



Origin of pre-Columbian guinea pigs from Caribbean archeological sites revealed through genetic analysis



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ABSTRACT

Sometime after A.D. 500, Ceramic Age populations traveling by canoe introduced domestic guinea pig (*Cavia porcellus*) from the mainland of South America to the Greater and Lesser Antilles as well as to the southern ABC (Aruba, Bonaire, Curaçao) islands. Pre-Columbian archeological specimens of guinea pig have been found on 18 sites on nine islands where disarticulated remains occur as food refuse. To identify the geographic origin of these animals, we extracted and analyzed ancient mitochondrial DNA of individual archeological guinea pigs (*C. porcellus*) from three sites. Two individuals each are from the sites of Finca Valencia and Tibes on Puerto Rico and one individual is from the Grand Bay site on Carriacou in the Lesser Antilles. The archeological contexts of the guinea pigs and the chronometric dates of these sites along with the genetic analysis lead us to hypothesize that guinea pigs were introduced initially to Puerto Rico from the modern-day region of Colombia. The genetic data, the first published on a pre-Columbian domestic animal from the Caribbean, allow us to infer direct human movement between the Caribbean Antilles and northwestern South America. These preliminary genetic data are parsimonious with archeological information regarding migration, exchange, and inter-island interaction that took place in the West Indies beginning approximately A.D. 600. These interactions contributed to the post-A.D. 500 cultural heterogeneity found in the Caribbean.

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1. Introduction

The guinea pig, along with the dog, was one of only two non-local domesticated animals introduced to the Caribbean archipelago during the pre-Columbian era. Sometime after A.D. 500, people transported domestic guinea pigs (*Cavia porcellus*)—via canoe—from the mainland of South America to the Greater and Lesser Antilles as well as to the southern ABC islands (Aruba, Bonaire, and Curaçao). The geographic homeland of these animals has previously been unknown. Here we present the results of a pilot study of the ancient DNA (aDNA) analysis of archeological guinea pig (*C. porcellus*) from three Caribbean sites to identify the probable origin of this intentionally translocated animal.

In addition to determining the likely geographic provenance of the Caribbean guinea pig, we examine the timing and probable trajectory of these introductions.

Previous phylogenetic and morphological research on guinea pigs using ancient and modern samples from South America and Europe indicate that domestic guinea pigs (*C. porcellus*), including those introduced to Europe following colonization of the Americas, all derive from a single domestication of wild guinea pigs (*Cavia tschudii*) in the western Central Andes, specifically the Peruvian highlands over 2500 years ago (see Spotorno et al., 2004, 2006, Spotorno et al. 2007; Walker et al., 2014; Wing, 1986). Building on the well-established evolutionary history of the guinea pig, this research expands the geographic and historical scope of human movement of guinea pigs into the Caribbean. The ancient DNA analysis of the five archeological guinea pigs reported here are from two sites on Puerto Rico, Finca Valencia and Tibes, as well as the site of Grand Bay located on the island of Carriacou in the Grenadines (southern Lesser Antilles) (Fig. 1).

Since the evolutionary history has established that domestic guinea pigs originate from South America, and the Central Andes specifically, we postulated two different scenarios to explain the distribution of

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Fig. 1. Location of Caribbean islands with sampled archeological assemblages. Red circles indicate northwestern South American region of modern-day Colombia and northeastern Orinoco River delta. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

guinea pigs in the West Indies. Because the Puerto Rican sites contain both the greatest quantities of guinea pig remains and the earliest occurrences of guinea pigs thus far known, one working hypothesis was that the Puerto Rican cohort constituted a founder population and that all guinea pigs were introduced to other islands from Puerto Rico. In this scenario, guinea pigs were introduced once to the West Indies and then people transported them to other locales. If this were the case, the genetic analysis should indicate that all of the guinea pigs are closely related, with possible minor genetic mutations evident in the populations most distant from Puerto Rico. The second working hypothesis postulates that guinea pigs were introduced from South America multiple times during the Ceramic Age to different islands. If this was the scenario, the genetic composition of the guinea pigs might differ significantly between the islands because different South American mainland populations of guinea pigs could potentially have been transported to the Caribbean. Spotorno et al. (2006) indicate that post-European colonization of the Americas and introduction of guinea pigs to Europe as well as modern breed improvements result in distinct guinea pig genetic compositions over relatively short periods of time (e.g., less than 100 years). Therefore, ancient Caribbean guinea pigs might exhibit a high rate of genetic diversity if multiple introductions took place.

As we describe here, all five samples produced results that clarify the history and probable routes of movement. The results place the five West Indian specimens in a lineage with published guinea pig genetic sequences primarily from Colombia, thus allowing us to hypothesize that the West Indian guinea pigs originated from there. In addition to providing a genetic baseline for the domestic guinea pig in the Caribbean archipelago, our study elucidates pre-Columbian human mobility, interaction, and probable sources of post-A.D. 500 cultural heterogeneity in the Caribbean. These data also complement aDNA studies of faunal remains from elsewhere in the Caribbean (e.g., rice rats; Brace et al., 2015) and in other island settings where phylogenetic information has proven valuable in revealing diachronic patterns of human movement and interactions, particularly across larger bodies of water (e.g., Greig et al., 2015; Larson et al., 2007; Matisoo-Smith and Robins, 2004; Storey et al., 2007, 2012). Following the presentation of our data, we discuss analysis plans that include the temporal and geographic expansion of

analyzed specimens as well as the proposed use of next generation sequencing for future samples.

