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Changes in parrot diversity after human arrival to the Caribbean

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Humans did not arrive on most of the world's islands until relatively recently, making islands favorable places for disentangling the timing and magnitude of natural and anthropogenic impacts on species diversity and distributions. Here, we focus on *Amazona* parrots in the Caribbean, which have close relationships with humans (e.g., as pets as well as sources of meat and colorful feathers). Caribbean parrots also have substantial fossil and archaeological records that span the Holocene. We leverage this exemplary record to showcase how combining ancient and modern DNA, along with radiometric dating, can shed light on diversification and extinction dynamics and answer long-standing questions about the magnitude of human impacts in the region. Our results reveal a striking loss of parrot diversity, much of which took place during human occupation of the islands. The most widespread species, the Cuban Parrot, exhibits interisland divergences throughout the Pleistocene. Within this radiation, we identified an extinct, genetically distinct lineage that survived on the Turks and Caicos until Indigenous human settlement of the islands. We also found that the narrowly distributed Hispaniolan Parrot had a natural range that once included The Bahamas; it thus became "endemic" to Hispaniola during the late Holocene. The Hispaniolan Parrot also likely was introduced by Indigenous people to Grand Turk and Montserrat, two islands where it is now also extirpated. Our research demonstrates that genetic information spanning paleontological, archaeological, and modern contexts is essential to understand the role of humans in altering the diversity and distribution of biota.

extinction | extirpation | biogeography | Anthropocene | *Amazona*

Significance

Integrating across paleontological, archaeological, and neontological resources is critical for understanding millennial-scale impacts of humans on biodiversity. Here, we showcase this valuable integration, with a focus on Caribbean parrot extirpation and cross-Holocene redistribution. We utilize modern and ancient DNA and radiocarbon data to determine historical distribution and diversity of the Cuban Parrot (*Amazona leucocephala*) and Hispaniolan Parrot (*A. ventralis*) and place this information in the context of extant diversity. Both species diversified during the Pleistocene and were more widespread and genetically diverse earlier in the Holocene than today. Results reveal a history of extirpations and translocations that began with Indigenous (Amerindian) occupation of the islands and continued with European colonization. Understanding these long-term dynamics is critical for Caribbean ecosystem restoration.

The Caribbean (Greater Antilles, The Bahamas, Turks and Caicos, and Lesser Antilles) is an ideal focal biogeographic region to understand the human role in shaping diversity and distributions of taxa across the Holocene. Caribbean islands are home to late Quaternary (late Pleistocene and Holocene) fossil (blue holes and caves, e.g., ref. 1) and archeological sites that record human colonization and illuminate human interactions with the native fauna (e.g., refs. 2 and 3). Based on these data, many of the abundant losses of birds and nonvolant mammals across the region date to the mid- or late Holocene, well after the last glacial/interglacial transition, and closely track Indigenous human settlement of the archipelago from ~6,000 to ~1,000 y ago (4–10). During this time frame, some species successfully adapted to human landscapes and/or lived in close contact with humans (11). Others were selected for translocation and introduction beyond their endemic ranges, with the potential for disruptive effects on native ecosystems (12–14). A second period of diversity loss began with European colonization at the end of the 15th century (2).

Within the Caribbean, parrots (Psittaciformes) have a deep history of cultural value and human manipulation of diversity and distribution during the Holocene. Fossils (prehuman) and archaeological specimens of parrots predating European colonization of the Caribbean, as well as observations and specimens compiled during the past ~500 y, outline a record of rapidly changing diversity through time. Ethnohistoric accounts (e.g., refs. 15 and 16) indicate that parrots were a food source, were kept in dwellings, their feathers used for personal adornment, and were popular trade items among Indigenous communities within the islands and beyond (e.g., continental South America; refs. 17 and 18). Similarly, across portions of South America and southwestern United States and northern Mexico archaeological evidence indicates that several parrot species (e.g., *Amazona aestiva, Ara ararauna, Ara macao,* and *Rhynchopsitta pachyrhyncha*) were reared and traded long distances based on the social value of their colorful feathers, their roles in ritual sacrifice, placement in burials, and as foci of artistic expression (e.g., refs. 19–24).

Between 14 and 23 species of Caribbean parrots have become extinct since the late Pleistocene, with some of the losses occurring during the 19th century (1, 10, 25, 26) (*SI Appendix*, Tables S1 and S2 and Fig. 1). Conservatively, the Greater Antilles lost four species of macaw (*Ara*) during the Holocene. The Bahamas lost a parrotlet (*Forpus*)

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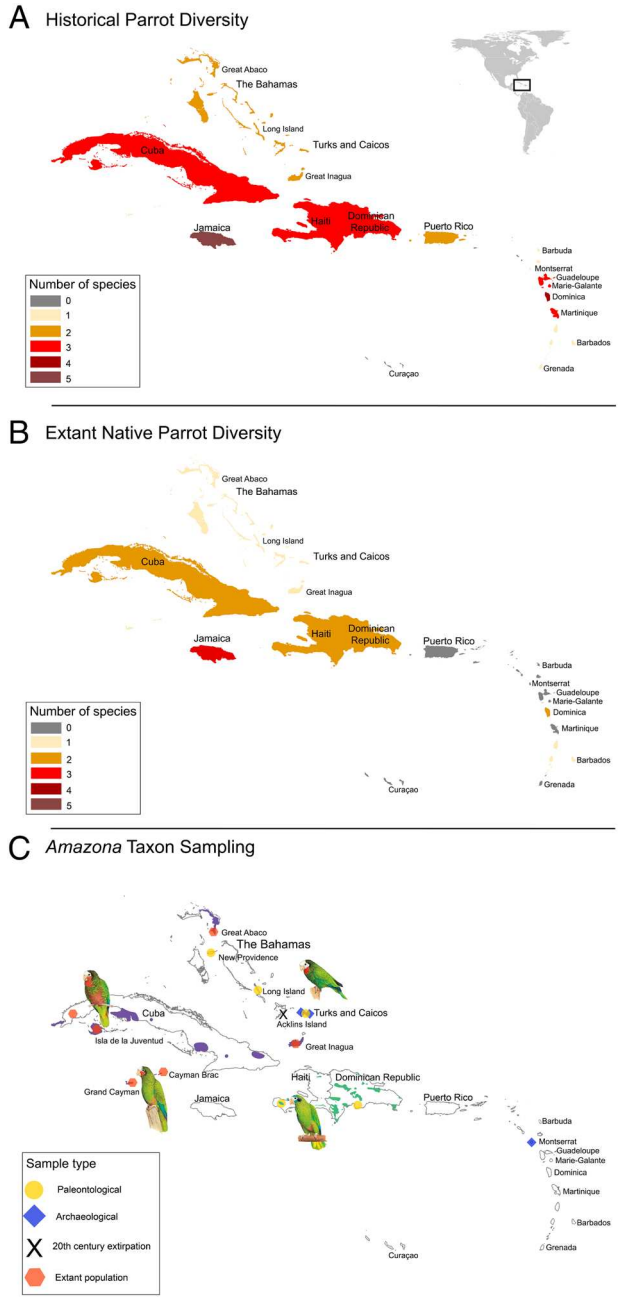

Fig. 1. Native Caribbean parrot diversity changed (in terms of species number) across the Holocene. Map of historical (extirpated, extinct) (*A*) and modern (extant) (*B*) native parrot diversity. The conservative minimum number of extinct Caribbean parrot species is based on fossil records (no human association), archaeological records, and historic explorer accounts (*SI Appendix*, Tables S1 and S2). (*C*) depicts the temporal and spatial genetic sampling of two paleontological and archaeological rich Greater Antillean species: *Amazona leucocephala* (current distribution in purple) and *Amazona ventralis* (current distribution in green). *Amazona leucocephala* is currently composed of four subspecies: *A. l. leucocephala* (Cuba), *A. l. bahamensis* (The Bahamas), *A. l. caymanensis* (Grand Cayman), and *A. l. hesterna* (Cayman Brac) (27). Note that Grand Cayman and Cayman Brac are each home to named subspecies of *A. leucocephala* that are phenotypically similar; however, Cayman Brac (*A. l. hesterna*) individuals are overall smaller and darker and can have a slightly larger dark pink-red belly patch (28). Herein, they are represented by a single illustration in the figure. All Illustrations are shared with permission by Lynx Publishing and Cornell Birds of the World (29, 30, 31).

(1, 10). In the Lesser Antilles, psittacid diversity was also much higher in the past, with three macaws (*Ara*), three parakeets (*Psittacara*), and four amazons (*Amazona*) becoming extinct (refs. 25 and 26, and *SI Appendix*, Table S1). Most of what we know about the extinct species is based on colonial explorers' accounts or bones from Indigenous archaeological middens that do not represent living species (17, 26).

Today, Caribbean islands sustain 12 native and 12 human-introduced species of parrots (32). No native species of macaws (*Ara*) or parrotlets (*Forpus*) live on the islands, and only three native species of parakeets (*Psittacara*, *Eupsittula; formerly Aratinga*) are extant. Determining the extent to which both extinct and extant species were translocated or negatively impacted by Indigenous peoples in the insular Caribbean requires fossils from prehuman contexts to confirm natural distributions. Herein, we focus on the most widespread and species-rich parrot genus in the Caribbean, *Amazona*, with nine extant species and robust paleontological and archaeological records.

The Cuban Parrot (*Amazona leucocephala*) remains the most widespread Antillean parrot even though it has undergone numerous extirpations, based on paleontological, archaeological, and historical accounts (10, 26). It occurs today in Cuba, Isla de la Juventud, Grand Cayman, Cayman Brac, and the Bahamian Islands of Abaco and Great Inagua. It has been extirpated from Little Cayman (33) and on many Bahamian islands including Grand Turk, Middle Caicos, Eleuthera, Andros, New Providence, Crooked Island, Long Island, Fortune Island, New Providence, and Acklins (10, 34, 35). Today four phenotypically defined subspecies are recognized (27, 28, 35). This widespread Caribbean endemic occupies a variety of habitats including palm and pine savanna, dry broadleaf woodlands, limestone forest, plantations, mangroves, and gardens (29). This species was once so abundant in The Bahamas that Columbus noted that they would "obscure the sun" (15), although today the Cuban Parrot is considered "near-threatened" across its range (29).

