**Electronic Supplementary Material for:**

**Oligocene divergence of frogmouth birds (Podargidae) across Wallace’s line**

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*Library amplification and mitochondrial enrichment*

At the end of the library preparation protocol, each library was amplified using PCR in eight separate reactions to reduce amplification bias. Each reaction contained 1 × PCR buffer, 2.5 mM MgCl2, 1 mM dNTPs, 0.5 mM primer, 1.25 U AmpliTaq Gold, 2 μL DNA library template. Reactions were subjected to the following thermocycling regime: 94 °C 12 min; 12 or 16 cycles (for tissues or toepads, respectively) of 94 °C for 30 s, 60 °C for 30 s, 72 °C for 40 s; and a final extension of 72 °C for 10 min. Individual PCR products for each library were pooled following amplification, and purified using 1.8x AxyPrep magnetic beads (Axygen). Libraries generated from the toepad samples were enriched for bird mitochondrial DNA using hybridisation enrichment with the RNA probes described by [17] following the protocol outlined by Cole *et al.* [18]. All libraries were then subjected to a final round of PCR as described above (five reactions, seven cycles for tissue samples and 15 cycles for toepads) but using fusion primers to add full-length indexed Illumina sequencing adapters for sequencing [15].

*Mitochondrial genome assembly*

As no full-length mitochondrial sequence was available on online databases for any member of Podargidae, we first iteratively mapped merged reads from B28111 to a published COX1 sequence from the common potoo (*Nyctibius* *griseus*; HM746792), another caprimulgiform, using the Geneious Read Mapper [21] in Geneious v9.1.6 (http://www.geneious.com) with the following parameter values: maximum mismatches per read = 10%, minimum mapping quality = Phred 30, maximum gaps per read = 10% read length, maximum gap length = 10 bp, minimum overlap = 25 bp, word length = 8. We continued mapping and extending iteratively using these parameter values until no additional reads were obtained, at which point the 1,551 bp COX1 seed had been expanded to 16,029 bp spanning all of the mitochondrial coding regions. We then created a 75% majority consensus sequence after checking the alignment by eye. Having created a reference for one member of Podargidae, we then made genus-specific references for our podargid samples by mapping merged reads from B03102 and B55782 against the B28111 consensus using the Geneious Read Mapper (with the same parameters described above). Our *Podargus* samples were all quite divergent, so we made sample-specific references as above, mapping the reads from B29946, B57671, and Z45285 against B55782. To create final consensus sequences, we used BWA aln [22] (settings aln -l 1024, -n 0.04, -o 2) to re-map merged reads from all samples to their respective reference sequence. After mapping with BWA, reads with a mapping quality Phred score >30 were selected using the SAMtools v1.4 [22] view command (-q 30), and duplicate reads were discarded using ‘FilterUniqueSAMCons.py’ [24]. A final 75% majority consensus sequence was then generated for each sample and checked by eye in Geneious, calling nucleotides for sites with a minimum depth-of-coverage of 1x (Z45285), 2x (B57671), or 3x (all other samples)."

**Table S1.** Podargidae mitochondrial genome specimen numbers and assembly statistics.

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Species | *B. cornutus* | *R. inexpectata* | *P. ocellatus* | *P. ocellatus* | *P. strigoides* | *P. papuensis* |
| Collection | NMV | ANWC | ANWC | NMV | ANWC | ANWC |
| Tissue Number | B28111 | B03102 | B57671 | Z45285 | B55782 | B29946 |
| GenBank | MT  180470 | MT  180465 | MT  180469 | MT  180468 | MT  180466 | MT  180467 |
| Total number of merged reads | 1,236,904 | 648,719 | 144,919 | 336,776 | 224,786 | 304,003 |
| Total number of mapped de-duplicated reads | 289,855 | 167,158 | 2,114 | 1,246 | 8,055 | 4,046 |
| Consensus length (bp) | 15,982 | 15,006 | 14,962 | 14,891 | 15,039 | 15,053 |
| Consensus coverage (%) | 100 | 100 | 99.8 | 99.2 | 100 | 100 |
| Mean read length ± standard deviation (bp) | 76.7 ± 19.7 | 70.2 ± 19.4 | 101.6 ± 17.8 | 94.4 ± 20.4 | 95.4 ± 19.4 | 95.6 ± 19.7 |
| Mean depth of coverage ± standard deviation (X) | 1,399.4 ± 934.8 | 779.2 ± 406.5 | 14.3 ± 5.1 | 7.9 ± 3.5 | 51.0 ± 17.0 | 25.6 ± 7.9 |

