Review of Palaeobotany and Palynology 324 (2024) 105091

Contents lists available at ScienceDirect

Review of Palaeobotany and Palynology

journal homepage: www.elsevier.com/locate/revpalbo

Morphometry of Lamiaceae pollen grains from the archaeological site of Kastrì (Epirus-Greece; 15th–16th cent. AD)

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ARTICLE INFO

Keywords:
Bireticulate exine
Exine morphometry
KNN classification
Salvia
Mentha
Thymus

ABSTRACT

Pollen analysis of a late medieval layer (15th–16th century AD) recovered in the archaeological site of Kastrì (Epirus, Greece) highlighted the occurrence of large amounts of stephanocolpate Lamiaceae pollen grains. Morphometric analysis, by means of Light and Scanning Electron Microscopy, allowed three different pollen types to be identified within the family. In particular, the structure of the bireticulate exine, which is only visible under SEM observation, revealed to be a diagnostic feature for genus and even species identification. This was possible thanks to comparison with modern Lamiaceae species that were selected on the basis of pollen morphological similarity and geographical distribution. The results of comparison, achieved through K-nearest neighbour classification, led to ascribe the subfossil pollen types to the genera *Salvia*, *Mentha* and *Thymus* and to propose *S. nemorosa/glutinosa/pratensis*, *M. arvensis* and *T. praecox* as the most probable species to be associated with the subfossil grains. The significance of such a large quantity of Lamiaceae pollen is not easy to decipher. However, the co-occurrence in the sample of grazing indicators would suggest that these aromatic herbs were probably related to feeding or caring for flocks, in line with the archaeological interpretation of the investigated medieval structure as a shelter for shepherds as well as with the current use of these plants as forage supplements.

1. Introduction

The ancient site of Kastrì is located in Epirus, the northernmost region of Greece bordering the Ionian Sea and a very peculiar territory in terms of geomorphology, archaeology and culture (Besonen et al., 2003). Archaeological evidence attests the interest for this region from the third millennium BC; since then it was hotly contended by different populations (Hammond, 1967; Georgiadis et al., 2000) but the predominantly mountainous nature of its territory was the cause of a weak ethnic cohesion. Indeed, the history of this region has been influenced by a certain isolation, with respect to the rest of the country, due to the presence of the Pindus Mountains that separate Epirus from eastern and southern Greece. During the 3rd-2nd century BC, the political cohesion of the region was due to the Molossian dynasty, of which King Pyrrhus was a member (Dakaris, 1973). In the medieval period, Epirus was occupied by various populations (Saracens, Bulgarians, Normans) until the 15th century, when it became part of the Ottoman Empire up to the 20th century (Veikou, 2012).

Kastrì was founded in the 4th century BC in the lower Acheron Valley, in central-western Epirus, and in particular on a low hill (about 80 m altitude) adjacent to the river. The elevated position above the plain represented a privileged point for the control of the surrounding territory that was well known by the 6th century BC (Georgiadis et al., 2000). The site, currently under investigation, shows two phases of settlement. The earliest phase, dated to the 3rd-2nd century BC, was a large fortified residential structure belonged to the Molossian dynasty (Roubis, 2024; https://www.project-delta.eu/). The latest phase, dating from the 12th–15th centuries, consists of a defensive wall perimeter including towers (Roubis, 2024; https://www.project-delta.eu/).

The site is located along the transhumance routes that represent,

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https://doi.org/10.1016/j.revpalbo.2024.105091
Received 12 February 2024; Received in revised form 4 March 2024; Accepted 5 March 2024
Available online 8 March 2024