The Hispaniolan Parrot (*Amazona ventralis*) is another relatively well-represented species in archaeological and paleontological sites. Endemic today to Hispaniola, the Hispaniola Parrot (*A. ventralis*) has a fragmented distribution across the island with most of its current range in the Dominican Republic. It occurs in montane humid evergreen forest up to 1,500 m, woodlands, and lowland palm savanna (30, 36). This species has undergone severe declines from the pet trade and deforestation; it is regarded as "vulnerable" (30).

The Lesser Antillean island of Montserrat sustains no parrot species today. However, five bones of an unknown *Amazona* species were recovered from the Trants archaeological site on the island (26). Indigenous pottery from Trants is classified as Saladoid, indicating that the site may have been occupied as early as ~500 BC (37). This unknown species of *Amazona* is much smaller than any living Lesser Antillean species of *Amazona*, resembling extant Greater Antillean species in size (37). With these morphological affinities to Greater Antillean species of *Amazona*, the archaeological samples from Trants may represent either an extinct species once found on Montserrat or a human-translocated Greater Antillean species.

Our analyses consider the extent to which fossils and archaeological bones of Amazon parrots add to the overall diversity of Caribbean *Amazona* as understood by molecular genetics of extant species. We examine the relationships of described taxa from modern populations within the *A. leucocephala* species complex using nuclear (ultraconserved elements; UCEs) and mitochondrial genome data in the context of a species-level phylogeny. We then compare mitochondrial ancient DNA (aDNA) from accelerator mass spectrometry (AMS) radiocarbon-dated specimens of Caribbean species of *Amazona* with modern data to evaluate changes in distribution and genetic diversity across the Holocene. These data allow us to ask the following questions: What are the phylogenetic relationships of extant Caribbean *Amazona* as well

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as recently extinct/extirpated taxa? Which species did Indigenous peoples likely translocate to different islands? Ultimately, addressing these questions will reveal how humans have shaped modern diversity and distributions of Caribbean parrots with relevance to contemporary conservation.

Results

Sampling.

Modern samples. DNA was sampled from modern (tissues) or historical (toe pad, bone) specimens of each named *Amazona leucocephala* subspecies (27). Currently, *A. leucocephala* comprises four named lineages: *A. l. leucocephala* (Cuba, Isla de la Juventud), *A. l. bahamensis* (Abaco, Great Inagua), *A. l. caymanensis* (Grand Cayman), and *A. l. hesterna* (Cayman Brac) (27). In morphological and plumage characters, the three modern Bahamian populations of *A. leucocephala* differ from each other as much as from non-Bahamian named lineages (35). Samples included herein are from Cayman Brac (*A. l. hesterna*), Isla de la Juventud (*A. l. leucocephala*), Cuba (*A. l. leucocephala*), Grand Cayman (*A. l. caymanensis*), and *A. l. bahamensis* from Abaco, Great Inagua, and from the extirpated (ca. 1940) population on Acklins Island (Table 1 and Fig. 1). DNA libraries were produced for these samples and enriched with ultraconserved elements (UCE; nuclear genome loci) and *Amazona*-specific mitochondrial genome bait sets.

Fossil and archaeological samples. From early/mid Holocene fossil contexts, we extracted aDNA from specimens morphologically identified as *Amazona leucocephala* from New Providence, Bahamas (UF 416285), Long Island, Bahamas (UF 540224), and Middle Caicos, Turks and Caicos (Holocene, ~1,600 y old, UF 218598;10), as well as an archaeological bone from Grand Turk, Turks and Caicos (~1,300 y old, Table 2, and Fig. 1). We sampled two Holocene fossils of *A. ventralis* for aDNA, one from Haiti (Trouing Nicolas; UF 323777) and one from the Dominican Republic (Cueva de las Abejas; UF 322045, Table 2, Fig. 1, specimens identified by DWS). From the Trants archaeological site on Montserrat, a single *Amazona* sp. bone was sampled for aDNA. aDNA libraries were prepared for these samples, and each was enriched with an *Amazona*-specific mitochondrial genome bait set. Previously undated fossils also were submitted for radiocarbon dating (see below).

Radiocarbon dates. The chronology of our aDNA samples ranged from early Holocene to late Holocene (Table 2). Five of the eight paleontological/archaeological samples had previously determined chronological information. We submitted small mammal (Bahamian hutia *Geocapromys ingrahami*) fossils from Hanging

Table 1. Modern samples of *Amazona* included in our dataset and sequence data information

Museum Catalog Number	Species	Subspecies	Collection Island	Specific locality	Date collected	Sample type	Read Pairs	UCE loci	Average UCE locus length	NCBI SRA Accession Number (UCE and Mitochondrial genome raw data)	Mitochondrial genome NCBI GenBank Accession Number
UF 25789	*A. leucocephala*	*hesterna*	Cayman Brac		1985	Bone	1,185,365	4,615	439 (184)	SAMN32316663	OR048930
UF 8362	*A. leucocephala*	*leucocephala*	Isla de la Juventud	Caleta Grande	1958	Toe pad	558,637	4,606	384 (167)	SAMN32316665	OR048934
UF 8364	*A. leucocephala*	*leucocephala*	Cuba	Pinar del Rio Providence, Cayuco, 15 km NNW	1958	Toe pad	3,181,898	4,569	385 (169)	SAMN32316667	OR048936
UF 37653	*A. leucocephala*	*caymanensis*	Grand Cayman	North Side, 2 mi S	1961	Toe pad	262,174	4,342	349 (159)	SAMN32316666	OR048932
UF 46992	*A. leucocephala*	*bahamensis*	Abaco	Murphytown	2008	Tissue	6,068,725	4,616	486 (203)	SAMN32316668	OR048931
UF 42477	*A. leucocephala*	*bahamensis*	Great Inagua		1995	Bone	2,279,872	4,616	449 (187)	SAMN32316664	OR048933
CM P30889	*A. leucocephala*	*bahamensis*	Acklins	Pompey Bay	1909	Toe pad	384,348	4,084	355 (155)	SAMN32316669	OR048943

Subspecies follow Clements et al. (27). These samples were used to produce two phylogenies one based on UCE loci and a second based on mitochondrial genome data. Raw data of UCE and mitochondrial genome bait–enriched samples are available on NCBI SRA (PRJNA913959) (38). Annotated mitochondrial genome data from that sequencing effort are available on GenBank.

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Table 2. Paleontological and archaeological samples of *Amazona* yielding aDNA

Sample	Site	Chronology	Site or sample Reference	Number of on-target mitochondrial genome reads	Percent of mitochondrial genome recovered	NCBI SRA Accession Number (raw data)	Mitochondrial genome NCBI GenBank Accession Number
UF 323777	Haiti: Trouing Nicolas, lower part of the cave (17 m)	1012–491 BC*	herein	23,638,136	98	SAMN32316670	OR048929
UF 322045	Dominican Republic: Cueva de las Abejas	Sample failed	herein	2,331	88.6	SAMN32316671	OR048937
UF 540224	Bahamas: Long Island, Hanging Garden Cave, unit 2, level 3	Early to mid-Holocene; Prehuman (see text)	herein	456	83.4	SAMN32316672	OR048941
UF 416285	Bahamas: New Providence, Banana Hole	7486–6440 BC‡	(39)	265	67.7	SAMN32316673	OR048942
GT3-FS-345	Turks and Caicos: Grand Turk, GT3	771–965 AD	(10)	306,816	86.3	SAMN32316674	OR048939
GT3-FS-224	Turks and Caicos: Grand Turk, GT3	771–965 AD	(10)	576,926	62.9	SAMN32316675	OR048940
PN 4977	Montserrat: Trants	500 BC–400 AD (Ceramic Age)	(37)	44,017	87.9	SAMN32316676	OR048938
UF 218598	Turks and Caicos: Middle Caicos, MC37, unit 4	10–770 AD	(10)	4,697,589	99.9	SAMN32316677	OR048935

*Denotes samples that were dated based on bioapatite. See the Results section for special considerations of this dating technique. See *SI Appendix*, Tables S3 and Table S5 for extended data. These data were combined with mitochondrial genome data generated from our efforts, Kolchanova et al. (40), and from mitochondrial bycatch from samples produced by Smith et al. (41) and Olekysyk et al. (42) to produce a mitochondrial genome phylogeny. Raw data for each of these ancient samples are available on NCBI SRA (PRJNA913959) (38), and annotated mitochondrial genome data are accessioned on NCBI GenBank.
‡Calibrated date based on application of IntCal 2020 (43) to uncalibrated radiocarbon age date published in Oswald et al. (39).

Garden Cave, Long Island, Bahamas, for radiocarbon dating in lieu of bird fossils as mammal fossils are more robust. Only two of the seven submitted *Geocapromys ingrahami* samples had enough collagen to radiocarbon date the organic fraction of the sample (*SI Appendix*, Table S3). The other five *Geocapromys* samples from Hanging Garden Cave (levels 2, 5, and 6) were also radiocarbon dated using their bioapatite (inorganic) fraction. Collagen extracted from *G. ingrahami* fossils from levels 1 and 2 yielded dates of 640 +/– 20 y BP and 1,940 +/– 25 y BP, respectively (UGAMS 61127, 61129, and *SI Appendix*, Table S3). A second *G. ingrahami* fossil from level 2 was dated with bioapatite at 6,540 y BP. The two *G. ingrahami* samples that recovered collagen (UGAMS 61127 from level 1, UGAMS 61129 from level 2) were also dated with bioapatite to investigate contamination. For each sample, the bioapatite dates were similar to the collagen dates. The level 1 sample recovered a bioapatite date of 570 +/- 25 yr BP and the level 2 recovered a bioapatite date of 2,140 +/– 25 y BP (UGAMS 61127a, 61129a, and *SI Appendix*, Table S3). The *Amazona ventralis* fossil from Long Island (UF 540224) is from level 3. Therefore, based on stratigraphic context, this fossil predates human arrival to the region, which was estimated to be ~1,300 y ago (44–46). The bioapatite dates from levels 5 and 6 were late Pleistocene in age, 10,400 and 12,650 +/– 30 y BP, respectively (*SI Appendix*, Table S3).

