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RESEARCH ARTICLE

Two cases of cystic echinococcosis reported from al-Andalus cemeteries (southern Iberia): Insights into zoonotic diseases in Islamic Medieval Europe

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Abstract

The identification of a calcified hydatid cyst in the bioarcheological record is key to the diagnosis of cystic echinococcosis (i.e., hydatid disease), a parasitic infection caused by the tapeworm *Echinococcus granulosus*. This zoonosis can be linked to human–canid interactions, given the reproductive cycle of the parasite (from canids to herbivores/humans as intermediate hosts), and it is commonly associated with agropastoral communities in both the past and present. However, it is not easy to identify a calcified hydatid cyst in the archeological record because of preservation problems and its similarity with other calcifications of biological and geological origin. We report the presence of two fragmented ovoidal calcified formations associated with human remains in different medieval Islamic cemeteries from the Kingdom of Granada in al-Andalus (Southern Iberia, 13th–15th centuries AD). These formations were analyzed by scanning electron microscopy and energy-dispersive X-ray spectroscopy. The localization, morphology, and composition of the calcifications indicate that they are hydatid cysts caused by *E. granulosus*, representing the first evidence of cystic echinococcosis in Islamic Medieval Europe. Our results are in line with archeological and historical records of human–animal interactions and agropastoral practices in al-Andalus, and they highlight the importance of analyzing calcified masses in the osteoarcheological record.

KEYWORDS
bioarcheology, calcified formations, Islamic Europe, medieval burials, paleoparasitology, zoonosis

1 | INTRODUCTION

The study of calcified formations associated with osteoarcheological remains provides key information about the bioarcheological and geological context (Aufderheide & Rodríguez-Martín, 2011; Rowland & Hamdan, 2012). Formations associated with human remains may be related not only to taphonomical and geological postdepositional processes (e.g., Boness & Goren, 2017) but also to pathologies that involve calcified masses, including tumors, and non-neoplastic diseases (Komar & Buikstra, 2003; Monge-Calleja et al., 2017) that have been identified in the osteoarcheological record (Antikas & Wynn-Antikas, 2016; Buikstra, 2019). However, calcified formations may also be linked to zoonosis, revealing complex human–animal interactions in the past, although their

evaluation is hampered by poor preservation, difficult macroscopic recognition, and their similarity with geological calcifications (Fornaciari et al., 2020). Their identification in the archeological record is therefore facilitated by a combination of integrated osteoarcheological study, microscopic morphological characterization, and element composition analysis.

The aim of this study is to investigate the nature of two calcified structures associated with skeletal remains in two Islamic cemeteries from the Granada Kingdom in al-Andalus (southern Iberia) during the late Middle Ages in order to determine the underlying pathological condition. Results of macroscopic and microscopic examination and elemental composition analysis and study of their localization in the skeletal anatomy indicate that the calcified formations are hydatid cysts, a parasitic calcification diagnosed as cystic echinococcosis. These findings provide the first evidence in Islamic Medieval Europe (al-Andalus) of parasitic infection by *Echinococcus granulosus*, a zoonosis linked to agropastoral communities.

1.1 | Paleoparasitology and calcified hydatid cysts

Certain parasites are key pathogens to reconstruct present and past human–animal interactions linked to zoonotic parasite infections, defined by evolutionary, cultural, and ecological factors (Ledger & Mitchell, 2022). In the case of cestode infection by *E. granulosus* (i.e., hydatid worm or dog tapeworm), adult parasites are typically found in dogs and other canids, with mammals as intermediate hosts (including humans as occasional dead-end host) (Roberts et al., 2013; Tamarozzi et al., 2022).

Humans can be accidentally infected by ingesting *E. granulosus* eggs from contaminated soil, leading to the development of a hydatid cyst as an anatomical lesion in the larval stage of the parasite (Tamarozzi et al., 2022). Once in the intermediate host, the cyst can undergo a process of degeneration and calcification (Conchedda et al., 2018; Hosch et al., 2007). Its presence is linked to close human-animal interactions (Tamarozzi et al., 2020), and, from a socioeconomic perspective, it has been related to pastoralism (Aufderheide & Rodríguez-Martín, 2011; Roberts & Manchester, 2010) in current (Bosco et al., 2021) and ancient (e.g., Fornaciari et al., 2020) populations. Paleoparasitological studies have detected eggs of *Echinococcus* sp. in soil samples (e.g., Maicher et al., 2019; Nezamabadi et al., 2013), indicating contamination of the environment.

