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RESOURCES

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The Ratón Pérez collection: Modern deciduous human teeth at the Centro Nacional de Investigación sobre la Evolución Humana (Burgos, Spain)

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Abstract

Objectives: The aim of this report is to present the large deciduous tooth collection of identified children that is housed at the National Research Center on Human Evolution (CENIEH) in Burgos, Spain.

Methods: Yearly, members of the Dental Anthropology Group of the CENIEH are in charge of collecting the teeth and registering all the relevant information from the donors at the time of collection. In compliance with Spanish Law 14/2007 of July 3, 2007, on Biomedical Research (BOE-A-2007-12945), all individuals are guaranteed anonymity and confidentiality. When the donor hands in the tooth, they fill out a Donor Information Form and sign the Informed Consent Form. At the same time, another person completes the data label for the transparent polyethylene zip lock bag where the tooth is temporarily stored. All teeth are then transferred to the CENIEH Restoration lab, where the specialists apply the same protocol as for the fossil remains.

Results: Although the sample is still growing, from the first collection campaign in 2014 to date it comprises 2977 teeth of children whose ages of tooth loss are between 2 and 15 years. Each tooth is associated with basic information of the individuals and their parents and grandparents (sex, date, and place of birth, ancestry, country of residence), as well as important data about early life history (pregnancy duration, breastfeeding, bottle-feeding) and other relevant information provided by the donors (such as if they are twins, dental loss, or dental extraction).

Conclusions: Due to the scarcity of deciduous dental samples available, the Ratón Pérez collection represents a highly valuable sample for a wide range of disciplines such as forensic, dental, and anthropological fields among others.

KEYWORDS
deciduous teeth, forensic odontology, human biology, identified specimens, reference sample

1 | INTRODUCTION

Personal identification of an unknown deceased individual is crucial in our society for both legal and humanitarian reasons. Thus, human skeletal collections are an important source of information for physical anthropologists to establish and develop rigorous methods to obtain reliable data from the individual understudy. There are some dental and osteological reference samples (Dayal et al., 2009; Voisin et al., 2012), but due to the currently low early mortality rate, none of these contain a large number of infants and young children from the 21st century.

Due to their hardness, teeth are commonly the best-preserved skeletal remains in natural conditions (burials) as well as in fatalities (accidents, armed conflicts, violent crimes) or natural disasters (earthquakes, floods). Each individual possesses unique dental characteristics that may be used to recover useful information for both anthropological and paleoanthropological fields (e.g., age, sex, stress events, diet) relevant for individual identification (Krishan et al., 2015; Martín-Francés et al., 2014). Unlike bones, teeth are not subject to remodeling processes; once the enamel and dentine are formed, the size and shape of the crown will only be modified by wear processes and dental diseases. Furthermore, tooth development in general and dental morphology, in particular, are under strong hereditary control (Scott & Turner II, 1997), allowing to obtain important data for the identification of an unknown individual.

Therefore, in most of the cases where human bones are destroyed due to fires or mass fatalities, teeth are usually the only evidence that remain to try identifying the victims. Thus, in forensic odontology, the identification of humans is based on the antemortem dental records (e.g., medical history, x-rays, dental biometrics) of the deceased. Given that teeth vary in size and shape among individuals, this scientific discipline tries to identify an unknown deceased by means of the particular characteristics present in the teeth of each individual. Furthermore, dental anomalies, pathologies, and restorations are other features that may help the identification process (Pretty, 2007; Tinoco et al., 2010).

In particular, sex estimation is one of the most important aspects in the reconstruction of the biological profiles of human remains, so its accuracy is an important point in several disciplines. Forensic specialists have developed different techniques to discriminate males from females. Some skeletal elements, such as the pelvis and cranium are considered the most reliable ones to this end (Luo, 1995). However, these bones are usually fragmentary, making sex estimation difficult. In this context, teeth provide a good alternative for this purpose. Several researchers agree that the human permanent dentition is sexually dimorphic (García-Campos et al., 2018; Schwartz & Dean, 2005). Although some investigations have already been done (Hernaiz García, 2017; López-Lázaro et al., 2018), now with this collection we will assess whether deciduous dentition exhibits the same dimorphic features as permanent ones.

