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Molecular Evidence for the Systematic Position of *Urocynchramus pylzowi*

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Urocynchramus pylzowi, known variously as Przewalski's Rosefinch, Pink-tailed Rosefinch, and Pink-tailed Bunting, is a finch-like bird of uncertain systematic affinities that is endemic to the mountains of western China. In many linear classifications (e.g. Sharpe 1909, Vaurie 1959, Peters 1968, Morony et al. 1975), it is listed next to the Long-tailed Rosefinch (*Uragus sibiricus*) in the Carduelinae, to which it is similar in size and several plumage characters. *Urocynchramus* has a much thinner bill than *Uragus,* but both species have disproportionately long tails compared with the rest of the cardueline finches, and the males are bright pink on the throat, breast, and belly. Females of both species are streaked, sparrow-like, and lack the bright pink coloration. *Uragus* and *Urocynchramus* also are exclusively Asiatic and live at high elevations near the center of species diversity of rosefinches in the genus *Carpodacus,* to which a close relationship with *Uragus* has never been questioned. The few available accounts of its ecology and behavior (Przewalski 1876 [recounted *in* Vaurie 1956], Schäfer 1938, B. King pers. comm.) state that the habitat associations, patterns of flight, and postures of *Urocynchramus* are similar to those of *Uragus.*

Despite its resemblance to *Uragus, Urocynchramus* frequently has been classified as an emberizid bunting (Sharpe 1888, Dresser 1902, Sibley and Monroe 1990). Przewalski (1876), who first collected and described the bird, noted the similarity of its bill structure to that of buntings and regarded its song as similar to that of the bunting *Cynchramus* (=*Emberiza*) *schoeniclus.* Sushkin (1927) considered the external anatomy and structure of the horny palette of *Urocynchramus* to be most like that of buntings. Zusi (1978) found that features of its interorbital septum were unlike those of cardueline finches, but he could neither confirm nor reject its possible relationship to buntings.

The most perplexing aspect of *Urocynchramus* is the condition of its outer primary (P10), which is about two-thirds the length of P9. Although a well-developed P10 such as this is characteristic of many oscine families, all fringillids and emberizids are "nine-primaried" in that P10 is vestigial. Although the nine-primaried condition has been derived often in oscines, no taxon within a nine-primaried group is known to have undergone a character reversal to secondarily derive the "ten-primaried" state (see Groth 1998). Paynter (*in* Peters 1968) doubted the inclusion of *Urocynchramus* with carduelines because of its ten-primaried condition and because the red coloration in its tail set it apart from rosefinches. The ten-primaried state prompted at least one author (Domaniewski 1918) to argue for classifying the bird in its own family, the Urocynchramidae.

Placing *Urocynchramus* among other oscine families has been frustrated by the absence of both skeletal and fluid-preserved specimens of this bird. Information on basic life-history attributes such as mating behavior, nest structure, and vocalizations generally is lacking, adding to the difficulties of producing convincing arguments for its taxonomic assignment. Nevertheless, it is now possible to extract DNA from dried museum skins that are decades old; these techniques allow acquisition of numerous characters that are useful in analyzing systematic relationships of problematic taxa when fresh biological material is not available. A prerequisite for such an analysis is comparable DNA sequence information from an appropriate set of other taxa.

Phylogenetic analyses of complete mitochondrial cytochrome-*b* (cyt *b*) genes show a well-supported separation between cardueline finches and emberizid sparrows (Groth 1998). If *Urocynchramus* is either a cardueline or an emberizid, then its cyt-*b* sequence should allow discrimination between these two possibilities. My analysis (Groth 1998) also included a wide range of other oscine groups, thus providing a broad phylogenetic context for placement of *Urocynchramus* if it is neither a cardueline nor an emberizid.