**Table S2.** Outgroup sequences used in full mitochondrial genome alignment.

|  |  |  |
| --- | --- | --- |
| **Species** | **Common name** | **GenBank accession** |
| *Aegotheles cristatus* | Australian owlet-nightjar | EU344979 |
| *Amazilia brevirostris* | White-chested emerald | KP722043 |
| *Amazilia millerii* | - | KP722042 |
| *Amazilia rondoniae* | Blue-fronted emerald | KP722041 |
| *Amazilia versicolor* | Versicolored emerald | KF624601 |
| *Apus apus* | Common swift | NC\_008540 |
| *Archilochus colubris* | Ruby-throated hummingbird | EF532935 |
| *Calliphlox amethystina* | Amethyst woodstar | KP853095 |
| *Caprimulgus indicus* | Jungle nightjar | KM272749 |
| *Chaetura pelagica* | Chimney swift | KT809406 |
| *Chrysolampis mosquitus* | Ruby-topaz hummingbird | KJ619585 |
| *Cypseloides fumigatus* | Sooty swift | KY688216 |
| *Florisuga fusca* | Black jacobin | KP853096 |
| *Florisuga mellivora* | White-necked jacobin | KJ619588 |
| *Glaucis hirsutus* | Rufous-breasted hermit | KT265275 |
| *Heliodoxa aurescens* | Gould’s jewelfront | KP853094 |
| *Hylocharis cyanus* | White-chinned sapphire | KJ619586 |
| *Lophornis magnificus* | Frilled coquette | KT265276 |
| *Nyctibius grandis* | Great potoo | EU344977 |
| *Oreotrochilus melanogaster* | Black-breasted hillstar | KJ619587 |
| *Phaethornis malari* | Great-billed hermit | KP853097 |

**Table S3.** Partitioning scheme and substitution models for analyses in IQ-TREE.

|  |  |  |  |
| --- | --- | --- | --- |
| **Subset** | **Best Model** | **Subset Partitions** | **Subset Sites** |
| 1 | SYM+I+G | CO1\_1, CO2\_1, CO3\_1, ND1\_1 | 1-315, 663-1178, 1179-1405, 1686-1946 |
| 2 | GTR+I+G | ATP6\_1, ATP8\_1, ATP8\_2, CYTB\_1, ND2\_1, ND3\_1, ND4L\_1, ND4\_1, ND5\_1 | 316-662, 1406-1458, 1459-1685, 1947-2062, 2063-2158, 2159-2614, 2615-3214, 3215-3594, 5000-5052 |
| 3 | HKY+I+G | CO1\_2, CO2\_2, CO3\_2, CYTB\_2, ND1\_2 | 3595-3909, 4257-4772, 4773-4999, 5280-5540, 6809-7188 |
| 4 | TVM+I+G | ATP6\_2, ND2\_2, ND3\_2, ND4L\_2, ND4\_2, ND5\_2 | 3910-4256, 5053-5279, 5541-5656, 5657-5752, 5753-6208, 6209-6808 |