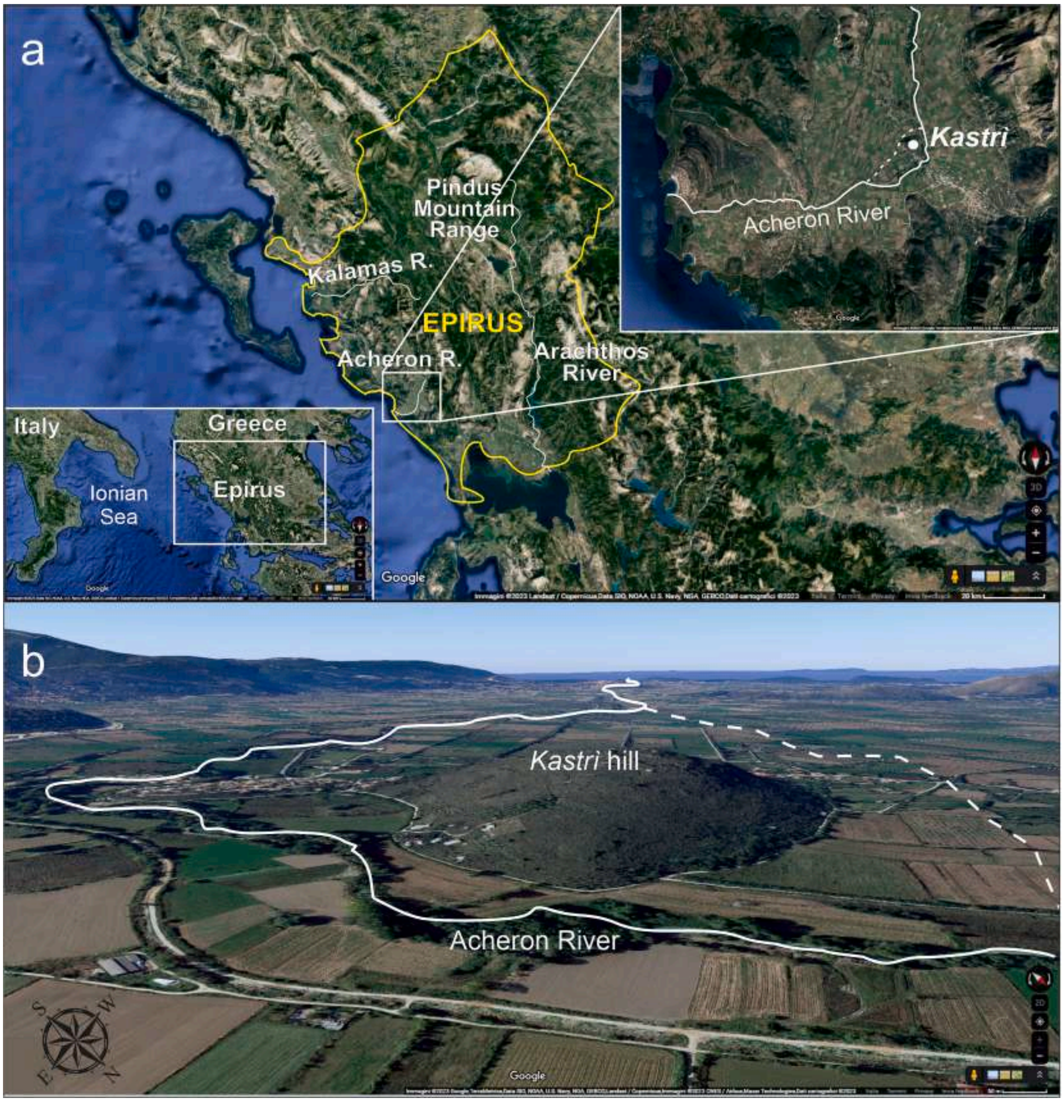

Fig. 1. a) Geographical position of Kastrì in the Epirus region, Greece; b) the Kastrì hill in the lower Acheron Valley. The dashed line indicate the presumed old course of the river (modified from GoogleMaps).

today as in the past, important ways of communication (Chang, 1999). The tradition of transhumance in Greece, and particularly in Epirus, has been practised since the Neolithic period (Ingold, 1980; Caftanzoglou, 1998; Bartosiewicz, 1999; Chang, 1999; Green and King, 2007; Hadjigeorgiou, 2011; Papayiannis, 2017; Picuno et al., 2017). Actually, the first archaeological studies in Epirus highlighted the absence of roads between sites but, at the same time, came across impressive transhumance flows that must have followed the communication routes used over the millennia to connect the various settlements (Papayiannis, 2017).

During the last excavation campaign at Kastrì (2021), a structure interpreted as a waste conduit developed in an earlier *intramuro* latrine was unearthed in the medieval wall perimeter. The stratification of the sediments therein contained was investigated and the best-preserved layer, pertaining to the 15th–16th century, was sampled for palynological investigations. The main purpose being to obtain data on land use in and around the site, special attention was paid to recognising anthropogenic indicators. The rich bibliography available and the numerous studies carried out are essential for the interpretation of their value in the sample (Brun, 2011; Ejarque et al., 2011; Mercuri et al., 2002, 2013a, 2013b, 2019; Torri et al., 2011; Florenzano et al., 2012, 2015; Florenzano, 2019; Deza-Araujo et al., 2020).

A particular category of anthropogenic indicators, defined as Local Pastoral Pollen Indicators (after Mazier et al., 2006; Mazier, 2007), provides useful information on livestock breeding and pastoralism. This plant group includes herbaceous taxa belonging to families such as Asteraceae, Ranunculaceae and Fabaceae, which are typical of grazing areas (Behre, 1981; Mercuri et al., 2010; Florenzano et al., 2015; Florenzano, 2019). Recently, also the Ericaceae were added to this group thanks to the work of Servera-Vives et al. (2023) on the Balearic Islands. The co-presence in the sample of coprophilous fungal spores represents further evidence of the presence of herbivores grazing in the area (López-Sáez and López-Merino, 2007; Mazier et al., 2009; Cugny et al., 2010). This has supported the interpretation of certain herb taxa, such as Fabaceae, as fodder supplements (Mercuri et al., 2013b; Miras et al., 2018; Di Lorenzo et al., 2021; Corbino et al., 2023).