During the 19th century, researchers showed the importance of teeth as a source of information for ontogenetic and phylogenetic studies, developing investigations in the field of histology and dental morphology. The first theories of morphogenesis and phylogenetic development of teeth came from Cope, Osborn, and Gregory (Cope, 1883; Gregory, 1922; Osborn, 1895). In the 20th century, Hrdlička and Dahlberg conducted some of the pioneering contributions to the field of dental anthropology using morphological features as a tool for the study of populations. The first morphometric studies were developed by Hrdlička (Hrdlička, 1921) and years later, Dahlberg created a classification system of different morphological traits by means of reference plates (Dahlberg, 1956). But recently, an important advance in the study of dental morphology came from Turner et al. (1991). These researchers developed a method to score the dental morphological traits known as the Arizona State University Dental Anthropology System

TABLE 1 Number of donors and teeth collected in different cities between 2014 and 2020

Ratón Pérez tooth collection campaings (2014–2020)			
Year	**City**	**Donors**	**Teeth**
2014	Burgos	**197**	**498**
2015–2017	Burgos	**584**	**748**
2018	Burgos	171	361
	Madrid	13	13
	Cáceres	34	39
	Mallorca	16	28
	Santander	70	270
	Avilés	30	80
	Oviedo	26	74
	Gijón	30	33
	Jerez	12	20
		403	**918**
2019	Burgos	115	160
	Madrid	18	92
	Mallorca	8	31
	Santander	22	43
	Avilés	14	17
	Alcalá	6	50
	Barcelona	23	114
	Jaén	12	24
	Córdoba	74	98
	Galicia	8	15
		300	**644**
2020	Burgos	89	129
	Córdoba	20	26
		109	**155**
TOTAL		**1593**	**2963**

Note: In bold we show the total number per year.

(ASUDAS). Since then, this method has been systematically used in the analysis and characterization of extinct and extant human populations (Martínez de Pinillos et al., 2014; Martinón-Torres et al., 2012). Because teeth are one of the most abundant and well-preserved fossil remains, the majority of the paleoanthropological studies are based on them (Bermúdez de Castro et al., 2019; Martínez de Pinillos et al., 2020). Thus, the Ratón Pérez Collection will be very useful for the scientific community that will be able to compare this deciduous teeth from *Homo sapiens* with other extinct humans and get new variables with taxonomic and phylogenetic potential (Bermúdez de Castro et al., 2017; Martínez de Pinillos et al., 2017).

For all this and since most of the time the deciduous teeth are disregarded, the main purpose of this project was to create a large reference collection that could be used by scientists from several disciplines such as archaeology, forensic anthropology, dental and oral medicine, and paleoanthropological studies among others, to obtain relevant information. This communication aims to make the research

