

Short Communication

Relicts of the lost arc: High-throughput sequencing of the *Eutrichomyias rowleyi* (Aves: Passeriformes) holotype uncovers an ancient biogeographic link between the Philippines and Fiji



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ABSTRACT

Molecular studies have revealed a number of cases in which traditional assessments of evolutionary relationships have been incorrect. This has implications not only for systematics and taxonomy but also for our understanding of how diversity patterns on Earth have been formed. Here, we use high-throughput sequencing technology to obtain molecular data from the holotype specimen of the elusive *Eutrichomyias rowleyi*, which is endemic to the Indonesian island of Sangihe. We show that *E. rowleyi* unexpectedly is a member of the family Lamproliidae, which dates back some 20 Million years and only include two other species, *Lamprolia victorae* from Fiji and *Chaetorhynchus papuensis* from New Guinea. Tectonic reconstructions suggest that the Melanesian island arc, which included land masses on the northern edge of the Australian plate (present day New Guinea) stretched as a string of islands from the Philippines (including proto-Sangihe) to Fiji from 25 to 20 My. Consequently, our results are indicative of an ancient distribution along the Melanesian island arc followed by relictualization, which led to members of the Lamproliidae to be distributed on widely separated islands across the Indo-Pacific.

1. Introduction

Natural history collections are unparalleled archives for the study of biodiversity and evolution (Suarez and Tsutsui, 2004) and have thus been harvested for genetic resources for decades (e.g. Ellegren, 1991). However, the fragmented nature of DNA in archaic samples has made it difficult, time consuming and costly to obtain more than limited numbers of loci with traditional amplicon-based Sanger-sequencing. Next-generation sequencing (NGS) technologies, however, specifically target millions of short DNA-fragments and the steady decline in sequencing costs has now made museomics a rising field in biology (e.g. Bi et al., 2013). As such, we can now evaluate the evolutionary and biogeographic history for rare species that were collected many decades ago and for which modern day samples are hard to obtain or are no longer available due to recent extinction.

The Cerulean Paradise-flycatcher (*Eutrichomyias rowleyi*) is an elusive species, endemic to the Indonesian island of Sangihe, a small isolated island between the Philippines and Sulawesi. A single specimen collected in 1873 was formally described in 1878 and placed in the genus *Zeocephus* (Meyer, 1878). The species was after its discovery not seen for more than a hundred years and feared extinct. Two

observations by M. D. Bruce in December 1978 (White and Bruce, 1986); and J. O. H. Small on 11 September 1995 (Riley, 1997) preceded the official rediscovery in October 1998 when a bird was mistnetted. During a survey lasting from October 1998–December 1999 in five valleys on Mount Sahendaruman a minimum of 19 individuals were observed (Riley and Wardill, 2001).

Meyer and Wigglesworth (1898) included the Cerulean Paradise-flycatcher in *Hypothymis* because of its similarity to *Hypothymis puella* [= *H. azurea puella*] from the neighbouring island of Sulawesi, while Meise (1939) erected the monotypic genus *Eutrichomyias* for the species based on morphological differences. Although Meise (1939) considered the closest relative of *Eutrichomyias* to be *Hypothymis* he also noted similarities to *Terpsiphone*. In a footnote to the same paper Stresemann agreed with this conclusion, which was later also supported by Watson et al. (1986).

The rediscovery of *Eutrichomyias rowleyi* has allowed for further taxonomic assessment based on plumage observations, behavioural observations and vocalization. This additional information led the authors to conclude that *Eutrichomyias* shows characters of both *Hypothymis* and *Terpsiphone*, although it is perhaps more closely allied to the former.