Methods.—I extracted DNA from a 1-mm^2 flake of toe skin from an adult male (American Museum of Natural History 782951; collected by Ernst Shäfer at Kham, western China, 18 October 1934) using the methods of Mundy et al. (1997). The entire cyt-*b* gene was amplified in five fragments using a combination of 10 primers (Table 1) designed for oscine birds. Five of the primers had been used in other studies, but I obtained fresh stocks from the manufacturer (Operon Technologies, Inc.) as a precaution against contamination. Further precautions included performing DNA extraction and assembly of reaction tubes in an isolated room away from the main laboratory using dedicated "ancient DNA" pipettors and aerosol-barrier pipet tips. I used Amplitaq Gold kits (Applied Biosystems) according to manufacturers' instructions for amplification reactions, but they were scaled down to total volumes of 10 μL. All kit components (except enzyme), water, and disposable

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TABLE 1. List of primers used for *Urocynchramus* cytochrome-*b* sequencing.

Primer[a]	Sequence[b]
L14851[c]	CCTACTTAGGATCATTCGCCCT
L15068	CTAGCCATACACTACACAGCAGA
L15410[c]	TGAGGAGGATTCTCAGTAGACAA
L15656[c]	CCAAACCTACTACTAGGAGACCCAGA
L15848	CCAAACTACGATCAATAACCTTCCG
H15103	TGCCGAGACGTACAATTCGGCTGA
H15460	ATCGTAGGCCTCACACTAGTCCAC
H15710[c]	GGCATAGGCGAATAGAAAGTATC
H15934	GGCTAGTTGGCCGATGATGATGAA
H16141[c]	AACATACCAGCTTTGGGAG

[a] L and H refer to extension products on the light and heavy strands, respectively. Numbers indicate the position of the 3′ base of the primer according to the system used for *Gallus* (Desjardins and Morais 1990).

[b] Primers are listed in the 5′ to 3′ direction.

[c] Previously cited by Groth (1998).

plastics were irradiated with UV light before use. Thermocycling was done in a Perkin-Elmer ABI 9600 machine with an initial hold at 94°C for 10 min followed by 40 cycles consisting of 95°C for 20 s, 52°C for 20 s, and 72°C for 30 s. To test for contamination, I also assembled negative control reactions containing all components except *Urocynchramus* DNA. Reaction products were then processed further and sequenced on an ABI 377 automated sequencer according to methods in Groth (1998).

I assembled and edited the sequences using Sequencher software (Gene Codes). After scoring all bases, which included the entire cyt-*b* gene, I added it to the cyt-*b* data of 53 other oscines (1,143 base pairs each; see Groth 1998). This data set included four putatively divergent lineages (genera) of cardueline finches, one Hawaiian honeycreeper (*Himatione*), five emberizid genera of which one (*Melophus*) is Asiatic, and nine other members of the "larger" Emberizidae (as formerly recognized by AOU [1983], which I refer to as the superfamily Emberizoidea). One additional entire cyt-*b* sequence, that from frozen liver tissue of *Uragus sibiricus* (Russia, Republic of Buryatia, 55 km W and 30 km S of Ulan-Ude, 16 June 1993; University of Washington Burke Museum CDS 4888), was generated using the laboratory methods of Groth (1998) and added to the data set for a total of 55 taxa compared. I analyzed the data using both parsimony- and distance-based methods in PAUP* version 4.0b2 (Swofford 1999). Following arguments I made earlier (Groth 1998), I eliminated transition (A↔G and C↔T) changes at third positions of codons (using the search and replace function in MacClade version 3.0.6; Maddison and Maddison 1992) to reduce "noise" due to saturation effects. To find the shortest parsimony trees, I conducted 500 heuristic searches with random taxon addition and TBR branch swapping. Nodal support was estimated with 500 bootstrap (Felsenstein 1985) replicates, with each replicate containing five separate heuristic searches with random-taxon addition. I depicted distance relationships with a neighbor-joining tree (Saitou and Nei 1987) generated using modified genetic distances (third-position transitions eliminated). Trees were rooted using data from four members of the Corvida (sensu Sibley and Monroe 1990).

