Molecular Phylogenetics and Evolution 124 (2018) 100–105

Contents lists available at ScienceDirect

Molecular Phylogenetics and Evolution

journal homepage: www.elsevier.com/locate/ympev

Short Communication

Molecular phylogenetics and species limits in a cryptically coloured radiation of Australo-Papuan passerine birds (Pachycephalidae: *Colluricincla*)

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ARTICLE INFO

Keywords:
Passerine birds
Corvides
Australia
New Guinea
Cryptic species
Species delimitation

ABSTRACT

Detailed knowledge of species limits is an essential component of the study of biodiversity. Although accurate species delimitation usually requires detailed knowledge of both genetic and phenotypic variation, such variation may be limited or unavailable for some groups. In this study, we reconstruct a molecular phylogeny for all currently recognized species and subspecies of Australasian shrikethrushes (*Colluricincla*), including the first sequences of the poorly known *C. tenebrosa*. Using a novel method for species delimitation, the multi-rate Poisson Tree Process (mPTP), in concordance with the phylogenetic data, we estimate species limits in this genetically diverse, but phenotypically subtly differentiated complex of birds. In line with previous studies, we find that one species, the little shrikethrush (*C. megarhyncha*) is characterized by deep divergences among populations. Delimitation results suggest that these clades represent distinct species and we consequently propose a new classification. Furthermore, our findings suggest that *C. megarhyncha melanorhyncha* of Biak Island does not belong in this genus, but is nested within the whistlers (*Pachycephala*) as sister to *P. phaionota*. This study represents a useful example of species delimitation when phenotypic variation is limited or poorly defined.

1. Introduction

Species are the fundamental taxonomic units of biological classification and it is therefore natural that the delimitation of species is an essential component in studies of biodiversity and related disciplines. Examples of fields that are critically dependent on a rigorous and consistent species delimitation include ecological studies of community composition and assembly (Webb et al., 2002), the modelling of evolutionary diversification dynamics through time and space (Etienne and Rosindell, 2011) and conservation (Mace, 2004; Fujita et al., 2012). Although the definition of species is still contentious (de Queiroz, 2007), species delimitation remains an active field of research as is evident from the multitude of species delimitation methods that have become available in recent years (e.g. Pons et al., 2006; Tobias et al., 2010; Reid and Carstens, 2012; Zhang et al., 2013; Solís-Lemus et al., 2015). The majority of these methods take advantage of the dramatic recent increases in genetic data and associated molecular phylogenies. An underlying assumption of such approaches is that phylogenetic branching patterns can be divided into speciation and extinction processes that operate between species and the coalescent population processes that occur within species. As such, the delimitation methods aim to establish the threshold at which the shift from one process to another occurs. Here we use such delimitation approaches to resolve species limits in a genus of passerine birds with a long and convoluted taxonomic history.

Colluricincla shrikethrushes are confined to Australia, New Guinea and the nearest smaller islands and represent a clade of corvoid passerine birds in the family Pachyecphalidae (whistlers). One species in particular, the little shrikethrush (*C. megarhyncha*) has been suspected of harbouring significant unrecognized species diversity (Deiner et al., 2011; Beehler and Pratt, 2016). Historically, more than 30 subspecies were described for *C. megarhyncha*, reflecting the sometimes slight, but noticeable variation in morphology among local populations. Accordingly, its taxonomy has gained considerable interest by ornithologists, many of whom have suggested that *C. megarhyncha* represents multiple distinct species (e.g. Mayr, 1944; Ford, 1979; Deiner et al., 2011; Beehler and Pratt, 2016). This notion has been exacerbated by recent molecular studies, which have demonstrated significant genetic

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https://doi.org/10.1016/j.ympev.2018.02.029
Received 18 May 2017; Received in revised form 26 January 2018; Accepted 27 February 2018
Available online 08 March 2018
1055-7903/

divergence among lineages comparable to other, highly divergent species or even genera (Deiner et al., 2011). Despite these findings, no attempts have been made to redefine species limits in this group, presumably due to the lack of concordance between genetic and morphological data, and limited morphological variation between populations.

Here we assess species limits within *Colluricincla* by combining existing molecular data with newly generated DNA sequences. First, we infer a molecular phylogeny of all currently recognized species and subspecies within the genus *Colluricincla*, including for the first time the poorly known sooty shrikethrush (*C. tenebrosa*). Second, we investigate species limits in this particular group using a novel and improved method for species delimitation, the multi-rate Poisson Tree Process (mPTP, Kapli et al., 2017) as well as the more well-established Generalised Mixed Yule Coalescent (GMYC) method (Pons et al., 2006). Finally, finding that *C. megarhyncha* is comprised of seven highly divergent clades that are suggested to represent distinct species, we propose a new classification for the group.

2. Materials and methods

2.1. Taxon sampling

We included molecular data for all five species (264 individuals) of *Colluricincla* shrikethrushes (mainly sourced from Deiner et al., 2011; Nyari and Joseph, 2013; Jønsson et al., 2010) following the IOC World Bird List v7.2 (Gill and Donsker, 2017). As outgroups, we included *Pseudorectes ferrugineus* and *Melanorectes nigrescens*. We sequenced an additional 24 ND2, 5 GAPDH, 5 ODC and 5 Myo2 sequences using standard protocols. Our final dataset included the mitochondrial gene ND2 for all individuals and three nuclear introns (GAPDH, ODC and Myo-2) for 12 individuals representing all five currently recognized species and outgroups. All new sequences have been uploaded on GenBank under accession numbers MG288640-MG288673 (Appendix A).