Given the absence of eggs in human individuals, osteoarcheology plays a crucial role in detecting *E. granulosus* in human remains as calcified hydatid cysts (Aufderheide & Rodríguez-Martín, 2011). In this way, evidence of this parasitosis in the past primarily derives from observations of calcified hydatid cysts in association with human remains (e.g., Antikas & Wynn-Antikas, 2016; Fornaciari et al., 2020; Monge-Calleja et al., 2017; Mowlavi et al., 2022).

2 | MATERIALS AND METHODS

2.1 | The archeological sites

The Nasrid Kingdom of Granada (i.e., Emirate of Granada) is considered the last Western European Islamic realm of al-Andalus in the southern Iberian Peninsula during the Late Middle Ages (1232–1492 AD) (Fábregas, 2020). The two samples of calcified masses in the present study were obtained from two urban medieval cemeteries in the Kingdom of Granada (Figure 1a).

A calcified formation was found in Tomb 132 of the Sahl ben Malik cemetery, one of the most important in Granada (Figure 1b) and in use between the 11th and 15th centuries AD (López-López, 1998). Thanks to its geographical location and abundance of water sources (Malpica Cuello, 2018), Granada was one of the wealthiest urban centers in al-Andalus and situated alongside extensive areas of fertile fields (San José, 2005). Tomb 132 contained the poorly preserved skeletal remains of an individual dated to the 13th century AD (López-López, 1998).

Another calcified formation was found in Tomb 55 of the Baza Mancoba cemetery (Figure 1c), radiocarbon-dated to the 14th century AD (1345–1396 cal AD) (Bronk Ramsey, 2009), when Baza occupied a strategic position in the Kingdom, being close to the Castilian Christian frontier (Santiago-Zaragoza et al., 2021). Tomb 55 contained a well-preserved skeleton.

It is difficult to determine the social status of the two individuals, given the Islamic tradition of egalitarian burials (Halevi, 2011). Their tombs did not differ from the others found in these cemeteries, with no distinguishing features.

Both calcified masses were discovered in the laboratory during the processing of bags that contained the vertebrae, ribs, and sediment from each tomb. They had not been detected during the excavation (in the context of rescue archeology), which may be attributable to the fragility and difficult identification of hydatid cysts, rarely reported in human remains (Fornaciari et al., 2020).

2.2 | Anthropological study and calcified formations analysis

In the anthropological study, age at death was estimated using established methods, considering morphological changes in the auricular surface of the ilium (Lovejoy et al., 1985), pubic symphysis (Brooks & Suchey, 1990), and sternal rib ends (Işcan et al., 1984). Sex was estimated by evaluating dimorphic features of the cranium and innominate bones, following Buikstra and Ubelaker (1994). In addition, bones were examined for osteological unspecific lesions that in rare case (1–2%) can be associated with echinococcosis (e.g., osteolytic long-bone epiphyseal lesion) (Aufderheide & Rodríguez-Martín, 2011; Vlok et al., 2022).

The calcified formations were analyzed by direct observation; by scanning electron microscopy (SEM, *AURIGA Zeiss*), to obtain