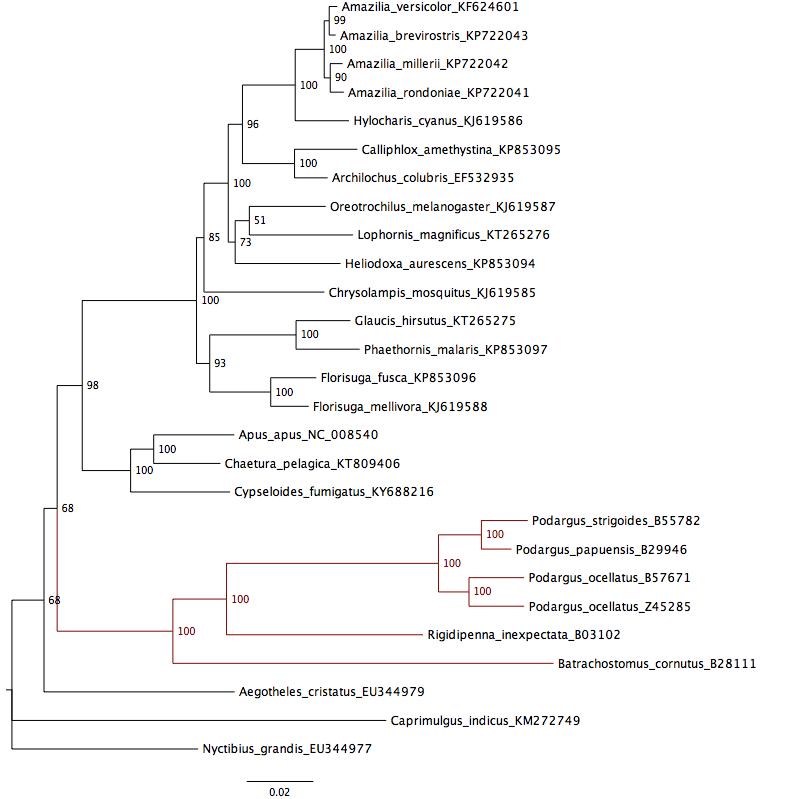
**Table S4.** Partitioning scheme and substitution models for analyses in MrBayes.

|  |  |  |  |
| --- | --- | --- | --- |
| **Subset** | **Best Model** | **Subset Partitions** | **Subset Sites** |
| 1 | GTR+I+G | CO1\_1, CO2\_1, CO3\_1, CYTB\_1, ND1\_1 | 1-315, 663-1178, 1179-1405, 1686-1946, 3215-3594 |
| 2 | GTR+I+G | ATP6\_1, ATP8\_1, ND2\_1, ND3\_1, ND4L\_1, ND4\_1, ND5\_1 | 316-662, 1406-1458, 1459-1685, 1947-2062, 2063-2158, 2159-2614, 2615-3214 |
| 3 | HKY+I+G | CO1\_2, CO2\_2, CO3\_2, CYTB\_2, ND1\_2 | 3595-3909, 4257-4772, 4773-4999, 5280-5540, 6809-7188 |
| 4 | GTR+I+G | ATP6\_2, ATP8\_2, ND2\_2, ND3\_2, ND4L\_2, ND4\_2, ND5\_2 | 3910-4256, 5000-5052, 5053-5279, 5541-5656, 5657-5752, 5753-6208, 6209-6808 |

