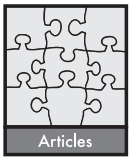

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The timing of diversification within the most divergent parrot clade

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The Strigopidae are an ancient parrot (Psittaciformes) family consisting of three extant species placed in two genera (*Nestor*, *Strigops*) and restricted to New Zealand. Their evolutionary history is clouded because the timing of divergence events within this family has variously been attributed to Pleistocene climate change or much earlier earth-historic events. Here we examine new psittaciform DNA sequence data, and combine them with previously published sequences, to shed light on the poorly understood timing of diversification within the Strigopidae. Using calibrations indirectly derived from both psittaciform and non-psittaciform fossils, our data indicate a Late Pliocene or Early Pleistocene (ca 1.2–3.6 mya) differentiation between the two *Nestor* species (kea and kaka), possibly in response to shifts in habitat distribution associated with sea level fluctuations. The unique, monotypic, nocturnal and flightless genus *Strigops* (kakapo) is shown to have diverged from the *Nestor* lineage probably ca 28–29 mya, coinciding with the potential Oligocene submergence of Zealandia when much of its landmass may have been fragmented into smaller islands, providing a setting for allopatric diversification.

The rifting of Gondwana has been implicated as a major driver of early parrot (Psittaciformes) and perching bird (Passeriformes) diversification, largely based on the geographic restriction to New Zealand (NZ) of extant basal clade members from both these orders (Ericson et al. 2002, Barker et al. 2004, Wright et al. 2008). The two orders also appear to be sister groups (Hackett et al. 2008, Suh et al. 2011, McCormack et al. 2013). Within Psittaciformes, the earliest divergence is between the NZ-restricted family Strigopidae (kakapo, kea and kaka) and other parrots, followed by divergence between the Australasian cockatoos (Cacatuidae) and remaining parrots (Psittacidae) (de Kloet and de Kloet 2005, Tavares et al. 2006, Wright et al. 2008; for family-level classification see Christidis and Boles 2008). Accordingly, the split between the Strigopidae and other parrots has been suggested to have resulted from vicariance associated with the Cretaceous rifting of the microcontinent 'Zealandia' from Gondwana ca 83–85 mya (McLoughlin 2001, Wright et al. 2008). However, the timing of subsequent diversification events within Strigopidae is still poorly known, and its Cretaceous origin has been the subject of controversy: using the proposed timing of the separation of Zealandia from Gondwana to date the divergence between the Strigopidae and other parrots has been suggested to be a circular argument because the divergence time in question is the same as the geologic vicariance event used for calibration (Schweizer et al. 2011).

Fossils provide an alternative approach for calibrating the dating of parrot diversification. The psittaciform fossil record mostly consists of Pleistocene and Pliocene finds that can be identified to the level of extant genera (Tonni and Noriega 1996, Boles 1998, Stidham 2009, Tambussi et al. 2009). The only older parrot fossils include a Miocene (ca 16 mya) cacatuid fragment which is identifiable to modern genus level (Boles 1993) and Eocene to mid-Miocene members of extinct taxa that have shed much light on early parrot evolution but are often difficult to place on the phylogenetic tree (Wetmore 1926, Mayr 1998, 2007, Mlíkovsky 1998, Cheneval 2000, Mayr and Göhlich 2004, Waterhouse et al. 2008), although some of them were conclusively identified as members of stem-group parrots (Mayr 1998, 2007, Waterhouse et al. 2008). On account of this sparse parrot fossil record, combinations of both psittaciform and non-psittaciform fossils have been used to provide calibration points for estimating the basal split between Strigopidae and other parrots (Pacheco et al. 2011, Schweizer et al. 2011, White et al. 2011). Using six avian fossil-calibrated points in combination with full mitochondrial genome sequences, White et al. (2011) produced estimates of the basal split

between Strigopidae and other Psittaciformes at ca 36–59 mya. Pacheco et al. (2011), using three fossil calibration points and >70 avian mitochondrial genomes, obtained a date of 57–61 mya for the same split. Importantly, the results of Pacheco et al. (2011) were not changed when an 85 mya separation of Zealandia from Gondwana was postulated as a fourth calibration point for the most basal split within the perching birds included in their analysis. Schweizer et al. (2011), using a dataset comprising three nuclear genes in combination with three fossil points, one of which differed from Pacheco et al. (2011), obtained a date of 45–72 mya for the basal split of Strigopidae and other Psittaciformes.

The fossil-based estimates for psittaciform diversification events reported in these studies (Pacheco et al. 2011, Schweizer et al. 2011, White et al. 2011) are in good agreement with estimates of the Cacatuidae–Psittacidae split obtained from studies examining Neoavian diversification utilizing >20 independent avian fossil calibrations and a spectrum of sequence data and analytical methods (Ericson et al. 2006, Brown et al. 2007, 2008). These Paleocene to Eocene divergence estimates have been interpreted as supporting strigopid overwater dispersal to NZ long after Zealandia had separated from Gondwana at 83–85 mya (Waters and Craw 2006, Goldberg et al. 2008, Schweizer et al. 2010, 2011).

