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The stuffed crocodile of "Castel Nuovo" in Naples (Italy): new insights from ancient DNA and radiocarbon

T. FIORAVANTI[1], E. CASAFREDDA[2], A. SPLENDIANI[1], & V. CAPUTO BARUCCHI[1]*

[1]*Dipartimento di Scienze della Vita e dell'Ambiente (DiSVA), Università Politecnica delle Marche, Ancona, Italy, and* [2]*Accademia di Belle Arti di Napoli, Napoli, Italy*

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Abstract
In the present study, a stuffed crocodile displayed for centuries at the "Castel Nuovo" (New Castle) in Naples was radiocarbon dated and examined using ancient DNA analysis. The specimen was classified as *Crocodylus niloticus* based on its large body size and the mitochondrial DNA haplotype obtained, already described for a living population in Lake Nasser (Egypt). Radiocarbon analysis indicated an age of 585 ± 40 ^{14}C year BP, which coincides with the end of the Middle Ages. These results are commented in the light of Medieval religious symbolism and ancient legends which link the crocodile to the history of Naples during the mid-fifteenth century. The data obtained seem to confirm the explanation found in an old guidebook to Naples, according to which the stuffed crocodile was offered by a soldier returning from Egypt as an *ex-voto* to the image of the "Madonna del Parto" in the chapel of the castle. In addition, the radiocarbon dating shows that the individual analysed could be the oldest taxidermied vertebrate in Europe.

Keywords: *Ancient DNA,* Crocodylus*, radiocarbon dating, species identification, taxidermy*

Introduction

For several centuries, a stuffed crocodile was displayed at the top of the triumphal arch of "Castel Nuovo" (New Castle), the residence of the Angevin and Aragonese Kings of Naples (Figure 1). Many chilling legends, which are intertwined with the city's history and inhabitants, have developed around the presence of this relic. In Western cultures, crocodiles have generally had a negative reputation; not only does Pliny the Elder bear witness to this (*Natural History*, book VIII, 37, 89–90) but the same superstition also emerges in Medieval bestiaries, starting from the oldest manuscripts in the Latin *Physiologus* (eighth century after Christ), in which the crocodile is considered a symbol of death and hell, "qui inimicus est Domini Salvatoris nostri" (which is the enemy of our Lord and Saviour) (see Morini 1996; Pastoureau 2012; Zambon 2018). Correlated to this religious symbolism there was the habit, documented in Europe from the fifteenth century (Bertelli 2018), of exhibiting the remains of large stuffed crocodiles in Marian churches. These reptiles were considered a symbol of the Devil's submission to God and the Virgin Mary but at the same time, a tangible sign of the presence and activity of the Devil in the world (Bertelli 2005).

The crocodile specimen from the Neapolitan castle is not mentioned in recent literature on the topic, which is not surprising. In fact, in 1875 the crocodile was moved to the city museum (currently known as the "Chartreuse and National Museum of San Martino"), where it was long forgotten in

*Correspondence: V. Caputo Barucchi, Dipartimento di Scienze della Vita e dell'Ambiente (DiSVA), Università Politecnica delle Marche, Ancona 60131, Italy. Email: v.caputo@univpm.it

Figure 1. An original photograph of the crocodile displayed at the top of the triumphal arch of "Castel Nuovo" in Naples (about 1870). Digital image courtesy of the Getty's Open Content Program.

a repository. However, tales about the crocodile lived on in the popular imagination. The Neapolitan historian Benedetto Croce reported an ancient legend according to which the crocodile came from Egypt in the wake of a ship and settled in the castle moat. The beast used to sneak into the dungeons, where prisoners were locked up, drag them into the water and devour them. When the cause of the mysterious disappearance of the convicts was finally discovered, the crocodile was deliberately used to eliminate troublesome prisoners but when the crocodile was no longer of any use it was killed, stuffed and hung above the castle gateway (Croce 1919). A second legend (Settembrini 1907) tells how King Ferdinand I of Naples (1424—1494) used the crocodile to do away with his enemies, while another version reports that Queen Joanna II of Naples (1373–1435) found a way to get rid of her many lovers by feeding them to the crocodile (see Bausilio 2005). Instead, according to Dumas (1862), the crocodile was used to eliminate the family of the revolutionary Masaniello after the Neapolitan uprisings in 1647.