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To assess the taxonomic affinity of the critically endangered Cerulean Paradise-flycatcher *E. rowleyi* from the only existing museum specimen, the Dresden holotype from 1873 (specimen data in [Eck and Quaiser \(2004\)](#)), we used NGS in order to recover the complete mitogenome and select nuclear markers. Our finding that *E. rowleyi* is a member of Lamproliidae whose other two members are endemic to New Guinea and Fiji is not only unexpected from a taxonomic point of view, but also suggests a link along the entire Melanesian island arc, which connected these far apart islands as far back as 25 Mya. Thus our study adds an important component to our understanding of species distributions and biogeography across vast island settings.

2. Material and methods

2.1. Library preparation and sequencing

DNA was extracted from a footpad sample from the type specimen of *E. rowleyi* following the precautions described in [Irestedt et al. \(2006\)](#). The genome library preparation followed the protocol published by [Meyer and Kircher \(2010\)](#). The library was pooled with one other species and sequenced on a single Illumina HiSeq X lane.

2.2. Bioinformatics

Illumina sequencing reads were processed using a custom designed workflow that is available at <https://github.com/mozesblom>. The workflow removes adapter contamination, low-quality bases and low-complexity reads. Trimming and adapter removal was done using TRIMMOMATIC (v.0.32 [[Bolger et al., 2014](#)]; default settings) and overall quality and length distribution of sequence reads was inspected prior and post the clean-up workflow using FASTQC (v.0.11.5 [[Andrews, 2010](#)]).

2.3. Assembly

Since phylogenetic placement remained uncertain and a suitable reference for direct mapping therefore absent, we assembled the *E. rowleyi* mitochondrial genome with an iterative baiting and mapping approach (MITObim v1.8 [[Hahn et al., 2013](#)]; default settings). MITObim finds initial regions of similarity between a target library and a distant reference, and then uses an iterative mapping strategy to find reads that overlap with these initial segments without using the initial reference. This reference free strategy is repeated until the complete mitochondrial genome is assembled or if no further overlapping reads are identified. We randomly subsampled five million paired-end reads and used a manually generated consensus sequence as initial reference seed. This reference was based on seven existing mitochondrial genomes from members within the Corvidae (Genbank accession numbers: Y18522.2, NC_015824.1, JQ423932.1, NC_020426.1, KJ598623.1, NC_025927.1 and KR057957.1). The resulting mitochondrial assembly was subsequently corrected and validated by mapping all sequence reads against the inferred mitogenome using the BWA mem aligner ([Li and Durbin, 2009](#); default settings). We first identified and removed PCR duplicates with Picard tools (MarkDuplicates) and used GATK ([McKenna et al., 2010](#)) to call any potential variable sites between reads and reference (HaplotypeCaller) and to ultimately generate a consensus sequence (FastaAlternateReferenceMaker). We then mapped cleaned reads directly against a select collection of traditional nuclear loci and called genotypes if read depth exceeded 3x coverage using the same mapping approach as described above.

2.4. Phylogenetics

We blasted the recovered mtDNA genes from *E. rowleyi* to the GenBank database and established that *E. rowleyi* is closely related to Lamproliidae. Consequently, we focused our efforts on five nuclear

introns and the mitochondrial gene ND2 that are available for the two known members of Lamproliidae as well as for a broad selection of other closely related corvid bird families (Supplemental Table 1).

DNA sequences were aligned for each gene individually and subsequently inspected in SEAVIEW. Each of the six gene partitions (ND2, GAPDH, ODC, Myo2, tgf2 and Fib-5) was then analyzed separately in BEAST ([Drummond et al., 2012](#)) applying the most appropriate model of nucleotide evolution as determined by Modeltest 3.7 (ND2: GTR+I+ Γ ; Myo: GTR+ Γ ; GAPDH: GTR+ Γ ; ODC: GTR+I+ Γ ; Tgf2: GTR+ Γ ; Fib5: GTR+ Γ) ([Posada and Crandall, 1998](#)) following the Akaike Information Criterion. We then analyzed the concatenated dataset using the same six nucleotide substitution partitions. To obtain absolute dates we applied to our ND2 partition a rate of 0.0145 substitutions per site per lineage (2.9%) per Myr following [Lerner et al. \(2011\)](#). In the same analysis we used a craticid fossil from the Early Miocene (16.3–23 Mya) deposits of Australia (*Kurrartapu johnnguyeni*; [Nguyen et al., 2013](#)). This calibration point was used to date the divergence leading to *Artamus* and *Peltops* (offset = 16.3, mean = 2, 95% HPD interval = 16.35–23.68 Mya).