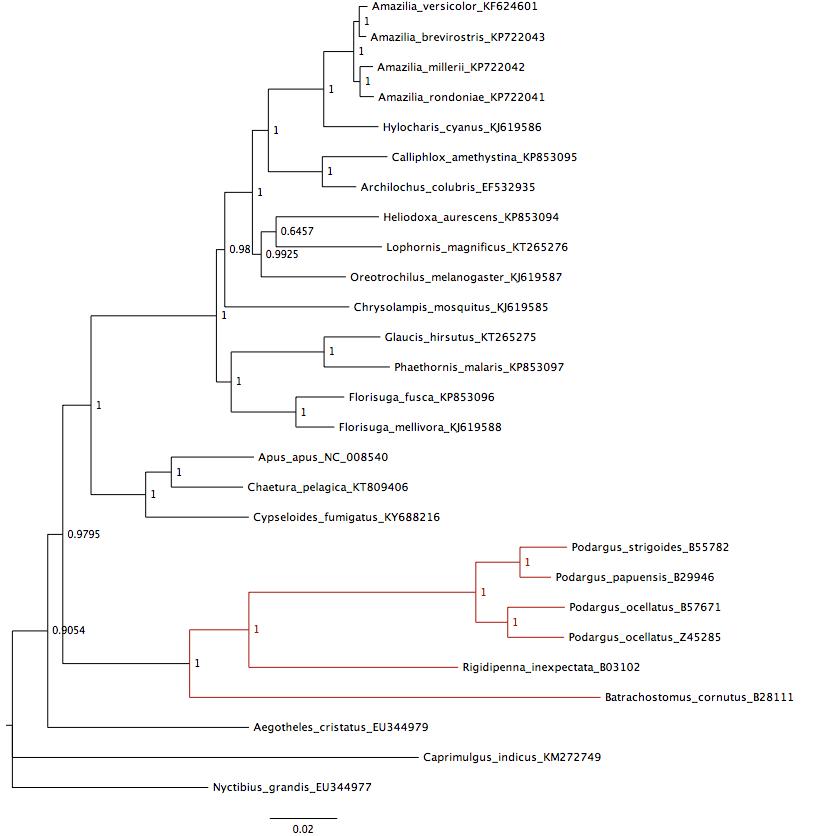
**Table S5.** Caprimulgiformes supermatrix dating tree sample information. Dataset modified from Hugall and Stuart-Fox (2012), deleting 10 Caprimulgidae taxa and incorporating new Podargidae data (highlighted bold), including mitogenome data from this study. Further information available at doi:10.1038/nature11050. The nomenclature has been updated to IOC World Bird List v 9.2 (dx.doi.org/10.14344/IOC.ML.9.2).