2. Domestic guinea pig and Caribbean island records

Native to Andean South America, the domesticated guinea pig (*C. porcellus*) is a small to medium sized, hystricomorph rodent. Modern species estimates range from six to eight taxa (Wilson and Reeder 2005; Nowak, 1999). Guinea pigs are interpreted as having been domesticated by approximately 2500 B.C. in the highland region of Ayacucho, Peru in the Central Andes Mountains (Wing, 1986). Genetic analysis of the wild species, *C. tschudii* and *C. aperea*, indicates that *C. tschudii* is the ancestor of *C. porcellus* (Spotorno et al., 2004) with further genetic manipulation (e.g., production of laboratory animals and recent selective breeding for larger-sized animals) after the animals were introduced outside of the Americas following European colonization (Spotorno et al., 2006).

Based on archeological evidence, domestic guinea pig in the Central Andes were consumed as food and also served ritual and social purposes (Moseley et al., 2005; Rofes, 2000, 2004; Rofes and Wheeler, 2003; Sandweiss and Wing, 1997). Among the Inca, guinea pig were associated with high social status community members and used as ritual items in mortuary offerings at Machu Picchu (de la Vega, 1966; Miller, 2003). In addition to Peru, archeological guinea pig has been documented from Andean sites in Ecuador and Colombia (see Stahl, 2008). In Ecuador, elite use of the animal was likely tied to trade and exchange networks associated with access to thorny oyster shells (*Spondylus* spp.) (Stahl, 2003). Guinea pigs are reported from highland plain sites in Colombia (Garcia, 2012; Izjereef, 1978; Uribe, 1977–1978), but have not been reported from its Caribbean coast. Archeological records of domestic guinea pig from coastal Venezuela are uncertain, with one recorded from the northern inland site of Turen (Garson, 1980). Eight individuals identified as *C. aperea* are reported from the Palmasola site located on or near the northwestern coast of Venezuela (Sýkora, 2006).

The distribution of ancient guinea pig remains in the Caribbean consists of only 218 bones and bone fragments from 18 archeological sites on nine islands representing at least 64 individual animals (LeFebvre and deFrance, 2014) (Table 1, Fig. 2). The earliest and greatest concentration of guinea pig remains is on Puerto Rico, where 156 bones

Table 1
Pre-Columbian Guinea Pig (*Cavia porcellus*) records in the Caribbean.

Island	Site	Reported date ranges of specimen(s)	NISP	MNI	Source
Jamaica	Green Castle, STM25	> A.D. 1223; A.D. 1420–1616	4	3	1
Dominican Republic	Anadel	Unavailable	2	2	2
	Andres, Boca Chica	Unavailable	1	–	3
	Cerro de Monte, Constanza	Unavailable		–	3
Puerto Rico	Finca Valencia, NCS-1	A.D. 690–1281; A.D. 1050–1383; A.D. 1226–1405; A.D. 1173–1400/A.D. 1271–1620/A.D. 1048–1399; A.D. 1270–1415/post A.D. 1400 A.D. 1000–post 1400; post A.D. 1400	121	20	6, 13, 17
	Jácana, PO-29	A.D. 650–900/A.D. 1300–European Contact; A.D. 650–900; A.D. 1300–European Contact	22	10	9
	Tibes	A.D. 600–900; A.D. 900–1200	8	4	7
	Hacienda Grande	A.D. 400–1500	3	1	5
	Río Tanamá, AR-38	A.D. 1320–135, A.D. 1390–1490	1	1	4
	Paso del Indio	A.D. 1275–1420	1	1	8
Vieques	Luján	A.D. 1000–1300	19	4	10
St. Johns	Cinnamon Bay	A.D. 1300–1485; post A.D. 1000	25	11	11
Antigua	Mill Reef	post A.D. 1150	–	2	14
	Indian Creek	A.D. 825, 840, 880, 950	–	1	5, 6, 13
	Coconut Hall, PE-15	A.D. 1035, A.D. 1045	2	1	12
St. Lucia	Giraudy	A.D. 1200–1400	1	1	15
Carriacou	Grand Bay	A.D. 985–1030	4	1	16
Curaçao	Santa Barbara	“Late prehistory”	4	1	6, 13
			218	64	

Hash mark (—) denotes unrecorded or unavailable data.

References:

- 1: Allgood (2000); Allsworth-Jones and Wesler (2001).
- 2: Miller (1929 as cited by Wing (1996)).
- 3: Rimoli (1976 as cited by Wing 1996).
- 4: Carlson (2008).
- 5: Wing (1990).
- 6: Quitmyer and Kozuch (1996); Wing (1996).
- 7: deFrance et al. (2010).
- 8: Singleton (2012).
- 9: DuChemin et al. (2010).
- 10: Quitmyer and Wing (2001).
- 11: Data on file Florida Museum of Natural History; Quitmyer (2003); Wild (1999).
- 12: Healy et al. (2003); data on file with authors.
- 13: Data on file Florida Museum of Natural History.
- 14: Wing et al. (1968).
- 15: Phulgence (2007).
- 16: Giovas et al. (2011).
- 17: Solís Magaña and Rodríguez (2000).

occur at six sites. Two sites on the nearby islands of Vieques and St. John's in the U.S. Virgin Islands account for an additional 44 specimens. The concentration of guinea pigs on and around Puerto Rico led Wing (2000) to suggest the animals may have been first introduced here and then transported elsewhere. Aside from Puerto Rican sites, guinea pig remains also occur on Jamaica and Hispaniola to the west, and Vieques, St. John, Antigua, Saint Lucia, Nevis, Carriacou, and Curaçao to the east and south. Their remains have not been identified thus far from Bahamian island sites. Both cranial and post-cranial skeletal elements are reported without evidence of butchering, although some specimens are burned. At most of the Caribbean sites, guinea pig remains are from non-descript midden contexts or features not readily associated with ritual, supra-culinary, or socially-conscripted activities; however, there are exceptions to this pattern.