Results and discussion.—Several observations suggest that the resulting sequence was authentic *Urocynchramus* cyt *b* and not a contaminant. In experiments using DNA from museum skins, all negative control reactions were blank when visualized on EtBr-stained agarose gels, whereas reactions with *Urocynchramus* DNA were positive. Nevertheless, even though the negative control reactions showed no amplification products, it is possible that the extract from *Urocynchramus* was contaminated with DNA from another species. If this were the case, "double" sequences, containing ambiguous base calls from a mixture of both *Urocynchramus* and the contaminant, might have resulted for some fragments. However, double sequences did not occur. Further support for the authenticity of the sequence was that the five separate target DNA fragments, ranging in size from 277 to 391 bases (excluding primer sites), showed no sites in disagreement within areas of overlap, suggesting that only one source was responsible for all amplification. It would have been highly unlikely for the same putative contaminant to have been amplified cleanly for all five fragments to the exclusion of any amplification from the *Urocynchramus* DNA, which certainly predominated in the extract. Similarly, I doubt that the sequence was that of a nuclear pseudogene because it is unlikely that all five pairs of primers preferentially amplified a nuclear product to the exclusion of the much more abundant mitochondrial cyt *b* in the DNA extract. When compared with all other cyt-*b* sequences I had previously generated, the final presumptive *Urocynchramus* sequence was unique and highly divergent from all others.

Parsimony analysis found five equally short trees (length = 2,290; not shown), all of which showed monophyly for both the Fringillidae and the "emberizoids." Not one of the trees linked *Urocynchramus* within or as the sister group of these two clades. These results clearly show that no phylogenetic relationship exists between *Urocynchramus* and *Uragus*. Instead, the branch to *Urocynchramus* originated from a more basal position in all trees, whereas *Uragus* was embedded within the other cardueline finches. A strict consensus of the five trees (not shown) placed *Urocynchramus* within a clade of "passeroid" (sensu Sibley and Monroe 1990) taxa; that is, in no trees was it linked to "sylvioids" (*Regulus, Abroscopus, Garrulax,* and *Zosterops*), larks (Alaudidae), or members of the Corvida.