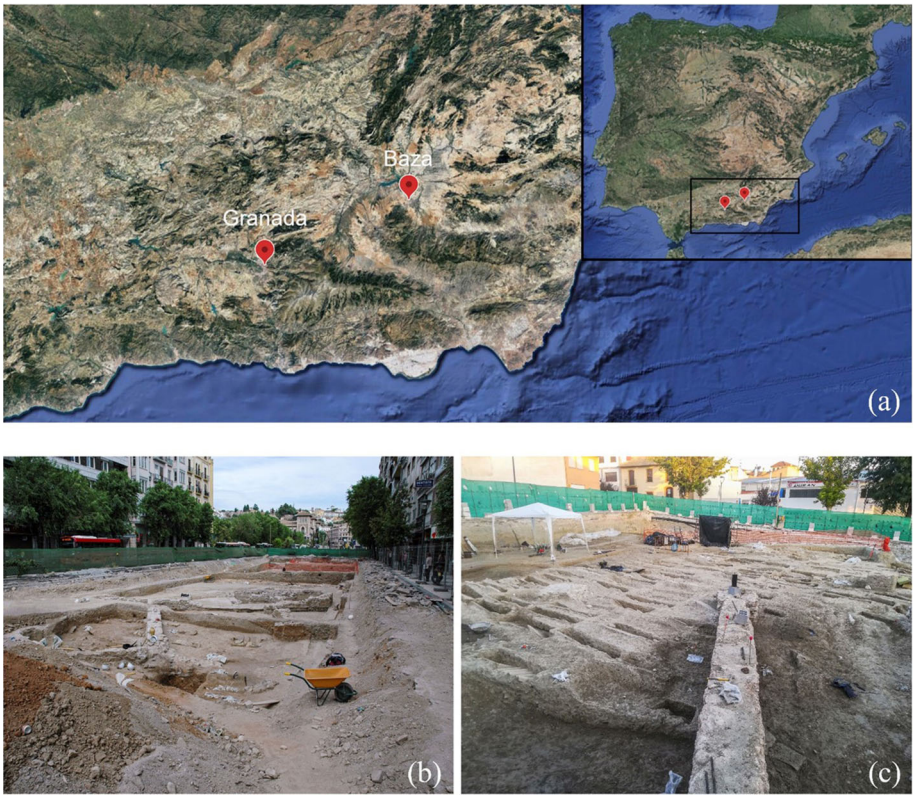

FIGURE 1 (a) Map showing the location of the city of Granada (37°10′55″N; 3°36′12″W) and Baza (37°28′58″N; 2°46′36″W) (Google Earth V 9.180.0.125 [April 19, 2023]. Andalusian region, Granada, Spain, eye altitude 247.03 km. Google 2023). Images of the excavation campaigns in Sahl ben Malik cemetery (Granada) (b) and Mancoba cemetery (Baza) (c). Images in (b) and (c) were edited using AI technology to remove individuals and safeguard their privacy. [Colour figure can be viewed at wileyonlinelibrary.com]

topographic and chemical images of their walls; and by energy-dispersive X-Ray spectrometry (EDX, *Oxford Instruments*), to determine their chemical composition.

3 | RESULTS

3.1 | Anthropological and pathological analysis

Based on the anthropological study, the individual in the tomb from Sahl ben Malik cemetery was identified as a middle-aged adult male (aged 45–50 years) and the individual in the tomb from Mancoba cemetery as an old adult male (aged 60–69 years). Pathological examination of the bones revealed no lesion compatible with *E. granulosus* infection.

3.2 | Macroscopic observations

Both incomplete calcified masses are semi-spheric and hollow with central hole and chamber (Figure 2). The inner surfaces have both smooth and rough areas, while theouter surfaces show small perforations but no vascular impressions. Dimensions are 31.17 × 26 mm for Mass A and 44.30 × 30.88 mm for Mass B. The wall is 1–2 mm thick in both masses. Their color and texture are similar those of bone, and the loss of calcified substance is associated with post-depositional taphonomic damage.

3.3 | Microscopic observations and chemical analysis

SEM study showed that both masses have triple-layered walls (Figure 3 and Supporting Information S1 and S2). EDX study of the composition of the layered structure revealed low concentrations of various elements and high concentrations of calcium (Ca) and phosphorus (P) (Figure 4 and Supporting Information S1 and S2 for raw data). The three layers did not differ in density.

4 | DISCUSSION

This study examined two calcified masses associated with the thoracic region of two individuals in a medieval Iberian Islamic funerary context. They were diagnosed as hydatid cysts associated with cystic echinococcosis, based on macroscopic and microscopic observations and chemical characterization. The identification and understanding of calcified formations in an osteoarcheological context should consider taphonomical, geological, geographical, and bioarcheological factors, including pathological conditions (see Boness & Goren, 2017; Monge-Calleja et al., 2017; Rowland & Hamdan, 2012).