Three of the 18 sites with guinea pig remains may include specimens that are associated with ceremonial or non-quotidian contexts. At the site of Tibes in south-central Puerto Rico one of the eight guinea pig specimens is from a midden associated with the remains of a structure of undetermined size. Based on the identification of a large post, Curet and Pestle (2010:424–425) postulated that the structure was communal and religious in function, and that the guinea pig remains represent possible high status food. Due to the lack of precision regarding the

identification of this context's function and the recovery of other guinea pig remains in general midden refuse, deFrance (2010) interpreted the Tibes guinea pigs as non-elite food that was probably communally shared. At the Jácana site, also in south-central Puerto Rico, DuChemin (2013) suggests that the recovery of 22 guinea pig bones in close spatial proximity to a *batey* (West Indian ball court) has social significance beyond subsistence, possibly as feast fare or special consumption food refuse. At the Cinnamon Bay site, located on St. John in the U.S. Virgin Islands, some guinea pig remains are associated with a context interpreted as representing Taíno elite ceremonial structure or *caney* (Quitmyer, 2003; Wild, 1999). In contrast to some Andean sites, Caribbean guinea pigs have not been recovered from mortuary contexts, as obvious offerings, or interments.

Relying on relative dating techniques and artifact associations, guinea pig remains from the Puerto Rican archeological sites of Finca Valencia, Jácana, and Tibes represent the earliest known occurrences of the animal, and suggest that guinea pig was introduced to the Caribbean islands after A.D. 500 and became established before A.D. 900 (LeFebvre and deFrance, 2014). Guinea pig has been reported from six sites on Puerto Rico, the most among the Caribbean islands, with occupations spanning from A.D. 600 to post A.D. 1400. None of the known guinea pig remains are from sites or contexts that post-date Spanish



Fig. 2. Islands with identified archeological specimens of guinea pig, *Cavia porcellus*.

colonization of the Caribbean. Captive guinea pigs are present today in the northwestern mountainous interior in the communities of Lares and San Sebastián in Puerto Rico where they are used for food; however, it is not known if these animals are remnant populations from the original introductions or a population of animals that was reintroduced to the island after European colonization. Modern feral populations of domestic guinea pigs are present in eastern Cuba, and some farmers reportedly raise guinea pigs for their meat (Borrito-Páez, 2009:2285). As with the modern guinea pigs in Puerto Rico, it is not known if these are remnant pre-Columbian populations or reintroduced populations. There are no known paleontological records of fossil guinea pigs that predate human arrival in the archipelago.

3. Site descriptions

3.1. Finca Valencia

Located in north-central Puerto Rico at the base of a limestone hill, Finca Valencia is a multi-component site with evidence of occupation from the Saladoid (ca. 500 B.C.–A.D. 500) and Ostionoid (A.D. 500–1500) pre-Columbian periods, as well as historical occupation dating to the 18th and 19th centuries (Solís Magaña and Rodríguez, 2000). The site began as a small short-lived Saladoid hamlet. The site later featured a larger Ostionoid village occupied between ca. A.D. 1000–1400. Excavations primarily of the Ostionoid component recovered material evidence for ritual activity and social complexity, including the recovery of *cemís* (small, three-pointed stone objects that represents an ancestor or spirit), stone collar fragment, shell ornaments and an associated infant burial, as well as large boulders likely associated with a ballcourt.

The guinea pig remains from Finca Valencia were recovered exclusively from the Ostionoid component of the site and represent the largest known pre-Columbian guinea pig assemblage in the Caribbean. Wing (in Solís Magaña and Rodríguez, 2000) suggests that the guinea pig remains at the site may be indicative of ritual activity and use. Citing the rarity of guinea pig remains across Caribbean island sites and the abundance of remains recovered from Finca Valencia, Wing further

suggests that the site may have been a source of guinea pig distribution to other Caribbean islands.

3.2. Tibes

The site of Tibes is a large ceremonial center (~16 ha) located on the Portugués River near the south coast of Puerto Rico. A small village was founded there sometime between A.D. 300–400 by horticulturalists who practiced tropical gardening and also hunted, fished, and collected a variety of shellfish from the coast approximately 8 km away. These populations are associated with the Saladoid culture, horticulturalists who migrated into the Caribbean beginning at ~500 B.C. The site underwent major architectural and spatial reorganization between A.D. 1000–1200 when the communal plazas, ballcourts, and causeways were constructed and a ranked society presumably emerged. This political and social transformation from a simple village to a civic-ceremonial center occurred during the Ostionoid culture period (A.D. 600–1500). Tibes is the earliest known civic center in the Greater Antilles and antecedent to the hierarchical societies that eventually emerged as the Taíno chiefdoms identified by Spanish ethnohistorians.

Excavations at Tibes focused on understanding how and why Tibes underwent this major social, architectural, and political reorganization sometime after A.D. 900 (Curet et al., 2006). Between 1995 and 2003, researchers excavated deposits dating to both the initial Saladoid (~A.D. 300–A.D. 500) and the later Ostionoid (~A.D. 500–A.D. 1492) time periods, with more contexts associated with the later occupation. The purpose of excavations was to identify the occupational history of the site, the variation in site contexts (e.g. residential, ceremonial deposits), and the nature of civic and ceremonial architecture. Excavations consisted of shovel tests, 1 × 1 m, 2 × 2 m, contiguous trenches, and a 3 × 3 m block. The archeological contexts include five domestic trash middens, a probable structure of undefined function, two Saladoid period burials, and deposits of rock rubble and refuse interpreted as refuse associated with the construction of the plazas and ballcourts (Curet et al., 2006). The plaza and ballcourt areas appear to have been kept clean as little refuse accumulated near them.

The guinea pig remains are from midden contexts dating to the Ostionoid period.

