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PHYLOGENETIC PLACEMENT OF THE PO'OULI, *MELAMPROSOPS PHAEOSOMA*, BASED ON MITOCHONDRIAL DNA SEQUENCE AND OSTEOLOGICAL CHARACTERS

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Abstract. The Po'ouli (*Melamprosops phaeosoma*) is a small oscine songbird first discovered on Maui in the early 1970s and originally described as a member of the Drepanidini (Hawaiian honeycreepers). A recent study suggested that the Po'ouli may not be a drepanidine because it lacks most of a small set of drepanidine synapomorphies (e.g., specialized tongue morphology and distinctive odor). We conducted phylogenetic analyses of the Po'ouli and a number of drepanidine and potentially related songbird taxa. Our character sets included mitochondrial DNA sequences (obtained for *Melamprosops* via PCR of DNA isolated from museum specimens) and osteological characters. Analyses support the placement of the Po'ouli within the drepanidine clade, although the position of the Po'ouli within the clade is not strongly supported by either data set. Our results indicate that the Po'ouli is relatively distinct phylogenetically among drepanidines. If a goal of biodiversity conservation is to retain as much genetic diversity as possible then the Po'ouli should be considered a species of very high priority for conservation efforts.

Key Words: ancient DNA; Drepanidini; *Melamprosops phaeosoma*; mitochondrial DNA; osteology; phylogeny; Po'ouli.

In 1973 a new genus and species of Hawaiian bird was discovered by a group of student researchers in a small area of rainforest on the north slope of Haleakalā Volcano on Maui. It was described from two collected specimens as the first new, living species of Hawaiian honeycreeper (Drepanidini) to be found in over 50 years (Casey and Jacobi 1974). Later, however, doubts arose concerning whether this small, brown, snail-eating bird is a drepanidine or some other type of songbird (Pratt 1992a). It was given the scientific name *Melamprosops phaesoma,* and the common Hawaiian name Po'ouli (which means "black-faced" in reference to its prominent black mask). The Po'ouli is now on the verge of extinction. Recent and intensive efforts to locate the species has resulted in detection (and marking) of only three individuals (S. Reilly and M. Collins, pers. comm.; Reynolds et al. *this volume*). It is possible that this number represents the entire living population for the species.

Although the Po'ouli differs in morphology, behavior and ecology from other living Hawaiian birds (Pratt et al. 1997b), its phylogenetic uniqueness and closest relatives remain uncertain (Bock 1978, Pratt 1992a). According to Pratt (1992a), *Melamprosops* completely lacks the few synapomorphies that define the Drepanidini, most notably the unique musty odor and specialized tongue characteristics. It also differs from all known drepanidines in plumage color and pattern, bill morphology, vocalizations, diet (i.e., specialization on snails), and other aspects of behavior (Pratt 1992a). Knowledge about the relationships and phylogenetic uniqueness of the Po'ouli will help in deciding how much effort should be expended to recover the species (Faith 1992, Krajewski 1994). Here we present cladistic analyses of mitochondrial DNA sequences and skeletal morphology that indicate that this troubling (and troubled) little bird is a Hawaiian honeycreeper, albeit an extremely distinctive one.

METHODS

SAMPLED TAXA

We compared DNA and skeletal characters of *Melamprosops* to a sampling of taxa from within the Drepanidini, Carduelini, Fringillini, Emberizinae, and other outgroups. Common and scientific names of North American and Hawaiian taxa follow the AOU Checklist (1998). Common and scientific names of other taxa, and subfamily classifications, are from Monroe and Sibley (1993).

Drepanidini analyzed for mtDNA sequence or osteology (see Figs. 1 and 2) include Nihoa Finch, *Telespiza ultima*; Laysan Finch, *T. cantans*; Palila, *Loxioides bailleui*; 'Ō'ū, *Psittirostra psittacea*; Lāna'i Hookbill, *Dysmorodrepanis munroi*; Maui Parrotbill, *Pseudonestor xanthophrys*; Kaua'i Creeper, *Oreomystis bairdi*; Hawai'i Creeper, *O. mana*; Maui 'Alauahio, *Paroreomyza montana*; 'Akeke'e, *Loxops caeruleirostris*; 'Ākepa, *L. coccineus*; 'Akiapōlā'au, *Hemignathus wilsoni*; Hawai'i 'Akialoa, *H. obscurus*; 'Anianiau, *H. parvus*; Kaua'i 'Amakihi, *H. kauaiensis*; O'ahu 'Amakihi, *H. flavus*; Maui 'Amakihi, *H. virens wilsoni*; Hawai'i 'Amakihi, *H. v. virens*; 'I'iwi, *Vestiaria coccinea*; Hawai'i Mamo, *Drepanis pacifica*; 'Apapane, *Himatione sanguinea*; and 'Ākohekohe, *Palmeria dolei.*