A further complication in elucidating the timing of strigopid diversification is that evidence for continuous land in present-day NZ throughout the Oligocene is equivocal (Trewick et al. 2006, Waters and Craw 2006, Goldberg et al. 2008, Landis et al. 2008, Trewick and Gibb 2010). While present-day NZ may have been largely submerged during the 'Oligocene drowning' it is possible that large landmasses were present in currently-submerged oceanic parts of Zealandia during that time (Meffre et al. 2006). While 'Oligocene drowning' remains controversial, it has been invoked to suggest that the present-day terrestrial fauna and flora of NZ may have descended from arrivals dispersing here over the past 23 million yr (Trewick et al. 2006, Waters and Craw 2006, Goldberg et al. 2008, Trewick and Gibb 2010).

We combine novel and published DNA data to better elucidate the timing of diversification within the Strigopidae. Our study reports new sequence data for 14 psittaciform species, including all three members of the Strigopidae, from the nuclear arylalkylamine *N*-acetyltransferase (AANAT) gene (ca 1.7–2.5 kb depending on intron length), encompassing three coding exons and two introns (Supplementary material Appendix 1, Fig. A1). This gene was previously used successfully in avian phylogenetic research (Fidler et al. 2004). We have also generated additional psittaciform mitochondrial (mtDNA) cytochrome c oxidase I (COI) partial coding sequences. These new datasets are combined with previously reported sequences of four nuclear introns and one mtDNA gene to comprise a total of seven loci and up to 7.25 kb of concatenated sequence (of which 44.4% are newly generated), and analyzed using calibrations derived from previous fossil-based enquiries of the timing of parrot diversification. The divergence estimates obtained from this study are interpreted against a range of possible earth-historic events.

Material and methods

Genomic DNA preparation, sequencing and sampling strategy

Following Fidler et al. (2004) (strigopid taxa) or de Kloet and de Kloet (2005) (all remaining taxa), genomic DNA was isolated from blood or feather samples of individuals from 14 psittaciform species spanning all three families, including the three extant species of the Strigopidae, three Cacatuidae and eight Psittacidae (Table 1). AANAT was amplified from all 14 genomes, while COI was amplified from four species (Table 1). We sourced from the literature sequence data for the following genes (Table 1): [mtDNA] COI (Russello and Amato 2004, Wright et al. 2008), NADH dehydrogenase subunit 2 (ND2) (Eberhard and Bermingham 2004, Joseph et al. 2008, Wright et al. 2008); [nuclear DNA] rhodopsin intron 1 (Rho1), transforming growth factor β2 intron 5 (TGFB), tropomyosin intron (Trop), spindlin intron III (SpinIII) (de Kloet and de Kloet 2005, Hackett et al. 2008, Wright et al. 2008). The full dataset therefore included two mtDNA and five nuclear loci, although *Nestor meridionalis* was only represented by AANAT, COI and spindlin (Table 1). For seven genera (*Ara*, *Amazona*, *Aratinga*, *Psittacula*, *Cacatua*, *Lorius*, *Platycercus*), sequences for some of the loci were only available for closely related congeners of the species for which we generated AANAT sequences (Table 1). In these cases we utilized such congeneric sequences only when there is no doubt about generic allocation. This was only an issue for some non-strigopid lineages that were not the main focus of this study.

Amplification of AANAT and COI sequences

For AANAT amplification, the following primers were used in combination with the GC-rich PCR system (Roche Diagnostics, Germany): 5′-RGCGCGKTGCCKTTCCTGAAGCC-3′ (forward); 5′-CGCTGTTCCTGCGCATGAAGGCATG-3′ (reverse). We used the following reaction conditions: 95°C/3 min; 95°C/30 s, 58°C/30 s, 72°C/90 s, ten cycles; 95°C/50 s, 62°C/30 s, 72°C/90 s increasing 5 s per cycle, 25 cycles; 72°C/7 min, 15°C/hold. Partial COI sequences were amplified using the primers LCOIp and HCOIp of Wright et al. (2008) at 0.8 μM, in combination with 2× BioMix (Bioline, UK) and the following thermocycling conditions: 94°C/2 min; 94°C/30 s, 45°C/30 s then ramping to 72°C at 0.2°C/s, 72°C/60 s, five cycles; 94°C/30 s, 55°C/30 s, 72°C/60 s, 35 cycles; 72°C/7 min, 15°C/hold. Note that our partial COI sequence is not the COI fragment commonly used in barcoding studies (Vernooy et al. 2010). Amplified products were sequenced following procedures described by Fidler et al. (2004). Sequences were deposited on GenBank with the accession numbers summarized in Table 1.