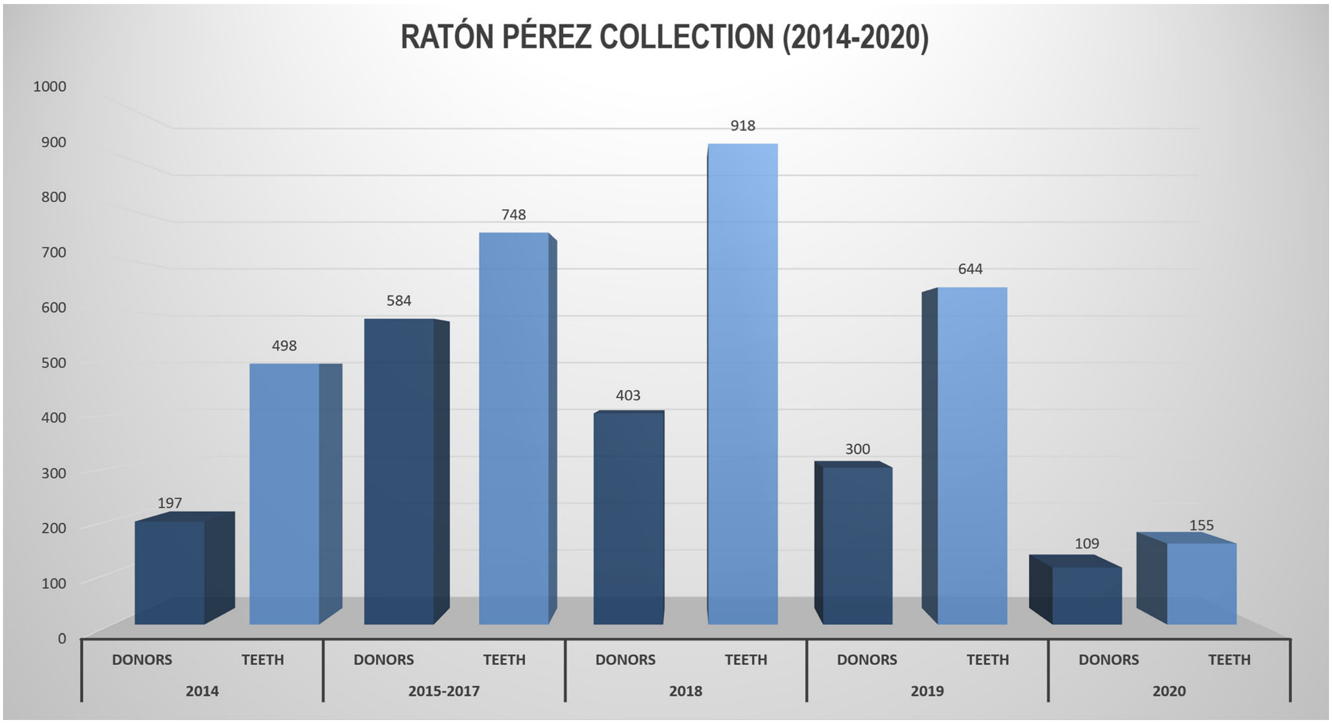

FIGURE 1 Total number of donors and teeth per year collected between 2014 and 2020

community aware of the existence and availability of this deciduous dental collection housed at the Centro Nacional de Investigación sobre la Evolución Humana (National Research Center on Human Evolution, CENIEH) in Burgos, Spain, of identified children that were born during the 21st century.

2 | GENERATING A REFERENCE SAMPLE

2.1 | History of the collection

Attending to the need of complete our knowledge about immature dentition, the Dental Anthropology Group of CENIEH talked with the head of the Scientific Culture and Innovation Department of this institution to organize a campaign to collect deciduous teeth. Our main goal was not only to gather an important reference collection of deciduous teeth for comparative studies that could be used by scientists from different fields but also to involve the community in this project. Thus, the first collection campaign took place in 2014 at the CENIEH installations during the European Researches' Night, where 498 teeth were collected in 3 h (following the protocol as described in Section 2.2). Additionally, during the event, we also conducted a dental anthropology workshop for children to teach them the basic function of teeth and how we get the most relevant data from them after their analysis in the laboratory. Between 2015 and 2017, another 745 teeth were added to the dental collection. Then, in 2018 and 2019, eight Spanish Regional Communities (Andalucía, Asturias, Baleares, Cantabria, Cataluña, Extremadura, Galicia, and Madrid) joined the project, thus further enlarging the collection with teeth from other origins. Despite the current situation of the pandemic, we have continued with the campaign. For security measurements, we developed the safety protocol (mask, hydroalcoholic gel, COVID survey, maximum two persons per appointment) for collecting teeth over 20 days instead of at the European Researches' Night 2020. In this way, through an appointment system, donors had to attend every 10-min time slot to avoid contact with others. Finally, 89 families came to donate teeth, providing us with more than 129 teeth (Table 1 and Figure 1).

2.2 | Gathering, cleaning, and processing the teeth for the database

Yearly, members of the Dental Anthropology Group of the CENIEH are in charge of collecting the teeth and registering all the relevant information from the donors at the time of collection. When the donor gives the tooth/teeth, they fill out a Donor Information Form (Figure 2). At the same time, another person completes the data label for the transparent polyethylene zip lock bags where the tooth is temporarily stored. Once the donor completes the Information Form, they receive a diploma, a toothbrush and a ticket to visit the Casa Museo del Ratón Pérez (Madrid) to thank them for their collaboration.

All teeth are then transferred to the CENIEH Restoration lab, where the specialists apply the same protocol as for the fossil remains, carrying out preventive conservation measures in order to safeguard the dental collection for futures studies. First, for preliminary cleaning, the teeth are carefully washed with a mixture of purified water and ethanol (1:1), removing any organic material adhered to them. Once the tooth is dry, it is consolidated with diluted resin (3% Acryloid B72 in acetone) to solidify and ensure their conservation. Each tooth is

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FICHA INFORMATIVA – PROYECTO RECOGIDA DE DIENTES DECIDUOS (RP2021- GAD)

☐ El donante ya ha aportado piezas dentales en anteriores campañas.