3.3. Grand Bay

The Grand Bay site is a large coastal Amerindian village on the eastern side of Carriacou, the largest island (32 km²) in the Grenadines chain. The site extends for more than 120 m along the coast where dense midden deposits, trash pits, human burials, and other domestic activities can be seen in the eroding profile. Since 2003, intensive archeological investigation has focused on establishing when Grand Bay was first settled prehistorically, how subsistence strategies may have changed over time, and the role that Carriacou played in inter-regional interaction systems. Results of more than a decade of research demonstrates that Carriacou was first settled during the Terminal Saladoid period ca. A.D. 400 and occupied continuously thereafter during the Troumassoid period up until A.D. 1400. Evidence from both Grand Bay and another nearby site, Sabazan, indicates that people here were engaged in extensive exchange with groups on the South American mainland and various islands in the Lesser Antilles. A rich array of artifacts made from bone, stone, shell, and clay show local variations, but also engagement with other islands based on stylistic similarities of artifacts (Fitzpatrick et al., 2009) and petrographic analysis of pottery (Pavia et al., 2013). Numerous faunal remains, dominated by nearshore marine species (LeFebvre, 2007), also include non-native mammals such as the armadillo (*Dasypos sp.*), agouti (*Dasyprocta sp.*), opossum (*Didelphis sp.*), peccary (*Tayassu/Pecari*), guinea pig

(*C. porcellus*) and deer (*Cervidae*) (see Giovas et al., 2011) that testify to the purposeful introduction of a large suite of animals for consumption, producing objects, and possibly other roles. The guinea pig remains reported for Grand Bay were recovered from an extensive midden and associated with other vertebrate and invertebrate remains, as well as stone and clay artifacts.

4. Materials and methods

4.1. Sample selection

The five specimens selected for aDNA analysis consist of two mandibles from the Finca Valencia site in North Central Puerto Rico, two mandibles from the Tibes site in South Central Puerto Rico, and one maxilla from the Grand Bay site on Carriacou (Figs. 3 and 4; Table 2). The elements were selected based on condition of preservation and the presence of molars. The archeological specimens were identified through comparisons with modern domesticated guinea pig skeletal materials in the Environmental Archeology Laboratory at the Florida Museum of Natural History, Gainesville. Morphometric information on the samples are provided in Table 2.

All five specimens are from archeological contexts dating to the later Ceramic Age (post A.D. 500). As chronologically broad a sample as possible was selected in order to cover the known time span of domestic guinea pig use during pre-Columbian Caribbean history. Direct dating of the Grand Bay specimen yielded an AMS radiocarbon date of cal. A.D. 985–1030 (Giovas et al., 2011). At this time, only relative dates



Fig. 3. Location of Finca Valencia and Tibes archeological sites on Puerto Rico; location of Grand Bay site on Carriacou.



Fig. 4. The archeological *Cavia porcellus* specimens analyzed for ancient DNA. Top row, left to right: NCS 1A, Tibes A; Bottom row, left to right: NCS 1B, Tibes B; Middle right: Grand Bay A.

are available for the Puerto Rican specimens with a range of A.D. 600 to post A.D. 1400, but they do overlap with the Carriacou sample.

4.2. Sample preparation

Archeological guinea pig samples were extracted in a laboratory in a building separate from the laboratory where the samples were cloned and sequenced. This extraction laboratory has positive air pressure and had never been used for any previous guinea pig DNA studies. No positive PCR controls were used in order to eliminate the possibility of modern contamination. Disposable gloves, hairnets and lab coats were used when handling samples. All reagents were either certified DNA free or tested by PCR to be free of exogenous DNA prior to extraction. All surfaces were cleaned with 20% dilute bleach (1.2% sodium hypochlorite) in between samples.

The outer layer of bone and any discolorations were removed with a new sterile sandpaper prior to extraction. The mortar and pestle used to grind samples was cleaned between samples first with detergent and water, followed by submersion in Nochromix sulfuric acid overnight, then rinsed and soaked in 20% dilute bleach for 15 min and finally dried after 3 rinses in DNA free water.

4.3. DNA extraction

The mandible and maxilla samples were extracted using the following protocol [modified from (Rohland and Hofreiter, 2007, Rohland et al., 2010)]: DNA from 130 to 165 mg of bone or tooth powder was extracted overnight in 5 ml extraction buffer containing 0.45 M EDTA pH 8.0 and 0.25 mg proteinase K/ml. DNA was then bound to silica [prepared as in (Boom et al., 1990)] for 3.5 h at room temperature at pH 5 after adding 3.75 ml of a buffer containing 5.0 M guanidine thiocyanate and 0.25 M sodium chloride. After washing the silica twice, once with the above buffer and once with a solution of 51.3% ethanol, 125 mM sodium chloride, 10 mM Tris and 1 mM EDTA, the DNA was eluted in 100 μ l TE pH 8.0. One extraction blank with all reagents except bone was processed in parallel with each sample.

4.4. PCR amplification

Primers were designed with the Primer3 program (Rozen and Skaletsky, 2000). The primers are named based on the cytochrome b guinea pig reference sequence NC_000884.1 (D'Erchia et al., 1996) deposited in GenBank. Primer pairs (Table 4) were designed to cover

Table 2

Cavia porcellus specimens sampled for aDNA analysis. All measurements are in millimeters (mm)^a.