The two *Amazona ventralis* fossils from Hispaniola sampled for aDNA (UF 323777, UF 322045) were also submitted for radiocarbon dating. UF 322045 from Cueva de las Abejas did not yield adequate collagen or bioapatite and thus was not dated. UF 323777 from the Trouing Nicolas site on Haiti recovered a bioapatite age of 2,640 +/– 90 y BP (UGAMS 61125 and *SI Appendix*, Table S3). As outlined in the Methods section, this date should be interpreted with caution as the abiotic conditions of the site are unknown.

UCE statistics. Our modern *Amazona leucocephala* samples were collected between 1909 and 2008 (Table 1). Raw data read pairs ranged from 262,174 (Grand Cayman sample from 1961) to >6 million from the tissue sample salvaged from Abaco in 2008. UCE loci from these samples ranged from 4,084 from the oldest sample from Acklins to 4,616 from the two most modern samples from Great Inagua (UF 42477, 1995) and Abaco (UF 46992, 2008). Of note was the recovery of a high number of read pairs and loci from UF 42477, for which DNA was extracted from vertebrae. The average locus length across samples ranged from 349 to 449 bp.

UCE phylogeny. The UCE dataset recovered a topology for Greater Antillean *Amazona* that was consistent with previous studies in that these species were a monophyletic group (refs. 40–41, 46 and *SI Appendix*, Fig. S1). However, Greater and Lesser Antillean *Amazona* species did not form a monophyletic group indicating multiple colonizations of the Caribbean. The Lesser Antillean taxa also did not form a clade but *A. arausiaca* (Dominica) and *A. versicolor* (St. Lucia) were sister taxa (ref. 47 and *SI Appendix*, Fig. S1). The Greater Antillean *Amazona* were a well-supported (bootstrap [BS] = 100) clade that was sister to two Mexican and Central American pine savanna and dry woodland species, *A. xantholora* and *A. albifrons* (*SI Appendix*, Fig. S1). Regarding focal taxa, *A. leucocephala* was sister to a clade composed of *A. vittata* and *A. ventralis*. Within *A. leucocephala*, only the Grand Cayman taxon (*A. l. caymanensis*, UF 37653) was a well-supported clade.

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All other samples represented a poorly supported (BS = 51) clade with shallow internodes relative to species-level comparisons.

The time-calibrated UCE phylogeny of *Amazona* suggested that the genus diversified in the late Miocene from 10.9 to 8.2 million y ago (mya, *SI Appendix*, Fig. S2). The Greater Antillean *Amazona* shared a most recent common ancestor (mrca) with the Mexican *A. xantholora* and Mexican-Central American *A. albifrons* during the Pliocene (6.4 to 3.0 mya, Fig. 2, and *SI Appendix*, Fig. S2). Among the Greater Antillean species, the most divergent was the Jamaican endemic *A. agilis* (divergence time 5.4 to 2.4 mya). The other Jamaican endemic, *A. collaria*, shared an mrca with the other Greater Antillean *Amazona* during the late Pliocene to mid-Pleistocene (3.3 to 1.4 mya). The remaining Greater Antillean taxa, *A. vittata*, *A. ventralis*, and *A. leucocephala*, shared an mrca in the Pleistocene 2.8 to 1.2 mya. The sister taxa *A. vittata* and *A. ventralis* diverged between 2.5 and 1.0 mya. BS node support was 100% for all Greater Antillean species and their sister taxa *A. xantholora* and *A. albifrons*. The phylogenetic relationships of populations within *A. leucocephala* had very low BS support (Fig. 2 and *SI Appendix*, Fig. S2). Divergences within *Amazona leucocephala* likely occurred within the Pleistocene (2.2 to 0.9 mya), but the lower quality of these samples limited our ability to infer more detailed patterns on spatial and temporal diversification within this group.

Mitochondrial Data Statistics. Modern *A. leucocephala* samples recovered 715 to 1,039,416 mitochondrial reads (average read number: 382,952, Table 1, and *SI Appendix*, Table S4). The low number of reads from the Cuban *A. leucocephala* sample (715) was likely because the mitochondrial baits were not successfully spiked into the UCE bait set, even though the reads recovered 81.3% of the mitochondrial genome for this sample. The other Greater Antillean *Amazona* taxa also varied greatly in sequenced mitochondrial data from 287 to 13,539 reads (*SI Appendix*, Table S4). *Amazona ventralis* and *A. vittata* both recovered read data for >89% of the mitochondrial genome. The lowest data yields were from the two Jamaican species: *A. agilis* (921 reads; 75.8% mitochondrial genome recovery) and *A. collaria* (287 reads; 50% mitochondrial genome recovery). Accordingly, we instead used *A. agilis* and *A. collaria* mitochondrial genome data from Kolchanova et al. (39) in our dataset (*SI Appendix*, Supplementary File S1).

Ancient *Amazona* samples recovered from 265 to 23,638,136 on-target reads. The samples that recovered the fewest reads were also the oldest (UF 540224, UF 416285). The mitochondrial genome coverage ranged from 63 to 100% (Table 2 and *SI Appendix*, Table S5).

Mitochondrial Genome Topology. The mitochondrial genome data recovered the same species-level topology as the UCE data of focal Greater Antillean and sister taxa (Fig. 3 and *SI Appendix*, Fig. S3). Relative to the UCE tree, there were some well-supported phylogenetic relationships within *Amazona leucocephala*. The ~1,200-y-old paleontological and archaeological samples from Middle Caicos (UF 218598) and Grand Turk (GT3-FS-224), respectively, formed a well-supported clade, although the other Grand Turk sample (GT3-FS-345) was among five other *A. "leucocephala"* and *A. ventralis* samples that were sister to *A. vittata*. Modern samples from three Bahamas islands (Abaco, Acklins, and Great Inagua) and Cuba formed a well-supported clade (BS = 82). A fourth well-supported clade (BS = 100) included the Isla de la Juventud and Cayman Brac samples. The Bahamas, Cuba (mainland), Cayman Brac, and Isla de la Juventud samples formed a clade with 90% bootstrap support. The deeper level relationships among taxa within the *A. leucocephala* clade were not determined because of the low bootstrap support of these internodes.

The *Amazona* sp. from the Trants archaeological site on Montserrat was part of a well-supported clade (BS = 100) of seven *Amazona ventralis* samples (Fig. 3). This clade consisted of two fossils from Hispaniola originally identified as *A. ventralis*, a

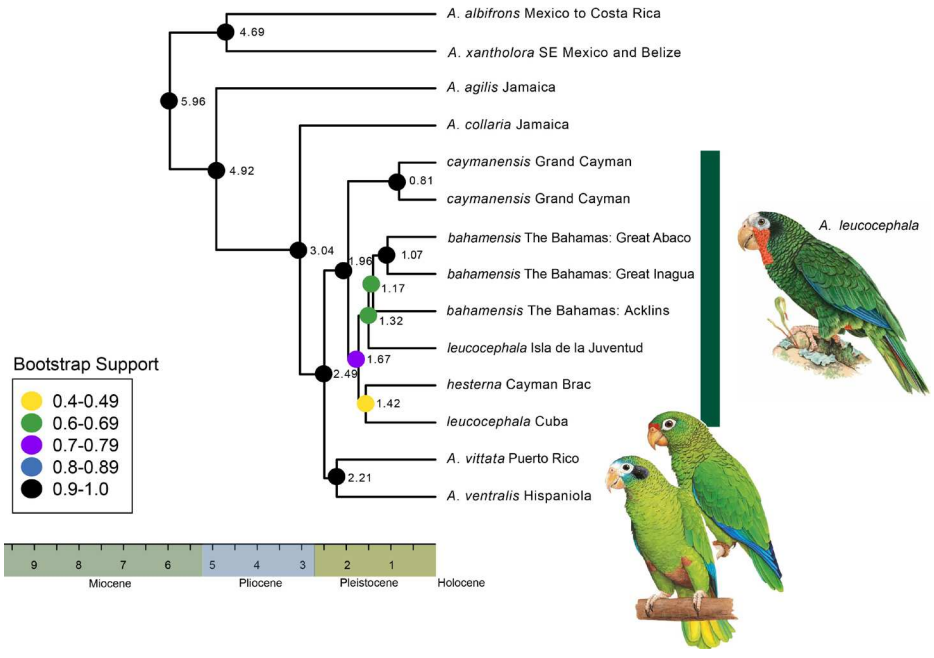

Fig. 2. UCE time tree of Greater Antillean focal taxa and mainland sister taxa. Bootstrap support (BS) is represented by color-coded circles on the nodes. Numbers to the right of the nodes represent the median estimated divergence times of these lineages. Note that the Miocene extends back to 23 mya. See *SI Appendix*, Fig. S2 for the time tree for all *Amazona* in our study and outgroup taxa that includes the range of estimated divergence time per node. *A. vittata* and *A. ventralis* illustrations are used with permission of Lynx and Cornell Lab of Ornithology Birds of the World Online (30, 31). The illustration of *A. leucocephala* is used with permission of artist Nils Navarro and Birds Caribbean.

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modern tissue of *A. ventralis*, and three Bahamian samples originally identified as *A. leucocephala*, namely two early-mid Holocene fossils from New Providence (UF 416285) and Long Island (UF 540224), and an archaeological bone from Grand Turk (~1,100 y old; GT3-FS-345). Fragmentary skeletal elements of *A. ventralis* and *A. leucocephala* are difficult to distinguish morphologically (37), perhaps not surprising given their close genetic affinities.

