Electronic Supplementary Information for

Non-destructive descriptions of carotenoids in feathers using Raman spectroscopy

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Table S1 Specimens analysed in the current study.

Species name	Common name	Pigment (spectra)[1]	Sex	Provenance	Collection year[2]	Catalogue number
Acanthisitta chloris	Tītipounamu	Yellow (3|3)	Male	New Zealand	Unknown	USNM[3] 124635
Acanthisitta chloris	Tītipounamu	Yellow (3|3)	Female	New Zealand	Unknown	USNM 124636
Apaloderma narina	Narina trogon	Red (1|3)	Male	Tanzania	2004	USNM 634596
Bombycilla cedrorum	Cedar waxwing	Yellow (1|3)	Unknown	USA, South Dakota	1988	USNM 623482
Cardinalis cardinalis	Northern cardinal	Red (1|3)	Male	USA, Kauai Island	2010	USNM 643555
Cardinalis sinuatus	Pyrrhuloxia	Red (1|3)	Male	USA, Texas	2009	USNM 642143
Carduelis chloris	European goldfinch	Yellow (1|3)	Male	Greece	2006	USNM 637389
Coereba flaveola	Bananaquit	Yellow (1|3)	Male	Guyana	2007	USNM 639172
Colaptes auratus	Northern flicker	Yellow (1|3)	Female	USA, Maryland	2002	USNM 623435
Cotinga cotinga	Purple-breasted cotinga	Purple (2|6)	Male	Guyana	2004	USNM 632564
Emberiza melanocephala	Black-headed bunting	Yellow (1|3)	Female	Greece	2006	USNM 637386
Euphonia laniirostris	Thick-billed euphonia	Yellow (1|3)	Male	Peru	2009	USNM 643899
Euphonia saturata	Orange-crowned euphonia	Yellow (1|3)	Male	Peru	2009	USNM 643992
Euplectes capensis	Yellow bishop	Yellow (1|3)	Female	Tanzania	2004	USNM 636396
Fringilla montifringilla	Brambling	Yellow (1|3)	Male	USA, Alaska	2010	USNM 645120
Icterus galbula	Baltimore oriole	Yellow (1|3)	Male	USA, Maryland	2005	USNM 623444
Icterus icterus	Venezuelan troupial	Yellow (1|3)	Male	Guyana	2004	USNM 632598
Leiothrix lutea	Red-billed leiothrix	Orange (1|3)	Unknown	USA, Hawaii	2010	USNM 643393
Melanerpes formicivorus	Acorn woodpecker	Red (1|3)	Male	USA, California	2008	USNM 641593
Mohoua ochrocephala	Mohua	Yellow (3|3)	Male	New Zealand	1872	USNM 66290
Mohoua ochrocephala	Mohua	Yellow (3|3)	Male	New Zealand	1872	USNM 66291
Mohoua ochrocephala	Mohua	Yellow (3|3)	Female	New Zealand	Unknown	USNM 109195
Notiomystis cincta	Hihi	Yellow (3|3)	Male	New Zealand	1892	USNM 172402
Notiomystis cincta	Hihi	Yellow (3|3)	Male	New Zealand	Unknown	USNM 174651
Oreothlypis ruficapilla	Nashville warbler	Yellow (1|3)	Male	USA, California	2007	USNM 637605
Paroaria coronata	Red-crested cardinal	Red (1|3)	Female	USA, Hawaii	2010	USNM 643469
Parus major	Great tit	Yellow (1|3)	Female	Greece	2006	USNM 637484
Petroica boodang	Scarlet robin	Red (3|3)	Male	Australia	1910	USNM 213052
Petroica boodang	Scarlet robin	Red (3|3)	Male	Australia	Unknown	USNM 71598
Petroica boodang	Scarlet robin	Red (3|3)	Male	Australia	1921	USNM 279221
Petroica goodenovii	Red-capped robin	Red (3|3)	Male	Australia	1903	USNM 236605
Petroica goodenovii	Red-capped robin	Red (3|3)	Male	Australia	1883	USNM 121275
Petroica goodenovii	Red-capped robin	Red (3|3)	Male	Australia	1920	USNM 278883
Petroica macrocephala	Miromiro	Yellow (3|3)	Male	New Zealand	Unknown	USNM 109208
Petroica macrocephala	Miromiro	Yellow (3|3)	Male	New Zealand	Unknown	USNM 148490
Petroica macrocephala	Miromiro	Yellow (3|3)	Male	New Zealand	1873	USNM 66293
Petroica multicolor	Pacific robin	Red (3|3)	Male	Australia	1883	USNM 325485
Petroica multicolor	Pacific robin	Red (3|3)	Male	Australia	1920	USNM 279219
Petroica phoenicea	Flame robin	Orange-red (3|3)	Male	Australia	Unknown	USNM 127140
Petroica phoenicea	Flame robin	Orange-red (3|3)	Male	Australia	1938	USNM 425214
Petroica phoenicea	Flame robin	Orange-red (3|3)	Male	Australia	1921	USNM 279223
Petroica rosea	Rose robin	Pink (3|3)	Male	Australia	Unknown	USNM121328
Petroica rosea	Rose robin	Pink (3|3)	Male	Australia	Unknown	USNM121276
Phaethon rubricauda	Red-tailed tropicbird	Red (1|3)	Male	Johnston Atoll	2003	USNM 632100
Phoeniconaias minor	Lesser flamingo	Pink (1|3)	Male	Tanzania	2004	USNM 634731
Picoides villosus	Hairy woodpecker	Red (1|3)	Male	USA, Alaska	2005	USNM 639056
Picumnus exilis	Golden-spangled piculet	Red (1|3)	Male	Guyana	2007	USNM 639369
Piranga flava	Red tanager	Yellow (1|3)	Male	Peru	2009	USNM 643860
Piranga ludoviciana	Western tanager	Yellow (1|3)	Male	USA, California	2005	USNM 634993
Platalea ajaja	Roseate spoonbill	Pink (2|6)	Male	Uruguay	2005	USNM 635736
Ploceus bicolor	Dark-backed weaver	Yellow (1|3)	Male	Tanzania	2004	USNM 634631
Ploceus velatus	Southern masked weaver	Yellow (1|3)	Male	Namibia	2009	USNM 642356
Pteroglossus aracari	Black-necked araçari	Red (1|3)	Female	Guyana	2006	USNM 637112

