Short Communication

Revising the phylogenetic position of the extinct Mascarene Parrot
Mascarinus mascarin (Linnaeus 1771) (Aves: Psittaciformes: Psittacidae)

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ARTICLE INFO

Article history:
Received 4 August 2016
Revised 25 November 2016
Accepted 20 December 2016
Available online 22 December 2016

Keywords:
Mascarinus
Mascarin
Coracopsis
Psittacula
Extinction
Indian Ocean parrots

ABSTRACT

The phylogenetic position of the extinct Mascarene Parrot Mascarinus mascarin from La Réunion has been unresolved for centuries. A recent molecular study unexpectedly placed M. mascarin within the clade of phenotypically very different Vasa parrots Coracopsis. Based on DNA extracted from the only other preserved Mascarinus specimen, we show that the previously obtained cytb sequence is probably an artificial composite of partial sequences from two other parrot species and that M. mascarin is indeed a part of the Psittacula diversification, placed close to P. eupatria and P. wardi.

1. Introduction

The Mascarene Parrot or Mascarin, Mascarinus mascarin (Linnaeus, 1771), was one of a series of bird species from the Mascarene Islands (Mauritius, La Réunion and Rodriguez) to face extinction soon after the islands became first visited by humans. Hunting and the introduction of predators badly impacted populations of native animals, most famously, the Dodo Raphus cucullatus. The Mascarin from La Réunion was firstly mentioned 1674 by Dubois, and the last Mascarins were seen in the wild in the 1770s. Captive specimens in Europe were mentioned up to the 1780s, thus very probably the species became extinct before 1800 (Hume, 2007). Only two specimens are held in scientific collections, one in Paris (MNHN 211), the other one in Vienna (NMW 50.688).

The species name has been spelled alternatively “M. mascarin” or “M. mascarinus” as a consequence of the different interpretation of the use of periods at the end of species epithets in Linnaeus’ (1771) original description. Here we follow the interpretation that “mascarin” was not intended as an abbreviation of “mascarinus” as stated explicitly by Peterson (2013) and Dickinson and Remsen (2013) and applied by del Hoyo and Collar (2014). For taxonomic names above the species level we follow the system proposed by Joseph et al. (2012).

The scarcity of specimens and poor descriptions hampered a straightforward taxonomic assessment for a long time. The morphological appearance of Mascarinus superficially resembles that of the Psittaculini (including, among others, the genera Tanygnathus and Psittacula), especially in having a large red bill which is a distinctive trait of that tribe and there are also shared osteological traits (Hume, 2007; Cheke and Hume, 2008). Concerning other traits, the Mascarin was phenotypically distinct from other Psittaculini not only because of its enormous bill size and its relatively short tail, but also by having a conspicuous black mask on the forehead and foreparts of the facial region, a mainly blackish-brown plumage that lacks a black semi-collar on the sides of the lower facial region and, as in some Psittacula, a nuchal collar (although a sharp demarcation between the colours of the head and dorsal surface as often seen in Psittacula is evident in paintings) and obvious patches on wing coverts (Hume, 2007; Hume and van Grouw, 2014).

A recent study (Kundu et al., 2012) provides the first putative DNA sequence of a Mascarin (GenBank accession no. GQ996499), reported as a fragment of mitochondrial cytochrome b (cytb) gene derived from a tissue sample from the Paris specimen. In contrast
to previous classifications (Forshaw, 1989; Juniper and Parr, 1998), the DNA-based tree of Kundu et al. (2012) clearly placed Mascarinus among the Vasa parrots, genus Coracopsis (tribes Psittacini or Coracopseinae, respectively) and, most surprisingly, actually nested within the different subspecies of C. nigra. This conclusion has been debated on several occasions (Hume and Wolters, 2012; Joseph et al., 2012; Safford and Hawkins, 2013), in particular, as published phylogenies of parrots with a broad taxon sampling (Wright et al., 2008; Schweizer et al., 2010, 2011) show that Coracopsis and Psittaculini are only distantly related.

The clear morphological distinctiveness between the Mascarin and the Vasa parrots and reservations expressed by Joseph et al. (2012) about the reliability of the DNA sequence provided by Kundu et al. (2012) prompted us to re-evaluate the phylogenetic position of the Mascarin by repeating a cytb sequence analysis with a sample from the other available specimen from the Natural History Museum of Vienna.