For all analyses we ran multiple independent Markov chain Monte Carlo (MCMC) chains for 100 million generations using a relaxed uncorrelated lognormal distribution for the molecular clock model and assuming a Yule speciation process for the tree prior. Convergence diagnostics were assessed using TRACER ([Rambaut and Drummond, 2007](#)) and a maximum Clade Credibility (MCC) tree was summarized using TreeAnnotator ([Drummond et al., 2012](#)).

3. Results and discussion

The molecular data (both mitochondrial and nuclear) unequivocally places *E. rowleyi* as sister to *Lamprolia victoriae* from Fiji within the recently described subfamily Lamproliinae ([Fig. 1](#)), which also includes *Chaetorhynchus papuensis* of New Guinea ([Schodde and Christidis, 2014](#)). While this clade was described as a subfamily within the family Rhipiduridae by [Schodde and Christidis \(2014\)](#), its great temporal divergence from core fantails (*Rhipidura*) probably warrants recognition at family level ([Holt and Jønsson, 2014](#); [Jønsson et al., 2016](#)). Thus, we refer to this group as Lamproliidae. This systematic affinity is novel, as *E. rowleyi* was thought to belong within passerine families present on nearby islands, either within “true” flycatchers (Muscicapidae) or within monarch flycatchers (Monarchidae, which includes both *Hypothymis* and *Terpsiphone*) ([Meise, 1939](#); [Meyer, 1878](#); [Meyer and Wigglesworth, 1898](#)).

The recent finding that *Lamprolia victoriae* and *Chaetorhynchus papuensis* are sister taxa ([Irestedt et al., 2008](#)) was itself surprising because of their disjunct distributions and different morphological appearances. The addition of *E. rowleyi* to Lamproliidae adds to both the morphological and the biogeographical complexity of this subfamily and makes the evolutionary history of this clade even more intriguing. Given the rather old divergence dating back some 20 My, [Irestedt et al. \(2008\)](#) put forth two plausible scenarios to explain the distributions of Lamproliidae, either via long-distance dispersal or relictualization of a previously widespread taxon across the whole island arc. Some authors have claimed that there was not sufficient land available for a terrestrial fauna to persist outside of Australia at that time ([Moyle et al., 2016](#); [Schodde and Christidis, 2014](#)), which would imply that an ancestral Australian Lamproliidae diversified into three species, which then later on dispersed to Sangihe, New Guinea and Fiji.

The Melanesian archipelagos together with the northern mountain ranges of New Guinea are part of a volcanic island arc system at the subduction zone between the Australian and Pacific plates ([Hall, 1998, 2002, 2011, 2012, 2013](#)). This arc system was nearly continuous from the Philippines to Fiji from ca 25 Mya, until ca 12 Mya onwards, when the opening of the North Fiji basin commenced, creating a gap between Fiji and Vanuatu ([Hall, 2002](#); [Schellart et al., 2006](#)).