Table 1. GenBank accession numbers of the sequences used for phylogenetic analysis. Underlined numbers reference sequences generated for this study, while the remaining accession numbers are from previously published studies as described in the Material and methods section. For *Amazona oratrix, Ara militaris, Aratinga erythrogenys, Psittacula derbiana, Platycercus elegans, Lorius garrulus* and *Cacatua galerita*, sequences for some loci were unavailable (Methods) and were substituted with orthologous sequences from close congeners, the names of which are given in the corresponding fields. Abbreviations: AANAT-arylalkylamine *N*-acetyltransferase (nuclear); COI-cytochrome c oxidase I (mitochondrial); ND2 – NADH dehydrogenase subunit 2 (mitochondrial); Trop –tropomyosin intron (nuclear); TGFB-transforming growth factor β 2 intron 5 (nuclear); Rho1 – rhodopsin intron 1 (nuclear), SpinIII – spindlin intron III (nuclear).

Species	AANAT	COI	ND2	Trop	TGFB	Rho1	SpinIII
Strigops habroptilus	**AY997623**	EU621663, **HQ616635**	EU327667	EU665631	EU660301	EU665557	AY741649
Nestor notabilis	**AY997622**	EU621637, **HQ616634**	EU327641	EU665606	EU660276	EU665536	AY741642
Nestor meridionalis	**AY997621**	**HQ616633**	–	–	–	–	AY741653
Cacatua galerita	**EU247791**	**HQ616632**	EU327605 (*C. sulphurea*)	EU665571 (*C. sulphurea*)	EU660243 (*C. sulphurea*)	EU665509 (*C. sulphurea*)	AY741620 (*C. sulphurea*)
Eolophus roseicapillus	**AY487932**	EU621617	EU327621	EU665587	EU660258	EU665522	AY741612
Nymphicus hollandicus	**EU247792**	EU621639	EU327643	EU665608	EU660278	EU665538	AY936562
Psittacus erithacus	**AY487936**	EU621657	EU327661	EU665625	EU660295	–	AY095503
Agapornis roseicollis	**AY487929**	EU621593	EU327596	EU665562	EU660234	EU665501	AY741609
Lorius garrulus	**AY487933**	EU621628 (*L. albidinuchus*)	EU327632 (*L. albidinuchus*)	EU665597 (*L. albidinuchus*)	EU660268 (*L. albidinuchus*)	EU665528 (*L. albidinuchus*)	AY741616 (*L. lory*)
Platycercus elegans	**AY487934**	EU621647 (*P. adscitus*)	EU407704	EU737583	EU737436	EU665544 (*P. adscitus*)	AY741654
Psittacula derbiana	**AY487935**	EU621655 (*P. columboides*)	EU327659 (*P. columboides*)	EU665623 (*P. columboides*)	EU660293 (*P. columboides*)	EU665550 (*P. columboides*)	AY741619 (*P. himalayana*)
Aratinga erythrogenys	**AY487931**	EU621597 (*A. pertinax*)	EU327600 (*A. pertinax*)	EU665566 (*A. pertinax*)	EU660238 (*A. pertinax*)	EU665505 (*A. pertinax*)	AY741625
Ara militaris	**AY487930**	EU621598 (*A. macao*)	EU327601 (*A. macao*)	EU665567 (*A. macao*)	EU660239 (*A. macao*)	EU665506 (*A. macao*)	AY741607 (*A. ararauna*)
Amazona oratrix	**AY499338**	AY301454	AY194465 (*A. ochrocephala*)	EU665564 (*A. viridigenalis*)	EU660236 (*A. viridigenalis*)	EU665503 (*A. viridigenalis*)	AY741645 (*A. ochrocephala*)

Phylogenetic and dating analysis

AANAT sequencing generated a ca 1.7–2.5 kb region of the AANAT gene encompassing three coding exons and two introns of variable length. The presence of up to seven larger indels accounted for variation in AANAT length among parrot species (Supplementary material Appendix 1, Fig. A1). Analysis of AANAT sequences with the program RepeatMasker (< www.repeatmasker.org/cgi-bin/WEBRepeatMasker >) identified *In3*-I-1 and *In3*-I-2 (Supplementary material Appendix 1, Fig. A1) as being LINE retroelements of the chicken repeat 1 class.

On account of these indels, sequence alignment was carried out manually by a single person (FER; Supplementary material Appendix 1, Table A1).

We utilized BEAST ver. 1.5.3 (Drummond and Rambaut 2007) to generate a phylogeny and date specific nodes on it. We unlinked substitution and clock models among loci while keeping tree parameters linked. Coding sequences were divided into three codon positions. The Akaike information criterion as implemented in jModelTest (Posada 2008) was used to evaluate the best fit among 88 evolutionary models for each locus. For BEAST runs, we specified each model as given by jModelTest (Table 2).

