst. Biol.* **53**: 140–153.
- Stamatakis, A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* **22**: 2688–2690.
- Steadman, D.W. 1981. A re-examination of *Palaeostruthus hatcheri* (Shufeldt), a late Miocene sparrow from Kansas. *J. Vertebr. Paleontol.* **1**: 171–173.
- Steadman, D.W. & McKittrick, M.C. 1982. A Pliocene bunting from Chihuahua, Mexico. *Condor* **84**: 240–241.
- Stone, W. 1933. Zoological results of the Dolan West China expedition of 1931. Part 1, Birds. *Proc. Acad. Natl Sci. Philadelphia* **85**: 165–222.
- Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony method. *Mol. Biol. Evol.* **28**: 2731–2739.
- Tietze, D.T., Päckert, M., Martens, J., Lehmann, H. & Sun, Y.-H. 2013. Complete phylogeny and historical biogeography of true rosefinches (Aves: *Carpodacus*). *Zool. J. Linn. Soc.* **169**: 215–234.
- Töpfer, T., Haring, E., Birkhead, T.R., Lopes, R.J., Liu Severinghaus, L., Martens, J. & Päckert, M. 2011. A molecular phylogeny of bullfinches *Pyrrhula* Brisson, 1760 (Aves: Fringillidae). *Mol. Phylogenet. Evol.* **58**: 271–282.
- Tugarinow, A. & Stegmann, B. 1929. *Urocynchramus pylzowi coloratus* subsp. nova. *Ornithol. Monatsber.* **37**: 116–117.
- Vaurie, C. 1956. Systematic notes on Palearctic birds, no. 20. Fringillidae: the genera *Leucosticte*, *Rhodopechys*, *Carpodacus*, *Pinicola*, *Loxia*, *Uragus*, *Urocynchramus* and *Propyrrhula*. *Am. Mus. Novit.* **1786**: 34–36.
- Vaurie, C. 1959. *The Birds of the Palearctic Fauna, a Systematic Reference, Order Passeriformes*: 671–709.
- Vaurie, C. 1972. *Tibet and its Birds*. London: H.F. & G. Witherby.
- Weir, J.T. & Schluter, D. 2008. Calibrating the avian molecular clock. *Mol. Ecol.* **17**: 2321–2328.
- Wolters, H.E. 1975–1982. *Die Vogelarten der Erde*. Hamburg: Paul Parey.
- Yang, S.J., Lei, F.M. & Yin, Z.H. 2006. Molecular phylogeny of rosefinches and rose bunting (Passeriformes, Fringillidae, Urocynchramidae). *Act. Zool. Sin.* **31**: 453–458.
- Yuri, T. & Mindell, D.P. 2002. Molecular phylogenetic analysis of Fringillidae, ‘New World nine-primaried oscines’ (Aves: Passeriformes). *Mol. Phylogenet. Evol.* **23**: 229–243.
- Zuccon, D., Prys-Jones, R., Rasmussen, P. & Ericson, P. 2012. The phylogenetic relationships and generic limits of finches (Fringillidae). *Mol. Phylogenet. Evol.* **62**: 581–596.
- Zusi, R.L. 1978. The interorbital septum in cardueline finches. *Bull. Br. Ornithol. Club* **98**: 5–10.

Received 8 December 2015;  
revision accepted 20 April 2016.  
Associate Editor: Martin Collinson.

## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Multi-locus phylogeny of Passeroidea based on six markers with all 100 taxa shown (cytb: 1041 bp; ND2: 1041 bp; 16S rRNA: 856 bp; myoglobin: 746 bp; ODC: 543 bp; fib7: 705 bp). Support from posterior probabilities is indicated at nodes; grey boxes indicate constraint nodes, numbers refer to the calibration points in Table 1 (except node 1 which is the root of the tree, the root taxon *Acanthisitta chloris* was pruned from the tree).

**Figure S2.** Phylogeny of Passeroidea based on (a) three mitochondrial genes (cytb, ND2, 16S rRNA) and (b) three nuclear introns (myo, ODC, fib7); likelihood trees were reconstructed with RAxML including bootstrap support and support from posterior probabilities indicated at nodes, full node support (BI = 1.0, ML = 100) indicated by an asterisk; – node in the Bayesian tree differing from the RAxML tree; the trees were rooted with *Acanthisitta chloris* (pruned from the trees).

**Table S1.** Material and sequences used for phylogenetic reconstructions.

**Table S2.** ESS values for all model parameters resulting from Bayesian inference of phylogeny using BEAST after 50 000 000 generations.

**Table S3.** ESS values for all model parameters resulting from Bayesian inference of phylogeny using MrBayes after 10 000 000 generations.