Site	Island	Date range	Specimen ^b	Element	Wt (g)	TL	L tooth row	Ht mand post M ₃	Ht mand ant PM	Dias L	Ht ascend. ramus
Finca Valencia	Puerto Rico	Post A.D. 1400	NCS-1 A	rt mandible	0.9	31.42	11.81	8.02	6.01	11.02	11.93
Finca Valencia	Puerto Rico	A.D. 1000–post A.D. 1400	NCS-1 B	rt mandible	0.8	30.24	12.19	8.36	6.58	na	na
Tibes	Puerto Rico	A.D. 900–1200	Tibes A	rt mandible	0.9	34.31	12.27	8.69	5.51	10.79	na
Tibes	Puerto Rico	A.D. 600–900	Tibes B	lf mandible	0.6	26.4	na	na	7.2	11.43	na
Grand Bay	Carriacou	A.D. 985–1030	Grand Bay A	rt maxilla	0.7	20.97	15.55	3.96	5.82	na	na

TL = total length refers to the longest proximal to distal measurement possible of specimen present.

L tooth row = cheektooth row measured along the longest alveoli length, when tooth row complete.

Ht mand post M₃ = height of mandible posterior to the M₃, medial side along edge of alveolus.

Ht mand ant PM = height of mandible anterior of PM, medial side along the edge of alveolus.

Dias L = length of diastema taken from PM alveolus to the top of the incisor alveolus, diastema complete.

Ht ascend. ramus = height of ascending ramus.

^a Measurements adapted from von den Dreisch (1976).

^b Finca Valencia, NCS-1 specimens, are Florida Museum of Natural History, Catalog # 0547.

variable regions of the cytochrome b gene, with the primers situated in conserved regions. The amplification products ranged from 85 to 119 base pairs. In addition, we designed primers to amplify a short fragment (121 basepairs) of the d-loop. PCR conditions were as follows: After an initial 7 min heating step at 94 °C, the reactions were cycled 55 times at 94 °C for 30 s, at the appropriate annealing temperature for 1 min, and at 72 °C for 1 min, followed by a final extension of 7 min. Sample volume was 1 µl for guinea pig samples from Tibes, Puerto Rico, but 1 µl of a 1:10 dilution were used for guinea pig samples from Finca Valencia, Puerto Rico and Grand Bay, Carriacou to minimize inhibition. Amplification products were visualized and photographed after electrophoresis on 1.5% agarose gels containing 50 µg ethidium bromide/100 ml agarose solution.

4.5. Sequence analysis

PCR amplification products were cloned into a TOPO TA vector (Invitrogen) following the manufacturers' recommendations. Three to five colonies from each cytochrome b amplification product were sequenced and analyzed on an ABI310 sequencer (Life Technologies), following the manufacturers' recommended protocol for sequencing. Preliminary data for the d-loop are based on sequencing of one to three colonies. Sequences were aligned with the Bioedit program and primer sequences removed. The obtained consensus sequences were concatenated for phylogenetic analysis. Unique *C. porcellus* cytochrome b sequences (among accession numbers NC000884, AF490405, AY228361-63, AY245094-97, AY382793, DQ017037-47, GU136732-33, HM447146-86) and *C. tschudii* (accession numbers AY 245099, AY382792, DQ017048-53 and GU136727-31) were obtained from GenBank (D'Erchia et al., 1996, Spotorno et al., 2002, Spotorno et al., 2003 deposited to GenBank, Dunnun and Salazar-Bravo, 2010, Spotorno et al., 2006, Burgoz-Paz et al., 2010 deposited to GenBank) and compared with the archeological sequences. The Caribbean sequences were also compared to the only available South American d-loop sequences (accession numbers AF491743, AF491744, AF491746 submitted to GenBank by Spotorno et al., 2002); and to KP100656 direct submission to GenBank by Cui (2014) and NC000884.

Phylogenetic relationships among sequences were estimated by maximum likelihood tree construction with PhyML 3.1/3.0 aLRT with the substitution model HKY85 and the SH-like approximate likelihood-ratio test (aLRT) for statistical branch support using the website www.phylogeny.fr (Dereeper et al., 2008). Trees were rendered with TreeDyn (v198.3) (Chevenet et al., 2006).

5. Results of aDNA analysis

The archeological samples were well preserved and all five samples yielded aDNA sequence data. The extracts from Finca Valencia and Grand Bay contained PCR inhibiting substances, but it was possible to overcome the inhibition by diluting the extract 1:10. The samples from Tibes did not show inhibition and both undiluted and 1:10 diluted extracts yielded PCR amplicons, also indicating that many copies of template DNA were present in the extract. Although the specimens appeared well-preserved, it was only possible to amplify short fragments varying in size between 85 and 121 base pairs including primers, as is generally the case for ancient DNA (Pääbo, 1989). Attempts to amplify a 236 base pair segment were unsuccessful. Thus, even though preservation was good, there was evidence of DNA damage. One common artifact of DNA damage is deamination of cytosine resulting in C to T transitions (Hofreiter et al., 2001). We sequenced between 3 and 5 clones for each of our cytochrome b PCR products to minimize the impact of such damage. Nevertheless, the majority of the differences we see between the archeological samples are C to T transitions, so it is possible that some variants are due to DNA damage and that the variation between samples is less than what we found. However, DNA damage