Anthropogenic indicators can be determined at different levels of taxonomic identification depending on the employed methodology and geographic setting (regional vs local indicators; Behre, 1981, 1990; Rull, 2012; Mercuri et al., 2013b; Deza-Araujo et al., 2020; Servera-Vives et al., 2023). Taxonomic precision can provide useful information on the development and use of plants and the evolution of vegetation (Huntley and Webb, 1988) in order to better understand and track the dynamics of the human-plant relationship, even in particular contexts such as the

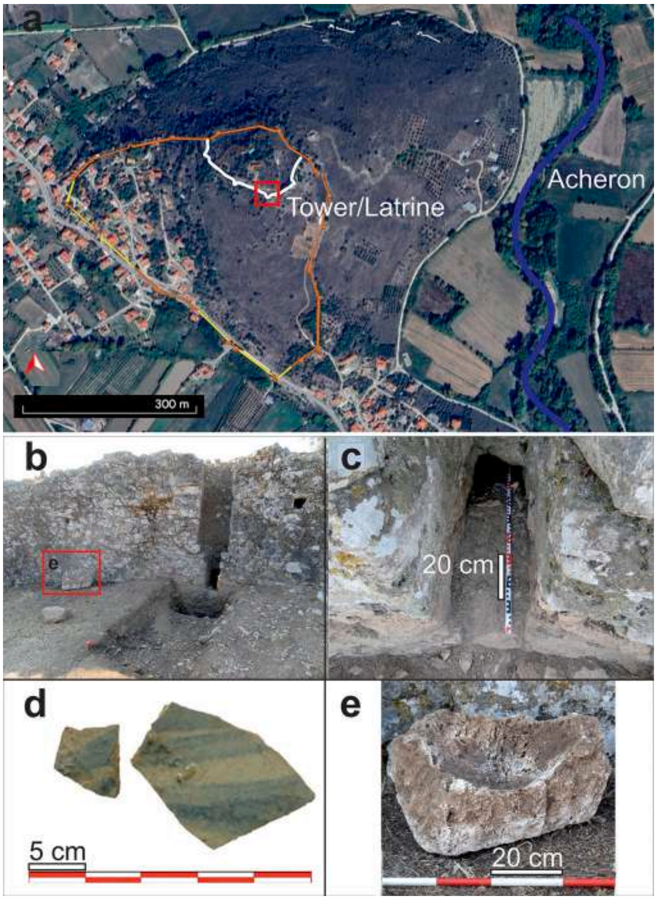

Fig. 2. a) The medieval defensive wall perimeter of Kastrì (in white) and the tower in which the latrine was found; b) the *intramuro* latrine; c) US 29 in the latrine duct; d) diagnostic potteries from US 29; e) mortar found during the archaeological excavation, in front of the latrine.

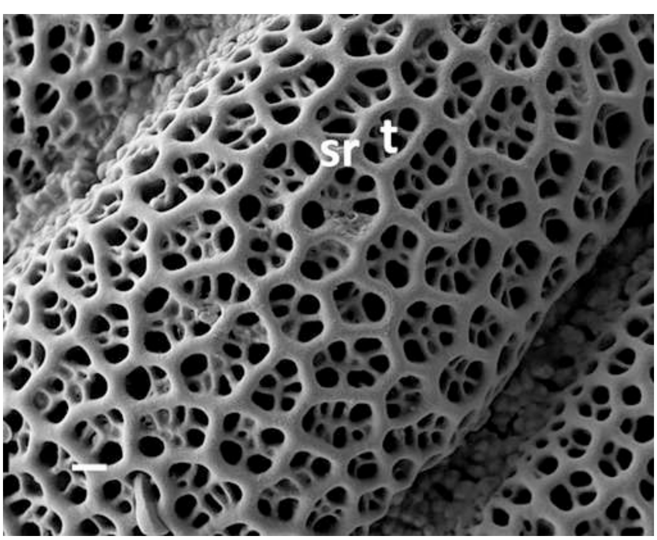

Fig. 3. Bireticulate exine of Lamiaceae at SEM, showing a *suprareticulum* (sr) supported by a microreticulate *tectum* (t). Scale bar = 1 μm.

archaeological ones. These insights are crucial when attempting to interpret past land use and human-induced changes in vegetation (Servera-Vives et al., 2023).

When a certain indicator is strangely abundant or is represented by exotic entities, this could be interpreted as the evidence of a specific human action. At this stage, the aim of further research is not only to identify the species, using different methods of investigation, but also to understand the purposes that led people to make a specific choice. In recent years, several studies have focused their attention on the identification of "anomalous" taxa in both archaeological and anthropized contexts (Mercuri et al., 2002; Russo Ermolli et al., 2014, 2022; Barone Lumaga et al., 2020; Deza-Araujo et al., 2022). The approaches used to identify the species are different, ranging from simple morphometric evaluation, with Light Microscopy (LM) images, to more sophisticated measurements of the exine features, both at the LM and Scanning Electron Microscopy (SEM). All instances, the identification of species allows better understanding the selection of plants for specific purposes. In fact, examples of selection for food have been identified in concomitance to cultural expansions (Mercuri et al., 2002; Russo Ermolli et al., 2014, 2022). There is also evidence of precise aesthetic or medical choices, as in the case of plants used for the production of drugs or to embellish green spaces (Ciaraldi, 2000; Barone Lumaga et al., 2020). These studies, characterised by a multidisciplinary approach, concur in outlining the constant technological improvement in the use of plants.

In the case of the present study, the anomalously abundant presence of Lamiaceae pollen, found in the sampled late medieval layer, gave us the opportunity to further investigate the morphological characteristics of the grains.

On the basis of these assumptions, the main aims of this work are: 1) to propose an innovative method based on morphometric analysis of pollen grains aimed at identifying genus/species within the Lamiaceae family; 2) to provide the first palynological data for the Epirus region, with a special focus on land use in medieval times.