Carduelini analyzed include the White-browed Rosefinch (*Carpodacus thura,* Genbank number

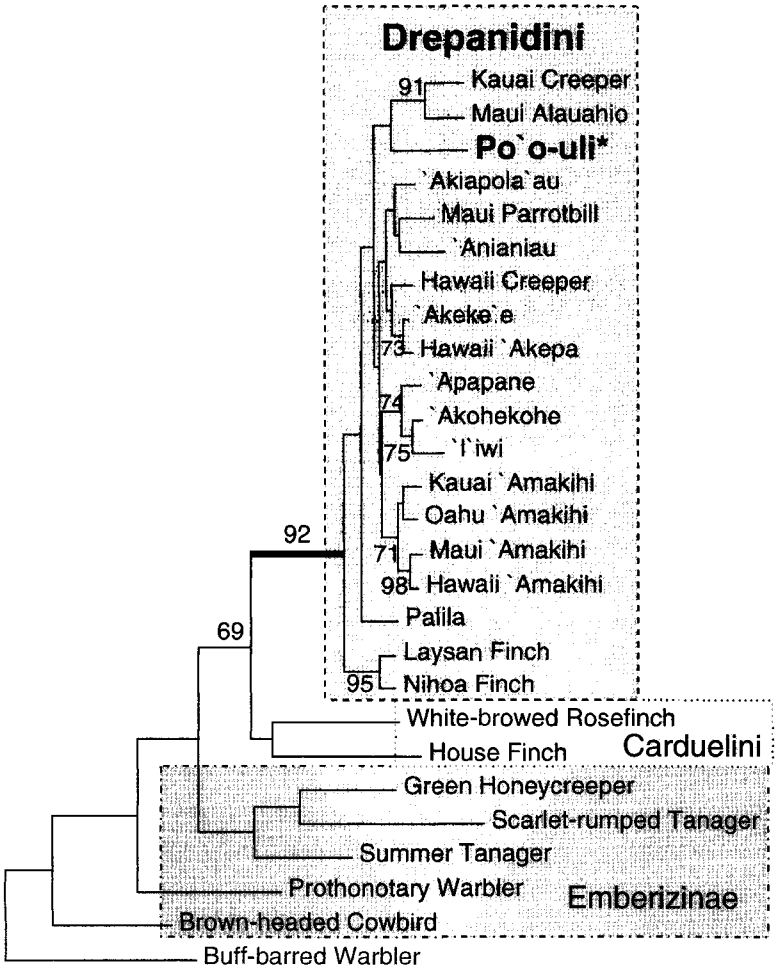

FIGURE 1. Phylogenetic tree constructed using a maximum parsimony criterion from mitochondrial DNA cytochrome *b* sequences. The phylogram is one of two maximum parsimony trees of (weighted) length 1255 and CI of 0.53. The numbers at particular nodes are the percentage of trees containing the node following a 500 repetition bootstrap. Nodes with percentages below 50% are not noted. These nodes are assumed to be unresolved and their branches collapse to a polytomy. See Methods for scientific names of taxa exhibited here.

AF015765), House Finch (*C. mexicanus*; Fleischer et al. 1998), Common Rosefinch (*C. erythrinus*), Purple Finch (*C. purpureus*), Spot-winged Grosbeak (*Mycerobas melanozanthos*), Evening Grosbeak (*Hesperiphona vespertina*), Desert Finch (*Rhodopechys obsoleta*), Golden-winged Grosbeak (*Rhynchostruthus socotranus*), European Greenfinch (*Carduelis chloris*), Pine Siskin (*C. pinus*) Red Crossbill (*Loxia curvirostra*), Yellow-fronted Canary (*Serinus mozambicus*), Grey-headed Bullfinch (*Pyrrhula erythraca*), Pine Grosbeak (*Pinicola enucleator*), and Asian Rosy Finch (*Leucosticte arctoa*). The Common Chaffinch (*Fringilla coelebs*) is a fringilline outgroup.