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  |  | **Locus accessions/samples** | | | | |
| **Taxon** | **Family** | **RAG1** | **v-myc** | **GH1** | **cytb** | **COI** |
| Aegotheles\_albertisi | Aegothelidae | AY233362 | FJ588484 | na | X95764 | na |
| Aegotheles\_albertisi.archboldi | Aegothelidae | na | na | na | AY090670 | na |
| Aegotheles\_bennettii | Aegothelidae | na | FJ588482 | na | X95774 | na |
| Aegotheles\_crinifrons | Aegothelidae | na | na | na | AY090682 | na |
| Aegotheles\_cristatus | Aegothelidae | na | FJ588483 | na | X95775 | NC011718 |
| Aegotheles\_insignis | Aegothelidae | DQ482636 | EU738243 | EF521432 | FJ588456 | na |
| Aegotheles\_novaezealandiae | Aegothelidae | na | na | na | AY090698 | na |
| Aegotheles\_savesi | Aegothelidae | na | na | na | AY090691 | na |
| Aegotheles\_tatei | Aegothelidae | na | na | na | AY090693 | na |
| Aegotheles\_wallacii | Aegothelidae | na | na | na | AY090697 | na |
| Antrostomus\_carolinensis | Caprimulgidae | DQ482627 | GU586572 | GU586503 | FJ588442 | DQ432814 |
| Antrostomus\_ridgwayi | Caprimulgidae | na | GU586590 | GU586521 | GU586653 | na |
| Antrostomus\_rufus | Caprimulgidae | na | GU586592 | GU586523 | GU586655 | na |
| Antrostomus\_salvini | Caprimulgidae | na | GU586593 | GU586524 | GU586656 | na |
| Antrostomus\_saturatus | Caprimulgidae | na | GU586594 | GU586525 | GU586657 | na |
| Antrostomus\_vociferus | Caprimulgidae | DQ482628 | GU586570 | GU586526 | GU586658 | AY666179 |
| Caprimulgus\_aegyptius | Caprimulgidae | na | GU586565 | GU586496 | GU586631 | na |
| Caprimulgus\_batesi | Caprimulgidae | na | GU586571 | GU586502 | GU586636 | na |
| Caprimulgus\_climacurus | Caprimulgidae | DQ482633 | GU586626 | GU586560 | GU586687 | na |
| Caprimulgus\_europaeus | Caprimulgidae | DQ482634 | GU586575 | GU586506 | GU586638 | GQ481447 |
| Caprimulgus\_fossii | Caprimulgidae | na | GU586576 | GU586507 | GU586639 | na |
| Caprimulgus\_indicus | Caprimulgidae | na | GU586577 | GU586508 | GU586640 | GQ481449 |
| Caprimulgus\_macrurus | Caprimulgidae | DQ482632 | GU586580 | GU586511 | GU586644 | na |
| Caprimulgus\_nigriscapularis | Caprimulgidae | na | GU586584 | GU586515 | GU586647 | na |
| Caprimulgus\_poliocephalus | Caprimulgidae | na | GU586588 | GU586519 | GU586651 | na |
| Caprimulgus\_vexillarius | Caprimulgidae | DQ482635 | GU586615 | GU586547 | GU586674 | na |
| Chordeiles\_acutipennis | Caprimulgidae | DQ482629 | GU586600 | GU586532 | GU586663 | DQ432851 |
| Chordeiles\_minor | Caprimulgidae | na | GU586602 | GU586533 | EU166983 | DQ433506 |
| Chordeiles\_nacunda | Caprimulgidae | DQ482630 | GU586624 | GU586558 | GU586685 | FJ028124 |
| Chordeiles\_pusillus | Caprimulgidae | na | GU586603 | GU586534 | GU586665 | na |
| Eleothreptus\_anomalus | Caprimulgidae | na | GU586605 | GU586536 | GU586666 | FJ027545 |
| Eurostopodus\_argus | Caprimulgidae | na | GU586606 | GU586537 | GU586667 | na |
| Eurostopodus\_mystacalis | Caprimulgidae | na | GU586607 | GU586539 | GU586668 | na |
| Eurostopodus\_papuensis | Caprimulgidae | na | GU586608 | GU586540 | X95780 | na |
| Gactornis\_enarratus | Caprimulgidae | na | GU586574 | GU586505 | GU586637 | na |
| Hydropsalis\_climacocerca | Caprimulgidae | DQ482620 | GU586610 | GU586542 | GU586670 | na |
| Hydropsalis\_maculicaudus | Caprimulgidae | DQ482622 | GU586581 | GU586512 | GU586645 | na |
| Hydropsalis\_torquata | Caprimulgidae | na | GU586609 | GU586541 | GU586669 | FJ027659 |
| Lurocalis\_semitorquatus | Caprimulgidae | DQ482616 | GU586611 | GU586545 | GU586672 | na |
| Lyncornis\_macrotis | Caprimulgidae | DQ482615 | EU738292 | EF521481 | FJ588447 | na |
| Nyctidromus\_albicollis | Caprimulgidae | DQ482618 | GU586616 | GU586550 | GU586677 | FJ027915 |
| Nyctidromus\_anthonyi | Caprimulgidae | na | GU586568 | GU586499 | GU586633 | na |
| Nyctiphrynus\_mcleodii | Caprimulgidae | DQ482624 | GU586622 | GU586556 | GU586683 | AY275857 |
| Nyctiphrynus\_ocellatus | Caprimulgidae | DQ482625 | GU586617 | GU586551 | GU586678 | na |