From about 25 Mya, this arc system was pushed westwards in front

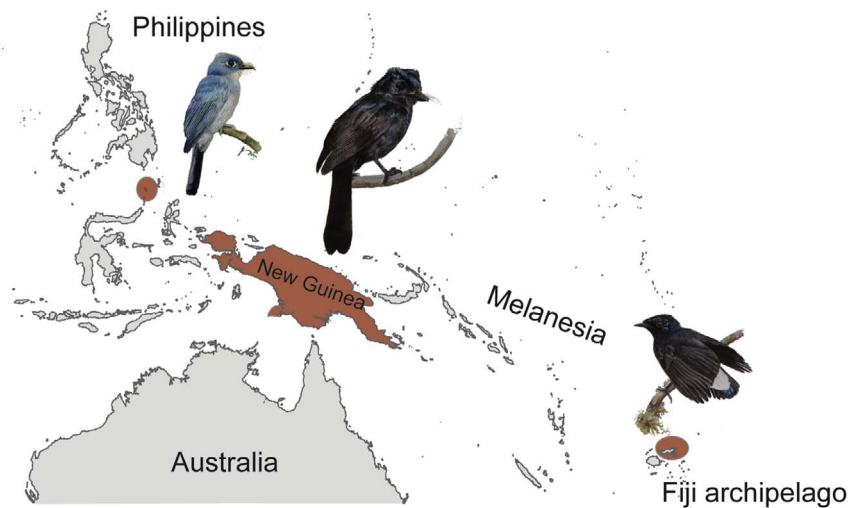
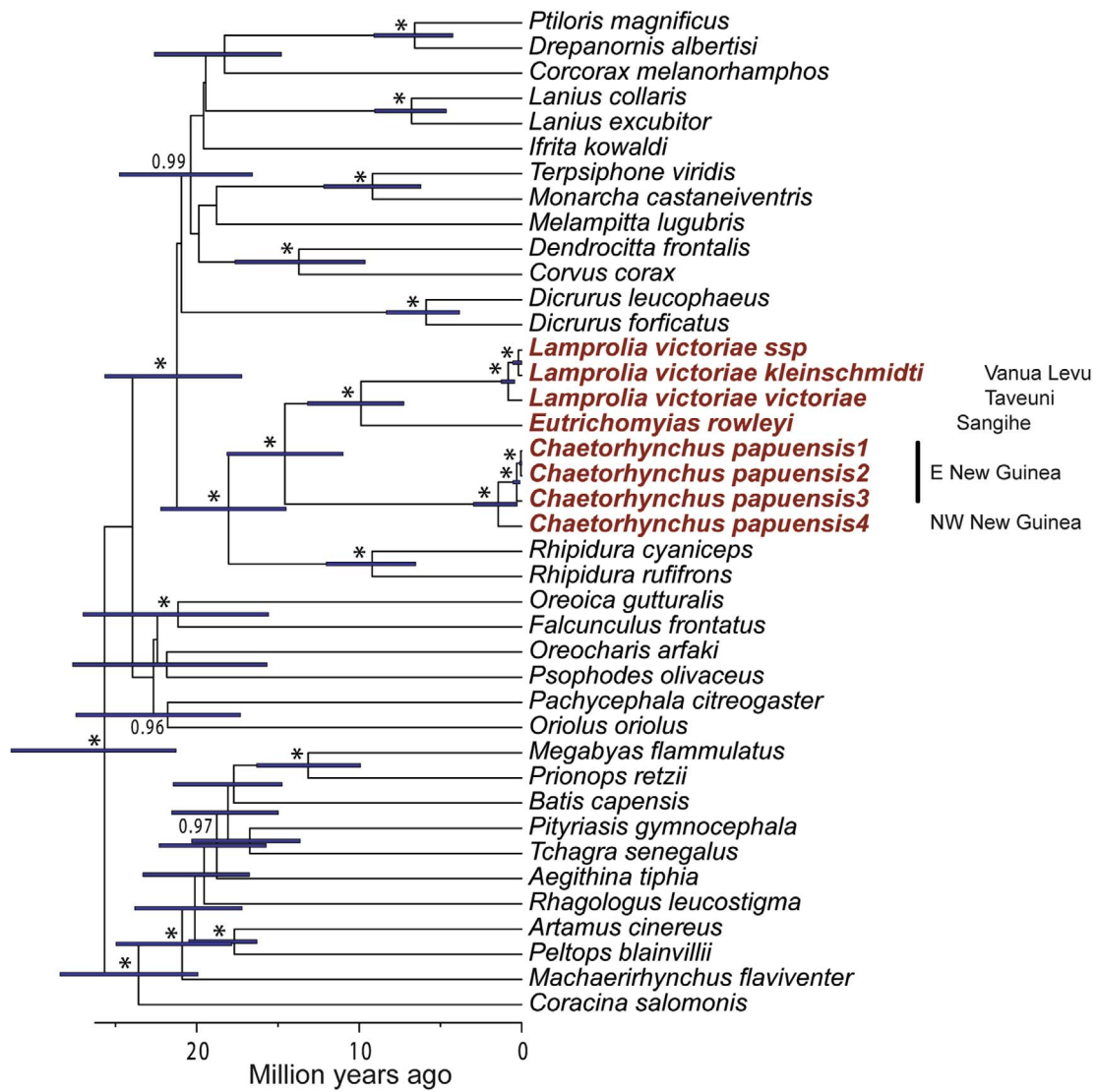