2. Materials and methods

A small foot pad tissue sample was taken from the Mascarin specimen (NMW 50.688) held at the Natural History Museum in Vienna. DNA isolation was performed using the QiAmp DNA micro kit (Qiagen, Hilde, Germany), especially suited to samples with minimal amounts of DNA. We followed the manufacturers protocol for animal tissues, opting for the addition of carrier RNA and choosing an elution volume of 30 μl. Digestion was commenced by adding 20 μl proteinase K, followed by overnight incubation at room temperature. We then added a second volume of 20 μl Proteinase K and the sample was incubated for additional 3 h at room temperature. PCR was done using a restorative mix, including 1 μl restorative (Sigma, Germany) and 3 μl DNA in a final 50 μl mastermix.

PCR conditions comprised a 30 min first step at 37 °C (allowing restorative to repair single strand breaks in double strand DNA), a 5 min amplification step at 72 °C and an initial denaturation step of 30 s at 94 °C. This initial procedure was followed by 40 cycles of 30 s at 94 °C (denaturation), 60 s at 48 °C (primer annealing) and 90 s at 72 °C (primer extension). A final elongation step was set for 2 min at 72 °C. We used two pairs of PCR primers previously developed for the amplification of two non-overlapping cytb fragments from parrot DNA (Manegold and Podosiadlowski, 2014): cytb-f1: 5’-CCTGATGAAACCTTAATTGGCTCC-3’, cytb-r1: 5’-CTCGGCGGCTA TGTTGAGTGGT-3’ (220 bp); cytb-f2: 5’-GGGATACCACTCCGTATGTA GAATGG-3’, cytb-r3: 5’-GGTAGGGTGGAAGTTTTCT-3’ (310 bp). As other primer pairs (cytb primer pairs from Sorensen et al., 1999; Manegold and Podosiadlowski, 2014) did not work with the Mascarin sample, we designed a third pair of primers, derived from the sequences obtained with those first two primer pairs in order to sequence the gap between the first two fragments: cytb-f5: 5’-GGTGACTAATTCGCCGAC-3’, cytb-r5: 3’-GGTAGGAGGAAGTGCAGGGGC-3’ (331 bp). PCR products were visualized on agarose gels. Successfully amplified fragments were purified with a column based PCR purification kit ( Macherey Nagel, Germany) and sequenced using the PCR primers as sequencing primers. All PCR products were sequenced at least two times.

Several precautions were taken to ensure that the sequences represent genuine DNA from the museum sample and not any kind of contamination. The DNA isolation procedure, PCR setup and PCR experiments were done in a special lab unit devoted to the isolation of DNA from ancient museum samples. Control PCR experiments were done in the absence of target DNA to exclude lab contaminations. All sequence reads were individually compared to external and internal databases (e.g. including the results of a previous study with parrots from the genus Agapornis) to detect possible contaminations before sequences were combined into contigs.

Overlapping sequences were combined applying the CAP contig assembly tool as implemented in BioEdit 7.0.9 (Hall, 1999). The final contiguous sequence consisted of 595 bp (NCBI GenBank accession number KX663839). For sequence comparison and computing phylogenetic trees, all available cyt b sequences from parrots (Psittaciformes) were obtained from the NCBI nucleotide database. Redundant sequences with identical or only minor differences were omitted from the taxon sampling resulting in a set of more than 300 cyt b sequences. A gap-free alignment was obtained using MAFFT, version 7.245 (Kato and Standley, 2013). Phylogenetic trees were computed with RAxML, v. 8.02 (Stamatakis, 2014), maximum likelihood analyses were performed by computing trees for 100 bootstrap pseudoreplicates, followed by final computation of the best tree. The selected model was GTR + CAT, the dataset was partitioned according to codon positions (first, second and third codon positions were modeled independently). In a first analysis we computed a tree for all parrot cyt b sequences available from GenBank, including the Mascarin sequences from both the Paris and the Vienna specimens. A second analysis was done only with the Vienna specimen sequence, all available members of the genus Psittacula and Tanygnathus and a selection of out-group taxa.

3. Results

PCR Amplification was successful in three overlapping segments. None of the fragments is 100% identical to any sequence in the NCBI nucleotide database, including the Mascarin sequence obtained from the Paris specimen. The resulting contiguous sequence of the Vienna specimen shows 48 mismatches in 545 aligned bp (about 9% divergence) with the sequence published by Kundu et al. (2012).