After more than a century of oblivion in a repository of the National Museum of San Martino, in 2018 the "Castel Nuovo" crocodile was brought to light again, in a poor state of conservation, and was therefore subjected to careful restoration work and some scientific investigations (Casafredda 2019). Among the latter, we performed an ancient DNA (aDNA) study in order to correctly assign the Neapolitan specimen to a *Crocodylus* species and, if possible, to identify its real geographic origin. In fact, recent genetic analyses on contemporary and museum crocodile specimens from several African regions have allowed the identification of two distinct African species, namely the Nile crocodile *Crocodylus niloticus* (Laurenti 1768) and the West African crocodile *C. suchus* (Saint-Hilaire 1807) (Hekkala et al. 2011). *Crocodylus niloticus* is currently found throughout Eastern and Southern Africa while *C. suchus* populations are concentrated in Western and Central Africa, although in the past the distribution of the two species was more overlapping (Hekkala et al. 2011; Shirley et al. 2015). Results obtained from Egyptian crocodile mummies and other museum samples have shown that the River Nile was inhabited in the past by both *Crocodylus* species (Hekkala et al. 2011). In addition to aDNA analysis, radiocarbon dating was performed to verify the age of the specimen, so as to clarify the mysterious origin of this important relic in the history of Naples.

Materials and methods

In order to evaluate the age of the crocodile specimen, a caudal osteoderm was radiocarbon dated at the "CEnter of applied physics, DAting and Diagnostics" (University of Salento, LE, Italy). The analysis was carried out using the Accelerator Mass Spectrometry (AMS) method (Calcagnile et al. 2005) and the calibrated age was obtained using the software OxCal Ver. 3.10 (Reimer et al. 2009). Description of the specimen and scale characters were taken according to Hoser (2012), and in addition, genetic analysis was performed to allow a more accurate species identification.

A substitution tooth was sampled and decontaminated prior to DNA extraction to reduce the presence of exogenous DNA and PCR inhibitors (Rohland & Hofreiter 2007). The decontamination of the tooth and DNA extraction was performed as proposed in Fioravanti et al. (2020). A fragment of 193 bp of the 12S rRNA and a fragment of 211 bp of the mitochondrial control region (D-loop) were amplified thanks to species-specific primer pairs previously designed (Hekkala et al. 2011). PCR reactions were carried out using the protocol proposed by Splendiani et al. (2016). The annealing temperature was set at 54°C as proposed by Hekkala et al. (2011). All amplicons were checked on 2% agarose gel stained with GelRed™ (Biotium) and send to the BMR genomics (Padova, Italy) for Sanger sequencing. They were purified by exoSAP-IT™ (Thermo Scientific) and sequenced in both directions using an automated sequencer, ABIPRISM 3730XL (Applied Biosystems). DNA extraction and PCR set up were performed in a laboratory dedicated to aDNA analysis. In order to detect contamination, negative controls were added during both extraction and amplification phases and, a second DNA extraction and amplification were performed to validate the result.

All the sequences from Hekkala et al. (2011), that include both the short 12S rRNA and D-loop, were downloaded from DRYAD repository (datadryad.org; DOI: 10.5061/dryad.s1m9h) and aligned in CLUSTALW (Larkin et al. 2007) with our one. Firstly, an alignment between our sequence and the two identifiers of *C. niloticus* and *C. suchus* (SAAF2 and SAAFedpool; see Hekkala et al. 2011) was performed. Secondly, an alignment was performed with all the sequences downloaded and, in order to highlight the relationship between our haplotype and those previously described (Hekkala et al. 2011), a Median-Joining network (with $\varepsilon = 0$) was built using the software Network 10 (Fluxus Technology Ltd., www.fluxus-engineering.com).