Pyrrhula pyrrhula	Eurasian bullfinch	Red (1|3)	Male	Greece	2006	USNM 637523
Ramphastos tucanus	White-throated toucan	Red (2|6)	Male	Guyana	2004	USNM 632532
Regulus regulus	Goldcrest	Yellow (1|3)	Male	Greece	2006	USNM 637526
Selenidera piperivora	Guianan toucanet	Red (1|3)	Male	Guyana	2004	USNM 632544
Serinus mozambicus	Yellow-fronted canary	Yellow (1|3)	Male	Tanzania	2005	USNM 636670
Setophaga petechia	American yellow warbler	Yellow (1|3)	Unknown	Nicaragua	2007	USNM 638043
Sicalis flaveola	Saffron finch	Yellow (1|3)	Female	Uruguay	2005	USNM 635754
Telophorus zeylonus	Bokmakierie	Yellow (1|3)	Female	Namibia	2009	USNM 642574
Trogon mesurus	Ecuadorian trogon	Red (1|3)	Male	Peru	2009	USNM 643987
Tyrannus vociferans	Cassin's kingbird	Red (1|3)	Male	USA, Texas	2009	USNM 642152
Uragus sibiricus	Long-tailed rosefinch	Red (1|3)	Male	Japan	2009	USNM 641788
Vestiaria coccinea	'I'iwi	Red (1|3)	Unknown	USA, Hawaii	1980	USNM 634051
Zosterops japonicus	Japanese white-eye	Yellow (1|3)	Female	Japan	2009	USNM 641812

[1]Analysed plumage colour and (number of feathers studied|total number of spectra collected).
[2]Year that birds were collected from the field.
[3]Specimens from the National Museum of Natural History, Smithsonian Institution, Washington, DC, USA.

Ancestral State Reconstruction

Ancestors of New Zealand *Petroica* robins likely immigrated from Australia.[1] Here we were interested in estimating the plumage colours of these ancestral birds. We can estimate ancestral states by modelling character evolution on phylogenetic trees reconstructed from molecular data.[2]

Relationships between many species of *Petroica*, and other species within Petroicidae, were recently interpreted from two mitochondrial genes (ND2, CO1) and one nuclear intron (β-Fibrinogen intron 5).[3] A phylogenetic tree constructed from the concatenated DNA sequences[3] was used here (Fig. S1) as the basis for ancestral state reconstruction. Branch lengths were estimated from the published figure and the tree was reconstructed in Mesquite 2.75.[4] Characters were coded as: (0), a visual absence of carotenoid-pigmented plumage; (1), yellow plumage colouration; (2), red/orange plumage colouration; (3), pink plumage colouration (Fig. S1). Character states 1–3 are each assumed to represent a distinct carotenoid as the major feather pigment; chemical confirmation of plumage carotenoids are not currently available for many taxa. Orange plumage colouration is assumed to be a mixture of red and yellow, and hence *Petroica phoenicea* produces character state 2. The pink carotenoid was not identified in our study, but is likely a keto-carotenoid similar to canthaxanthin, and therefore not homologous with character state 2. Ancestral state reconstruction was performed in Mesquite 2.75 using the Mk. 1 maximum likelihood model ("One-parameter Markov k-state model";[5] data were plotted in R 2.15.2 using functions from the 'ace' package).[6, 7]

Unresolved relationships within Petroicidae influenced the reconstruction of ancestral states, and interpretations may change with further research. From the data available at present, the most recent common ancestor of *Eugerygone* and *Petroica* was reconstructed with red feathers (proportional likelihood of 0.78 for character state 2; Fig. S1). We would therefore expect the yellow plumage coloration of miromiro (*Petroica macrocephala*) and toutouwai (*Petroica australis*) to have evolved within the *Petroica* crown-lineage. Given that yellow species of *Petroica* do not presently live in Australia, we propose that the evolution of yellow plumage occurred after the *Petroica* migrants arrived in New Zealand, and not before.