We applied an uncorrelated lognormal relaxed clock for each partition. Monophyly of several parrot clades (e.g. Strigopidae; [Cacatuidae+Psittacidae]; Cacatuidae; Psittacidae; Arini [incl. *Ara*, *Amazona* and *Aratinga*]) has been well-corroborated and is not in question (Wright et al. 2008, Schweizer et al. 2010). As BEAST analyses with locus-specific model parameters took too long to complete, we therefore constrained these five uncontroversial clades as monophyletic in the starting tree and in the taxon set parameters of BEAST analysis. However, we are confident that this practice did not introduce unwarranted assumptions as the topologies from preliminary unconstrained analyses (using simpler model specifications) were always identical and branch lengths were very similar (data not shown).

To obtain dates and 95% highest posterior density (HPD) intervals for important lineage divergences, BEAST was run based on calibration dates for three basal nodes in the psittaciform tree: 1) the age of the psittaciform ancestor, 2) the ancestor of the Cacatuidae and Psittacidae (minus Strigopidae), and 3) the ancestor of Cacatuidae. Based on the sparse psittaciform fossil record, only two parrot fossils are currently available that may serve as calibrations in our study (Boles 1993, Waterhouse et al. 2008), and one of these (Boles 1993) is identifiable at below the modern genus level, indicating that its use as a family-level calibration point is probably inappropriate. Hence, any calibration of psittaciform phylogenetic trees must additionally rely on fossils from outside the Psittaciformes. Since our sampling is restricted to parrots, our calibrations were based on three taxonomically more extensive studies that included fossil calibrations of other bird groups (Pacheco et al. 2011, Schweizer et al. 2011, White et al. 2011). Hence, our calibrations are indirectly based on fossil evidence insofar as they are estimates of psittaciform divergence times derived from studies that primarily used non-psittaciform fossil evidence to make these estimates. The first study

Table 2. Details on the evolutionary models computed by the program jModelTest as used in BEAST analyses.

	No. of substitution types	Distribution of rate variation across sites	Proportion of invariable sites	No. of rate categories for gamma distribution
AANAT	2	gamma	0	4
COI	6	gamma	0.645	4
ND2	6	gamma	0	4
Tropomyosin	6	gamma	0	4
Transforming Growth Factor β 2 intron 5	6	equal	0	n/a
Rhodopsin intron 1	6	equal	0.366	n/a
Spindlin intron III	6	gamma	0	4

(White et al. 2011) used six fossil calibrations, including fossils of early 1) Galloanserae, 2) Sphenisciformes, 3) the ancestor of Podicipediformes/Phoenicopteriformes, 4) Pandionidae/Accipitridae, 5) Apodidae/Trochilidae (for citations see White et al. 2011), as well as 6) a psittaciform fossil interpreted to be placed near the Cacatuidae/Psittacidae divergence (Boles 1993). The second study (Schweizer et al. 2011) used the first three of these fossils as calibration points. The third study (Pacheco et al. 2011) used the first two of the above fossils in addition to an Eocene psittaciform bone fragment unidentifiable to any modern genus and interpreted as the minimum age of the psittaciform ancestor (Waterhouse et al. 2008).

Pacheco et al. (2011) and White et al. (2011) both used mitogenomic datasets. As their taxon sampling and choice of fossil calibrations differed only slightly, their dating of major parrot divergences was largely similar. In contrast, Schweizer et al. (2011) used three nuclear genes and obtained different divergence estimates. We used both sets of calibrations separately to generate estimates derived from mitogenomic and nuclear data.

In BEAST, tree priors and priors for times since the most recent common ancestors were set as normal. Our first set of calibrations and their standard deviation ranges, based on Pacheco et al.'s (2011) and White et al.'s (2011) estimates, were as follows: 1) the ancestor of all Psittaciformes was constrained to 47 mya with a standard deviation of 7.5 leading to an approximate 95% HPD interval from 36–59 mya; 2) the ancestor of the Cacatuidae and Psittacidae (minus Strigopidae) was constrained to 41 mya with a standard deviation of 8 leading to an approximate 95% HPD interval from 29–53 mya; and 3) the ancestor of Cacatuidae was constrained to 28 mya with a standard deviation of seven leading to an approximate 95% HPD interval from 18–38 mya.

Our second set of calibrations and their standard deviation ranges, based on Schweizer et al. (2011), were as follows: 1) the psittaciform ancestor was constrained to 58 mya with a standard deviation of 8 leading to an approximate 95% HPD interval from 45–71 mya; 2) the ancestor of Cacatuidae and Psittacidae was constrained to 47 mya with a standard deviation of 7 leading to an approximate 95% HPD interval from 36–59 mya; and 3) the ancestor of Cacatuidae was constrained to 20 mya with a standard deviation of 6 leading to an approximate 95% HPD interval from 10–30 mya.