Results

The "Castel Nuovo" crocodile was radiocarbon dated (95.4% probability) to 1296–1419 cal yr AD (585 ± 40 ^{14}C yr BP). Concerning the meristic characters still detectable, the crocodile showed 6 post-occipital and 6 nuchal scales (Figure 2(a) and (b), respectively); 14 maxillary, 5 premaxillary (on the right side, being the left damaged) and 15 mandibular teeth (on both hemi-mandibular arch) (Figure 2(c) and (d), respectively); dorsal scales arranged from 6 to 8 rows. The incompleteness of the specimen prevented the observation of the ventral scales, diagnostic for the identification of *C. niloticus* (range = 16–20) and *C. suchus* (range = 12–15). However, the estimated total length (about 377 cm) based on the sole photograph of the specimen (Figure 1) suggested that the specimen could belong to the species *C. niloticus*, being *C. suchus* usually smaller-sized (respectively, 4–5 m versus 1.5–2.5 m, for adult males of both species) (Hoser 2012).

The analysis of aDNA allowed obtaining 153 bp of the 12S rRNA and 167 bp of the D-loop (GenBank accession numbers MT165582 and MT165583). The alignment between our sequence and those from

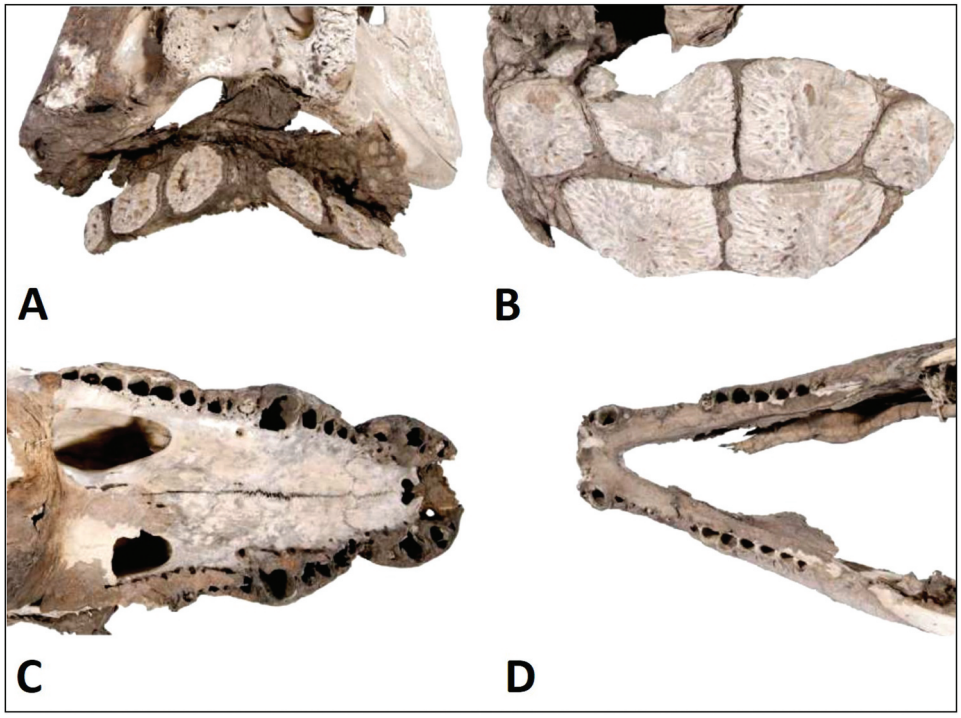

Figure 2. Some morphologic traits (not to scale) of the head of the "Castel Nuovo" crocodile. (A) post-occipital and (B) nuchal scales; (C) palate vault and (D) mandible in dorsal view.

Table I. Diagnostic sites obtained from the alignment between the sequences of the 12S rRNA + D-loop of *C. niloticus* and *C. suchus*.

	12S											D-loop												
	17	23	34	36	39	51	55	59	88	104	133	161	162	163	168	196	241	243	247	249	263	267	274	280
C. niloticus	T	A	G	A	T	T	T	G	A	T	A	G	C	T	C	G	A	G	C	G	G	C	T	C
CrocNA	T	A	G	A	T	T	T	G	A	T	A	G	C	T	C	G	A	G	C	G	G	C	T	C
C. suchus	A	G	A	C	C	A	C	A	T	C	G	A	T	C	T	A	T	A	-	A	T	T	C	T

C. niloticus and *C. suchus* (Table I) confirmed that the "Castel Nuovo" crocodile belongs to the species *C. niloticus*. In fact, the alignment shows the presence of 24 diagnostic sites that allow the genetic distinction between the two species (Hekkala et al. 2011). The comparison with all the complete sequences (12S rRNA+ D-loop) from Hekkala et al. (2011) revealed the presence of seven *C. niloticus* haplotypes and three *C. suchus* haplotypes (Figure 3). In addition, the network allows the assignment of the "Castel Nuovo" crocodile to a haplotype that was previously described only for Egyptian crocodiles (Figure 3), specifically individuals coming from Lake Nasser (Table S1).