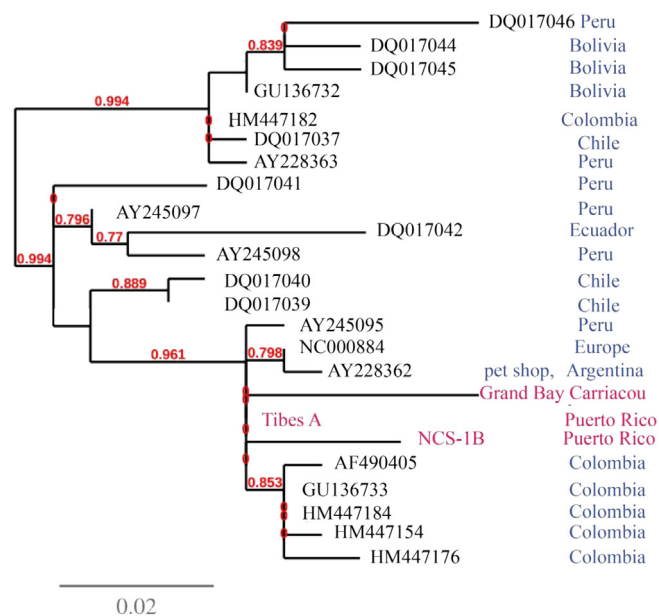


Fig. 5. Maximum likelihood tree of the phylogenetic relationship between archeological Caribbean and modern *C. porcellus*. The analysis is based on concatenated unique 288 base pair long cytochrome b sequences obtained from NCS-1B, Tibes A, Puerto Rico and Grand Bay, Carriacou. The archeological samples are labeled in red, and the modern samples are identified by their GenBank accession number. The red numbers indicate aLRT branch support. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

should not impact our conclusions about the origin of the samples as it would not affect the topology of the maximum likelihood tree, e.g. the approximate likelihood ratio value of 0.96 placing our longer archeological samples with Colombian samples show strong statistical support for this branch.

As no positive PCR controls were used and no guinea pig specimens had ever been analyzed in the laboratory, the guinea pig DNA is likely to derive only from the archeological specimens. Short amplicons in between the primers ranging in size from 43 to 74 base pairs in the variable regions in the mitochondrial cytochrome B gene were combined to form the final concatenated sequences that ranged in size from 215 to 288 base pairs. Phylogenetic analysis placed the three 288 base pair Caribbean guinea pig samples ((NCS-B, Tibes A, Grand Bay) in one tree branch together with published guinea pig sequences primarily from Colombia (Fig. 5). The branch with the archeological guinea pigs is well supported (branch support = 0.961) and ends in a polytomy that includes the archeological specimens, the European guinea pig, a guinea pig from a pet shop in Argentina, one domestic guinea pig sequence from Peru, and five Colombian sequences, but no domestic guinea pig sequences from Bolivia, Chile or Ecuador. The European specimens clearly originate from South America (see Spotorno et al., 2006); therefore, their inclusion in the branch with the Caribbean specimens suggests that European guinea pigs either originate from the Caribbean or possibly directly from Colombia. Other published Colombian guinea pig sequences were in the same branch as samples from Peru, Chile and Bolivia. Adding the two shorter (215 bps and 244 bps) archeological sequences (NCS-A and Tibes B) to the analysis placed all Caribbean samples in the same branch, albeit with less branch support (data not shown), suggesting that 215 bps has insufficient signal for phylogenetic resolution.

When the phylogenetic analysis included *C. tschudii*, one *C. tschudii* sequence from Ica, Peru fell in the same branch (branch support = 0.92) as the Caribbean guinea pig sequences and was identical to the Tibes A specimen (Fig. 6). This sequence was submitted to GenBank by Dunnun and Salazar-Bravo (2010) and their analysis of 1140 bp of the cytochrome b gene showed that it fell into a strongly supported

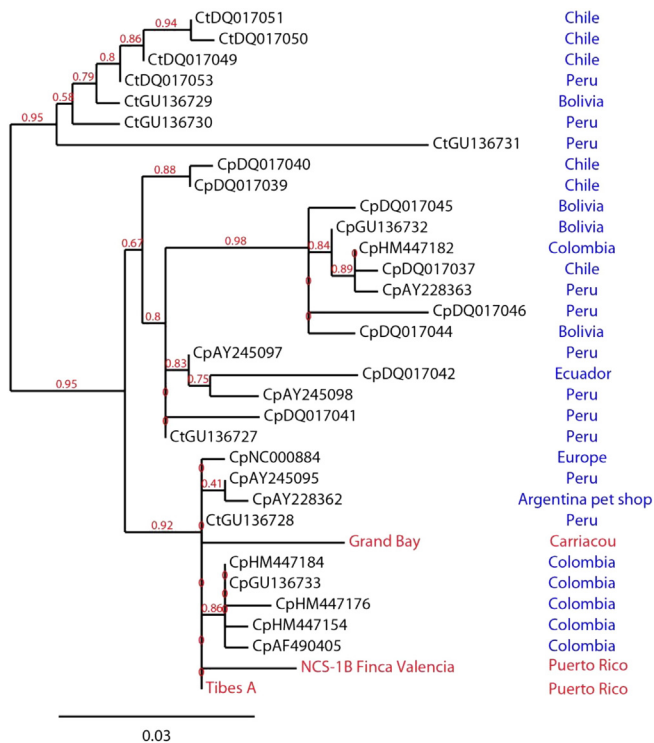


Fig. 6. Maximum likelihood tree of the phylogenetic relationship between archaeological Caribbean *C. porcellus*, and modern *C. porcellus* and *C. tschudii*. The analysis is based on concatenated unique 288 base pair long cytochrome b sequences obtained from NCS-1B, Tibes A, Puerto Rico and Grand Bay, Carriacou. The archaeological samples are labeled in red, and the modern samples are identified by Cp for *C. porcellus* and Ct for *C. tschudii* followed by their GenBank accession number. The red numbers indicate aLRT branch support. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

clade with *C. porcellus* from Colombia away from other *C. tschudii* specimens. The similarity in the sequence may indicate that the original domestication occurred near Ica, Peru from ancestors to both this wild *C. tschudii* specimen and Colombian and Caribbean domestic guinea pigs, or, alternatively, that this *C. tschudii* specimen was misidentified. Most other *C. tschudii* sequences clustered away from domestic guinea pig sequences, but one other Peruvian sequence was placed in a less supported branch with *C. porcellus* from several South American countries.