CENIEH-21/ INDIVIDUO Nº ___________	
Nombre y apellidos	
Fecha y lugar de nacimiento	......./............./................ en.. País: ....
Lugar de residencia habitual	en.. País: ....
Sexo	Femenino ☐ Masculino ☐
Edad de caída del diente (años*)	

*** Indicar la edad más exacta posible**

☐ **Extracción**

NACIMIENTO	☐ Prematuro (antes de las 37 semanas de gestación o del octavo mes)
	☐ A término (entre la semana 37 y la 41 de gestación o en el noveno mes)
	☐ Después de termino (después de semana 41 de gestación o del noveno mes)
ALIMENTACIÓN	☐ Lactancia materna
	☐ Lactancia artificial
	☐ Lactancia mixta (materna + artificial)

ASCENDENTES

Familiar	Lugar de nacimiento	Lugar de residencia habitual
Padre	en .... País:	en .... País: ....
Madre	en .... País:	en .... País: ....
Abuelo paterno	en .... País:	en .... País: ....
Abuela paterna	en .... País:	en .... País: ....
Abuelo materno	en .... País:	en .... País: ....
Abuela materna	en .... País:	en .... País: ....

OBSERVACIONES (otros datos relevantes que se quieran aportar)

PASEO SIERRA DE ATAPUERCA, 3 · 09002 BURGOS (España)
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FIGURE 2 Donor information form with all the data the donor should fill out

assigned an inventory number and stored individually inside a zip lock bag with the corresponding label. This label contains the inventory number of the tooth as well as an identification number of the donor, the tooth class, the sex, age of tooth loss, the box number where it is stored and a checkbox to indicate if the tooth has been scanned or not. Between 50 and 75 teeth inside their own plastic bags are kept in polystyrene transparent boxes, which are then placed in polypropylene containers (Figure 3). Finally, these containers are put inside compact cabinets at the CENIEH storage room, where the environmental parameters are fit for preservation at 50% relative humidity and 20°C.

At the moment, 798 teeth have been scanned with a microtomographic Phoenix v/tome/xs of GE Measurement, housed at the CENIEH with the following parameters: 100–120 kV, 110–140 μA and 0.2 mm Cu filter resulting in an isometric voxel size ranging between 14 and 18 μm. These parameters allow obtaining an accurate visualization and segmentation of the dental tissues through the application of an Imaging Software (Amira 6.3 Visage Imaging, Inc.) (Figure 4).

FIGURE 3 Tooth inside a polyethylene zip-lock bag with its corresponding label (upper left); polystyrene box with teeth (upper right); polypropylene containers with different boxes (lower)

2.3 | Legal requirements and ethical issues

In compliance with Spanish Law 14/2007 of July 3, 2007, on Biomedical Research (BOE-A-2007-12945), all individuals are guaranteed anonymity and confidentiality. Since most of the donors are underage, parents or legal representatives have to sign an Informed Consent Form after receiving the detailed Information Sheet with all the information about the collection project. Both documents are written in a language and in terms the participants can fully understand; describe the aims, methods, and implications of the research; explicitly state that participation is entirely voluntary and that anyone has the right to refuse to participate and to withdraw their participation, samples or data at any time without any consequences.

All the data provided in the Donor Information Form is transferred to a database, and their use for other purposes different from those the consent was given for is forbidden. The data collected are identified by an alphanumeric code, and to preserve the confidentiality of personal data, the access to the name and surname of the individuals is restricted only to the head of the collection project and the CENIEH Data Protection Officer. Consequently, despite giving access to relevant information for scientific studies, the identity of the donors and parents/legal representative will never be disclosed, keeping participants' anonymity always preserved. Therefore, no data shall be disclosed to third parties, unless required by Law or with the express written consent of the data subject.