Monophyly of all families of finches and allies was

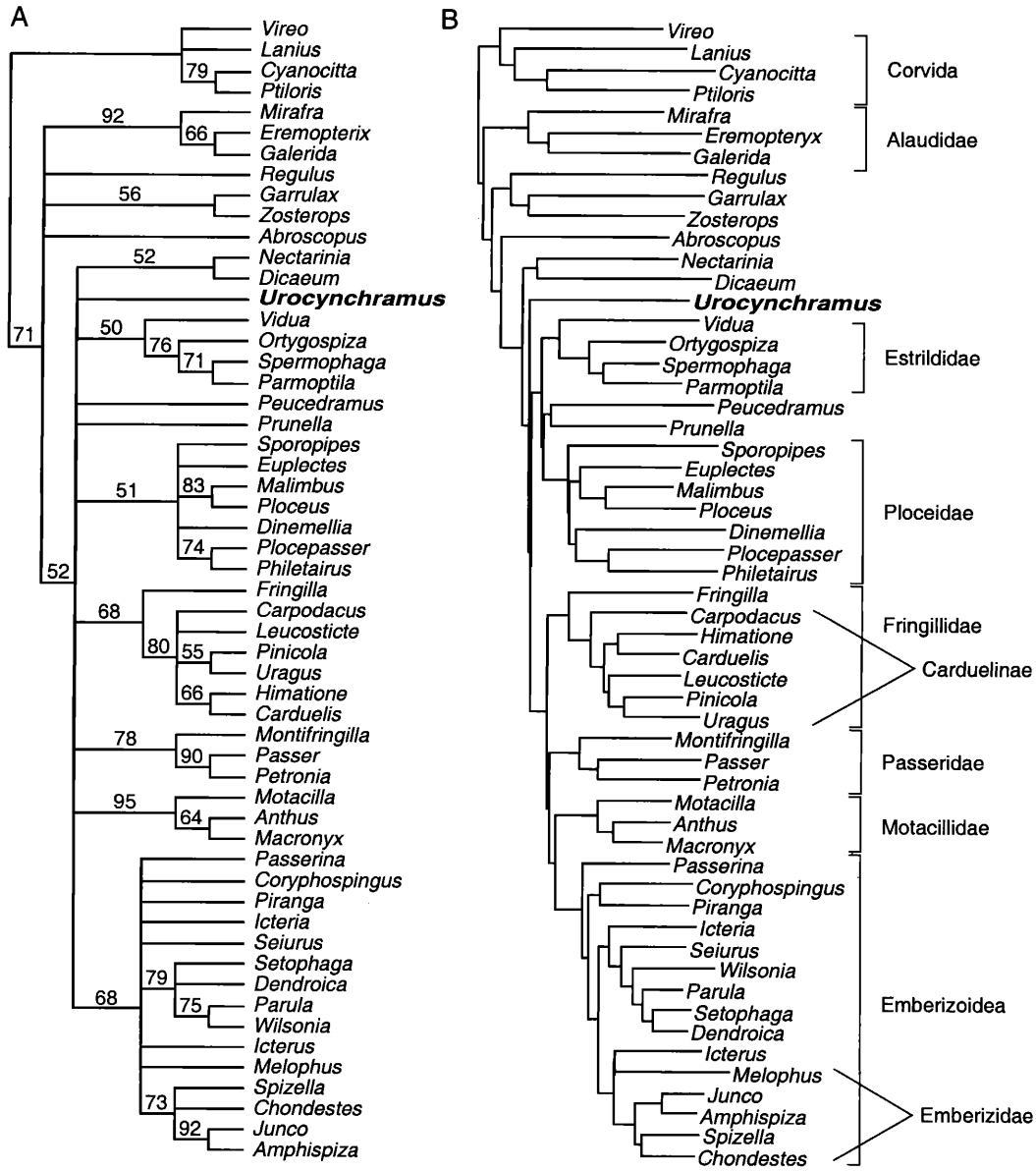

FIG. 1. Phylogenetic trees showing relationships of *Urocynchramus* to other finches and allies ("Emberizoidea" = Emberizidae of AOU1983). (A) Majority-rule tree showing only those nodes achieving a 50% or higher bootstrap value using parsimony. Numbers on branches are bootstrap percentages. (B) Neighbor-joining tree, in which branch lengths are drawn proportional to genetic distances.

corroborated by bootstrap analysis (Fig. 1A), but no support occurred for linking *Urocynchramus* to any of these families. Although it is clear that entire cyt-*b* sequences for this set of taxa are inadequate for resolving basal relationships among these families, and for assigning a sister group to *Urocynchramus* with robust estimates of nodal support, it is still possible to address the hypotheses that *Urocynchramus* is a cardueline finch or an emberizid sparrow. Tree-building using genetic distances (Fig. 1B) placed *Urocynchramus* on a long, basal branch in the passeroid assemblage and not near any other taxon in the analysis. The genetic distinctiveness of *Urocynchramus* can be further described using transversion distances, which have been considered to evolve in a clock-like manner (Brown et al. 1982). Pairwise

transversion distances between *Urocynchramus* and the fringillids ranged from 6.22 to 7.27%, and distances between *Urocynchramus* and "emberizoids" ranged from 6.04 to 7.09%. In contrast, the maximum pairwise transversion distance was 5.34% (4.55% between carduelines) between any two fringillids, and 5.07% (3.32% between emberizids) between any two "emberizoids." The lowest transversion distance between *Urocynchramus* and any other species that I analyzed was with *Parmoptila* (5.60%), a taxon clearly supported to be within the Estrildidae (and with only 2.45% transversion distance from *Spermophaga*).

Given that cardueline and emberizid finches can be eliminated as candidates for the nearest relatives of *Urocynchramus*, it is necessary to consider the remaining groups of finches and allies. The Passeridae and Motacillidae consist entirely of nine-primaried species; *Urocynchramus* is genetically divergent from all sampled members of both groups (6.13 to 7.01% transversion distance), and it did not link to either family in any of the phylogenetic trees. Alternatively, both the Ploceidae and the Estrildidae consist mainly of ten-primaried taxa, yet the mitochondrial DNA evidence does not support a direct sister-group relationship between *Urocynchramus* and either of these families.

