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Full genomes of all nine currently recognized lovebird species (genus *Agapornis*) sampled from wild populations

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African lovebirds are popular pet parrots commonly hybridized by breeders leading to genetic admixture. Trade escapees and land use change have now led lovebirds to genetically admix in the wild. Sampling origin is therefore of utmost importance when deriving genetic data from lovebird species to reconstruct phylogeny or historical demographic events as the inclusion of taxa of hybrid origin is a source of spurious results. Here we present complete genomes of all nine currently recognized lovebird species. Each species is represented by an archival geo-referenced individual collected within their natural range.

Background & Summary

Lovebirds are a group of small sub-Sahara African parrots that belong to the genus *Agapornis*. Currently nine species are recognized by avian checklists and the International Union of Conservation in Nature IUCN[1]. Multiple aspects of the phylogeny and historical biogeography of the genus remain poorly understood and genomics presents an opportunity to gain insights into a number of research questions regarding evolution and approaches to conservation[2]. Past phylogenetic studies based on genetics have either not included all known species[3], relied on only one or two molecular markers[3,4] or relied on samples from captive-bred individuals[5–10]. The most recent phylogenetics study[8] included archival individuals (i.e. specimens held in museum collections) from the wild but for most species the ancestral heritage and precise geographical provenance of the specimens used, are unknown, except three *A. canus*. The use of specimens from captivity or with uncertain provenance is problematic given that lovebirds hybridize readily, and crossbreeding has been a common practice in some captive collections. Naturalised populations of lovebirds have also been established from introductions outside their natural ranges in multiple localities. As a result, cryptic hybrids may exist within captive and introduced populations[11–13].

Here we present full genome data of all nine species of lovebird derived from archival geo-referenced individuals collected within the natural historical range of each species with the aim of presenting sequences of individuals with a high certainty of being free from artificial hybridisation. Each genome is linked to a voucher specimen housed in a recognized natural history collection (Table 1).

Methods

A toepad from representative wild-caught individuals (collected from within their natural range[2], Fig. 1) of each species was sampled from the Academy of Natural Sciences of Drexel University (ANSP) and the Field Museum of Natural History (FMNH). To keep the possibility of hybrid material due to past human interference minimal, we took the following into account when selecting samples. (1) Locality (Fig. 1) - samples were collected from sites that were remote and geographically separate from known centres of human habitation where the risk of hybridisation as result of escaped captive birds is low. Samples were also collected from the core of species range and not from possible hybrid zones or areas where the distribution of species overlap. *Agapornis swindernianus* and *A. pullarius* distributions do overlap (Fig. 1), however, these two species are ecologically separated and are not known to hybridize[12] (2) Age - most samples (apart from *A. canus*, collection in the 1996) were collected before the peak of exploitation and mass trade of lovebirds which occurred in 1980s. (3) Phenotypic characters

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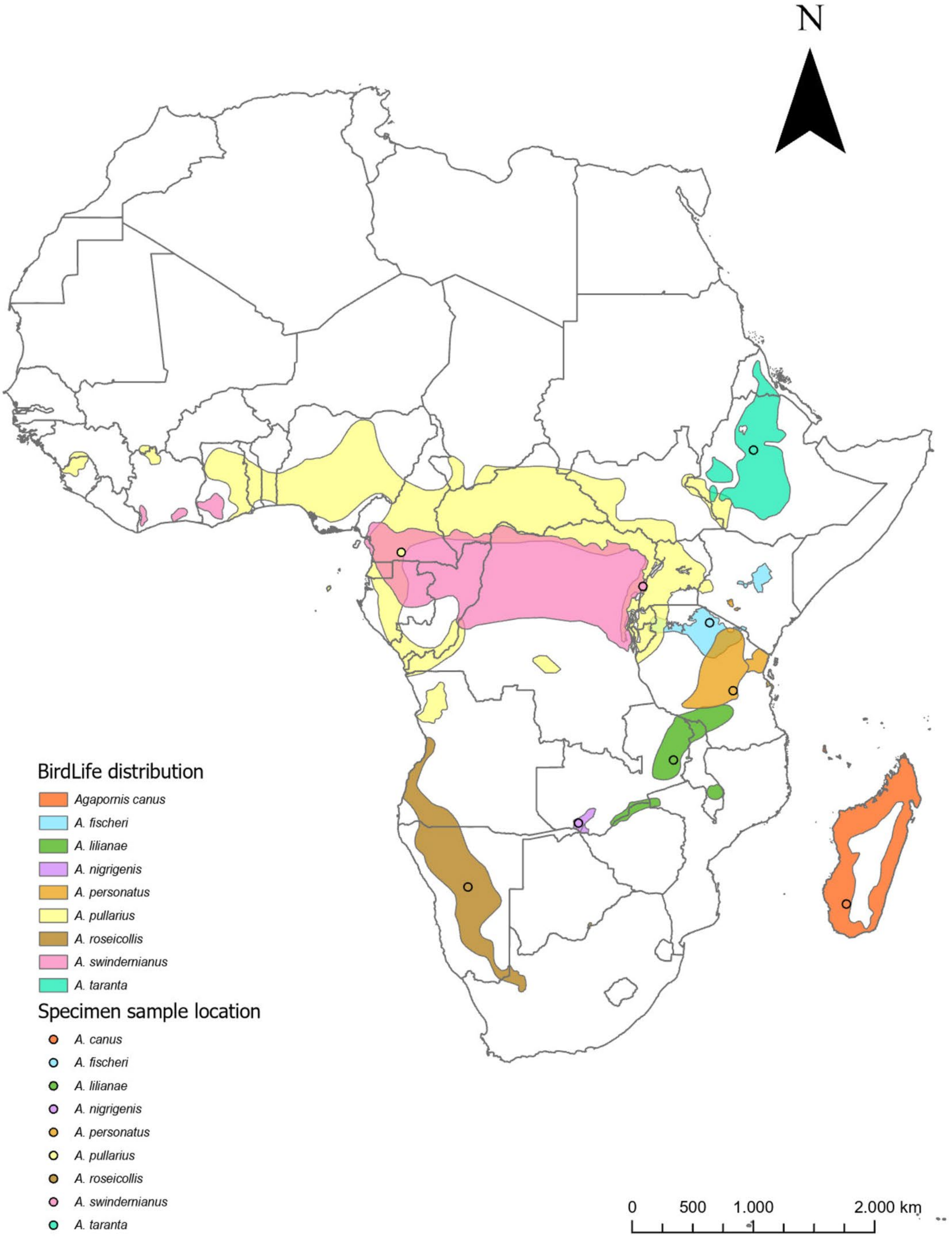