The formations are semi-spherical shaped hollow calcifications less than 45 mm in diameter with three-layered walls that have a uniocular structure and exhibit no vascular impressions on inner or outer surfaces. According to the degree of degeneration, cysts can have irregularities on their inner surfaces due to the possible

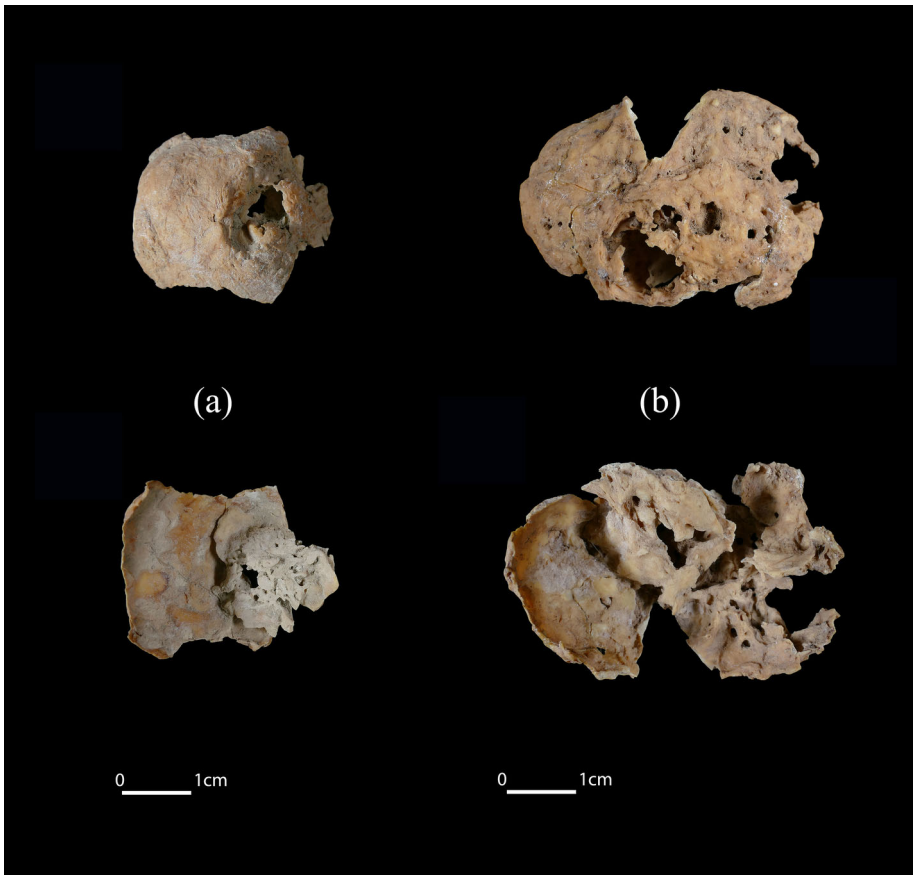

FIGURE 2 Outer and inner surface of calcified masses from Mancoba (a) and Sahl ben Malik (b) Islamic cemeteries in southern Iberia. [Colour figure can be viewed at wileyonlinelibrary.com]

calcification of elements they contain (Conchedda et al., 2016, 2018; Consul et al., 2020). Both formations have a hole, that is, depressions that connect with their interior, which might be explained by taphonomic processes or by partial calcification of the cyst wall (see Conchedda et al., 2018).

Chemically, the elemental composition shows a similar Ca:P ratio to that of hydroxyapatite (Skinner & Jahren, 2003). Alongside their localization in the thoraco-abdominal area, these findings suggest biological formation in vivo (i.e., biomineralization), consistent with hydatid cysts (see Aufderheide & Rodríguez-Martín, 2011; Monge-Calleja et al., 2017). In addition, the ages and sex of the individuals match the profile of those reported to have hydatid cysts in current epidemiological studies in Spain (Carmena et al., 2008).

We considered a wide range of possible origins of calcified masses in the osteoarcheological record. In this way, a taphonomic/geological interpretation was ruled out by the aforementioned finding of biomineralization. Differential diagnosis was conducted to establish the etiology of these masses (Fornaciari et al., 2020), given that calcification can be produced by various human diseases (Figure 5). The fact that both individuals are male allows the exclusion of calcifications associated with female-specific diseases (e.g., ovarian cysts). Consideration was taken of cysts that might be associated with vertebrae and ribs, such as hepatic and non-parasitical splenic cysts, which are often single and unilocular. However, hepatic cysts are generally smaller and are more frequently observed in females (Lewin, 2013), while non-parasitic splenic cysts are highly infrequent at any age and also more frequent in females (Pointer & Slakey, 2019).

Although neoplastic diseases can originate calcifications, these usually exhibit vascularization, resemble solid masses, or have a different shape to that of the present formations (Binder et al., 2016; Fornaciari & Giuffra, 2012; Miller et al., 2020).