Table 1
List of taxa and number of pollen grains counted in the US 29 sample. Taxa are grouped following their ecological or land use meaning.

Taxa	nb	Group
Quercus ilex	4	Mediterranean elements
Cistus	3	
Quercus deciduous	8	
Carpinus	4	Deciduous elements
Hedera	135	
Ostrya	5	
Alnus	3	Wet woodland
Corylus	5	
Abies	3	Montane
Castanea	17	
Juglans	3	Tree crops
Olea	10	
Vitis	3	
Cichoriae	20	
Asteroideae	18	Grazing
coprophilous fungi	48	
Lamiaceae	115	?
Apiaceae	3	
Plantago	1	
Amaranthaceae	4	Ruderals
Caryophyllaceae	10	
Geranium	2	
Myriophyllum	10	
Polypodium	1	Wet environments
Microalgae	29	
Trilete spores	15	Fire
Microcharcoals	146	

2. The study site

2.1. Geographical and geomorphological setting

The Epirus region, which covers about 7% of the Greek territory, is mainly characterised by a mountainous landscape with deep river valleys (e.g. Arachtos, Acherontas and Kalamas; Fig. 1). The main mountain

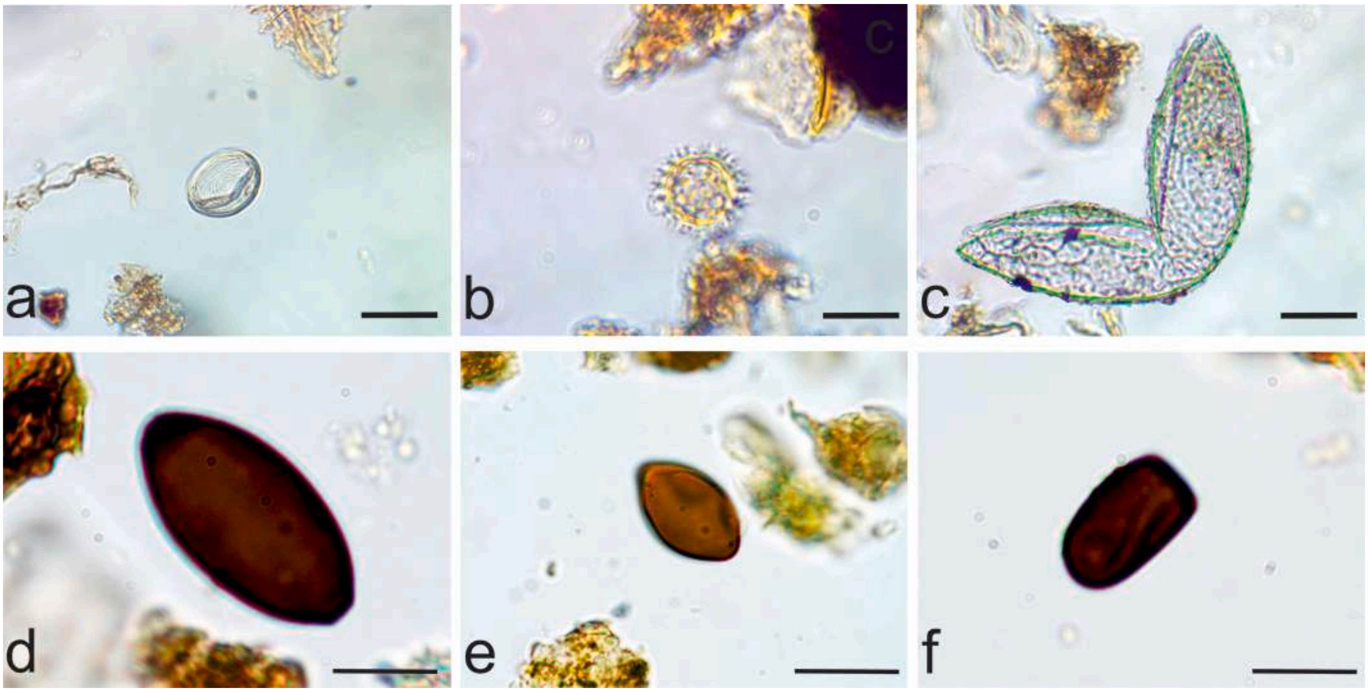

Fig. 4. NPPs recovered in US 29. Algae: a) *Pseudoschizaea* (Carrion et al., 2000); b) HdV 181/182 (Cugny et al., 2010); c) *Spirogyra* sp. (Pals et al., 1980). Scale bar = 20 μm. Coprophilous fungal spores: d–e) Sordariales (Cugny et al., 2010); f) *Sporomiella* (Cugny et al., 2010). Scale bar = 10 μm.