Mitochondrial Genome Pairwise Distance. The modern samples of Caribbean *Amazona* species (using UF 46992 to represent *A. leucocephala*) were ~5.3% divergent from their continental sister taxa (*A. xantholora*, *A. albifrons*, respectively, *SI Appendix*, Table S6). Within the Caribbean, the divergences of *A. agilis* and *A. collaria* from *A. leucocephala* (UF 46992) were 4.8% and 2.5%, respectively. UF 46992 was 1.7% divergent from *A. vittata* (SRS7124124) and A. *ventralis* (KU 8132), which together are sister to *A. leucocephala*. The divergence within *A. leucocephala* varied among clades. Within The Bahamas-Cuba clade, the Abaco sample (UF 46992) was 0.4% divergent from each sister lineage. UF 46992 was 0.8% divergent from the Cayman Brac-Isla de la Juventud clade, 0.8 to 1.3% divergent from the extinct Turks and Caicos lineage, and 0.9 to 1.3% divergent from the Grand Cayman samples. The modern *A. ventralis* sample (KU 8132) was 0.5% divergent from the archaeological *Amazona* sp. (Montserrat) and 0.5% divergent from the archaeological Grand Turk sample (GT3-FS-345). The Montserrat sample was 0.9 to 1.3% divergent from *A. leucocephala* samples. The fossils from New Providence and Long Island (Bahamas) were 0.2 to 0.4% divergent, respectively,

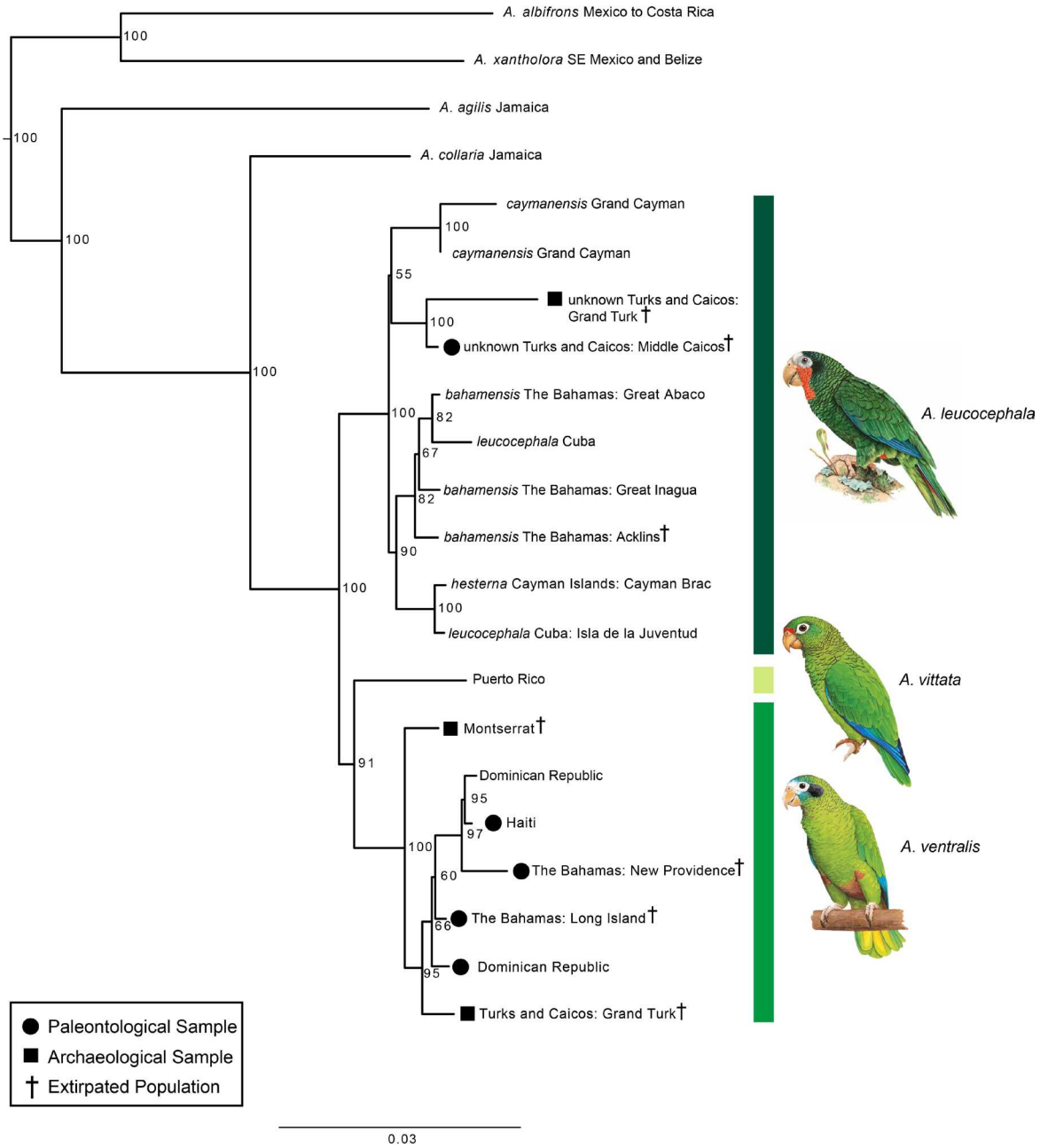

Fig. 3. Mitochondrial genome phylogeny of all ancient and modern samples of Caribbean *Amazona* and sister taxa. Current *A. leucocephala* subspecies names are included in the tip label with the sampling locality. Ancient sample type designations are included next to the sample tip. Archaeological samples (with a square symbol) that are extirpated (with a dagger symbol) may represent a human translocated individual. *A. vittata* and *A. ventralis* illustrations are used with permission of Lynx and Cornell Lab of Ornithology Birds of the World Online (30, 31). The illustration of *A. leucocephala* is used with permission of artist Nils Navarro and Birds Caribbean. See *SI Appendix*, Fig. S3 for the mitochondrial phylogeny for all *Amazona* in our study and outgroup taxa.

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from modern *A. ventralis* (KU 8132). The *A. ventralis* fossil samples (UF 322045, UF 323777) from Hispaniola were 0.1 to 0.3% divergent from the modern *A. ventralis* (KU 8132).

Discussion

Our work leverages paleontological, archaeological, and contemporary data from across the Holocene to evaluate the long-term (millennial-scale) influence of humans on the distribution of parrots in the Caribbean region. We established baselines of diversity of *Amazona* prior to human arrival by documenting a Pleistocene genetic divergence among the Hispaniolan (*A. ventralis*), Cuban (*A. leucocephala*), and Puerto Rican (*A. vittata*) species. We find evidence that *A. ventralis*, rather than being endemic to Hispaniola, also occupied Bahamian islands before human arrival. Additionally, *A. ventralis* was either native or translocated to Montserrat ~2,500 y ago and to the Turks and Caicos ~1,000 y ago. We uncovered an extinct *A. leucocephala* population on the Turks and Caicos and found relatively deep divergences within the widespread *A. leucocephala*, including a distinctive lineage based on mtDNA and UCEs on Grand Cayman.

Prehuman Diversity and Distributions of Caribbean Parrots. In geologically recent timeframes, but prior to human arrival, Caribbean biodiversity was shaped by the dynamic conditions of the Quaternary. Late Pleistocene–Holocene Bahamian fossils suggest that historical climate changes more often caused local, island-specific extirpations of Caribbean bird populations rather than species-level extinctions (8, 10, 48). Events during the Pleistocene also drove diversification among Greater Antillean *Amazona* species (41, 49, herein). The Cuban Parrot, *A. leucocephala*, has a relatively shallow (early Pleistocene) divergence from its extant sister clade, *A. ventralis* and *A. vittata* (40, 41, 49, herein). Geographic variation in the Cuban Parrot complex was delimited originally on plumage characters, with additional variation found recently among the Bahamian populations (*A. l. bahamensis,* ref. 35). Based on both UCE and mitochondrial genome data, the Grand Cayman taxon of Cuban Parrot (*A. l. caymanensis*) is the most divergent from other named lineages. The absence of people on Grand Cayman until European arrival in the 16th century (50, 51) likely allowed this parrot to survive. Based on nuclear genome data, the divergence of other named taxa is minor and thus relatively recent (Fig. 2). Despite the lack of structure in the UCE data, our mitochondrial data suggest two well-supported divergent populations in addition to the Grand Cayman taxon: one comprising phenotypically different named subspecies from Isla de la Juventud (*A. l. leucocephala*) and Cayman Brac (*A. l. hesterna*) and a second comprising all Bahamian (*A. l. bahamensis*) and Cuban (*A. l. leucocephala*) samples. During lower sea levels of the late Pleistocene, Cuba was only ~28 km from the Great Bahama Bank, which would have facilitated biotic dispersal between the two island groups.

Long-Term Indigenous Human Impacts on Parrot Distributions. The arrival in the Caribbean of Indigenous peoples and their associated nonnative plants and animals led to a cascade of changes across the region that continue today. Current archaeological findings indicate that Indigenous people introduced a diversity of animals across the region over millennia (2, 3). Mammals were the most widely transported class of vertebrates, including two domestic species (guinea pig *Cavia porcellus*, dog *Canis lupus familiaris*) and several wild mammals [e.g., agouti (*Dasyprocta* sp.), opossum (*Didelphis marsupialis*), and armadillo (*Dasypus* sp.)] from continental South America (52–54). Caribbean species, especially capromyid rodents (Capromyinae), were also the target of translocations and introductions beyond their native islands (14, 39). In line with these trends, our results suggest that parrot distributions were also influenced by Indigenous peoples. It may be that Indigenous peoples translocated Hispaniolan Parrot to Grand Turk 1,250 to 1,050 y ago and Monsterrat ~2,500 y ago, as this species has not been recovered from prehuman or nonhuman contexts from these islands. However, considering that the prehuman distribution of Hispaniolan Parrot included the central islands of The Bahamas (>700 km from the closest point on NW Hispaniola), it could be that Grand Turk (only ~165 km from northernmost Hispaniola) was also part of its native range. Anthropogenic impacts and native species losses accelerated with European colonization in the 16 century (2); tragically, even most populations of Indigenous peoples were lost (46).