Emberizines include the Green Honeycreeper (*Chlorophanes spiza*; Fleischer et al. 1998), Scarlet-rumped Tanager (*Ramphocelus passerinii*; U15717), Summer Tanager (*Piranga rubra*; U15725), Prothonotary Warbler (*Protonotaria citrea*; this study), Brown-headed Cowbird (*Molothrus ater*; this study), Northern Cardinal (*Cardinalis cardinalis*), Black-and-white Warbler (*Mniotilta varia*), Vesper Sparrow (*Pooecetes gramineus*), White-lined Tanager (*Tachyphonus rufus*), Red-winged Blackbird (*Agelaius phoeniceus*), and Saffron Finch (*Sicalis flaveola*). Outgroups are the House Sparrow (*Passer domesticus*) and the Buff-barred Warbler (*Phylloscopus pulcher*; Y10732).

Mitochondrial DNA

DNA was isolated from samples taken from the only two *Melamprosops* museum specimens that exist. The tip of one small secondary feather was removed from the B. P. Bishop Museum specimen (holotype: BBM-X147112; under the care of C. Kishinami and A. Allison), and a small piece of skin from the ventral open-

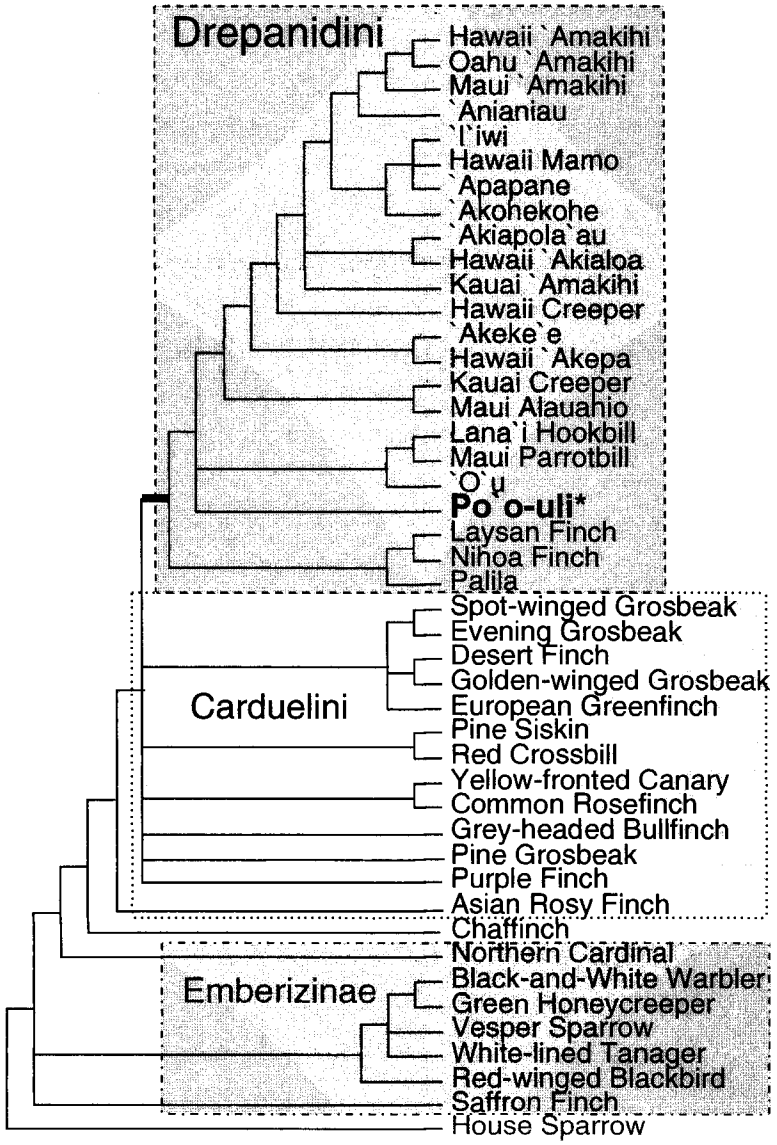

FIGURE 2. Phylogenetic tree constructed using a maximum parsimony criterion from a matrix of osteological characters. A strict consensus of 128 optimal trees found by repeated random searches of these data (500 replicates, closest addition sequence with ten trees held at each step, initial tree improved upon with TBR branch swapping; optimal tree length 286 steps). See Methods for scientific names of species included in the tree.

ing was taken from the American Museum of Natural History specimen (paratype: AMNH-810456; under the care of G. Barrowclough). Museum specimen DNA was isolated in a small laboratory dedicated to ancient DNA analyses using "ancient DNA" procedures (e.g., Cooper et al. 1996, Paxinos et al. 1997). Modern DNA analyses were conducted in a laboratory separated by >500 m from our ancient laboratory. Briefly, DNA was isolated by digesting skin or feather pulp overnight at 55° C in a DTT-SDS-EDTA buffer with proteinase K, followed by phenol and chloroform extractions and centrifugal dialysis to remove buffer and other solutes (as in Paxinos et al. 1997).