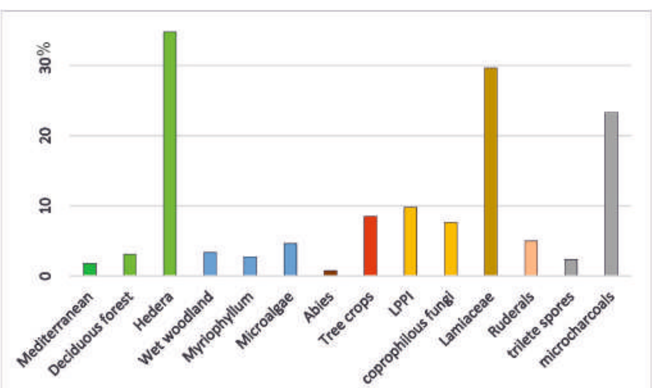

Fig. 5. Percentages of taxa groups. For group composition, see Table 1.

range is the Pindus, whose highest peak is at 2637 m asl, whereas plains are limited to the areas of Arta and Preveza and along the rivers Arachtos and Acheron (Acherontas; Ntokos, 2017). During the Holocene, the most significant geomorphological changes concerned the size of the *Glykys Limen* (today's Phanari-Ammoudia Bay), into which the Acheron River flows, the development of Lake Acherousia, which does not exist anymore, and the course of the Acheron River. To clarify these points, several core drillings were carried out in various locations of the valley (Besonen et al., 2003). The analysis of core samples provided significant evidence that these geomorphological changes occurred in the last 4000 years. In particular, the coastline of the *Glykys Limen* prograded by almost 6 km, at varying rates, and practically closed the bay (Besonen et al., 2003). Lake Acherousia developed relatively late, probably between the 8th and 5th centuries BC, and was ever since subject to continuous flooding that entirely filled it (Besonen et al., 2003). Moreover, it seems that the Acheron River, whose original course ran to the north of the Kastrì hill, moved to the south of it in the last 500 years (Fig. 1; Besonen et al., 2003).

2.2. Present climate and vegetation

The weather conditions of Epirus are very variable because the region includes a huge area, from the mountains of Pindus to the coastal areas of Arta and Preveza. The coastal plains are characterised by a typical Mediterranean climate with hot summers and mild winters. The average amount of annual precipitation is 842 mm, with December as the wettest month (130 mm) and August the driest (15 mm).

The Epirus region has the highest number of forest and endemic taxa in the whole Greece (Strid, 1995). This high vegetal biodiversity has been partly attributed to the abandonment of several urbanised areas, which resulted in the return of sub-original vegetation (Van Der Leeuw, 2004). Specifically, Epirus can be divided into two biogeographical areas (Strid, 2000). One is characterised by the temperate deciduous forest, the so-called "Illyrian deciduous forest", the other by the Mediterranean forest. The first is remarkable for the presence of beech (*Fagus sylvatica* L.) in association with hornbeam (*Carpinus orientalis* L. and *C. betulus* L.) and conifers, including silver fir (*Abies alba* Mill.; Strid and Tank, 1997).

In addition to forests, what distinguishes the Epirus region is the high and constant presence of flocks of sheep and goats. Therefore, vegetation in the territories dedicated to grazing, and subject to transhumance flows, remained unchanged for years, preserving its features (Van Der Leeuw, 2004). Moreover, it is worth focusing on the widespread occurrence of some aromatic and medical plants. Despite being native to the Mediterranean area (Pignatti, 1982), it is in Epirus that they have their highest rate of diffusion, due to assiduous cultivation. In fact, these species, particularly the ones belonging to the Lamiaceae family, that includes *Salvia officinalis* L., *Mentha spicata* L., *Rosmarinus* and *Thymus*, are still widely used in this region to treat various ailments and integrate both human and animal nutrition (Malamas and Marselos, 1992).

2.3. Archaeological setting

The Acheron valley is mentioned in several literary sources and, at least from the 6th century BC, the Acheron River represented a political border, dividing the territory of Thesprotia to the north from the one of