Ossified tendons and ligaments have a distinctive appearance, following the anatomical structure of the tissue, and calcified muscle tissues are usually continuous with bone and have a non-cyst-like morphology (DiMaio & Francis, 2001; Meyers et al., 2019). Hence, this etiology can be ruled out. Sarcoidosis, an inflammatory disorder that can cause eggshell calcification, can also be excluded because the formations are typically multiple and characterized by vascular impressions on their surface (Fornaciari et al., 2020). Hydatid cysts can also resemble some rare calcifications caused by tuberculosis (Azzaza et al., 2020), but this also tends to generate multiple calcifications with a different morphology (Biehler-Gomez et al., 2020; Gawne-Cain & Hansell, 1996). The morphology of calcified abscesses observed in rare cases of brucellosis infection is also very distinct, showing a characteristic snowflake shape or, less commonly, a concentric lamellar pattern with central calcification (Colmenero et al., 2002; Karaosmanoglu et al., 2021; Torres et al., 2015). Fungal infections such as histoplamosis or *Pneumocystis carinii* can also originate calcifications, but these are multiple and smaller (Consul et al., 2020).

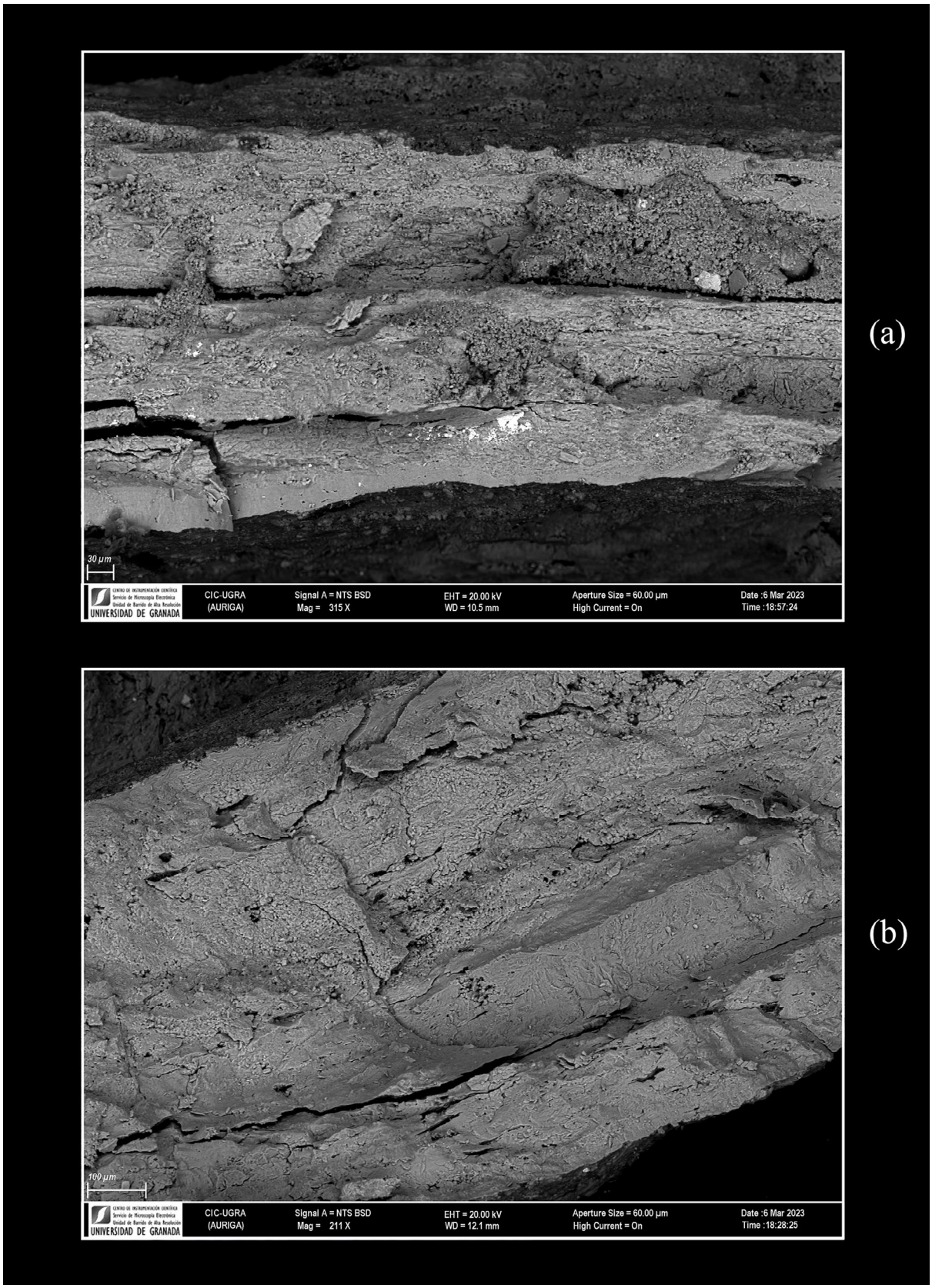

FIGURE 3 SEM characterization of the walls of the calcified masses from Mancoba (a) and Sahl ben Malik (b) cemeteries. The three-layered structure can be observed in both samples. See Supporting Information S1 and S2 for raw data. [Colour figure can be viewed at wileyonlinelibrary.com]