A preliminary phylogenetic analysis of a 79 base pair segment of the mitochondrial d-loop also places three Puerto Rican and the Carriacou sample together in one branch (Fig. 7). The d-loop data is based on sequencing of three clones from Finca Valencia NCS-1A and Tibes A, but only single clones were obtained from Tibes B and Carriacou, so the phylogeny is uncertain. Although the Caribbean samples are in a separate

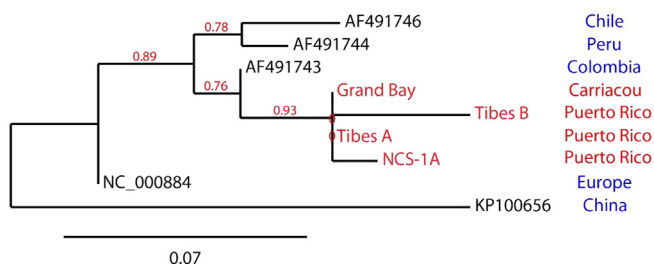


Fig. 7. Maximum likelihood tree of the phylogenetic relationship between guinea pigs based on 79 base pair sequences from the mitochondrial d-loop. The archaeological samples are labeled in red. The other sequences were obtained from GenBank and are identified by their accession number. The red numbers indicate aLRT branch support. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

branch from the available South American sequences, they have more sequence similarities with the Colombian sequence than with the other sequences.

Although the number of samples is small, we detected some sequence differences among the sites. The two samples from Tibes, Puerto Rico, differed from each other at two positions; the two samples from Finca Valencia, Puerto Rico, differed from each other at four positions, but the sample from Grand Bay, Carriacou, was the most divergent from the other Caribbean samples (Table 3). NCS-1A from Finca Valencia and the Tibes A specimen were identical for all positions for which sequence data were obtained. The variation between and within sites on Puerto Rico may be due to a diverse population of guinea pigs or changes over time, but a larger sample and more samples from sites located between Puerto Rico and Carriacou would be needed to differentiate between those possibilities. Analysis of a larger section of the mitochondrial d-loop, which accumulates more change over time, could also help determine the relationship between guinea pigs on the different islands. Even though the Carriacou guinea pig was most divergent from all *C. porcellus* sequences, it was still closely related to Colombian and Puerto Rican guinea pigs, indicating a common origin.

6. Discussion and conclusions

Our pilot analysis of ancient DNA from five domestic guinea pig individuals from two pre-Columbian archeological sites on Puerto Rico (Greater Antilles) and one on Carriacou (southern Lesser Antilles) helps to elucidate the probable continental origin of this animal and its evolutionary history. First, our data show that the Caribbean guinea pigs from the two islands are closely related, indicating that they likely were derived from the same original population. Second, our analysis allows us to hypothesize that Caribbean domestic guinea pig originated from the area of modern-day Colombia (see Fig. 1). Based on our results, it is possible that domestic guinea pig arrived in the Caribbean islands through multiple introductions or translocations from the same population or area of Colombia, or that domestic guinea pig arrived through a single introduction, possibly to Puerto Rico first, and subsequently was spread by human transport among the islands. At this time it is not possible to rule out an introduction of domestic guinea pigs from Venezuela into the Caribbean as there are no published Venezuelan sequences for comparison although there is scant archeological evidence for prehistoric use of domesticated guinea pig in or near Venezuela (e.g., Garson, 1980, Sýkora, 2006).

Our analysis contributes to both the evolutionary history of guinea pigs and an understanding of the probable routes of transport of the guinea pig outside of South America. The genetic analysis confirms the zooarchaeological identification of the analyzed specimens as domestic guinea pig and the importation of domestic rather than wild guinea pig into the Caribbean archipelago. These new data also support genetic models of the original domestication of *C. porcellus* from *C. tschudii* in Peru. The inclusion of two likely European guinea pigs in the same branch as Colombian and Caribbean guinea pigs also raises the possibility that the European guinea pigs were first obtained in the Caribbean, although Peru or Colombia are other possibilities (Table 3; Figs. 5 and 6). The genetic data along with the archeological contextual information allow us to hypothesize the following scenario. An original guinea pig domestication in Peru was followed by the introduction of domesticated guinea pigs to northwestern South America. From there a population or multiple populations of Colombian guinea pigs were brought into the Greater Antilles, and subsequently people moved them west, east, and south within the Caribbean. The genetic data do not allow us to confirm that a founder population was responsible for the West Indian cohort nor can we determine if guinea pigs arrived once or through multiple introductions from various locales in South America.

The genetic analysis of this small sample allows us to make only preliminary statements regarding the probable origin of guinea pigs; however, when the genetic data are combined with other archeological

Table 3
Puerto Rican and Carriacou cytochrome b variable sites identified in the sequencing compared to domestic *Cavia porcellus*, and closest matches in GenBank. Samples are numbered based on the GenBank reference sequence NC00084.

14,000 +	527	642	817	819	824	827	928	929	948	955	1159	1188
<i>Cavia porcellus</i> NC00084 Europe	A	T	C	C	C	C	C	C	C	C	C	C
<i>Cavia porcellus</i> HM447184 Columbia	G	T	C	C	C	C	C	C	C	C	C	C
<i>Cavia tschudii</i> GU136728 Peru	G	T	C	C	C	C	C	C	C	C	C	C
NCS-1A Finca Valencia, Puerto Rico	G	NA	C	C	C	C	C	C	C	C	C	C
Tibes B, Puerto Rico	G	C	C	C	C	C	NA	NA	NA	NA	C	T
Grand Bay, Carriacou	G	T	T	T	T	T	T	C	C	C	C	C
Tibes A, Puerto Rico	G	T	C	C	C	C	C	C	C	C	C	C
NCS-1B Finca Valencia, Puerto Rico	G	T	C	C	C	C	C	T	T	T	A	C