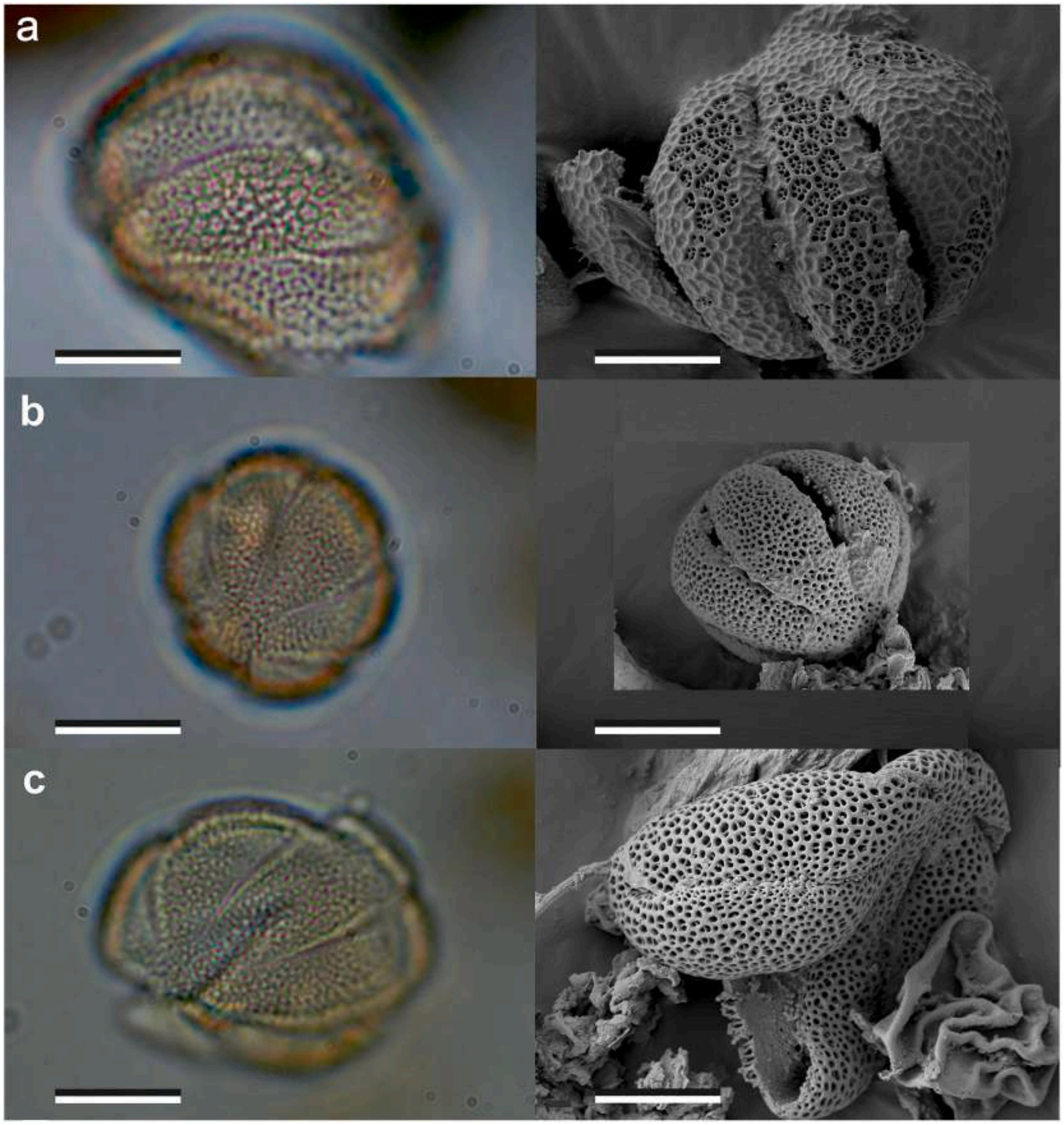

Fig. 6. LM images (sx) and SEM images (dx) of the three typologies of subfossil pollen grains; a) pollen type 1; b) pollen type 2; c) pollen type 3. Scale bar = 10 μm.

Table 2
Morphometric parameters of subfossil pollen grains under SEM.

Subfossil pollen	Type 1	Type 2	Type 3
Equatorial diameter range (μm)	25–40	15–20	20–35
Exine micromorphology	Bireticulate	Perforate–Bireticulate	Bireticulate
Average number of reticulum lumina per each suprareticulum lumen (μm)	5.6	2.4	2.7
Average length of suprareticulum lumina (μm)	1.57	0.84	0.87
Average width of suprareticulum lumina (μm)	1.08	0.47	0.61

Kassopea to the south (Georgiadis et al., 2000).

According to ancient literary sources (Demostene Or VIl, 32; Strabone Geog VII7, 5; Livio ab Urb. Con. VIII, 24; Pausania Per. Hellas I 17, 4–5; Tucidide Hist. I, 46, 4), among the main sites that developed on the hills by the river there were Ephyra and Kastrì-Pandosìa, which became very important.

Limited surface investigations were carried out on the latter settlement, mainly in the 1990s, as part of the Nicopolis Project. The Ephoreia of Antiquities of Preveza (Epirus, GR) also made emergency excavations at some points at the foot of the hill and in 2008, several research operations were realised on some remains of ancient buildings. Finally, since 2019, the Ephoreia of Preveza in collaboration with the University of Basilicata and the ISPC-CNR, have been conducting archaeological excavations in one of the few residential architectural complexes, in several points of the Classical and Medieval wall circuits and in a Byzantine cult building located on the acropolis.[1] The excavation of a large residential building allowed distinguishing layers of collapse and abandonment (2nd-1st century BC) and surfaces related to life phases that can be dated around the late 4th-3rd century BC and chronologically constrained between the Reign of Pyrrhus and the Roman conquest

[1] The archaeological missions are carried out by the Post Graduate School of Specialisation in Archaeology of the University of Basilicata and the ISPC-CNR in agreement with the Ephoreia alle Antichità (Superintendency) of Preveza – Greece and are directed by Anthi Angeli and Dimitris Roubis, the mediaeval phases of the settlement are under the scientific responsibility of Francesca Sogliani.

Table 3
Selection of genera to be used for comparison with subfossil pollen. A) found in Greece; B) with 6 colpi; C) equatorial diameter in the range of subfossil pollen; D) bireticulate or perforate-bireticulate exine as subfossil pollen; E) number of lumina in each suprareticulum lumen in the range of subfossil pollen; F) length of suprareticulum lumina in the range of subfossil pollen; G) width of suprareticulum lumina in the range of subfossil pollen.