Among parasites potentially responsible for calcified cystic formations (see Roberts et al., 2013), *Echinococcus multilocularis* and *Dracunculus medinensis* can be ruled out by the chronology and geographical location of our samples (Gaeta & Fornaciari, 2022), *Taenia solium* (Fornaciari et al., 2020) by their shape, and *Trichinella* sp. by their size (Machnicka et al., 2005). The position in the body also discounts the possible role of other parasites such as *Toxoplasma gondii* (Patel et al., 1996). Figure 5 depicts comparisons between our samples and other disease-related calcified formations. A previous study found that echinococcosis was associated with a single cystic mass in 80% of cases (Brunetti et al., 2010), as in the present individuals, unlike cysticercosis (Roberts et al., 2013). Our macro-microscopic observations, the anatomic localization of the calcified formations (Figure 5) and the differential diagnosis indicate that their most likely etiology is cystic echinococcosis.

Study limitations include the absence of microscopic observations of hooklets or aDNA evidence for a definitive diagnosis, which have also been absent in previous osteoarcheological examinations of hydatid cysts.

The presence of the tapeworm *E. granulosus* in osteoarcheological contexts is of interest for the reconstruction of past socio-economic activities, health conditions, and hygiene habits. For instance, this parasite has been associated with sheep, goat, and cattle breeding, among other economic activities, and with the use of dogs for their