Contemporary Conservation Implications. Even though one-third of the world's extant parrots are at risk of extinction today from human impacts (55, 56), understanding what drives extinction risks cannot be based purely on the present. Living parrot diversity reflects millennia of human manipulation and impacts and an unknown number of past extinctions. The long history of human translocation and extirpation of Caribbean parrots creates challenges for delimiting the prehuman parrot communities (2, 26). Nevertheless, genomic data from extinct and extirpated taxa provide important genetic baselines useful for better setting conservation goals and initiatives (57). Paleontology and archaeology are critical to estimate what the parrot communities, and their distribution would look like prior to the influence of people (e.g., ref. 22), thereby helping to guide conservation priorities and reintroduction strategies (58, 59). Conserving parrot diversity goes beyond simply protecting iconic, beloved species. Loss of parrots in ecosystems directly affects habitats and dependent species. For example, native parrots are important seed predators and dispersers in the Caribbean (60, 61). Many Caribbean islands sustained multiple native species of parrot before Indigenous human arrival, which might reflect higher plant diversity on these islands in the past. Only two Caribbean islands have two *Amazona* species today: 1) Jamaica with the Yellow-billed Parrot (*A. collaria*) and Black-billed Parrot (*A. agilis*) and 2) Dominica in the Lesser Antilles with the Imperial Parrot (*A. imperialis*) and Red-necked Parrot (*A. arausiaca*). The survival of these species may be linked to the rugged topography of these islands.

The Bahamas, and probably the Turks and Caicos, sustained two species of *Amazona* prior to human arrival: the Cuban Parrot and Hispaniolan Parrot. Despite extirpations, the Cuban Parrot has the widest distribution of any native Caribbean parrot today. This probably reflects its broad habitat preferences, which include pine savanna, limestone forest, dry mixed broadleaf woodlands, mangroves, plantations, gardens, and parks (29). Even with its extensive distribution and habitat preferences, Cuban Parrot populations on some individual islands are exceedingly small and number in the hundreds [between 299 and 430 (in 1991) on Cayman Brac] to thousands of individuals (1900 on Grand Cayman, ref. 29). In contrast, the Hispaniolan Parrot seems to have narrower habitat preferences than Cuban Parrot; it does not frequent human-associated areas as readily (e.g., gardens and parks), though it has been recorded eating crop plants (30). Today, the total population of this species is estimated at 6,000 to 15,000 individuals and in decline (30, 62).

Here, we have outlined the phylogenetic relationships and historical distributions of parrot species prior to human arrival and the ensuing losses of many populations. Ultimately, conservation and species reintroductions depend on dialogue and collaboration among archaeologists, paleontologists, molecular biologists, conservationists,

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and local stakeholders to mitigate the ongoing loss of species and ecosystem degradation. Such dialogue can highlight the natural heritage that has already been lost, in order to better understand present and future stakes. While humans have manipulated populations and caused the extinction of species for millennia, we also will be essential to save those species that have endured in the ongoing era of human domination.

Materials and Methods

Sample Information and Aims. Here, we generate UCE and mitochondrial genome data for *Amazona leucocephala* taxa based on samples from across this species distribution and include a single sample collected in 1909 from a now extirpated population (Table 1). These samples represent all the currently recognized *A. leucocephala* subspecies (27). Modern sample UCE and mitochondrial genome data from *Amazona leucocephala* were obtained from tissue samples (n = 1), toe pads from round skins (n = 4), and vertebrae (n = 2) from skeletal specimens. UCE data from these specimens were combined with 35 taxon dataset from Smith et al. (41), where all species within *Amazona* from across the Americas and the Caribbean are represented. This dataset is to provide a robust nuclear-loci phylogeny of extant or recently extirpated taxa. Ancient DNA samples (n = 8) of *Amazona leucocephala*, *A. ventralis*, and *Amazona* sp. from paleontological and archaeological material were collected from the Bahamas (New Providence, Long Island), the Turks and Caicos (Grand Turk and Middle Caicos), Hispaniola, and Montserrat (Table 2). These samples were radiocarbon dated (if not previously), library prepared, and enriched for mitochondrial genomes (see below). These data were combined with modern mitochondrial data to phylogenetically place extinct/extirpated taxa in a robust dataset.

Radiocarbon Dating. The *Amazona* fossils from Haiti and the Dominican Republic were sampled for aDNA and the remaining material was sent for AMS radiocarbon dating to the University of Georgia Center for Applied Isotope Studies (UGAMS). Bird fossils from Hanging Garden Cave (Long Island; Bahamas) have yet to be successfully dated (10). Therefore, instead of attempting to date the *Amazona* fossils from this site, we dated mandibles of Bahamian hutia (*Geocapromys ingrahami*) from site levels 1 (n = 1), 2 (n = 2), 5 (n = 2), and 6 (n = 2) at the UGAMS because of their robustness relative to bird fossils. If the samples lacked collagen, then bioapatite (inorganic carbon) from the specimen was dated (*SI Appendix*, Table S3). Bioapatite dating performs well in arid conditions but should be evaluated on a site-by-site basis, ideally with the bioapatite and collagen fractions of the same sample radiocarbon dated (63). The UGAMS pretreatment methods are outlined at www.cais.uga.edu. Methods for the radiocarbon chronology of other sites (New Providence, Bahamas; Grand Turk and Middle Caicos, Turks and Caicos Island; Montserrat) are found in references in Table 2.

Toe Pad, Bone, and Tissue DNA Extractions. *Amazona leucocephala* toe pads from Isla de la Juventud, Cuba, Grand Cayman, and Acklins Island, Bahamas, were extracted using a modified protocol based on Soares et al. (64) (Table 1). Toe pads were washed with 0.5 M EDTA for 5 min and then placed in a lysis solution composed of 160 μL buffer ATL (Qiagen), 20 μL 1 M dithiothreitol (DTT), and 20 μL of proteinase K. The samples were incubated at 56 °C and intermittently vortexed. If the sample was not lysed after 24 h, an additional 20 μL of proteinase K was added and incubated for another 24 h. Once lysed, a QIAquick Nucleotide Removal Kit (Qiagen) was used to purify the samples. Briefly, the sample was gently mixed with 1,320 μL buffer PNI. Then, the sample volume was split between two spin columns. The samples were centrifuged for 1 min at 6,000 RPM and then flow-through was discarded. Then, 750 μL of buffer PE was added to the filter and centrifuged for 1 min at 6,000 RPM with the flow-through discarded. The filter was dry spun at 13,000 RPM, and then the column was placed in a clean 1.5-mL tube. DNA was eluted with 50 μL of heated EB buffer and repeated for a second elution. DNA from *A. l. leucocephala* from Abaco (UF 46992) was extracted from preserved frozen tissues using a Qiagen DNeasy Blood and Tissue Kit following the protocol supplied by the kit. These extractions were performed in a lab separate from the ancient samples.

In a University of Florida (UF) lab dedicated to processing ancient DNA extractions, a single fossil or archaeological sample was processed per day with negative controls following Oswald et al. (39, 65, 66). The eight paleontological or archaeological samples included are from Haiti, Dominican Republic, Long Island, New Providence, Grand Turk (n = 2), Middle Caicos, and Montserrat (see Table 2, *SI Appendix*, Table S5 for site information). Each sample was soaked in a 5% bleach solution to remove surface contaminants for 5 min. The fossil was crushed in liquid nitrogen and combined with 949 μL of 0.5 M ethylenediaminetetraacetic acid (EDTA), 25 μL 20 mg/mL proteinase K, 21 μL of 10 mg/mL DTT, and 5 μL of 10% sodium dodecyl sulfate. Samples were incubated at 60 °C for 24 h and intermittently vortexed. Samples then were concentrated with an Amicon Ultra-4 Centrifugal Filter Unit, purified using a Qiagen QIAquick MinElute Kit, and eluted in 48 μL of EB buffer. DNA extractions were quantified with a Qubit® 2.0 Fluorometer to determine DNA yield.

Two modern/historical *Amazona leucocephala* specimens (UF 25789, *A. l. hesterna*, Cayman Brac; UF 42477, *A. l. bahamensis*, Great Inagua) were represented by skeletal material. Two vertebrae were photographed and then destructively sampled from each specimen. The DNA extraction protocol used for the fossils was also used for these specimens.

Library Preparation. Library preparation was performed with a Swift Biosciences Accel-NGS Methyl-Seq DNA library kit following the standard protocol but excluding the bisulfite conversion step. All library preparation steps were performed in a separate lab from the ancient DNA extractions. The DNA extraction from the tissue sample of UF 46992 was sheared to 200 base pairs (bp) according to kit specifications using a Covaris S220. The bead-to-sample ratios followed Oswald et al. (39, 65, 66). PCR cycles for the bone sample were seven and eight for toe pads and four for UF 46992. Library concentration was determined with a Qubit® 2.0 Fluorometer.

UCE and Mitochondrial Enrichments. A 1:250 ratio of mitochondrial baits (Arbor 303008.v5–*Amazona ventralis*) was mixed with UCE baits (Arbor myBaits UCE Tetrapods 5Kv1) to increase the recovery of mitochondrial genomes and UCEs. The modern sample enrichment protocol followed that on the Arbor 303008.v5 kit. During amplification, 16 PCR cycles increased overall library concentration prior to sequencing. Bead clean-ups were performed on the PCR products. DNA yield was quantified with a Qubit® 2.0 Fluorometer. Modern samples were quantified, cleaned, and pooled at the UF ICBR and sequenced on an Illumina MiSeq using a 2 × 150 platform.

Ancient Sample Mitochondrial Genome Enrichments. Genomic libraries were double-enriched following the myBaits high-sensitivity protocol (version 5) with mitochondrial baits (Arbor 303008.v5) designed for *Amazona*. The hybridization temperature was set to 60 °C; the sample was incubated for 12 to 16 h per enrichment round. Two 50-μL PCR reactions were prepared for the entire resuspended DNA library (30 μL total volume). Using the entire sample maximizes yield and can also control for PCR duplicates. The samples were subjected to 16 PCR cycles. The DNA concentrations of the resultant PCR products were combined and quantified with a Qubit® 2.0 Fluorometer. The PCR product was concentrated with a vacuum centrifuge. The concentrated product then had a second round of enrichment following the protocol except for the PCR step. In this step, to further inhibit PCR duplicates, the two 50 μL PCR reactions for this sample were split into four 25 μL reactions. Following PCR, the products were combined, bead cleaned, and quantified with a Qubit® 2.0 Fluorometer. Samples were quantified, cleaned, and pooled at the UF ICBR and sequenced first on an Illumina MiSeq 2 × 150 platform. After recovering few on-target reads, the same library pool was then sequenced on an Illumina NovaSeq, S4, (¼ lane) using a 2 × 150 platform.