We amplified and sequenced two regions of mtDNA from the museum and modern specimens (Fig. 1) using the polymerase chain reaction and specific primers: (1) 675 bp of the Cytochrome *b* (Cyt *b*) gene in two overlapping pieces (see Fleischer et al. 1998); and (2) 224 bases of the 5′ end of the mitochondrial control region (CR; Tarr 1995). Cyt *b* and CR sequences were also obtained for some non-drepanidine songbird species from Genbank (see Fig. 1). The Cyt *b* sequence was

amplified only from the AMNH specimen, and analyzed with 18 other drepanidine taxa as reported in Fleischer et al. (1998). The CR segment was amplified from the BPBM specimen only, and from an additional 6 drepanidine species. PCR controls were negative (i.e., no apparent product produced) for the study skin amplifications for both Cyt *b* and CR. Sequences were produced either manually as in Fleischer et al (1998) or on an ABI-373 automated DNA sequencer as in Greenberg et al. (1998), and were aligned with Sequencher 3.0. Phylogenetic reconstructions and other analyses utilized PAUP*4.0d64 (D. Swofford, pers. comm.) and MacClade 3.01 (Maddison and Maddison 1992), and are described in the results section below.

Osteology

A subset of data from a separate study of cranial osteology and phylogeny in the drepanidines (James 1998) was used to determine if the Po'ouli is supported as part of the drepanidine clade. The original study involved 72 characters and 55 species of drepanidines, including 17 fossil species that became extinct following human settlement of the archipelago less than two thousand years ago (James and Olson 1991). For the present study, the fossil taxa were excluded in order to specifically examine the phylogenetic placement of *Melamprosops* relative to extant or historically extinct drepanidines. Twenty-one other species of nine-primaried oscines were included so that other potential relationships might be revealed. *Passer domesticus* was included as an outgroup. The resulting matrix had 45 terminal taxa and 57 informative characters.

The osteological matrix was analyzed using a parsimony criterion. All characters were run as ordered characters except for seven multistate characters that were run as unordered because the states were not judged to be sequential. Ten characters had an essentially binary distribution of states except that a few taxa showed intermediate conditions. In these instances, the intermediate condition was scored as a third state, but the character was assigned a weight of 0.5 for the parsimony analyses, to prevent intermediate conditions from exerting an undue influence on tree length. All other characters were unweighted.

RESULTS

Mitochondrial DNA

Cladistic parsimony analyses of the Cyt *b* sequences consistently place the Po'ouli within the Drepanidini (Fig. 1). We initially ran a heuristic search in PAUP* with replicated, random addition and no character weighting, and obtained seven equally most parsimonious trees for drepanidines and carduelines. A maximum likelihood (ML) estimate of the transition-to-transversion ratio was then made using the tree with the lowest ML score (ts:tv ~ 4.0:1). This ratio was used to weight transversional changes, and a heuristic search generated two maximum parsimony trees of length 1255 (unweighted for the same topology is 685 steps) and a consistency index of 0.53 (Fig. 1). Placement of Melamprosops within the Drepanidini, however, occurs regardless of whether transversions are weighted 4.0:1, 10.0:1, or unweighted relative to transitions (although weighting and additional outgroup taxa does affect the topology of drepanidine relationships). Forcing the Po'ouli from the Drepanidini to the Carduelini (in MacClade; Maddison and Maddison 1992) increases the length of the tree (unweighted) in Figure 1 by 12 additional steps. This constrained tree is significantly longer than that of Figure 1 based on both parsimony (Kishino-Hawegawa test, $t = 2.69$, $P = 0.0072$; Kishino and Hasegawa 1989) and maximum likelihood ($G = 52.71$, $P < 0.001$; Felsenstein 1988) tests. Making Melamprosops the sister to each emberizine clade also significantly increases tree length (by 20–27 additional steps; Kishino-Hasegawa test, $t = 3.56$, $P < 0.001$).