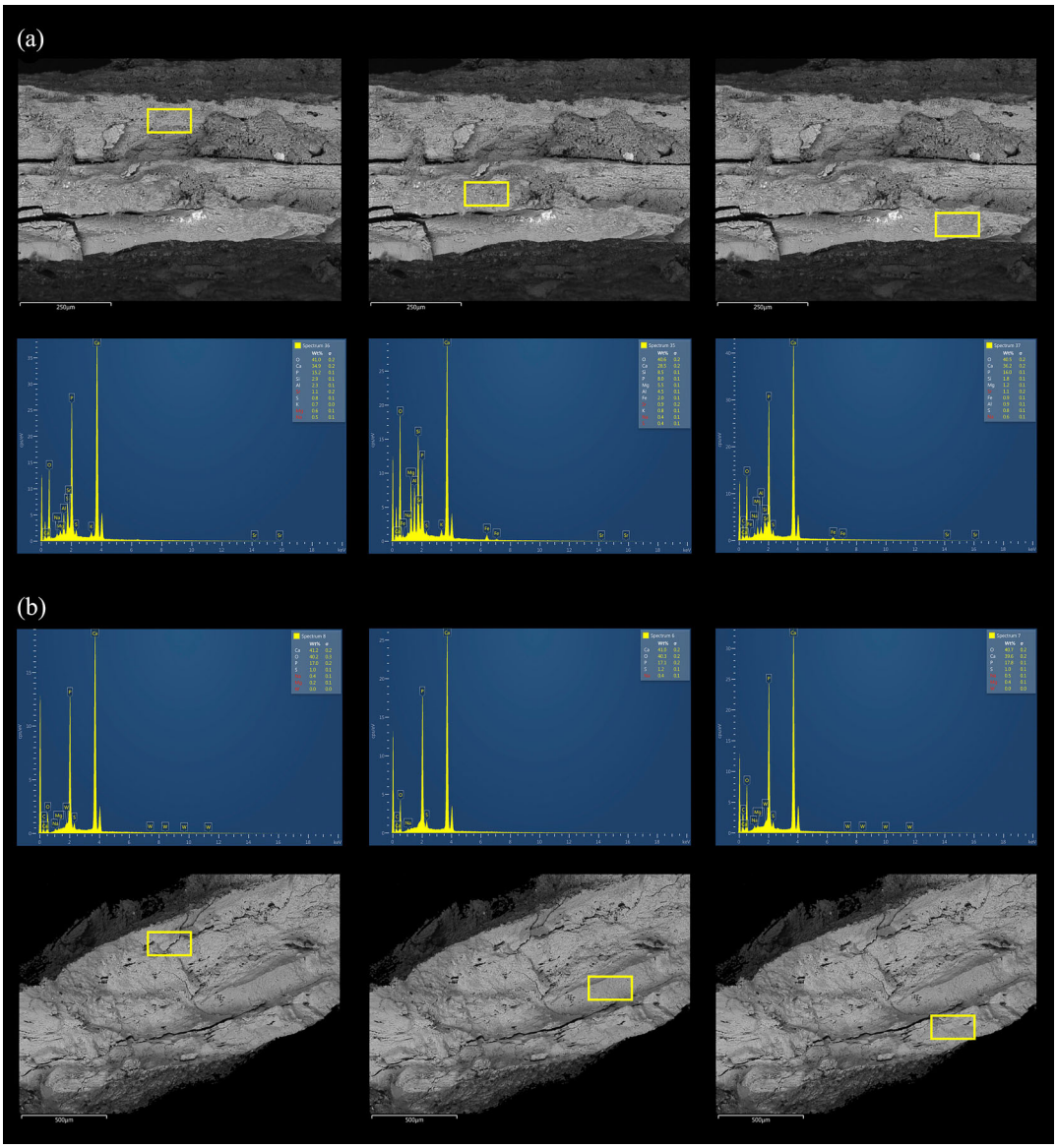

FIGURE 4 EDX-evaluated chemical elemental composition of the multi-layered structure of the masses from Mancoba (a) and Sahl ben Malik (b) cemeteries. High calcium and phosphorus levels are observed in both masses. See Supporting Information S1 and S2 for raw data. [Colour figure can be viewed at wileyonlinelibrary.com]