We used both the nuclear and mitogenomic-based calibrations in BEAST to carry out analyses aimed at two separate levels of evolution. The first analysis (called 'whole-strigopid analysis') aimed to date older (>10 mya) bifurcation events in the strigopid evolutionary history based on an extensive sampling of seven loci. The second analysis (called '*Nestor* analysis') aimed at specifically estimating a separation date for the two *Nestor* lineages using three loci.

For the whole-strigopid analysis, we used our full dataset minus *Nestor meridionalis* (i.e. 13 species and 7 loci). For each of the two calibration sets, we 'trained' operator settings using a BEAST run of 5 million generations, and applied these new settings to subsequent analyses. Then we conducted four final BEAST runs each for the nuclear and mitogenomic calibration set, two of which were run for 50 million generations, one for 150 million and one for 250 million, with tree sampling rates set at 1000. A tracer (Rambaut and Drummond 2008) run determined that 10%, 6.67% and 4% are an appropriate burn-in for the analyses that were run for 50, 150 and 250 million generations, respectively, regardless of calibration set. We log-combined all analyses for each calibration set into one dataset, resampling the trees at a frequency of 10 000. This step resulted in datasets with effective sample sizes of posteriors >100 at burn-in. Since individual trees of all runs were identical in topology and very similar in nodal ages, we are confident that our multiple runs have converged on the same signal.

The *Nestor* analysis was conducted using sequences from all 14 taxa (i.e. including *Nestor meridionalis*) but was restricted to three loci (AANAT, COI, SpinIII), using the same alignment for these loci as in the whole-strigopid analysis. We applied the same two calibration sets as split priors. Again we ran BEAST for 5 million generations to 'train' operator settings for subsequent runs. We conducted four final BEAST runs at 50, 50, 50 and 250 million generations, respectively, and used identical procedures and burn-in rates as in the first analysis to check the quality of runs and combine them.

Results

Our final AANAT alignment amounted to non-gapped sequence lengths ranging from 1716 bp (*Agapornis roseicollis*) to 2519 bp (*Amazona oratrix*; Table 1; Supplementary material Appendix 1 Table A1). We amplified 599 bp of COI sequence from three strigopid and one cacatuid species (Table 1). In combination with previously published psittaciform COI sequences and five additional loci (mtDNA: 1; nuclear: 4), our final concatenated alignment comprised

7250 bp, with variation in sequence length between taxa largely due to variable intron lengths (Table 1, Supplementary material Appendix 1, Fig. A1).

Our whole-strigopid analysis resulted in a fully resolved tree in which all nodes received maximum branch support (Fig. 1, 2). While five unequivocal nodes had been constrained to monophyly (Material and methods), our results provide corroborative evidence on several other less well-established phylogenetic arrangements.

Dates of bifurcating events based on both mitogenomic and nuclear-derived calibrations indicate a separation of the family Psittacidae into its present American, African and Australasian lineages mainly in the Oligocene, although a few events may have occurred in the Late Eocene to Early Miocene (see Fig. 1 and 2 for individual estimates). The Strigopidae separated into *Strigops* and *Nestor* some time between the Late Eocene and Early Miocene, most likely in the mid-Oligocene around 28–29 mya (95% HPD intervals from 18–38 mya; Fig. 1, 2; note that estimates in Fig. 3 and 4 are based on fewer loci, hence priority is given to estimates in Fig. 1 and 2).

The *Nestor* lineage divergence analysis, which was conducted on a dataset comprising all taxa but only three loci (AANAT, COI, SpinIII; Table 1), resulted in identically well-supported trees that, depending on calibration set, provided an estimate for the split between *Nestor meridionalis* and *N. notabilis* at ca 2.3–2.5 mya (95% HPD intervals from ca 1.2–3.9 mya; Fig. 3, 4) well within the Late Pliocene or Pleistocene.

Discussion

Psittaciform diversification

The relationships revealed herein are consistent with previously published psittaciform phylogenies (Fig. 1, 2; de Kloet and de Kloet 2005, Wright et al. 2008, Schweizer et al. 2010, 2011). While the greater part of the present arrangements are not generally disputed, we provide additional support for relationships in the early parrot radiation that have hitherto been shown by few studies (Wright et al. 2008,