A	B	C	D	E	F	G
Acinos	*Acinos*	*Acinos*				
Ajuga						
Ballota						
Betonica						
Calamintha	*Calamintha*	*Calamintha*	*Calamintha*	*Calamintha*	*Calamintha*	*Calamintha*
Clinopodium	*Clinopodium*	*Clinopodium*				
Galeobdolon						
Galeopsis						
Glechoma	*Glechoma*					
Hyssopus	*Hyssopus*	*Hyssopus*	*Hyssopus*			
Lamium						
Lavandula	*Lavandula*	*Lavandula*				
Leonorus						
Lycopus	*Lycopus*	*Lycopus*	*Lycopus*			
Marrubium						
Melissa	*Melissa*	*Melissa*				
Melittis						
Mentha	*Mentha*	*Mentha*	*Mentha*	*Mentha*	*Mentha*	*Mentha*
Micromeria	*Micromeria*	*Micromeria*	*Micromeria*	*Micromeria*	*Micromeria*	*Micromeria*
Moluccella						
Nepeta	*Nepeta*	*Nepeta*	*Nepeta*			
Origanum	*Origanum*	*Origanum*	*Origanum*	*Origanum*	*Origanum*	*Origanum*
Phlomis						
Prasium						
Prunella	*Prunella*	*Prunella*	*Prunella*			
Rosmarinus	*Rosmarinus*					
Salvia	*Salvia*	*Salvia*	*Salvia*	*Salvia*	*Salvia*	*Salvia*
Satureja	*Satureja*	*Satureja*	*Satureja*	*Satureja*	*Satureja*	*Satureja*
Scutellaria						
Sideritis						
Stachys						
Teucrium						
Thymbra						
Thymus	*Thymus*	*Thymus*	*Thymus*	*Thymus*	*Thymus*	*Thymus*
Ziziphora	*Ziziphora*	*Ziziphora*				

of the area. Another unearthed structure dates to the Medieval (Byzantine) period and pertains to a defensive wall perimeter. The chronological range of this complex covers a period that goes from the 12th to the 15th century. The most extensively investigated part of it is one of the fortified towers of the wall (Fig. 2a), which seems to have changed its usage several times during the centuries. The excavations have clarified that the primary function of the medieval tower was as a strategic point to guard the river, the marine landings and the access to the summit plateau. The structure has an internal dimension of 3.20 × 3.80 m and is preserved to a considerable height, over 5 m. The excavations also involved an *intramuro* latrine, i.e. a niche carved into the boundary wall and large enough for one person, located next to the tower (Fig. 2b). In the basement part of the latrine there is a 50 cm wide cavity, which crossed the entire width of the boundary wall and had the function of discharging the wastewater. Such conduit (Fig. 2c) was obstructed in post-medieval times, as indicated by the presence in the matrix of ceramic materials dated to the second half of 15th century (Fig. 2d).

The settlement dynamics between the late Byzantine period and the modern age, from the defunctionalisation of the tower and the latrine to its reoccupation in the early 20th century, are still being analysed, also considering the most recent findings from the excavation of the church on top of the hill, enclosed by the medieval walls. The settlement certainly underwent a ruralisation due to the changed geopolitical conditions that led the entire fortified enclosure at the top of the hill to lose its value as a defence bulwark. The discovery, in front of the latrine, of a large hollowed limestone boulder (Fig. 2e), interpreted as a mortar or an animal watering trough, could be connected to the change in the intended use of this area and to the presence of small temporary pastoral communities. This phase, therefore, probably involved the spoliation of part of the tower and its use as a shelter for flocks and shepherds during transhumance periods. It should be remembered that Kastrì is currently located along one of the transhumance routes that still characterise this part of the Greek territory.

3. Materials and methods

3.1. The sample

The sediment sample recovered for the study of subfossil pollen comes from a single archaeo-stratigraphic unit, namely US (Stratigraphic Unit) 29. It was collected during the most recent archaeological excavation at Kastrì (39°15′04″N–20°34′25″E), in the sector labelled as "*Fortificazione Sommitale*", a medieval fortified wall composed by gates, towers and a nearby wastewater outflow system, used by soldiers as a toilet (latrine). The layer 29 is located within the drainage channel of the fortification wall, interpreted as an intramoenia latrine, typical of medieval fortified structures. Following the abandonment and defunctionalisation of these defensive structures, the drainage channel was obstructed by earth and large stones coming from the latrine top. This fill formed because of the failure to maintain the conduit open, when the area no longer served its function of control and defence. So, US 29 is an earthy matrix layer with fine and dusty granulometry, including stones of different sizes, many small chipped and unworked, few large unworked (Fig. 2c). No clear evidence of dung or animal faeces could be found in it, its formation being the result of natural rainwater run-off in an area frequented by grazing animals.

The layer is 27 cm thick and was formed on a north–south gradient, following the slope of the conduit. The few diagnostic finds indicate the late-post medieval age as the approximate chronological interval for the defunctionalisation of the structure. In fact, the recovered ceramic