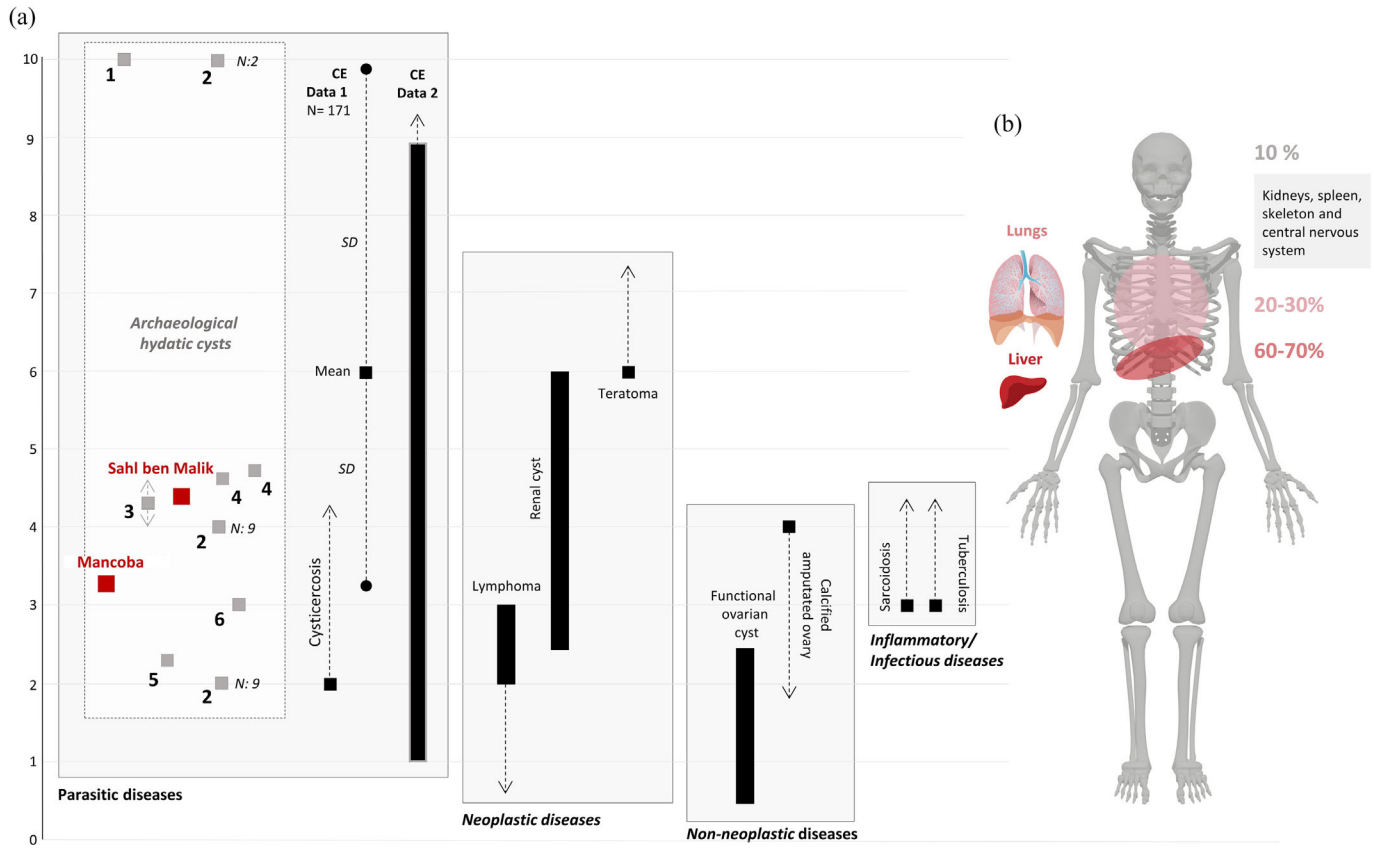

FIGURE 5 (a) Maximum length of cysts in different diseases (in mm). CE (cystic echinococcosis) Data 1 is extracted from Lissandrin et al. (2016) and CE Data 2 from Fornaciari et al. (2020) (as are all other measurements, including cysticercosis and cystic measurements for all other non-parasitic diseases. SD, standard deviation). Archeological data are extracted from (1) Antikas and Wynn-Antikas (2016); (2) Kristjánsdóttir and Collins (2011); (3) Minozzi et al. (2020); (4) Mowlavi et al. (2014); (5) Fornaciari et al. (2020); and (6) Monge-Calleja et al. (2017). (b) Main anatomic location of hydatid cysts according to Fornaciari et al. (2020). [Colour figure can be viewed at wileyonlinelibrary.com]

protection (Mitchell, 2017a, 2023). Dogs can contribute to the spread of this parasitosis (Sobrino et al., 2006), whose presence in al-Andalus is attested by the archeozoological record (Estaca-Gómez et al., 2019). The utilization of dogs would have been especially common in Baza due to the presence of wolves in the surrounding area (Rosas-Artola, 2020) and the importance of agropecuarian activities (Malpica Cuello, 2017; Sarr, 2015). Sheep and goat farming was of particular importance in the peninsular Islamic world (García-García, 2017), and Baza and Granada are both characterized by large fertile plains in which agrarian and pastoral activities coexisted (García-García, 2018). The presence of *E. granulosus* in canid feces during its dispersal phase can produce the contamination of vegetables used for human consumption (Cringoli et al., 2021). Hence, humans in Baza and Granada would have an increased risk of infection with this parasite.

Written sources can point to the presence of certain parasites in ancient populations (Mitchell, 2017b), and there are references to echinococcosis. In the present case, this zoonosis is attested in Islamic writings from the Iberian Peninsula, including the Kitāb al-Tasrīf (ca. 1000 AD) of al-Zahrawi (also known as Abulcasis) (Herrera-Carranza, 2022) and the manuscripts of Muhammad Aš-Šafra (13th–14th centuries AD) (Llavero Ruiz, 1988; Medrano Heredia, 2007).

5 | CONCLUSIONS

The identification of calcified formations in the osteoarcheological record is not common. It is important for archeologists to consider their possible presence in excavations and apply appropriate protocols for their recovery and identification. It is also necessary to conduct an exhaustive differential diagnosis to establish their etiology, as in the present study. Besides methodological issues, we present the first evidence of echinococcosis in medieval Islamic populations in Europe, based on the diagnosis of two hydatid cysts. These findings contribute to our understanding of health and disease in al-Andalus and may facilitate identification of these cysts in the future.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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