A thorough comparison of the sequence obtained by Kundu et al. (2012) with those of the Lesser Vasa Parrot Coracopsis nigra, of the Vienna Mascarin specimen and of selected Psittacula sequences confirms our suspicion and that of Joseph et al. (2012) of the artificial nature of sequence GQ996499 (Fig. 1). Two stretches of 240 bp and 84 bp are identical to sequence GQ996513 of C. nigra barklyi as well generated in the course of the study by Kundu et al. (2012), while the remaining middle segment is almost identical (only one mismatch in 230 bp) to a cytb sequence from Psittacula alexandri fasciata (GQ996507: again from the very same study by Kundu et al. (2012)). Such a clustered distribution of matches and mismatches is highly unexpected and unlikely with respect to common patterns of sequence variation, particularly between closely related species. Indeed, mismatches are much more randomly scattered within the genus Psittacula and also compared to the Vienna Mascarin sequence (Fig. 1).

The phylogenetic analysis reveals the position of the Vienna specimen sequence among the members of the genus Psittacula, whereas the sequence from Kundu et al. (2012) is found in a clade with Coracopsis (data not shown). However, due to the large number of OTUs (more than 200) compared to the small size of the alignment (600 bp), bootstrap percentages for many branches were below 50%. To get a better resolution and higher bootstrap support, we performed a second phylogenetic analysis with a smaller taxon sampling (52 taxa), including the Vienna Mascarin sequence and all available sequences of the genera Psittacula and Tanygnathus (in the first analysis nested within the Psittacula clade), as well as a set of outgroup taxa (Eclectus, Polytelis, Aprosimictus and Coracopsis). The resulting tree (Fig. 2) revealed the position of the Mascarin inside the Psittacula-Tanygnathus clade with a moderate bootstrap support of 80%. The exact position of Mascarin
is not well resolved in the tree, but there is weak bootstrap support of 67% for a clade consisting of *M. mascarin*, the widespread *Psittacula eupatria*, and of *P. wardi*, an extinct species formerly found on the Seychelles. *Psittacula* also turns out to be not monophyletic with respect to the genus *Tanygnathus*, which is nested within the *Psittacula* radiation, but with only weak bootstrap support.
4. Discussion and conclusion

Since Kundu et al. (2012) amplified their cyt b sequences in three separate PCRs with overlapping fragments and because they both amplified and sequenced C. nigra barklyi and P. alexandri fasciata for the very same study, we conclude that the previously published Mascarin sequence is an artefactual hybrid of two PCR fragments from C. n. barklyi and a third PCR product from P. alexandri fasciata. Consequently, a contamination during the laboratory procedures appears very likely since the combined sequence does not contain any information from M. mascarin at all, Moyle et al. (2013) have detected a similar case of chimeric sequences in Gallicolumba (Aves: Columbidae).

The erroneous phylogenetic placement of the Mascarin within the Vasa parrots is most probably due to the fact that about 60% of sequence GQ996499 is identical to a cyt b fragment from the Lesser Vasa parrot Coracopsis nigra.

Although the exact position of the Mascarin is not well resolved in our tree (Fig. 2), in any case the Mascarin belongs to the tribe Psittaculini (subfamily Psittaculinae) and should neither be incorporated into the traditional Psittaci nor into the recently established subfamily Coracopseinae (Joseph et al., 2012) as done by del Hoyo and Collar (2014; but see also Jackson et al., 2015). We tentatively consider the Mascarin as being part of the Psittacula radiation, although the weak bootstrap support from our analysis limits definitive statements about the exact position in the tree. Biogeographically, we interpret the Mascarin as a descendant from ancestral lineages of the Asian Psittacula eupatria group which dispersed towards the Mascarene islands across the Indian Ocean as suggested earlier (Hume, 2007; Jackson et al., 2015). Taxonomically we suggest to reexamine the delimitation of the genera Mascarinus, Tanygnathus and Psittacula to each other, probably resulting in merging all three to a single genus Psittacula.

Acknowledgements

We thank Anja Bodenheim, technical assistant at Institute for Evolutionary Biology in Bonn for developing the ancient DNA protocol used in this study. We also thank Leo Joseph (CSIRO, Canberra, Australia) and an anonymous reviewer for helpful comments to a previous version of this manuscript.

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