3 | THE DECIDUOUS TEETH COLLECTION

At present, from the overall collection we have processed 2157 deciduous teeth from 1160 Spanish children and 9 children from France, India, China, Russia, Dominican Republic, Netherlands, Australia, and Mexico. The individuals are of both sexes and with ages of tooth loss between 2 and 15 years (Table 2). The male sample is represented by a 46.36% (n = 1000) and the female by a 43.58% (n = 940), with

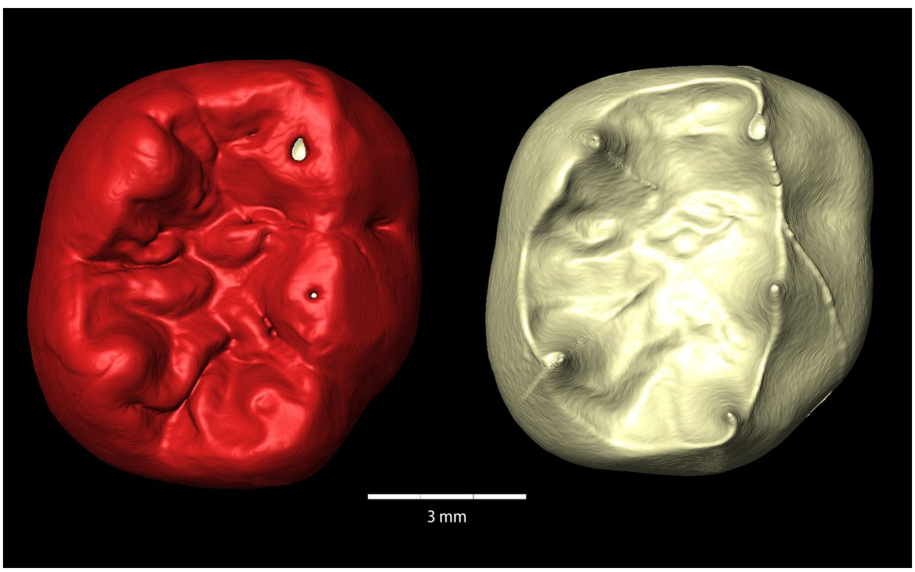

FIGURE 4 Virtual reconstruction of the enamel (red) and dentine (beige) surfaces of a molar based on microCT techniques

TABLE 2 Ratón Pérez sample separated by sex and ages of tooth loss (total number in bold)

	Male		Female		Indeterminate		Total	
Age of tooth loss	N	%	N	%	N	%	N	%
2	0	0.00	1	0.05	0	0.00	1	0.05
3	2	0.09	1	0.05	0	0.00	3	0.14
4	14	0.65	8	0.37	0	0.00	22	1.02
5	89	4.13	93	4.31	2	0.09	184	8.53
6	160	7.42	186	8.62	2	0.09	348	16.13
7	150	6.95	109	5.05	0	0.00	259	12.01
8	69	3.20	73	3.38	0	0.00	142	6.58
9	43	1.99	65	3.01	0	0.00	108	5.01
10	53	2.46	66	3.06	0	0.00	119	5.52
11	58	2.69	22	1.02	0	0.00	80	3.71
12	15	0.70	44	2.04	0	0.00	59	2.74
13	3	0.14	5	0.23	0	0.00	8	0.37
14	0	0.00	1	0.05	0	0.00	1	0.05
15	0	0.00	1	0.05	0	0.00	1	0.05
unknown	344	15.95	265	12.29	213	9.87	822	38.11
Total (*n*; %)	**1000**	46.36%	**940**	43.58%	**217**	10.06%	**2157**	100.00%

TABLE 3 Ratón Pérez sample separated by sex and tooth class (total number in bold)

	Male		Female		Indeterminate		Total	
Tooth class	*N*	%	*N*	%	*N*	%	*N*	%
Upper first incisor (i^1)	173	8.02%	141	6.54%	18	0.83%	332	15.39%
Upper second incisor (i^2)	137	6.35%	106	4.91%	22	1.02%	265	12.29%
Upper canine (c^-)	65	3.01%	58	2.69%	14	0.65%	137	6.35%
Upper first molar (m^1)	69	3.20%	71	3.29%	22	1.02%	162	7.51%
Upper second molar (m^2)	61	2.83%	69	3.20%	26	1.21%	156	7.23%
Lower first incisor (i_1)	140	6.49%	127	5.89%	25	1.16%	292	13.54%
Lower second incisor (i_2)	166	7.70%	148	6.86%	16	0.74%	330	15.30%
Lower canine (c_-)	70	3.25%	91	4.22%	27	1.25%	188	8.72%
Lower first molar (m_1)	65	3.01%	77	3.57%	24	1.11%	166	7.70%
Lower second molar (m_2)	54	2.50%	52	2.41%	23	1.07%	129	5.98%
Total (*n*; %)	**1000**	46.36%	**940**	43.58%	**217**	10.06%	**2157**	100.00%

10.06% ($n = 217$) indeterminate (Table 3). The indeterminate corresponds to donors that did not provide such information as teeth belonging to different individuals were mixed in the same bag.