evidence, the probable role of northwestern South America in Caribbean history is much stronger. Zooarchaeological and isotopic studies of animal life history and animal-derived artifact provenance (e.g., animal bone-derived pendants made from felines/jaguars and other non-local exotic animals) show that South American animals were readily imported to and moved throughout the Caribbean Antilles during the Ceramic Age (500 B.C.–post 1400), providing evidence of routes and networks of interaction and human mobility (Giovas et al., 2011; Laffoon, 2013; Laffoon et al., 2014). The geographic resolution of Isthmo-Colombian (Colombia, Panama, and Costa Rica) and island interactions and archeological artifact and material origins based on isotopic analysis alone has been limited to broad-level regional scopes of understanding. This is due in part to the geochemically complex nature of circum-Caribbean geologic formations and mineral compositions as well as a lack of comparative geochemical data from southern portions of Central America and northern South America (Garcia-Casco et al., 2013; Laffoon et al., 2014). As shown here, these genetic data not only augment these recent archeological interpretations that the Isthmo-Colombia region and northwestern South America were focal points of post-A.D. 500 trade, interaction, and population movement in the Caribbean (Rodríguez Ramos, 2010), but the genetic data also provide greater resolution in identifying the probable geographic provenance of a Caribbean-introduced animal in comparison to those derived from stable isotope analysis alone or studies of artifact provenance. The isotope studies and our genetic analysis support an ample and growing body of non-faunal (e.g., stone, pottery) archeological evidence demonstrating that during the Caribbean Ceramic Age, Pre-Columbian, human mobility and interaction among and between islands and the surrounding the circum-Caribbean mainland were multi-scalar across space and communities (Booden et al., 2008; Hofman et al., 2007, 2011; Hoogland et al., 2010; Pavia et al., 2013; Rodríguez Ramos, 2010, 2011).

Furthermore, Puerto Rico is characterized by a possible unique history that includes long-term sustained cultural influences from the Isthmo-Colombian and northwestern regions of South America (Rodríguez Ramos, 2010, Rodríguez Ramos, 2013). Even though the genetic sample is small, our study bolsters arguments for these sustained cultural connections. Previously, researchers suggested that archeological assemblages on Puerto Rico and the island of Vieques

that include Huecoid-style artifacts could be attributed to an Andean highland point of origin for either groups of people or cultural manifestations (Chanlatte Baik, 2013; Chanlatte Baik and Narganes Storde, 1980; Rodríguez Ramos, 2010). This suggestion is contrary to long-held views that northeastern South America and the Orinoco River basin were the main progenitors of pre-Columbian Caribbean people and culture (see Fig. 1). In contrast, arguments favoring the role of interaction with northwestern South America are increasingly gaining favor (Callaghan, 2003; Chanlatte Baik, 2013; Hofman et al., 2011; Laffoon et al., 2014; Rodríguez Ramos, 2011, Rodríguez Ramos, 2013). However, these ideas are not mutually exclusive.

The aDNA data also point to some intriguing avenues for future study. Although closely related, the genetic variability between the Puerto Rican and the Carriacou samples may reflect differentiation that developed through time or a later guinea pig introduction from the same Colombian region directly to the southern Lesser Antilles. At this time, it is not possible to rule out that domestic guinea pigs, genetically originating in Colombia, entered the Caribbean from continental areas other than Colombia. Future genetic and direct chronometric analyses of specimens from sites on islands in the northern Lesser Antilles and from archeological sites in Colombia and elsewhere in South America will help to assess whether or not a founder population of domestic guinea pig was introduced to Puerto Rico prior to other islands, as well as other possible routes of mainland-to-island and island-to-island movement of guinea pig. These future studies will also help to clarify the cultural relations between indigenous groups within the Caribbean as well as relationships with those who inhabited South America.

The ability to identify the probable geographic origin of Caribbean domestic guinea pigs to the region of modern-day Colombia is significant. Although more aDNA data are needed to confirm our current model, these genetic data provide a highly parsimonious explanation that the initial population of introduced West Indian guinea pigs originated in northwestern South America. The introduction of the guinea pig to the Caribbean by A.D. 600 coincides with a range of other social and cultural changes that took place at that time. As such, guinea pigs are an important proxy for understanding human movement and networks of interaction and cultural ties between sites, islands, and the South American mainland after A.D. 500.

The proposed next phase of our genetic research on guinea pig remains includes specimens from both other Caribbean islands and South American archeological contexts as well as next generation sequencing, which will help to refine our understanding of how, when, and why people translocated these animals to the Caribbean.

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Table 4
Cytochrome B primer sequences.

Name of primer	Sequence	T used in PCR °C
14468L	TTTACTACGGATCATACATTCC	56
14554R	ACCCTATGAATGCGGTAGCC	
14619L	TATCCCTACATCGGGACAA	56
14703R	GGCAAAGAATCGTGTAGGG	
14763L	CCACGAGACAGGATCAACA	53
14865R	AAATAAGGCTCTAAATGTCTT	
14886L	CCTAGTACTCTTACACCCGACCT	57
15004R	GGATTGCGTAGGCAATAAGA	
15120L	CCTCAGCAATGCCTCTAT	56
15222R	CAACTGGCAATGGTGATGT	

mandibles. Andrzej and Marlena Antczak provided a reference and information regarding guinea pig in northern Venezuela. Carmen Lager-Díaz provided information on the modern rearing of guinea pigs on Puerto Rico and their location. This study was funded in part by a 2013 Provost Research Award from Santa Fe College. The manuscript was improved with comments from Elizabeth Wing, David Steadman, Reniel Rodríguez Ramos, Christina Giovas, and Nicole Cannarozzi. We thank two anonymous reviewers and the editors.

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