2.2. Phylogenetic and dating analyses

DNA sequences were aligned for each gene individually using Seaview (Gouy et al., 2010). A large number of the ND2 sequences were identical and as this may bias some species delimitation approaches we removed these prior to further analyses, retaining ND2 sequences for 129 individuals of *Colluricincla* with dense geographical sampling (Fig. 1, Appendix A). We analysed both a concatenated dataset of all genes as well as a separate ND2 dataset using maximum likelihood (ML) as implemented in RAxML v8.2.4 (Stamatakis, 2014) and run on the CIPRES Science Gateway v3.3 (Miller et al., 2010) using default settings. For the concatenated analyses, we partitioned the data by codon position for the mitochondrial ND2 gene and by gene for the three nuclear introns. Using the rapid bootstrap technique we computed the most likely tree simultaneously with 100 bootstrap replicates, applying the default GTR + Γ substitution model to each partition.

We also analysed the ND2 and concatenated datasets using a Bayesian approach in BEAST v1.8.4 (Drummond et al., 2012). We used the same partitioning strategy as described for the ML analyses, but applied the most appropriate model of nucleotide substitutions to each partition as determined by jModelTest2 (Darriba et al., 2012), following the Bayesian Information Criterion (BIC). We thus used HKY + Γ for ND2 codons 1 and 3, HKY + I for ND2 codon 2, K80 for GAPDH and Myo2, and HKY + I for ODC. We unlinked clock models for all partitions except for ND2. Rates across the three ND2 codon partitions were linked and we applied a rate of 0.0145 substitutions per site per lineage (2.9%) per million years (Lerner et al., 2011). A relaxed uncorrelated lognormal distribution was used for the molecular clock model and we assumed a birth-death speciation process for the tree prior. We ran Markov Chain Monte Carlo (MCMC) chains for 100 million generations sampling every 10,000 generation for the analysis of the concatenated dataset, and for 50 million generations sampling every 5000 generation for the analysis of the ND2 alignment. Convergence diagnostics were assessed using Tracer v1.6 (Rambaut et al., 2014). Output trees were summarized as maximum clade credibility (MCC) trees using mean node heights after discarding 25% of generations as burnin using TreeAnnotator v1.8.4 (Drummond et al., 2012).

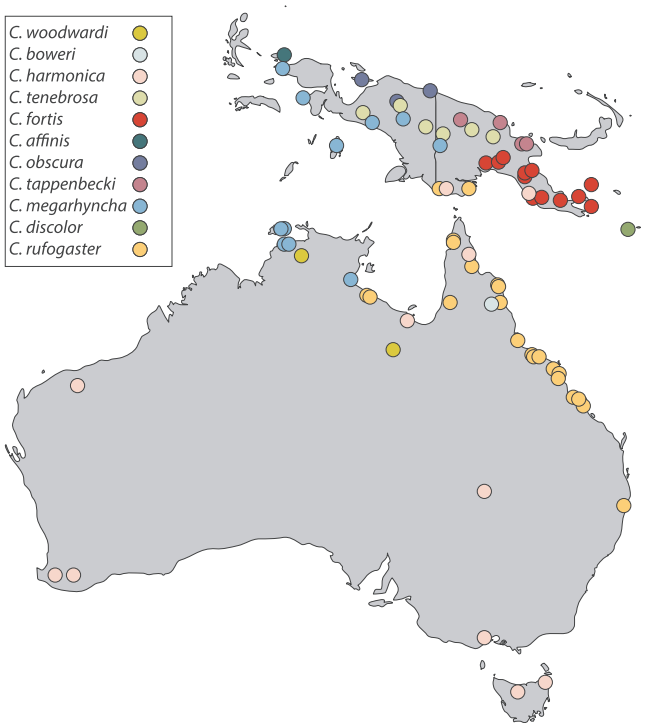

Fig. 1. Sampling localities for the 129 sequences included in this study. Colours and taxon names refer to the eleven species delimited in this study (this figure).

2.3. Single-locus species delimitation

To assess species limits in the little shrikethrush species complex, we applied the recently developed multi-rate Poisson Tree Process (mPTP) approach (Kapli et al., 2017). This method is an extension to the original PTP method introduced by Zhang et al. (2013), both of which attempt to delimit the transition from between- (speciation) to within-species (coalescence) processes. However, unlike PTP, which assumes that all species evolve with a single evolutionary rate, mPTP allows each species to have different rates. Using ML optimization, a separate parameter is estimated for the speciation and coalescence processes, respectively, and their fit to the data is evaluated. mPTP assumes that branching events within species will be more frequent than between species, with each substitution having a small probability of generating branching events. Unlike other species delimitation methods such as GMYC, mPTP does not require an ultrametric tree, thus eliminating potential errors and confounding effects associated with molecular dating.

We evaluated species limits with mPTP using the RAxML ND2 tree as input. As the presence of very similar sequences may confound the delimitation analyses leading to false positives (i.e. oversplitting), we calculated the minimum branch length to correct for this potential source of error. To assess the confidence of our ML species delimitation, we ran ten MCMC chains of 1 million steps each. Overall support for our ML estimate was then estimated by calculating average support values (ASV) across all ten MCMC runs following Kapli et al. (2017). Briefly, ASV values close to one indicate high support for the ML species delimitation. Convergence of each chain was assessed by calculating the