| Nyctiphrynus\_rosenbergi | Caprimulgidae | na | GU586619 | GU586553 | GU586680 | na |
| Nyctipolus\_nigrescens | Caprimulgidae | na | GU586583 | GU586514 | FJ588446 | na |
| Nyctiprogne\_leucopyga | Caprimulgidae | DQ482617 | GU586621 | GU586555 | GU586682 | na |
| Phalaenoptilus\_nuttallii | Caprimulgidae | DQ482626 | GU586623 | GU586557 | GU586684 | DQ433918 |
| Setopagis\_parvula | Caprimulgidae | DQ482623 | GU586586 | GU586517 | GU586649 | FJ027295 |
| Setopagis\_whitelyi | Caprimulgidae | na | GU586597 | GU586528 | GU586660 | na |
| Siphonorhis\_brewsteri | Caprimulgidae | na | GU586627 | GU586561 | GU586688 | na |
| Systellura\_longirostris | Caprimulgidae | DQ482621 | EU738263 | EF521453 | GU586641 | na |
| Uropsalis\_segmentata | Caprimulgidae | DQ482619 | GU586630 | GU586564 | GU586690 | na |
| Nyctibius\_aethereus | Nyctibiidae | AF294659 | FJ588473 | na | X95781 | na |
| Nyctibius\_bracteatus | Nyctibiidae | na | EU738327 | EF521514 | GU586675 | na |
| Nyctibius\_grandis | Nyctibiidae | DQ482612 | EU738328 | EF521515 | GU586676 | na |
| Nyctibius\_griseus | Nyctibiidae | na | FJ588472 | na | X95767 | FJ027913 |
| Nyctibius\_jamaicensis | Nyctibiidae | na | FJ588471 | na | FJ588449 | na |
| Nyctibius\_leucopterus | Nyctibiidae | na | FJ588469 | na | X95768 | na |
| Nyctibius\_maculosus | Nyctibiidae | na | FJ588470 | na | FJ588448 | na |
| Batrachostomus\_cornutus | Podargidae | na | na | na | X95776 | MT180470 |
| Batrachostomus\_moniliger | Podargidae | na | na | na | KJ456203 | na |
| Batrachostomus\_septimus | Podargidae | DQ482613 | EU738255 | EF521445 | EF100673 | na |
| Podargus\_ocellatus | Podargidae | na | FJ588474 | na | X95771 | MT180468 |
| Podargus\_ocellatus\_B57671 | Podargidae | na | na | na | B57671 | MT180469 |
| Podargus\_papuensis | Podargidae | na | FJ588475 | na | X95772 | MT180467 |
| Podargus\_strigoides | Podargidae | DQ482614 | GU586625 | EF521539 | GU586686 | JQ175917 |
| Rigidipenna\_inexpectata | Podargidae | na | na | na | EF100671 | MT180465 |
| Steatornis\_caripensis | Steatornithidae | DQ482611 | EU738373 | EF521558 | EF100675 | na |
| Aerodramus\_3n2m | Apodidae | AY056979 | EU738244 | EF521433 | HM626167 | HM594288 |
| Hemiprocne\_3n1m | Hemiprocnidae | DQ482637 | EU738306 | EF521495 | AY294483 | na |
| Grus\_3n2m | Gruidae | AF143732 | EU738303 | EF521492 | U27554 | AY666489 |
| Pandion\_2n2m | Pandionidae | AY461394 | na | EF521521 | AY987232 | NC008550 |
| Psittaciformes\_2n2m | Psittaciformes | DQ482642 | EU738357 | na | AY156278 | HM396335 |
| Strix\_2n2m | Strigidae | DQ482641 | EU738375 | na | AJ004056 | DQ434176 |
| Tauraco\_3n1m | Musophagidae | DQ482643 | EU738378 | EF521563 | na | U83784 |

**Figure S1.** Maximum Likelihood phylogeny for Caprimulgiformes estimated using IQ-TREE and our full mitochondrial genome dataset (7,188bp). Podargidae branches are highlighted in red. Scale is in number of substitutions. Values associated with nodes reflect Bootstrap support (%). All Podargidae mitochondrial genomes were newly generated in this study (see electronic supplementary material, table S1).



**Figure S2.** Bayesian phylogeny for Caprimulgiformes estimated using MrBayes and our full mitochondrial genome dataset (7,188bp). Podargidae branches are highlighted in red. Scale is in number of substitutions. Values associated with nodes reflect Bayesian posterior probabilities. All Podargidae mitochondrial genomes were newly generated in this study (see electronic supplementary material, table S1).



**Figure S3.** Chronogram for the Caprimulgiformes estimated using BEAST from a concatenated dataset of two nuclear and three mitochondrial genes. Scale axis is in millions of years before present. Podargidae branches are in red. Nodes at which topology was constrained are indicated with an asterisk. Bars associated with nodes reflect 95% Highest Posterior Densities of node age estimates. Values associated with branches reflect Bayesian posterior probabilities.