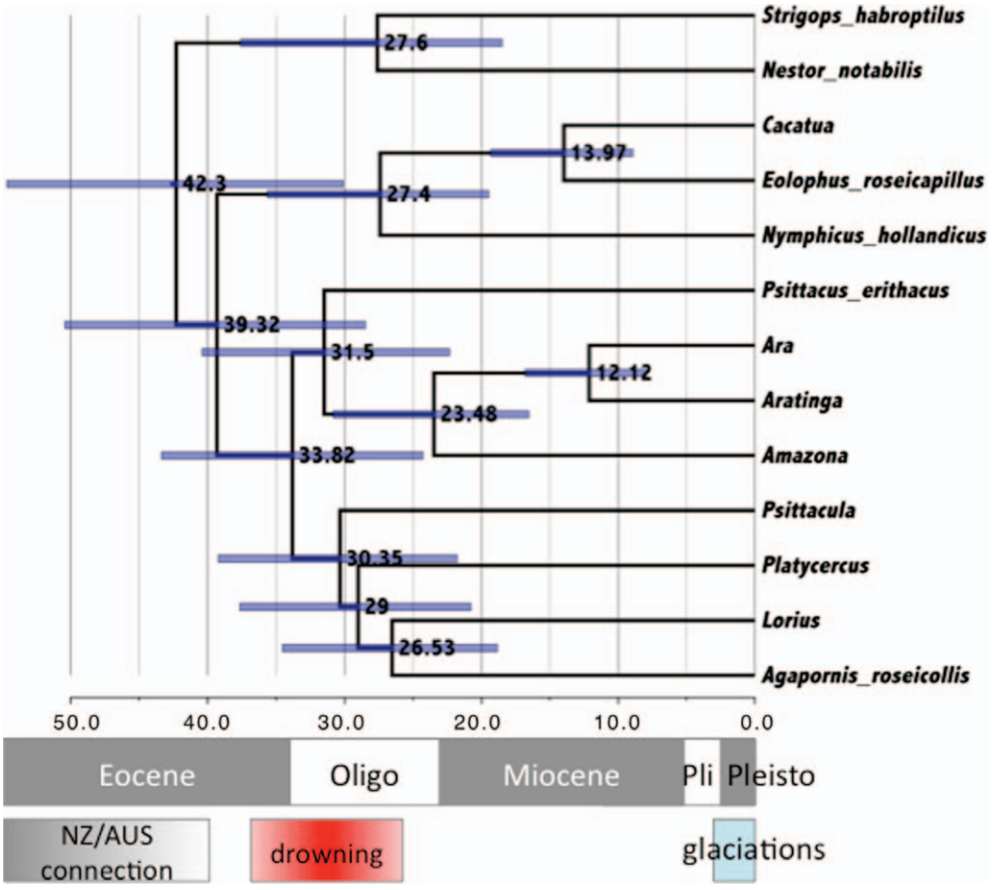

Figure 1. Dating of major psittaciform lineage divergence times using a 7-locus 13-genus sequence dataset (omitting *Nestor meridionalis*) and a calibration set based on mitogenomic studies (Pacheco et al. 2011, White et al. 2011) using independent fossil calibrations. All nodes were recovered with a posterior probability of 1. Numbers at nodes indicate estimated node age, with 95% highest posterior density intervals in purple. Scales below indicate 1) timing in millions of years before present, 2) major epochs and 3) major associated earth-historic events, such as a controversial assumption of New Zealand land connections with Australia in the Late Eocene, the more rigorously validated submergence ('drowning') of large parts of New Zealand around the Oligocene, and the current (Pleistocene) series of glacial and interglacial periods (see Discussion for these earth-historic events). Abbreviations: Oligo – Oligocene; Pli – Pliocene; Pleisto – Pleistocene; NZ – New Zealand; AUS – Australia.