Fig. 1 Specimen sample locations and BirdLife distribution for each of the 9 species[1].

– the specimens included in our study did not show phenotypic characteristics such as unusual plumage characters that might indicate hybridisation. DNA extraction was performed on the toe pad tissue using the Qiagen DNeasy genomic extraction kit (QIAGEN N.V.) using the standard protocol. Paired-end sequencing libraries were constructed using the Illumina TruSeq kit according to the manufacturer's instructions. The library was sequenced in 50–100x coverage on an Illumina Hi-Seq platform in paired-end (haploid), 2 × 150 bp format by GENEWIZ (Azenta Life Sciences). The resulting fastq files were trimmed of adapter/primer sequence and low-quality regions using Trimmomatic v0.33[14]. The trimmed sequences were then assembled using SPAdes v2.5[15] followed by a finishing step performed in Zanfona[16]. Zanfona[16] uses a series of related species that function as references for each other. The genome was assembled on scaffold-level. There was no single alignment where reads from one species were mapped to the chromosomes of another species.

Scientific name	Common name	Museum	BioSample	Collection country	Lat_Lon	Collection year
Agapornis canus[18,19]	Grey-headed Lovebird	FMNH	SAMN36687877	Madagascar	23.11 S 44.96 E	1996
A. fischeri[20,21]	Fischer's Lovebird	FMNH	SAMN37054379	Tanzania	2.31 S 34.83 E	1932
A. lilianae[22,23]	Nyasa Lovebird	FMNH	SAMN36798136	Zambia	12.45 S 32.15 E	1950
A. nigrigenis[24,25]	Black-cheeked Lovebird	ANSP	SAMN35444842	Zambia	17.12 S 25.11 E	1907
A. personatus[26,27]	Yellow-collared Lovebird	FMNH	SAMN36687878	Tanzania	7.32 S 36.57 E	1950
A. pullarius[28,29]	Red-headed Lovebird	FMNH	SAMN36687879	Cameroon	2.90 N 11.98 E	1932
A. roseicollis[30,31]	Rosy-faced Lovebird	FMNH	SAMN16756440	Namibia	21.85 S 16.92 E	1950
A. swindernianus[32,33]	Black-collared Lovebird	FMNH	SAMN37054378	Uganda	0.38 N 29.89 E	1946
A. taranta[34,35]	Black-winged Lovebird	FMNH	SAMN37054381	Ethiopia	10.47 N 38.08 E	1927

Table 1. Information on nine voucher specimens used to produce genomic data. ANSP (Academy of Natural Sciences of Drexel University), FMNH (Field Museum of Natural History).

Scientific name	Raw data	Assembled genome	Scaffold N50 (kb)	Contig N50 (kb)	Genome size (GB)	Genome coverage
Agapornis canus[18,19]	SRR31107690	JBAFWW000000000	26.9	13.7	1.1	60x
A. fischeri[20,21]	SRR25700511	JAVHNB000000000	19.1	5.5	1.1	60x
A. lilianae[22,23]	SRR26151489	JAWWTZ000000000	27.3	15.9	1.1	60x
A. nigrigenis[24,25]	SRR26073671	JAWWLU000000000	81.5	66.2	1.0	60x
A. personatus[26,27]	SRR25700512	JAVHNE000000000	27.9	15.9	1.1	60x
A. pullarius[28,29]	SRR25700514	JAVKLG000000000	366 bp	366 bp	1.4	60x
A. roseicollis[30,31]	SRR13753101	JANEXD000000000	19.2	3.9	1.2	130x
A. swindernianus[32,33]	SRR25700359	JAVHNG000000000	18.2	4.3	1.1	60x
A. taranta[34,35]	SRR25700513	JAVHND000000000	17.4	2.2	1.1	60x

Table 2. Genomic data for each of the nine recognized lovebird species sequenced for this study.

Data Records

All raw reads and assembled genomes have been deposited and are available on GenBank (National Center for Biotechnology Information NCBI, Table 2).

Technical Validation

The specimens selected for sequencing were collected from the wild and accompanied by precise geo-referenced information (including precise Latitude and Longitude to the nearest minute). We used the default parameters for SPAdes[15], Trimmomatic[14], and Zanfona[16] to assess the validity and quality of the data. Decontamination was performed using FSCR-gx[17] and the NCBI's in-house libraries. No gap closing was performed.

Code availability

Publicly available codes used are listed in Methods. No custom code was used for this study.

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Author contributions

All authors discussed the results and contributed to the final manuscript. Taylor Hains and Sascha Dueker selected specimens for inclusion in the study. Stacy Pirro carried DNA extraction and sequencing. Sascha Dueker wrote the manuscript with support from Sandi Willows-Munro and Rowan Martin.

Competing interests

The authors declare no competing interests.

Additional information

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