The only ten-primaried finch-like groups not already discussed are sunbirds (Nectariniidae) and accentors (Prunellidae). *Urocynchramus* clearly is unlike sunbirds in external morphology, and the mitochondrial evidence suggests no reason to consider the two taxa related. Some accentors, on the other hand, superficially are rather similar to *Urocynchramus* in body size, bill shape, and relative length of the tail. Additionally, the Prunellidae (consisting of a single genus, *Prunella*) is exclusively Palearctic, and the center of species diversity is near the range of *Urocynchramus*. Nevertheless, *Urocynchramus* and *Prunella* showed a high (6.04%) transversion distance, and the two taxa did not link as monophyletic in any of the shortest parsimony trees. Furthermore, no prunellids possess the bright pink body plumage characteristic of male *Urocynchramus*.

Phylogenetic analysis of mitochondrial sequences suggests that *Urocynchramus* is no more closely related to any single family than it is to several others. In other words, the sister group of *Urocynchramus* likely is a clade consisting of two or more oscine families. The best summary of the present genetic data is that *Urocynchramus* is a relict member of a lineage that is as old as, or older than, other families of finches. I agree with Domaniewski (1918) and Wolters (1979) that *Urocynchramus* belongs in its own family, the Urocynchramidae.

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Anatomical and Nutritional Adaptations of the Speckled Mousebird (*Colius striatus*)

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Folivory is a rare phenomenon in birds that has evolved independently in several lineages. It has been reported in ratites (Withers 1983, Herd and Dawson 1984), anatids (Buchsbaum et al. 1986, Dawson et al. 1989), ptarmigan (Gasaway et al. 1975), the Kakapo (*Strigops habroptilus*; Oliver 1955, Powlesland et al. 1992), and some species of *Saltator* (Bosque et al. 1999). The Hoatzin (*Opisthocomus hoazin*) is unique among birds in being the only documented foregut fermenter and the only obligate avian folivore (Grajal et al. 1989). Although folivory is common in mammals (Chivers and Langer 1994), the evolution of folivory in birds is constrained by body mass and the high mass-specific energy requirements of endothermy and flight, despite the apparent unlimited supply of fresh foliage in nature. Klasing (1998) defines an avian folivore as one that concentrates on leaves; however, the categories from an obligate folivore to a facultative folivore are poorly defined.

Folivory is associated with reduced food quality (Chivers and Langer 1994). Digestion costs for fermenting folivores are high owing to gut specializations (i.e. a fermentation chamber) and associated bacterial microflora for the breakdown of cellulose and release of volatile fatty acids (VFA; VanSoest 1983). Energy from a folivorous diet is released slowly, requiring a behavioral and physiological lifestyle that minimizes energy expenditure. Because energy requirements per unit body mass increase with decreasing body mass, small folivores have proportionally higher metabolic requirements relative to their gut capacity than do large folivores (Demment and VanSoest 1985). Consequently, small avian folivores have greater problems of energy acquisition than their larger avian counterparts and thus are expected to be rare.

Mousebirds, order Coliiformes, comprise six species that are endemic to sub-Saharan Africa (Maclean 1993). Despite the paucity of species, mousebirds are remarkably successful and have radiated into many habitats in Africa, occurring from harsh desert to moist savanna. The folivorous habits of these birds were first observed in the Speckled Mousebird (*Colius striatus*; Rowan 1967), although peculiarities of their thermoregulatory ability were noticed prior to this (McAtee 1947). Body temperatures of mousebirds are correlated with ambient temperature fluctuations, which is a putative reason for their nocturnal huddling behavior (Rowan 1967). Average body mass of each species is about 50 g, making mousebirds among the smallest of folivorous birds. Consequently, we investigated their gastrointestinal tract and digestive physiology and suggest how adaptations in these traits permit the combination of small size and folivory in birds. To determine the extent of morphological and physiological adaptations, we made outgroup comparisons with the Purple-crested Turaco (*Tauraco porphyreolophus*), which is a large forest frugivore, and with the Hoatzin.

Methods.—We collected 13 Speckled Mousebirds from Creighton (30°02'S, 29°46'E) and Pietermaritzburg (29°36'S, 30°24'E), South Africa, in May 1994

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