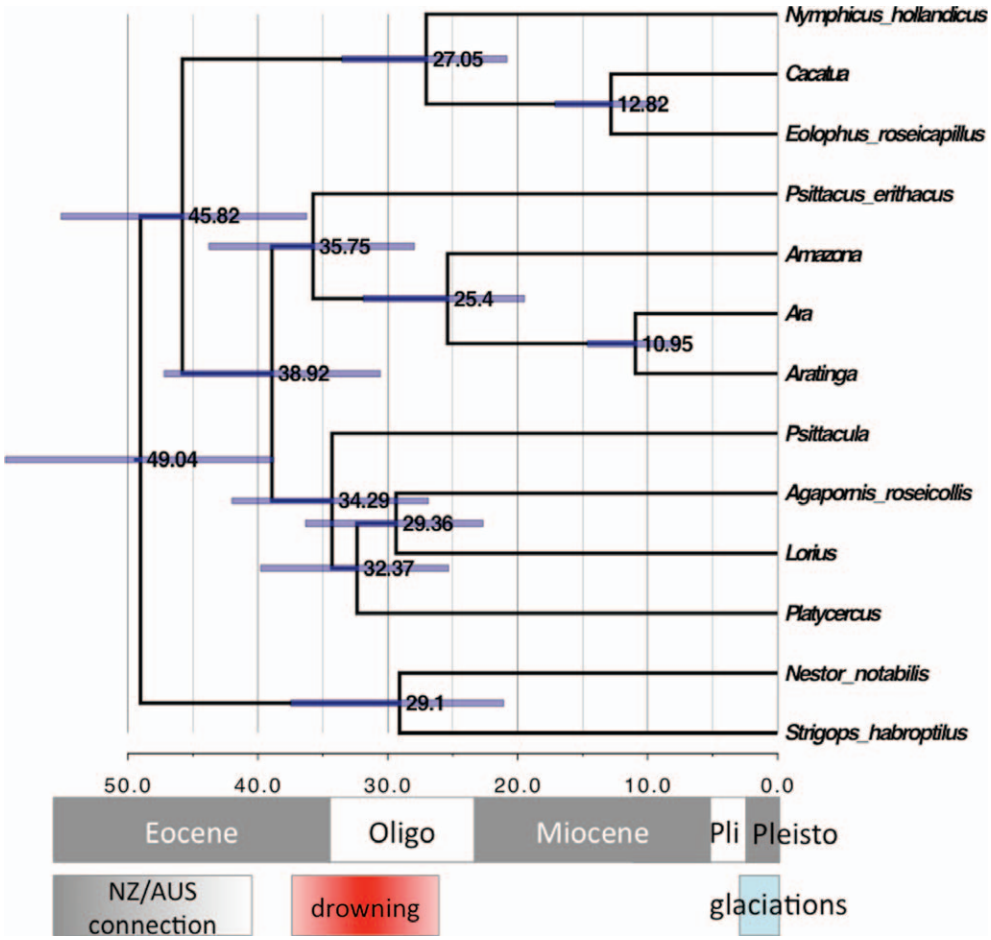

Figure 2. Dating of major psittaciform lineage divergence times using the same dataset as in Fig. 1, but with a calibration set based on a nuclear study (Schweizer et al. 2011) using independent fossil calibrations. For more details, see Fig. 1.

Schweizer et al. 2010). For example, we provide further support for a close relationship between African *Psittacus* and the Neotropical Arini (Fig. 1, 2). We also provide strong support for the African genus *Agapornis* (lovebirds) being more closely related to the Loriini (lorikeets; here represented by *Lorius*) than to the Australian broad-tailed parrots Platycercini (Fig. 1, 2), an arrangement that was well-supported by Schweizer et al. (2010) but was not retrieved in all analyses by Wright et al. (2008).

Evolution of *Strigops*

The genus *Strigops* has a single member, the critically endangered kakapo *Strigops habroptila*. Uniquely amongst parrots, the kakapo has evolved the combined traits of lek-breeding, complete nocturnality and flightlessness (Powlesland et al. 2006), the latter two traits probably a reflection of inhabiting remote islands devoid of terrestrial mammalian predators for >16 million yr (Worthy et al.

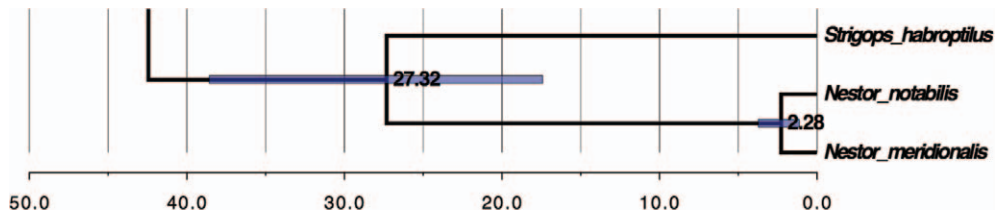

Figure 3. Dating of strigopid lineage divergence times using a 3-locus 14-taxon dataset (including *Nestor meridionalis*) and a calibration set based on mitogenomic studies (Pacheco et al. 2011, White et al. 2011) using independent fossil calibrations. All nodes were recovered with a posterior probability of 1. Numbers at nodes indicate estimated node age, with 95% highest posterior density intervals in purple. Scales below indicate 1) timing in millions of years before present, 2) major epochs and 3) major associated earth-historic events, such as a controversial assumption of New Zealand land connections with Australia in the Late Eocene, the more rigorously validated submergence ('drowning') of large parts of New Zealand around the Oligocene, and the current (Pleistocene) series of glacial and interglacial periods (see Discussion for these earth-historic events). Abbreviations: Oligo – Oligocene; Pli – Pliocene; Pleisto – Pleistocene; NZ – New Zealand; AUS – Australia.

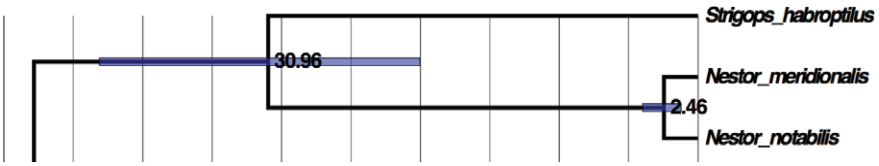

Figure 4. Dating of strigopid lineage divergence times using the same dataset as in Fig. 1, but with a calibration set based on a nuclear study (Schweizer et al. 2011) using independent fossil calibrations. For more details, see Fig. 3.