Discussion

Two previously tested mtDNA markers (12S rRNA and D-loop) were selected for genetic analysis in order to identify the "Castel Nuovo" crocodile at species level. They contained diagnostic sites that allow the distinction between *C. niloticus* and *C. suchus*, which lived in sympatry along the River Nile up to the 1920s (Hekkala et al. 2011). Contrary to what was previously supposed, molecular phylogeny has revealed that the two African species are evolutionarily distant. The closest relatives of the Nile crocodile (*C. niloticus*) are the American *Crocodylus* species, while *C. suchus* occupies a basal position in the clade of Nile and American crocodiles (Meredith et al. 2011). Our results clearly assigned the Neapolitan specimen to *C. niloticus* thanks to the 24 diagnostic sites (Table I) identified after the alignment, confirming the attribution based on the large size, estimated from a photograph dating back to around 1870 (Figure 1). In addition, the sequences obtained are identical to those of *C. niloticus* individuals from the artificial Lake Nasser in Egypt (Figure 3,

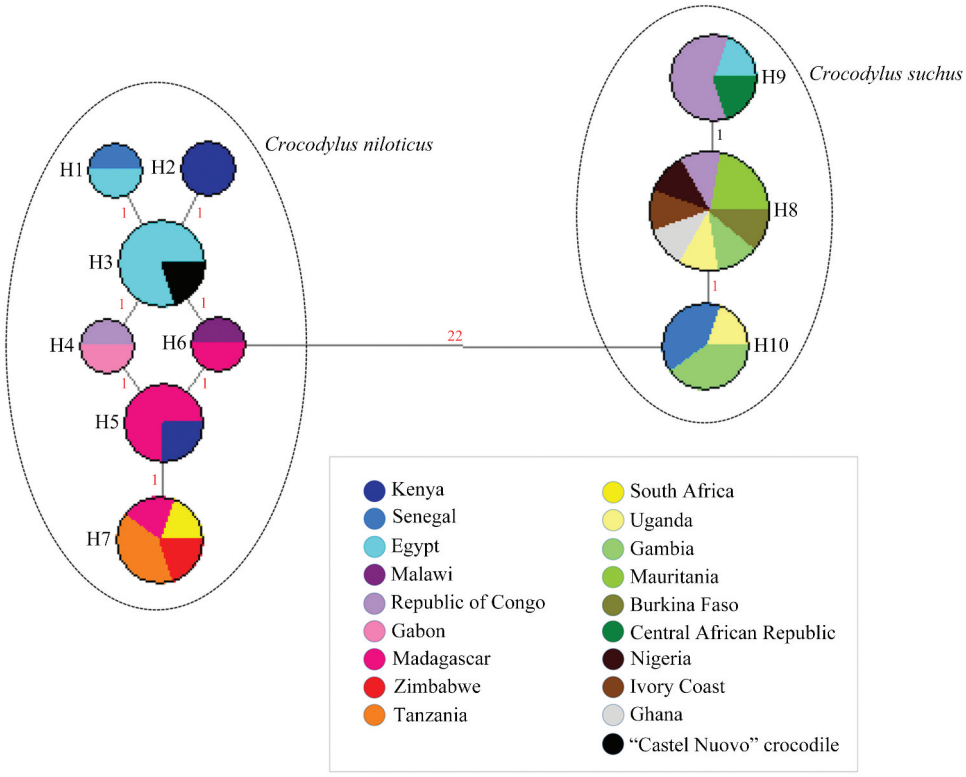

Figure 3. Median-Joining network showing the relationship between all the mtDNA sequences (12S rRNA + D-loop) from Hekkala et al. (2011) and the one obtained in this study. The different colours indicate the geographical origin of the haplotypes identified (Hekkala et al. 2011).