Distance analyses further support a drepanidine relationship for *Melamprosops.* Kimura 2-parameter and gamma-corrected distances were lower for comparisons of the Po'ouli and drepanidines (0.086 ± 0.002, range 0.062–0.102) than for comparisons of the Po'ouli and carduelines (0.147 ± 0.005, range 0.142–0.152) or emberizines (0.196 ± 0.010, range 0.170–0.218).

The CR sequence analyses also place the Po'ouli within the Drepanidini. First, three single-base deletions found in the Fringillini and Carduelini CR sequences do not occur in drepanidines nor in Melamprosops CR sequence (Table 1). Second, 1000 replication bootstraps of maximum parsimony trees (with gaps and transversions weighted 10:1 or 5:1 over transitions; heuristic search) reveal 88% and 90% support, respectively, for monophyly of the drepanidines, including the Po'ouli. Last, forcing the Po'ouli from the Drepanidini into the Carduelini (i.e., sister to *Carduelis chloris*) or Emberizinae (i.e., as a sister to *Melospiza georgiana*) increases unweighted tree length by 4 and 10 steps, respectively. The constrained trees are significantly longer (Kishino-Hasegawa test, $t = 2.15$, $P = 0.032$ when sister to *Carduelis*; $t = 2.32$, $P = 0.021$ when sister to *Melospiza*).

Osteology

Parsimony analysis produced 128 equally most parsimonious trees from which we derive a strict consensus tree (Fig. 2). The Po'ouli is nested within the drepanidine clade in all of the 128 trees. Moving the Po'ouli outside the drepanidine clade to a position as sister to either cardueline terminal taxa or cardueline resolved clades adds 9 to 23 additional steps to the total tree length. Making the Po'ouli a sister taxon to *Fringilla* or any emberizine outgroup adds 13 to 20.5 steps.

TABLE 1. LISTED ARE 67 VARIABLE NUCLEOTIDE SITES (OF 224 TOTAL) FROM THE 5′-END OR LEFT DOMAIN OF THE MITOCHONDRIAL CONTROL REGION ASSESSED FOR ONE EMBERIZINAE (*MELOSPIZA GEORGIANA*; GREENBERG ET AL. 1998) AND TEN FRINGILLINAE, INCLUDING THREE FRINGILLINI (*FRINGILLA*; MARSHALL AND BAKER 1997), ONE CARDUELINI (*CARDUELIS*; MARSHALL AND BAKER 1997), AND SIX EXTANT MEMBERS OF THE DREPANIDINI (TARR 1995)

	1 2 3 4 5 6
	12345678901234567890123456789012345678901234567890123456789012345678901234567
Melospiza georgiana	TAGCCACGACACCTTATTATGAA-CCACTAGTGA-A-AACACTCCCGTAGGTATATTCAATAGATAG
Fringilla teydea	....TGTA.-.T.....A.C..TA..T....A..-.-.G.TA..T...T.....-.GCTTC.TA.C..
Fringilla montifringilla	....T.TAG-..AC.....-..T.CC.GA.-.-.G.TA.....T.....-.GCTTC.TA.C..
Fringilla coelebs	C....G...T.....A..-A.T....A..-.-.G.TA.....T.....-.GCTTC.TAGC.A
Carduelis chloris	.CAAT.A....GT.....A.TAA.CT...GA.-G-.GA.A..T....ACAT-GCCTGCCTAGC..
Paroreomyza montana	.CA...A...GATC.....C.CTA.AC.AG.GAGG.TGG.....ACT.....C..C.T..C..
Loxioides balleui	.CA.T.A....G....C..C.CCAA.T.AC.C..G.G.G.....GT.....C...C.T.....
Telespiza cantans	.CA...A...GGT.C.C...ACCA.AC.A.....G.G.G.....NNNNN.....C...C.TT.C..
Hemignathus parvus	.CA...A...GA.....G.ATCAAAC.A..A..G.NNG.....AGT....G....TC.T..C..
Hemignathus kauaiensis	.CA.T.A...GA.....ACCCAAC.A..A..A.A.G...C...CAC....AG.C.TC.T..CC.
Himatione sanguinea	.C..TTA...G.....C.CTAAAC.ATCAC.G.NNG....A.T..T...CG..C.TC.T..C..
Melamprosops phaeosoma	.CA.T.A.....C.....TTC.AT.A..A..G.NNG...NNNNNNN.....GCTTC.T..C..