Ancient Data Processing. Adaptor removal, quality trimming, deduplication, and pairing of the MiSeq and NovaSeq reads were performed in fastp (67). These data were imported into Geneious Prime (www.geneious.com) and the 5′ and 3′ ends trimmed by a minimum of 8 bp and then mapped to the complete mitochondrial genome of *Amazona leucocephala* (UF 46992 or UF 42477) or *Amazona ventralis* (KU 8132). The mitogenomes of UF 46992 and UF 42477 sequenced for this project represent the most recent and high-quality specimens of extant populations of the Cuban Parrot in our dataset. Mapping settings were set to a 5% mismatch between reads and to produce no gaps within a single read. All alignments were evaluated by eye to examine sequence read depth. A depth of 1× was only allowed when the reads did not differ from the reference. A 2× coverage minimum was required for a SNP call. Further, SNPs at the ends of reads were removed and ends of reads were trimmed. Finally, a consensus sequence

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was produced with a 75% threshold (i.e., the base call was the SNP represented by greater than 75% of the SNPs at a particular site).

mapDamage. Reference-based alignments were exported as bam files. One ancient sample (UF 323777) had abundant on-target reads (~24 million), so it was subsampled to 2.5 million reads to preprocess it for mapDamage (68). mapDamage 2.2.0-80-g470506a was used to verify that the sample reads were aDNA reads (*SI Appendix*, Fig. S4). The read pile-ups from each sample were from both MiSeq and NovaSeq data accordingly mapDamage settings included a --merge-libraries command. For samples (GT3-FS-224, GTS-FS-345, PN4497, UF 322045), the read depth was reduced to a SD of <10 to reduce errors in the output due to heterogeneity in coverage (*SI Appendix*, Fig. S4).

UCE Processing. To obtain genome-wide markers across *Amazona*, we used a sequence-capture technique that targets highly conserved portions of the genome (i.e., ultraconserved elements; UCEs) that are informative at deep (69) and shallow (70) phylogenetic scales. UCEs have been particularly powerful and widely used in avian systematics to resolve relationships among living birds (e.g., refs. 71 and 72). We assembled a UCE dataset with modern geographic sampling in *A. leucocephala* and representatives from all *Amazona* species and two outgroup taxa, *Graydidascalus brachyurus* and *Alipiopsitta xanthops,* resulting in a 42-taxon dataset (*SI Appendix*, Table S4). The *A. leucocephala* UCE data were produced for this study, and the species-level *Amazona* data and outgroups were from Smith et al. (41). We used the same data-processing pipeline from Smith et al. (41). Briefly, FASTQ files were demultiplexed, and illumiprocessor v1 (73, 74) was used to remove low-quality bases and adaptor sequences. For a reference, we followed Smith et al. (41) and used the UCEs from a de novo contigs produced from ABySS v.1.5.2 (75) from a single taxon in our dataset *Amazona festiva*. This individual was selected because it produced the most complete assembly of UCE loci within the *Amazona* samples for which we produced data. We then mapped contigs to UCE probes and generated an index for the reference sequence and independently mapped reads from each sample using BWA v0.7.13-r1126 (76). SAM files produced from the BWA mapping were converted to BAM files and sorted with SAMtools v. 1.10 (77). Then, we used the *mpileup* function in SAMtools v. 1.10 (-C 30; -Q 20) to produce a VCF file in bcftools v. 1.12 (78), and vcfutils to call variant sites and filter sites based on coverage (<5× coverage per SNP) and quality score (<20), and convert FASTQ files to FASTA in seqtk. Only loci ≥100 bp were retained. Heterozygous sites were assigned IUPAC ambiguity codes. MAFFT v. 7.455 (79) was used to align loci, and the final alignments retained only loci for which 75% of the samples were present using default settings in PHYLUCE v.1.7.1 (80). A concatenated alignment of all loci was produced.

The threshold for retaining or masking variant sites is a key setting for potentially changing the information content in an alignment. We followed Smith et al. (41), which was the source of the nontarget *Amazona* taxa used in this study and masked all variant sites with less than 5× coverage. Previous work found that this level was an acceptable compromise in retaining variant sites in low-quality samples from historical museum specimens and rigorous enough to mask unreliable positions. To assess potential biases in phylogenetic inference caused by our coverage-threshold (5×), we repeated the pipeline described above and produced separate alignments where we included 1) all sites irrespective of coverage and 2) masked sites with less than 10× coverage. Additionally, we produced an alignment using an alternative UCE reference from *A. ochrocephala* with the 5× coverage-threshold to assess whether the inferred topology was influenced by which taxon we used as a reference. We summarized the information content across these treatments by estimating alignment summary statistics in AMAS (81).

Modern Samples—Mitochondrial Genome. Modern *Amazona leucocephala* samples were mapped to an *Amazona* mitochondrial genome in Geneious (v. 2022.2.2) to obtain mitochondrial sequence data. The Geneious map to reference settings were the Geneious Mapper, medium sensitivity/fast with fine-tuning set to iterate up to five times. The 35 samples from Smith et al. (41) were mapped to *A. barbadensis* (JX524615.1). The *A. leucocephala* samples sequenced for this project were mapped to *A. ventralis* (KX925977.1). Unsurprisingly, historical (toe pad) *A. leucocephala* samples had fewer mapped reads than modern species; therefore, these samples were mapped to the mitochondrial genome of UF 424777 (*A. leucocephala*, Great Inagua) to obtain more on-target reads. *Amazona vittata* (SRS7124124) genome data were downloaded from the NCBI SRA database, and 7.5 million reads were subsampled from this file and mapped to JX524615.1 with the same settings as above. Each pile-up was checked by eye to detect any mapping errors or contamination and a 75% consensus, and a highest assignment quality consensus sequence was generated for each sample. See *SI Appendix*, Table S4 for each *Amazona* taxon, *Graydidascalus brachyurus*, and *Alipiopsitta xanthops* sample in our dataset, reference taxon, and the number of reads mapped to the mitochondrial reference genome for each. This extant *Amazona* taxa mitochondrial genome dataset was then combined with the mitochondrial genome dataset of extinct *Amazona* taxa produced above.

UCE Data.

Phylogeny. To assess extant species relationships, IQTREE 2.1.2 (82) was used to obtain a phylogeny from the 42-sample (*Amazona* taxa, *Graydidascalus brachyurus*, *Alipiopsitta xanthops*, *SI Appendix*, Table S3) concatenated UCE alignment with 1,000 rapid bootstrap replicates. ModelFinder (implemented through IQTREE, ref. 83) was used to determine the best-fit substitution model for each partition (84). We also produced phylogenies from the alignments assembled for assessing coverage (all sites and 10× coverage) and reference (alternative reference: *A. ochrocephala*) biases. In addition to estimating 1,000 rapid bootstraps for the comparison across trees, we also estimated site concordance factors using 100 quartets. Site concordance factors are the percentage of informative sites supporting a node (82, 85). Although the information content varied across coverage thresholds and references (*SI Appendix*, Tables S7 and S8), we found that the topology remained stable across treatments (*SI Appendix*, Fig. S5). Highly supported nodes as determined from bootstrap values and site concordance factors had high support across the trees; the same pattern was observed for the weakly supported nodes. Across these trees, the topology differed only across nodes with weak support.

Time-calibrated phylogeny. To provide a temporal framework of divergence in Caribbean *Amazona*, we estimated a time-calibrated tree with the UCE (extant taxon) dataset. Because there were no internal fossil calibrations to calibrate nodes in the *Amazona* phylogeny, we leveraged a time tree for the order Psittaciformes that was calibrated with five non-*Amazona* fossils (41). We used ages from this tree to calibrate two key nodes for *Amazona*. The first was the divergence of *Amazona* from the outgroup in the phylogeny, *Alipiopsitta xanthops* (minimum age: 6.91 Ma; maximum age: 15.98 Ma). The second was the basal node in *Amazona* (minimum age: 4.3 Ma; maximum age: 12.4). To maintain consistency in how the branch lengths were converted to absolute time as the source of our calibrations, we used the same penalized-likelihood method, treePL (86). Our input phylogeny included 42 tips, where 40 samples belonged to *Amazona* and two outgroups (*Graydidascalus brachyurus* and *Alipiopsitta xanthops*). To estimate optimal parameter settings for 100 out of the 1,000 rapid bootstrap trees, we used the prime and thorough options and randomly sampled during the cross-validation over a range of smoothing parameters (1 × 10−7–1 × 104) for 10 iterations.

Mitochondrial Data.

Phylogeny. Mitochondrial genome consensus sequences from fossil, archaeological, and modern samples were combined and aligned in Geneious (v. 2022.2.2) using the Geneious Alignment tool. The resultant alignment was checked by eye and manually aligned as necessary in SeaView v5.0.4 (87, 88). IQTREE 2.1.2 (82) was used to obtain a phylogeny from the 50 taxa (extirpated and extant *Amazona* taxa, *Graydidascalus brachyurus*, *Alipiopsitta xanthops*) mitochondrial genome data alignment with 1,000 rapid nonparametric bootstrap replicates. ModelFinder (implemented through IQTREE, ref. 83) was used to determine the best-fit substitution model for the data (84).

Pairwise distance. The R v4.1.3 (89) packages pegas (90) and ape (91) were used to obtain a raw pairwise distance matrix for the Greater Antillean taxa, *A. albifrons*, and *A. xantholora* mitochondrial genome data.

Data, Materials, and Software Availability. Genomic data have been deposited in NCBI SRA; NCBI GenBank. Raw sequence reads generated for this project can be found on NCBI Sequence Read Archive (PRJNA913959 (38); SAMN32316663 (92) to SAMN32316677 (93)). Annotated mitochondrial genomes of ancient and modern sequences generated for this project are on NCBI GenBank (accession numbers OR048929 (94) to OR048943 (95); https://www.ncbi.nlm.nih.gov/genbank/). Raw data from Smith et al. (41) can be found on NCBI SRA (PRJNA692616) (96) and Dryad (http://dx.doi.org/10.5061/dryad.b5mkkwhfm) (97). Mitochondrial genome bycatch from Smith et al. (41), samples

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and *Amazona vittata* (SAMN02981494 (98) and ref. 42) and mitochondrial data from Kolchanova et al. (40) are provided in *SI Appendix*, Supplementary File 1].