Table S1), which is currently the northernmost known population of this species (Hekkala et al. 2010). This result is consistent with the possible Egyptian origin of the "Castel Nuovo" specimen and is also supported by the presence of other stuffed Nile crocodiles, dating back to the fifteenth or sixteenth centuries, in sites near the coasts and along the commercial routes to and from the East (Bertelli 2018). Furthermore, it is important to remember that the port of Alexandria played a key role for trade between Egypt and Europe during the Medieval period (Reimer 1997).

The radiocarbon dating performed shows that the individual analysed could be the oldest taxidermied animal specimen in Europe. The oldest taxidermied specimen known so far was the crocodile displayed in the "Santuario della Beata Vergine delle Grazie" (Curtatone, Mantova, Italy) dated back to 1420 ± 60 (Bertelli 2018), a date that makes it more recent than the "Castel Nuovo" crocodile (1296–1419 cal yr AD). This evidence is also supported by the rudimentary technique of taxidermy used to preserve the crocodile. Specific analyses showed that the skin had been treated with calcium hydroxide and the animal had been filled mainly with grasses of the genera *Phalaris*, *Agrostis* and *Triticum* (Casafredda 2019).

As regards the grim legends surrounding the Neapolitan crocodile, it is interesting to observe that radiocarbon analysis dates the specimen back to a period preceding most of these popular tales. The only exception is the connection with Queen Joanna II of Naples (who died in 1435), but this legend is not supported by historical evidence (e.g., Croce 1895; Cutolo 1968; Galasso 1992). The Queens's terrible reputation probably depends on the fact that she commissioned the murder of a nobleman called Giovanni Caracciolo, one of her lovers, who had become too powerful and influential in the Neapolitan court (e.g., Antonioli 2019). In addition, Joanna II was also considered mainly responsible for the political crisis which occurred during the transition between the Angevin and Aragonese dynasties in the mid-fifteenth century (Galasso 1992). However, it must be emphasized that this and other legends about the Neapolitan crocodile are more likely to have originated because of hostile propaganda directed against the Kings of Naples during the first half of the nineteenth century, when the unification of the Italian States were taken place (see Dumas 1862).

The true origin of the crocodile is probably the one mentioned in a tourist guidebook published in

1685 by the local historian Pompeo Sarnelli. This text specifically refers to the traditional votive offering of a stuffed crocodile to the image of the Virgin Mary. In fact, the guidebook explains (see pp. 40; Sarnelli 1685) that a Spanish soldier returning from Egypt offered the crocodile as an *ex-voto* to the "Madonna del Parto" (Madonna of Parturition, the image of the Virgin Mary that protects women during labour). It hung for many years in the Palatine Chapel of the castle and had the same symbolic meaning as the crocodiles found in other Marian Sanctuaries (Bertelli 2018). Nowadays, a marble bas-relief can still be admired in "Castel Nuovo"; it is called the "Madonna del Parto" and has been attributed to the Renaissance sculptor Antonio Rossellino (1427–1479), who was active in Naples between 1470 and 1475 (Janson 1995). Even if the crocodile seems to be older than the bas-relief, as demonstrated by the result of the radiocarbon dating, it was probably offered to the "Madonna del Parto" after the 1475. The bas-relief, now on an external portico, was located in the Palatine Chapel until 1834 (D'Afflitto 1834). Between 1543 and 1545, the chapel roof was rebuilt because it had collapsed during the explosion of a gunpowder warehouse (Filangieri 1940) and, the crocodile was removed from its original position and hung above the triumphal arch of the castle, at the foot of a sculpture depicting two angels holding a coat of arms. The crocodile remained there until 1875 and was then forgotten in a repository of the National Museum of San Martino, only coming to light again in 2018.

In conclusion, the genetic analysis performed on the "Castel Nuovo" crocodile has shown that the individual undoubtedly belongs to the species *C. niloticus* and probably originates from Egypt. This result together with that of the radiocarbon dating seem to validate the hypothesis that the crocodile was offered as an *ex-voto* to the "Madonna del Parto" by a Spanish soldier returning from Egypt. Moreover, the age of the crocodile makes it the oldest example of a taxidermied vertebrate in Europe, showing that the analyses carried out in this study, together with the restoration of the specimen, are extremely useful for the knowledge and conservation of an important piece of the history of Naples and of the taxidermy.

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Disclosure statement

No potential conflict of interest was reported by the authors.

Supplementary material

Supplemental data for this article can be accessed here

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