Note: *Melamprosops phaeosoma* sequence is from this study. A "." indicates identity of the nucleotide to the topmost base and an "N" indicates a base that could not be called. A "-" indicates a gap or deletion in the sequence. Note the three insertions found in all drepanidines relative to fringillines (at sites 35, 37, and 54). In addition, there are three drepanidine transversional synapomorphies (22, 26, and 29). See Fig. 1 for common names of drepanidine taxa.

DISCUSSION

In spite of Pratt's (1992a) assessment that the Po'ouli might not be a drepanidine, we find consistent evidence to the contrary. Pratt (1992a) notes that the Po'ouli should be considered a "nine-primaried oscine of uncertain affinities," and that it "does not look, smell, act, or sound like a Hawaiian honeycreeper." Our DNA evidence places *Melamprosops* within the drepanidines, and osteological characteristics indeed make the Po'ouli "look" like a honeycreeper. How does one reconcile the apparent morphological, ecological, and behavioral distinctiveness of the Po'ouli (Pratt 1992a; Pratt et al. 1997b, *this volume*) with our results? Two explanations may account for this: (1) some of the phenotypic traits that Pratt emphasizes (i.e., those associated with foraging mode and feeding) may be affected by adaptive radiation and thus we might expect to see wide diversity in their character states; and (2) some of the 17 extinct drepanidine species known only from fossils may have shared these traits with the Po'ouli, thus making it different only in the context of living or historically extinct taxa. We do not know what factors effected the evolution of the brownish coloration and the black facial mask, nor why *Melamprosops* (and apparently *Paroreomyza*; Pratt 1992b) lack the distinctive drepanidine odor.

While our results indicate that the Po'ouli is a Hawaiian honeycreeper, the relationships of the Po'ouli within the drepanidines are not well resolved by the mtDNA data (Fig. 1). Majority rule bootstrap analysis results in collapse of supporting branches such that *Melamprosops* becomes a basal drepanidine lineage. On the strict consensus for the morphological trees, the Po'ouli joins at a node proximal to the finch-like species but distal to most other living drepanidines. It is not depicted as the sister group of any living drepanidine species. Thus, in both mtDNA and osteological trees the Po'ouli appears to represent a unique drepanidine lineage. Its lineage may have diverged from other drepanidine lineages prior to evolution of the synapomorphic characters defined by Pratt (1992a).

How phylogenetically distinct is the Po'ouli among living drepanidines? To answer this we estimated the contribution of each taxon to the

total minimum evolution score for the Cyt *b* tree in Figure 1. In PAUP*, we constrained the tree topology, pruned a taxon from the tree, then recalculated the ME score. The process was repeated for each drepanidine taxon; each ME score was subtracted from the total ME score to provide a phylogenetic "distinctiveness" score (*U*) for the taxon (essentially that of Faith 1992). The Po'ouli had the highest *U* (0.044) among the 19 drepanidines (mean and SE of *U* for the other 18 taxa was 0.015 ± 0.002). To evaluate the Po'ouli's distinctiveness in the osteology-based tree we constrained the tree in Figure 2 in MacClade 3.01. A drepanidine taxon was removed and the length of the reduced tree was subtracted from the length of the total tree. The procedure was repeated for each of the 23 drepanidines, and revealed that the Po'ouli was the fourth most distinctive taxon based on osteology (after Maui and Kauai creepers and the 'Akiapōlā'au). Thus we consider the Po'ouli to be phylogenetically unique among the drepanidines, and the taxon that individually contributes most to extant drepanidine phylogenetic diversity.

The closest corrected genetic distance between the Po'ouli and other drepanidines is 0.062. Applying a corrected internal rate calibration for Cyt *b* in honeycreepers of about 0.016 ± 0.005/MY (from Fleischer et al. 1998) suggests that the Po'ouli split from its nearest living drepanidine relative about 3.8 ± 0.9 MY ago (fairly early in the drepanidine radiation; Tarr and Fleischer 1995, Fleischer et al. 1998). Of course extinct fossil drepanidines (James and Olson 1991) not included here, such as *Xestospiza,* may turn out to be more closely related genetically. Nonetheless, in comparison to other extant drepanidines, the Po'ouli has had a long, independent evolutionary history. This long period of independent evolution can perhaps explain some of *Melamprosops'* unique phenotypic characteristics. Such phylogenetic distinctiveness also increases the Po'ouli's conservation value, in that the species represents a significant fraction of the genetic diversity of the drepanidines (Faith 1992, Krajewski 1994). Along with its singular ecological, behavioral, and morphological characteristics, the Po'ouli's unique evolutionary history convinces us that serious efforts should be undertaken to avoid its impending extinction.

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