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1. D. W. Steadman *et al.*, Exceptionally well-preserved late Quaternary plant and vertebrate fossils from a blue hole on Abaco, Bahamas. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 19897–19902 (2007).
2. M. E. Kemp, A. M. Mychajliw, J. Wadman, A. Goldberg, 7000 years of turnover: Historical contingency and human niche construction shape the Caribbean's Anthropocene biota. *Proc. Biol. Soc.* **287**, 20200447 (2020).
3. L. A. Newsom, E. S. Wing, *On Land and Sea: Native American Uses of Biological Resources in the West Indies* (University of Alabama Press, 2004).
4. D. W. Steadman, G. K. Pregill, D. L. Olson SL, Fossil vertebrates from Antigua, Lesser Antilles: Evidence for late Holocene human-caused extinctions in the West Indies. *Proc. Natl. Acad. Sci. U.S.A.* **81**, 4448–4451 (1984).
5. D. W. Steadman *et al.*, Asynchronous extinction of late Quaternary sloths on continents and islands. *Proc. Natl. Acad. Sci. U.S.A.* **102**, 11763–11768 (2005).
6. T. Tyrberg, "Holocene avian extinctions" in *Holocene Extinctions*, S. Turvey, Ed. (Oxford University Press, 2009), pp. 63–106.
7. S. B. Cooke, L. M. Dávalos, A. M. Mychajliw, S. T. Turvey, N. S. Upham, Anthropogenic extinction dominated Holocene declines of West Indian mammals. *Annu. Rev. Ecol. Evol. Syst.* **48**, 301–327 (2017).
8. J. A. Oswald, D. W. Steadman, The late Quaternary bird community of New Providence, Bahamas. *Auk* **135**, 359–377 (2018).
9. M. F. Napolitano *et al.*, Reevaluating human colonization of the Caribbean using chronometric hygiene and Bayesian modeling. *Sci. Adv.* **5**, eaar7806 (2019).
10. D. W. Steadman, J. Franklin, Bird populations and species lost to Late Quaternary environmental change and human impact in the Bahamas. *Proc. Natl. Acad. Sci. U.S.A.* **117**, 26833–2684 (2020).
11. M. J. LeFebvre, S. D. deFrance, "Animal management and domestication in the realm of Ceramic Age farming" in *Thearchaeology of Caribbean and Circum-Caribbean Farmers (6000 BC – AD 1500)*, R. B. Routledge, Ed. (Routledge, 2018), pp. 149–170.
12. C. A. Hofman, T. C. Rick, Ancient biological invasions and island ecosystems: Tracking translocations of wild plants and animals. *J. Archaeol. Res.* **26**, 65–115 (2018).
13. C. M. Giovas, Pre-columbian amerindian lifeways at the sabazan site, carriacou, West Indies. *J. Island Coast. Archaeol.* **13**, 161–190 (2018).
14. M. J. LeFebvre, G. DuChemin, S. D. deFrance, W. F. Keegan, K. Walczesky, Bahamian hutia (*Geocapromys ingrahami*) in the Lucayan realm: Pre-Columbian exploitation and translocation. *Environ. Archaeol.* **24**, 115–131 (2019).
15. O. Dunn, A. E. Kelley, *The Diario of Christopher Columbus's First Voyage to America 1492–3: Abstract by Fray Bartolomé las Casas* (University of Oklahoma Press, 1989).
16. G. F. Oviedo, *Natural History of the West Indies*. S. A. Stoudemire, Ed. (The University of North Carolina Press, 1959).
17. S. L. Olson, W. B. Hilgartner, Fossil and subfossil birds from the Bahamas. *Smithson. Contrib. Paleobiol.* **48**, 22–56 (1982).
18. P. W. Stahl, Adventive vertebrates and historical ecology in the Pre-Columbian Neotropics. *Diversity*, 10.3390/d1020151 (2009).
19. J. M. Capriles *et al.*, Pre-Columbian transregional captive rearing of Amazonian parrots in the Atacama Desert. *Proc. Natl. Acad. Sci. U.S.A.* **118**, e2020020118 (2021).
20. D. Creel, C. McKusick, Prehistoric macaws and parrots in the Mimbres Area, New Mexico. *Am. Antiq.* **59**, 510–524 (1994).
21. R. J. George *et al.*, Archaeogenomic evidence from the southwestern US points to a pre-Hispanic scarlet macaw breeding colony. *Proc. Natl. Acad. Sci. U.S.A.* **115**, 8740–8745 (2018).
22. J. A. Moretti, An ancient Thick-billed Parrot (*Rhynchopsitta pachyrhyncha*) from southeastern New Mexico with a review of archaeological occurrences in the American Southwest. *Wilson J. Ornithol.* **134**, 438–445 (2022).
23. F. V. Ottalagano, Naturalistic parrots, stylized birds of prey: Visual symbolism of the human-animal relationship in Pre-Hispanic ceramic art of the Paraná River Lowlands, South America. *Camb. Archaeol. J.* **33**, 193–215 (2022).
24. C. W. Schwartz, A. D. Somerville, B. A. Nelson, K. J. Knudson, Investigating pre-Hispanic scarlet macaw origins through radiogenic strontium isotope analysis at Paquimé in Chihuahua, Mexico. *J. Anthropol. Archaeol.* **61**, 101256 (2021).
25. S. L. Olson, E. J. Maíz López, New evidence of *Ara autochthones* from an archeological site in Puerto Rico: A valid species of West Indian macaw of unknown geographical origin (Aves: Psittacidae). *Caribb. J. Sci.* **44**, 215–222 (2008).
26. M. I. Williams, D. W. Steadman, "The historic and prehistoric distribution of parrots (Psittacidae) in the West Indies" in *Biogeography of the West Indies: Patterns and Perspectives*, C. A. Woods, F. E. Sergile, Eds. (CRC Press, 2001), pp. 175–189.
27. J. F. Clements *et al.*, The eBird/Clements checklist of Birds of the World: v2022 (2022), https://www.birds.cornell.edu/clementschecklist/download/ (Accessed 1 November 2022).
28. T. Juniper, M. Parr, *Parrots: A Guide to the Parrots of the World* (Pica Press, 1998).
29. N. Collar, P. F. D. Boesman, G. M. Kirwan, C. J. Sharpe, "Cuban Parrot (*Amazona leucocephala*), version 1.0" in *Birds of the World*, J. del Hoyo, A. Elliott, J. Sargatal, D. A. Christie, E. de Juana, Eds. (Cornell Lab of Ornithology, 2020).
30. N. Collar, P. F. D. Boesman, C. J. Sharpe, "Hispaniolan Parrot (*Amazona ventralis*), version 1.0" in *Birds of the World*, J. del Hoyo, A. Elliott, J. Sargatal, D. A. Christie, E. de Juana, Eds. (Cornell Lab of Ornithology, 2020).
31. N. Collar, P. F. D. Boesman, C. J. Sharpe, "Puerto rican parrot (*Amazona vittata*), version 1.0" in *Birds of the World*, J. del Hoyo, A. Elliott, J. Sargatal, D. A. Christie, E. de Juana, Eds. (Cornell Lab of Ornithology, 2020).
32. G. M. Kirwan, A. Levesque, M. Oberle, C. J. Sharp, *Birds of the West Indies* (Lynx Edicions, 2019).
33. P. M. Bradley, *Birds of the Cayman Islands* (Caerulea Press, 1995).
34. H. Bryant, Additions to a list of birds seen at the Bahamas. *Proc. Boston Soc. Nat. Hist.* **11**, 63–70 (1867).
35. M. Bryant, J. Reynolds, W. K. Hayes, Conservation taxonomy of the Cuban Parrot (*Amazona leucocephala*): Variation in morphology and plumage. *J. Carib. Ornithol.* **22**, 1–18 (2009).
36. N. Arlott, *Birds of the West Indies* (Princeton University Press, 2010).
37. K. R. Reis, D. W. Steadman, Archaeology of Trants, Montserrat, Part 5. Prehistoric Avifauna. *Ann. Carnegie Mus.* **68**, 275–287 (1999).
38. J. A. Oswald *et al.*, PRJNA913959: Changes in parrot diversity after human arrival to the Caribbean. National Center for Biotechnology Information, Sequence Read Archive. https://www.ncbi.nlm.nih.gov/bioproject/PRJNA913959. Deposited 19 December 2022.
39. J. A. Oswald *et al.*, Ancient DNA and high-resolution chronometry reveal a long-term human role in the historical diversity and biogeography of the Bahamian hutia. *Sci. Rep.* **10**, 1373 (2020).
40. S. Kolchanova *et al.*, Molecular phylogeny and evolution of amazon parrots in the Greater Antilles. *Genes* **12**, 608 (2021).
41. B. T. Smith *et al.*, Phylogenetic analysis of parrots of the world distinguishes artifactual from biological sources of gene tree discordance. *Syst. Biol.* **72**, 228–241 (2023).
42. T. K. Oleksyk *et al.*, A locally funded Puerto Rican parrot (*Amazona vittata*) genome sequencing project increases avian data and advances young researcher education. *GigaScience* **1**, 2047–217X–1–14 (2012).
43. P. J. Reimer *et al.*, The IntCal20 northern hemisphere radiocarbon age calibration curve (0–55 cal kBP). *Radiocarbon* **62**, 725–757 (2020).
44. M. J. Berman, P. L. Gnivecki, M. P. Pateman, "The Bahama Archipelago" in *The Oxford Handbook of Caribbean Archaeology*, W. F. Keegan, C. L. Hofman, R. Rodríguez Ramos, Eds. (Oxford University Press, 2013), pp. 264–280.
45. L. A. Carlson, *Aftermath of a Feast: Human Colonization of the Southern Bahamian Archipelago and its Effects on the Indigenous Fauna* (University of Florida, Gainesville, FL, 1999).
46. W. F. Keegan, C. L. Hofman, *The Caribbean before Columbus* (Oxford University Press, 2017).
47. M. A. Russello, G. Amato, A molecular phylogeny of *Amazona*: Implications for Neotropical parrot biogeography, taxonomy, and conservation. *Mol. Phylogenet. Evol.* **30**, 421–437 (2004).
48. D. W. Steadman, J. Franklin, Origin, paleoecology, and extirpation of bluebirds and crossbills in the Bahamas across the last glacial-interglacial transition. *Proc. Natl. Acad. Sci. U.S.A.* **114**, 9924–9929 (2017).
49. S. Kolchanova *et al.*, Genomes of three closely related Caribbean amazons provide insight for species history and conservation. *Genes (Basel)* **10**, 54 (2019).
50. A. V. Stokes, W. F. Keegan, A reconnaissance for prehistoric archaeological sites on Grand Cayman. *Caribb. J. Sci.* **32**, 425–430 (1996).
51. P. L. Drewett, S. J. Scudder, I. R. Quitmyer, "Unoccupied islands? The Cayman Islands" in *Prehistoric Settlements in the Caribbean: Fieldwork in Barbados, Tortola and the Cayman Islands*, P. L. Drewett, Ed. (Archetype Publications for the Barbados Museum and Historical Society, 2000), pp. 5–16.
52. C. M. Giovas, The beasts at large – perennial questions and new paradigms for Caribbean translocation research. Part II: Mammalian introductions in cultural context. *Environ. Archaeol.* **24**, 294–305 (2019).
53. M. J. LeFebvre, S. D. deFrance, Guinea pigs in the Pre-Columbian West Indies. *J. Island Coast Archaeol.* **9**, 16–44 (2014).
54. E. Lord, C. Collins, S. deFrance, M. J. LeFebvre, E. Matisoo-Smith, Complete mitogenomes of ancient Caribbean guinea pigs (*Cavia porcellus*). *J. Archaeol. Sci. Rep.* **17**, 678–688 (2018).
55. G. Olah *et al.*, Ecological and socio-economical factors affecting extinction risk in parrots. *Biodivers. Conserv.* **25**, 205–223 (2016).
56. G. Olah, B. T. Smith, L. Joseph, S. C. Banks, R. Heinsohn, Advancing genetic methods in the study of parrot biology and conservation. *Diversity* **13**, 521 (2021).
57. C. J. Hogg *et al.*, Threatened species initiative: Empowering conservation action using genomic resources. *Proc. Natl. Acad. Sci. U.S.A.* **119**, e2115643118 (2022).
58. A. D. Barnosky *et al.*, Merging paleobiology with conservation biology to guide the future of terrestrial ecosystems. *Science* **355**, eaah4787 (2017).
59. A. Perino *et al.*, Rewilding complex ecosystems. *Science* **364**, eaav5570 (2019).
60. J. L. Tella *et al.*, Endangered plant-parrot mutualisms: Seed tolerance to predation makes parrots pervasive dispersers of the Parana pine. *Sci. Rep.* **6**, 31709 (2016).
61. S. Kim, L. Sales, D. Carreira, M. Galetti, Frugivore distributions are associated with plant dispersal syndrome diversity in the Caribbean archipelagos. *Divers. Distrib.* **8**, 2521–2533 (2022).

