Investigating bird strikes in Brazil through DNA barcoding

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Abstract
Background. Air aircraft collisions, or bird strikes, are frequent worldwide, causing huge material losses and endangering the lives of crew members. Identification of birds involved in such events can be important for many reasons, including the assistance of accident investigations and supporting management plans to reduce risks in critical areas. Bird strike remains are frequently fragmented or restricted to blood stains, which makes morphological identification impossible. In these situations, DNA barcoding can be used to assign unknown samples from reference samples by comparing cytochrome c oxidase (COI) gene (BOLD). The Barcode of Life Data System (BOLD) provides a reference database with authenticated sequences and a search tool used for species identification. Results: Between 2014 and 2016 the Brazilian Federal Police DNA Laboratory received for analysis 53 unidentified bird strike samples sent by CNPEN, the Brazilian Air Force unit responsible for the investigation of aeronautical accidents in Brazil. Using BOLD and DNA extraction data, 49 samples were classified, 44 species identified. Fourteen bird species or genera were identified. The Black Vulture (0.32%), the Southern Caracara (22.6%), the Magnificent Frigatebird (9.4%) and the Southern Lapwing (0.7%) are the four most frequent species, possibly a result of two independent impacts in the same part of the aircraft. Significance: Although a more extensive study is necessary to corroborate the results presented here, few species are more frequently involved in bird strikes. In order to be more cost effective, management plans should prioritize these species. As showed, the use of DNA barcoding associated to species occurrence is an efficient and sometimes the only way to identify bird strike remains, helping to improve flight safety in Brazil.

Introduction
Bird strikes are frequent worldwide, causing huge material losses and endangering the lives of crew and passengers. In Brazil, 1,733 bird strikes were registered in 20152, and civil aviation companies estimated expenses of 21 million dollars caused by this kind of event in 20093. The occurrence of bird strikes can be used to identify accidents and support management plans to reduce risks in critical areas, such as airports1,4. Removing some species and/or the resources used by them from airport sites (e.g., food, water) and the strict control of land use and economic activities in nearby areas may reduce the frequency of bird strikes. When biological remains from bird strikes are not suitable for morphological identification, barcoding methods can associate unknown samples to a reference sample by comparing DNA sequences that differ between species5. DNA barcoding was designed to be a universal system for cataloging and identifying animal species based on standard sequences of the COI gene. The system is based on a reference database with authenticated sequences and a search tool used for species identification (BOLD)6. DNA barcoding has already been used successfully to identify evidence from bird strikes5. The Brazilian Federal Police DNA Laboratory initiated a cooperative action with CNPEN, a unit of the Brazilian Air Force responsible for the investigation and prevention of aeronautical accidents in Brazil. Since then, unidentified bird strikes from all over the country are submitted to the Laboratory for identification. The first results of this cooperative action are presented here.

Material and Methods
From April 2014 to December 2016, the laboratory received 53 samples from 50 different bird strikes (three events with two impacts). Samples consisted of dried or ethanol preserved fragments of feathers, viscera, muscular tissue or swabs with biological fluids. DNA from sub-samples was extracted with PrepFiler Express DNA Extraction Kit in the AutoMate Express DNA Extraction System (Applied Biosystems). Fragments of 650 bp from the 5' region of the COI gene were amplified using FishFi/FishR1 and LCO1490/HCO2198 primers. PCR conditions and sequencing procedures are described elsewhere7. Consensus sequences were searched in BOLD Species Level Barcode Records database. Searching was based on BOLD species results and representativeness of the group in the database, associated with species occurrence data. Taxonomic information and sequence data followed IOC World Bird List8 and Sissigt (2009)9. A species identification was performed when the consensus sequence presented >99% similarity to a single BOLD species and all the known congeners were represented in the database; and (2) query sequence presented >99% similarity to more than one BOLD species and/or congeners are not represented in the database, but these candidate species do not occur at the bird strike site. Considering situation (2), a genus level identification was provided if these species do occur at the bird strike site.

Results
Twenty quality sequences were obtained from 49 samples (unsuccesful results were usually due to the lack of DNA). All samples presented more than 99% similarity with at least one bird species from BOLD. Based on the methodology, 49 samples (92.5%) were successfully matched to species levels of the BOLD database alone allowed a species level identification for 18 samples (Figure 1). Thirteen different bird species or genus were identified. Interestingly, one sample was associated to two different species (Table 1).

Discussion and Conclusions
The occurrence of bird strikes and the improvement of management plans and risk analysis should support prioritization. As showed here, the use of DNA barcoding associated to species occurrence is an efficient and sometimes the only way to identify these species, helping to improve flight safety in Brazil.

REFERENCES