References

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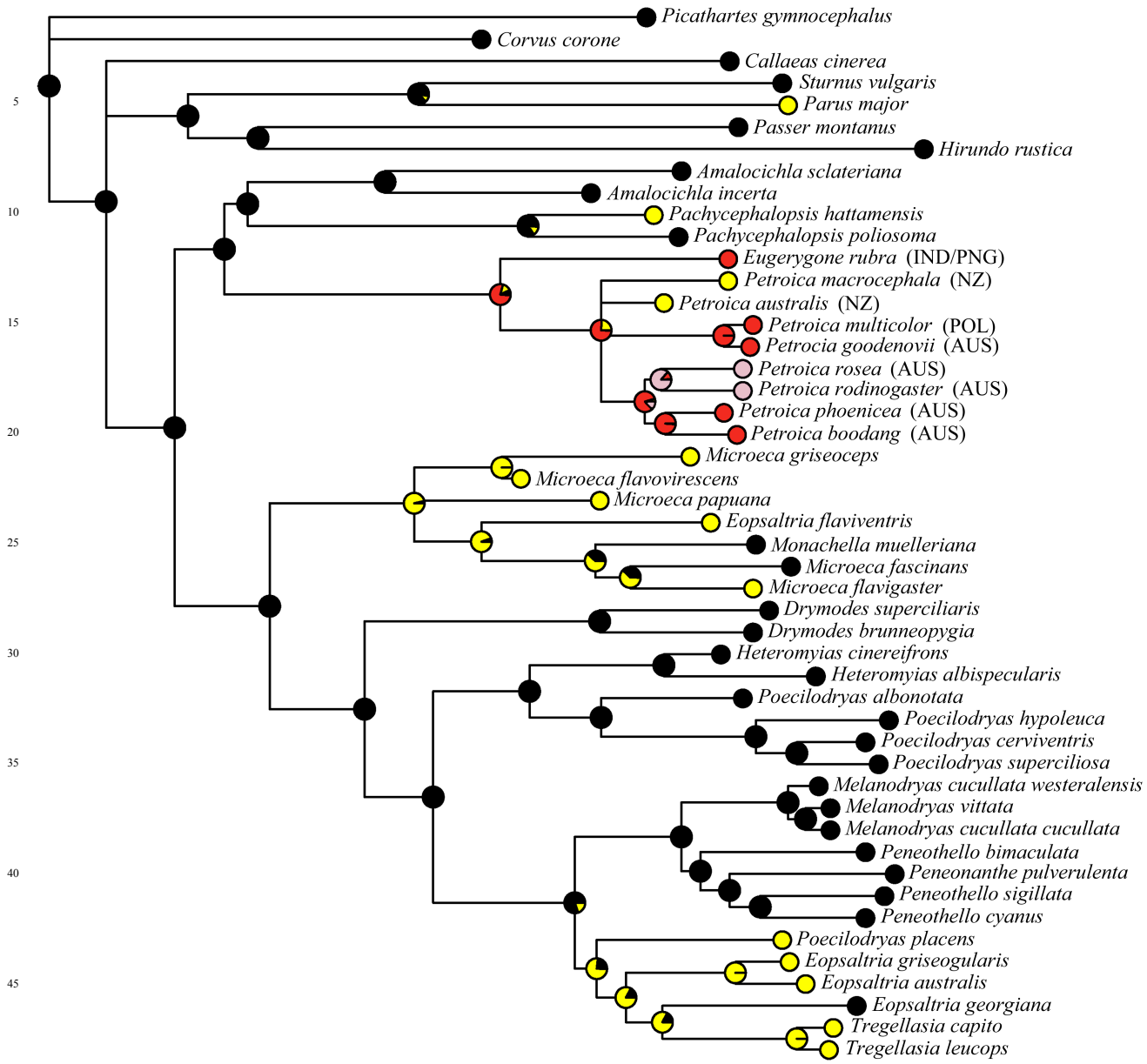

Fig. S1 Ancestral state reconstruction of carotenoid-based plumage pigmentation within Petroicidae. Pie charts show the proportional likelihood for each character state at each node. Characters are coded by colour: black = 0 (a visual absence of carotenoid-pigmented plumage); yellow = 1 (yellow plumage coloration); red = 2 (red/orange plumage coloration); pink = 3 (pink plumage coloration). The yellow plumage colours of miromiro (*Petroica macrocephala*) and toutouwai (*P. australis*) are most likely derived from a red-pigmented ancestor. Phylogeny from ref 3; figure 4. Geographic regions are identified in parentheses for select species: AUS = Australia; IND/PNG = Indonesia and Papua New Guinea; NZ = New Zealand; POL = Polynesia, Melanesia and Norfolk Island.