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62. BirdLife International, Species factsheet: *Amazona ventralis* (2023), http://datazone.birdlife.org/species/factsheet/hispaniolan-amazon-amazona-ventralis (Accessed 7 May 2023).
63. A. Zazzo, J.-F. Saliége, Radiocarbon dating of biological apatites: A review. *Palaeogeogr. Palaeoclimatol. Palaeoecol.* **310**, 52–61 (2010).
64. A. E. R. Soares *et al.*, Complete mitochondrial genomes of living and extinct pigeons revise the timing of the columbiform radiation. *BMC Evol. Biol.* **16**, 230 (2016).
65. J. A. Oswald *et al.*, Ancient DNA from a 2,500-year-old Caribbean fossil places an extinct bird (*Caracara creightoni*) in a phylogenetic context. *Mol. Phylogenet. Evol.* **140**, 106576 (2019).
66. J. A. Oswald *et al.*, Ancient DNA from the extinct flightless Haitian cave-rail (*Nesotrochis steganinos*) suggests a biogeographic connection of the Caribbean and Old World. *Biol. Lett.* **17**, 20200760 (2021).
67. S. Chen, Y. Zhous, Y. Chen, J. Gu, fastp: An ultra-fast all-in-one FASTQ processor. *Bioinform.* **34**, i884–i890 (2018).
68. H. Jónsson, A. Ginolhac, M. Schubert, P. L. F. Johnson, L. Orlando, mapDamage2.0: Fast approximate Bayesian estimates of ancient DNA damage parameters. *Bioinform.* **29**, 1682–1684 (2013).
69. B. C. Faircloth *et al.*, Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. *Syst. Biol.* **61**, 717–726 (2012).
70. B. T. Smith, M. G. Harvey, B. C. Faircloth, T. C. Glenn, R. T. Brumfield, Target capture and massively parallel sequencing of ultraconserved elements for comparative studies at shallow evolutionary time scales. *Syst. Biol.* **63**, 83–95 (2014).
71. C. H. Oliveros *et al.*, Earth history and the passerine superradiation. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 7916–7925 (2019).
72. M. G. Harvey *et al.*, The evolution of a tropical biodiversity hotspot. *Science* **370**, 1343–1348 (2020).
73. B. C. Faircloth, illumiprocessor: A trimmomatic wrapper for parallel adapter and quality trimming (2013), 10.6079/J9ILL.
74. A. M. Bolger, M. Lohse, B. Usadel, Trimmomatic: A flexible trimmer for Illumina sequence data. *Bioinform.* **30**, 2114–2120 (2014).
75. J. T. Simpson *et al.*, ABySS: A parallel assembler for short read sequence data. *Genome Res.* **19**, 1117–1123 (2009).
76. H. Li, R. Durbin, Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinform.* **25**, 1754–1760 (2009).
77. H. Li *et al.*, 1000 Genome project data processing subgroup. The sequence alignment/map format and SAMtools. *Bioinform.* **25**, 2078–2089 (2009).
78. P. Danecek *et al.*, Twelve years of SAMtools and BCFtools. *Gigascience* **10**, p.giab008 (2021).
79. K. Katoh, D. M. Standley, MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. *Mol. Biol. Evol.* **30**, 772–780 (2013).
80. B. C. Faircloth, PHYLUCE is a software package for the analysis of conserved genomic loci. *Bioinform.* **32**, 786–788 (2016).
81. M. L. Borowiec, AMAS: A fast tool for alignment manipulation and computing of summary statistics. *PeerJ* **4**, e1660 (2016).
82. B. Q. Minh, M. W. Hahn, R. Lanfear, New methods to calculate concordance factors for phylogenomic datasets. *Mol. Biol. Evol.* **37**, 2727–2733 (2020).
83. S. Kalyaanamoorthy, B. Q. Minh, T. K. F. Wong, A. von Haeseler, L. S. Jermiin, ModelFinder: Fast model selection for accurate phylogenetic estimates. *Nat. Methods* **14**, 587–589 (2017).
84. O. Chernomor, A. von Haeseler, B. Q. Minh, Terrace aware data structure for phylogenomic inference from supermatrices. *Syst. Biol.* **65**, 997–1008 (2016).
85. Y. K. Mo, R. Lanfear, M. W. Hahn, B. Q. Minh, Updated site concordance factors minimize effects of homoplasy and taxon sampling. *Bioinform* **39**, btac741 (2022).
86. S. A. Smith, B. C. O'Meara, treePL: Divergence time estimation using penalized likelihood for large phylogenies. *Bioinform* **28**, 2689–2690 (2012).
87. M. Gouy, S. Guindon, O. Gascuel, SeaView version 4: A multiplatform graphical user interface for sequence alignment and phylogenetic tree building. *Mol. Biol. Evol.* **27**, 221–224 (2010).
88. N. Galtier, M. Gouy, C. Gautier, SEAVIEW and PHYLO_WIN: Two graphic tools for sequence alignment and molecular phylogeny. *Comput. Appl. Biosci.* **12**, 543–548 (1996).
89. R Core Team, *R: A Language and Environment for Statistical Computing* (R Foundation for Statistical Computing, Vienna, Austria, 2022).
90. E. Paradis, pegas: An R package for population genetics with an integrated-modular approach. *Bioinform.* **26**, 419–420 (2010).
91. E. Paradis, K. Schliep, ape 5.0: An environment for modern phylogenetics and evolutionary analyses in R. *Bioinform.* **35**, 526–528 (2019).
92. J. A. Oswald *et al.*, SRX18979571: ultraconserved elements (UCEs), mitochondrial genome enriched libraries of UF25789_Amazona_leucocephala. National Center for Biotechnology Information, Sequence Read Archive. https://www.ncbi.nlm.nih.gov/sra/?term=SAMN32316663. Deposited 19 December 2022.
93. J. A. Oswald *et al.*, SAMN32316667, SRX18979580: ultra conserved elements (UCEs), mitochondrial genome enriched libraries of UF8364_Amazona_leucocephala. National Center for Biotechnology Information, Sequence Read Archive. https://www.ncbi.nlm.nih.gov/sra/?term=SAMN32316667. Deposited 19 December 2022.
94. J. A. Oswald *et al.*, Amazona ventralis mitochondrion, partial genome. GenBank. https://www.ncbi.nlm.nih.gov/nuccore/OR048929. Deposited 25 May 2023.
95. J. A. Oswald *et al.*, OR048943, Amazona leucocephala mitochondrion, partial genome. GenBank. https://www.ncbi.nlm.nih.gov/nuccore/OR048943. Deposited 25 May 2023.
96. B. T. Smith *et al.*, Dataset for: Phylogenomic analysis of the parrots of the world distinguishes artifactual from biological sources of gene tree discordance. National Center for Biotechnology Information, Sequence Read Archive. https://www.ncbi.nlm.nih.gov/bioproject/PRJNA692616/. Accessed 16 August 2022.
97. B. T. Smith, Dataset for: Smith et al., Phylogenomic analysis of the parrots of the world distinguishes artifactual from biological sources of gene tree discordance. Dryad. https://doi.org/10.5061/dryad.b5mkkwhfm. Accessed 16 August 2022.
98. T. K. Oleksyk *et al.*, SRX4935223: Filtered trimed [sic] paired end reads: A.vittata [sic]. National Center for Biotechnology Information, Sequence Read Archive. https://www.ncbi.nlm.nih.gov/sra/?term=SAMN02981494. Accessed 12 August 2021.

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