4 | RELEVANCE OF THE COLLECTION: ONGOING STUDIES AND FORTHCOMING RESEARCH

The entire dentition, in its gross morphology is governed strictly by the action of genes (Kraus & Furr, 1953), which makes teeth to be consider the "safe box" of the genetic code. This characteristic gives us the possibility to obtain important variables to reconstruct the specific biography of an individual, making teeth the best material to retrieve information of the individuals they belonged to. However, the lack of systematic studies in large and diverse human samples makes it difficult to identify consistent patterns of variation to explore their correlation with relevant biological parameters.

Although historically the deciduous dentition has received significantly less attention than the permanent dentition, some important investigations were done in the late 20th century in order to explore the main characteristics of this type of dentition (Black, 1978; Garn et al., 1980). According to Korenhof, the dentine morphology is more evolutionarily conservative than the enamel morphology because "the enamel-dentine partition is much more a genetic blueprint of the occlusal anatomy of the teeth" (Korenhof, 1982, p. 350). In the past, in order to study the dentine surface, it was necessary to destroy the enamel cap. But more recently, new microCT techniques have helped to get information on both the enamel and dentine surfaces in a non-destructive manner, maximizing the biological information that can be extracted from teeth. Thus, relevant investigations had been published regarding morphometric analysis (Becam & Chevalier, 2018), tissue proportions (Zanolli et al., 2017), tooth formation (Mahoney, 2011) pathologies (Arnaud et al., 2017), and sexual dimorphism (López-Lázaro et al., 2018) among others.

Dental tissue measurements and proportions can provide information about populations (Martín-Francés et al., 2018), sex (Schwartz & Dean, 2005), and age (Zilberman & Smith, 2001). Due to the scarcity of deciduous teeth, most of these studies were performed with permanent ones. Therefore, this collection will expand the scope of the studies as it represents an unprecedented source of information. In particular, the Ratón Pérez Collection represents a unique opportunity to study different aspects related to infant life history (such as pregnancy, breastfeeding, bottle-feeding, city/country of residence) as well as a comparative sample in paleoanthropological studies (e.g., morphometric aspects between species, teeth development). Although the collection is still growing, the dental sample has already been used as a comparative sample to study the measurements and the external and internal morphology of the Early Pleistocene hominin deciduous teeth from *Homo antecessor* (Bermúdez de Castro et al., 2017; Martínez de Pinillos et al., 2017). Moreover, since sexual dimorphism studies also provide information about the life history, the evolution, and behavior of a specific population (Grine, 2005; Plavcan, 2000), this collection was also the focus of one Master's dissertation which analyzed the potential sexual dimorphism based on the tissue proportions of the second lower molars (Hernaiz García, 2017). Furthermore, other projects are being carried out with this collection to obtain new and more accurate methodologies for sex and age estimations.

We highlight the significance of the Ratón Pérez collection for the scientific community to conduct dental research in several disciplines (such as forensic, anthropology, paleoanthropology) that will provide relevant information.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest. They have no affiliations with or involvement in any organization or entity with any financial or nonfinancial interest in the subject matter or materials discussed in this manuscript.

AUTHOR CONTRIBUTIONS

Marina Martínez de Pinillos: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; resources; software; supervision; validation; visualization; writing - original draft; writing-review & editing. **Ana Pantoja-Pérez:** Conceptualization; data curation; formal analysis; investigation; methodology; resources; validation; visualization; writing-review & editing. **Pilar Fernández-Colón:** Data curation; methodology; resources; validation; writing-review & editing. **Laura Martín-Francés:** Data curation; formal analysis; investigation; methodology; resources; software; visualization; writing-review & editing. **Cecilia García-Campos:** Investigation; methodology; resources; software. **Mario Modesto-Mata:** Methodology; resources; visualization. **Chitina Moreno-Torres:** Funding acquisition; project administration. **José Maria Bermúdez de Castro:** Funding acquisition; project administration; writing-review & editing. **María Martinón-Torres:** Funding acquisition; project administration; writing-review & editing.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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