Fig. 1. Chronogram of the Corvidae with particular emphasis on the subfamily Lamproliidae and closely related clades. Posterior probabilities > 0.95 are indicated to the left of nodes (PP = 1.00 denoted with an asterisk). Blue bars represent 95% highest posterior density (HPD) intervals. Terminal taxa coloured red are members of the Lamproliidae. At the bottom the map indicates the distributions of the three members of the Lamproliidae consistent with an ancestral distribution along the entire Melanesian island arc, which stretched from the Philippines via New Guinea to Fiji, followed by relictualization. Illustrations of the three members of the Lamproliidae by Jon Fjeldså. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

of the advancing Australo-Papuan plate, leading to a collision between the island arc and the first subaerial areas at the northern edge of the Australasian plate (present day New Guinea). In this process, several terranes were accreted to the northern margin of the Australian plate, forming the northern coastal mountain ranges of New Guinea (Hall, 2002). Knowledge of the actual extent of land during different periods is uncertain, but parts of the Melanesian Arc have no doubt been continuously above water and during periods of low sea levels, partial and intermittent land connections may have been formed along the chain, facilitating dispersal of organisms (see Jönsson et al., 2017).

The origin of Lamproliidae and its sister family Rhipiduridae is well-established to be Papuan (Nyári et al., 2009; Jönsson et al., 2011) and it appears that the ancestral Lamproliidae may thus successfully have colonised the entire length of this island arc, some 20 Mya, followed by differentiation/speciation, extinction and finally relictualisation leaving divergent sister taxa on far apart islands. We find this latter explanation more parsimonious than the independent dispersal scenario. A similar island origin scenario is also proposed for the entire corvid passerine bird radiation (Jönsson et al., 2011) and the current distribution of Lamproliidae may be best explained in light of taxon cycles (Ricklefs and Bermingham, 2002; Wilson, 1961) in which an ancestral taxon expanded its range, differentiated and then contracted as individual island populations/species went extinct. Such a scenario is also evident from other groups within corvid passerine birds (e.g. Jönsson et al., 2014). The expectation being that relictual species are assumed to persist the longest on larger islands, as species on smaller islands are at a higher risk of going extinct. In the case of Lamproliidae, two relictual forms occur on relatively small islands (Greater Sangihe, Vanua Levu and Taveuni), but the islands in the Fiji archipelago may have been connected at times of low sea-levels and have combined formed a larger area in the past.

4. Conclusions

This particular case constitutes an exceptional example of a bird species whose true affinity was for a long time completely misinterpreted and how this affects biogeographical and evolutionary interpretations. The present study further demonstrates how contemporary sequencing technologies and natural history collections can be jointly utilized to shed further light on the evolutionary history of elusive or rare taxa. Such studies can improve our understanding of the processes that determine biodiversity over long periods of time and the biogeographic history of the regions where these taxa occur.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.jmpev.2017.11.021>.

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