2006). Our study indicates that *Strigops* diverged from its sister lineage *Nestor* at ca 28–29 mya, *albeit* with a wide 95% probability margin of ca 18–38 mya (Fig. 1, 2). Such a date places the divergence of *Strigops* and *Nestor* in the Oligocene or Early Miocene, a period when the 'Zealandia' microcontinent is thought to have been reduced in size or, potentially, entirely submerged (Trewick et al. 2006, Waters and Craw 2006, Goldberg et al. 2008, Landis et al. 2008, Trewick and Gibb 2010).

The ancestor of Strigopidae probably dates back to the Eocene (>40 mya; Fig. 1). If there was a complete Oligocene submergence of Zealandia's landmass, strigopids would have colonized NZ after the submergence while going extinct everywhere else where they occurred. Given that other studies have similarly revealed the presence in NZ of ancient endemic bird families before the Oligocene (e.g. moas and kiwis; Phillips et al. 2010), we consider it more likely that parts of the Zealandia/NZ archipelago remained above water during the Oligocene although post-Oligocene overwater colonization cannot be ruled out. Fossil evidence also suggests that remnant Gondwanan terrestrial mammals survived in NZ into the early Miocene (ca 16–19 mya; Worthy et al. 2006), implying that the Oligocene submergence of Zealandia may not have been complete. Indeed, rather than simply being an agent of extinction, Oligocene land submergences and the associated land fragmentation may have facilitated the allopatric differentiation of *Nestor* and *Strigops*, and perhaps other avian lineages.

It should be noted that the unusual *Strigops* traits of nocturnality and flightlessness may have evolved long after its divergence from *Nestor*. Flightlessness may have evolved once terrestrial mammalian predators became extinct in NZ after the Early Miocene (≤ 16–19 mya; Worthy et al. 2006), while nocturnality may have evolved as recently as the last two million years in response to the evolution of very large diurnal predators such as Haast's eagle (*Harpagornis moorei*) (Bunce et al. 2005).

Diversification within *Nestor*

In contrast to monotypic *Strigops*, the diurnal and volant genus *Nestor* diversified into four modern species. Two of those inhabited isolated islands in the vicinity of NZ, the Chatham Islands (*Nestor* sp. nov.) and Norfolk Island (*N. productus*), and have gone extinct within the last 500 yr following human settlement (Dawson 1959, Holdaway and Anderson 2001). Although not included in this analysis, these two extinct *Nestor* species are considered closely related to the *N. meridionalis*, one of the two extant *Nestor* species now present in NZ.

On the South Island, the two extant *Nestor* species, the kaka (*N. meridionalis*) and the kea (*N. notabilis*), live in broad sympatry, although they occupy distinct niches (Diamond and Bond 1999). Our results are consistent with an existing hypothesis that kea and kaka diverged relatively recently within the Late Pliocene or Pleistocene (ca 2.3–2.5 mya; Fig. 3, 4; Diamond and Bond 1999). This timing coincides with the onset of a period of recurrent global glaciations along with global fluctuations in sea levels (Lambeck and Chappell 2001, Siddall et al. 2003, Bintanja et al. 2005, Thompson and Goldstein 2005, Caputo 2007). In NZ, the associated climatic fluctuations drove recurrent expansions and retreats of glaciers with concomitant shifts in habitats and cycles of land expansion and submergence, coupled with a narrowing and widening of the Cook Strait as global sea levels fluctuated by up to 130 m (Coates 2002). Such repeated climatic changes may have caused fragmentation of the ancestral *Nestor* species into allopatric populations (Diamond and Bond 1999) during interglacial periods of high sea levels when the North and South Islands are separated, with subsequent range expansions and secondary contact during periods of low sea levels when the two islands are nearly connected. While kaka and kea presently overlap only on the South Island, it would be erroneous to suggest that the North Island served as a refuge exclusively for kaka, as fossil evidence indicates that kea inhabited the North Island until 10 000 yr ago (Holdaway and Worthy 1993).

Evolutionary history of Strigopidae

The Strigopidae have diversified into a mere handful of modern species, which is only a fraction of the present-day diversity of the other two parrot families: Psittacidae (ca 350 species) and Cacatuidae (ca 25 species). Strigopid speciation may have been restricted to two periods when the Zealandia/NZ archipelago went through major biogeographic upheaval: the Oligocene, when much of Zealandia/NZ's landmass may have been submerged underwater, and the Late Pliocene and Pleistocene, when the current regime of repeated glaciations started to lead to profound cyclical changes in NZ's land distribution and associated habitats.

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Supplementary material (Appendix JAV-00200 at <www.oikosoffice.lu.se/appendix>. Appendix 1.