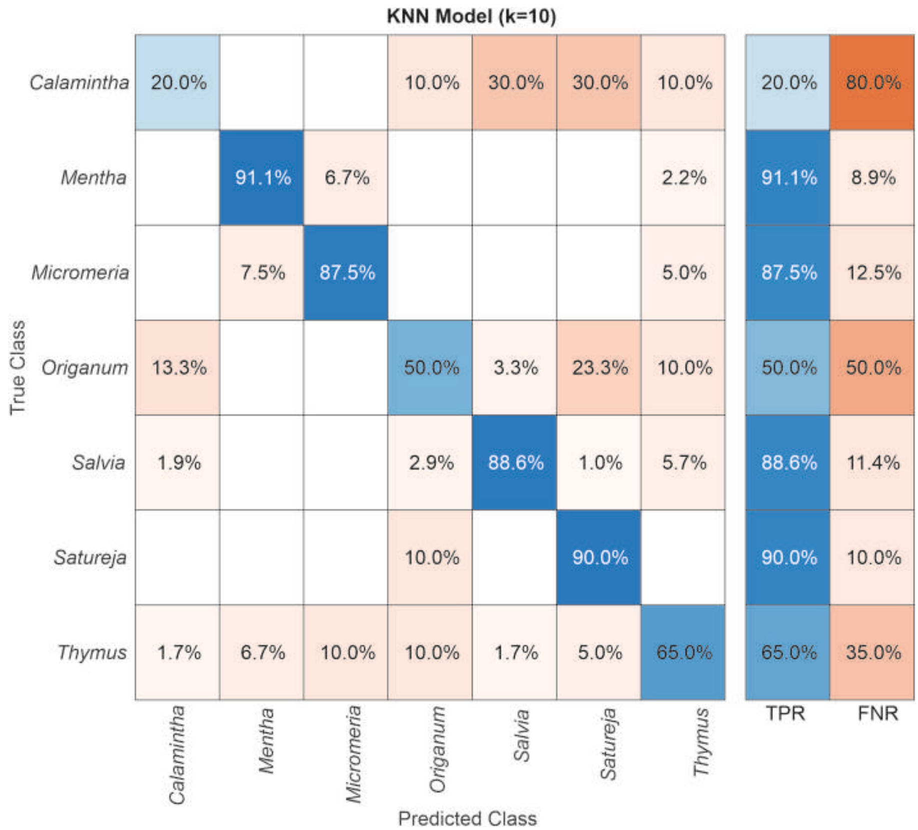

Fig. 7. KNN confusion matrix for the seven genera included in the training dataset. TPR: true positive rates; FNR: false negative rates.

Table 4
Results of the classification method applied to subfossil grains.

Subfossil type	Grain	Predicted class
Pollen type 1	Grain 1	*Salvia*
	Grain 2	*Salvia*
	Grain 3	*Thymus*
	Grain 4	*Salvia*
	Grain 5	*Salvia*
Pollen type 2	Grain 1	*Mentha*
	Grain 2	*Mentha*
	Grain 3	*Mentha*
Pollen type 3	Grain 1	*Thymus*
	Grain 2	*Thymus*
	Grain 3	*Thymus*
	Grain 4	*Thymus*

sherds have a glazed surface with blue decorations, which is attested to have been widespread from the second half of the 15th century (Fig. 2d).

3.2. Pollen analysis

Five grams of dry sediment were treated with chemical (HCl 20%, HF 40%, hot HCl 10%) and physical (10 μm ≤ sieving ≤ 200 μm, $ZnCl_2$ floating) procedures in order to concentrate pollen grains in the residue. A further treatment with acetolysis was made to remove the pollen cement (*pollenkitt* after Knoll, 1930) present on some pollen grains. One *Lycopodium* tablet was added in order to calculate pollen concentration. Slides were mounted in glycerine; determinations and counts were carried out under a LM at 500x and 1000x magnification, with the support of pollen atlases (Reille, 1992, 1995; Beug, 2015) and reference pollen material. The results of pollen analysis are presented in a table and in a synthetic percentage graph where taxa are grouped following their ecological or land use meaning.

3.3. Reference pollen material

The large number of stephanocolpate Lamiaceae pollen grains in sample US 29 suggested proceeding with further investigation in order to identify the genera and, if possible, the species of plants which were present in the study site. With this aim, a comparison was made with selected reference pollen material.

The Lamiaceae family contains more than 7000 species distributed in the world and is one of the largest angiosperm families (Celenk et al., 2008; Özler et al., 2011; Bendiksby et al., 2011; Azzazy, 2016; Li et al., 2016). For this reason, the selection of genera and then modern species for comparison consisted of successive exclusion criteria that took into account geographic distribution of plants and morphological similarity of modern pollen to subfossil grains. Since the presence of synonymies was frequently found, particular attention was paid also to this aspect. Only the species found in the "Greek Flora" (IPNI and portal.cybertaxonomy.org/flora-greece/intro) were taken into consideration.

Pollen of selected modern species (obtained from the Herbarium Neapolitanum-NAP) was processed with acetolysis before LM observation, or directly placed on stubs, coated with gold or gold/palladium to ca. 30 nm, for observation under a Zeiss Merlin VP Compact SEM or a Nova NanoSEM 450.

Modern pollen grains were photographed as part of this work in order to obtain uniform comparison material. In fact, other available images from articles or websites show a considerable degree of variability in the dimension of the polar and equatorial axes, depending on the process used for the preparation of samples. In particular, a remarkable variation in the pollen grains' size was noted by comparing dehydrated, hydrated or acetolysed samples (PalDat, 2000 onwards, www.paldat.org; Celenk et al., 2008; Özler et al., 2011; Azzazy, 2016). On the contrary, a uniformity in the ornamentation of the exine of the species observed with SEM was noted both in this study and in the available works on Lamiaceae species (PalDat, 2000 onwards, www.

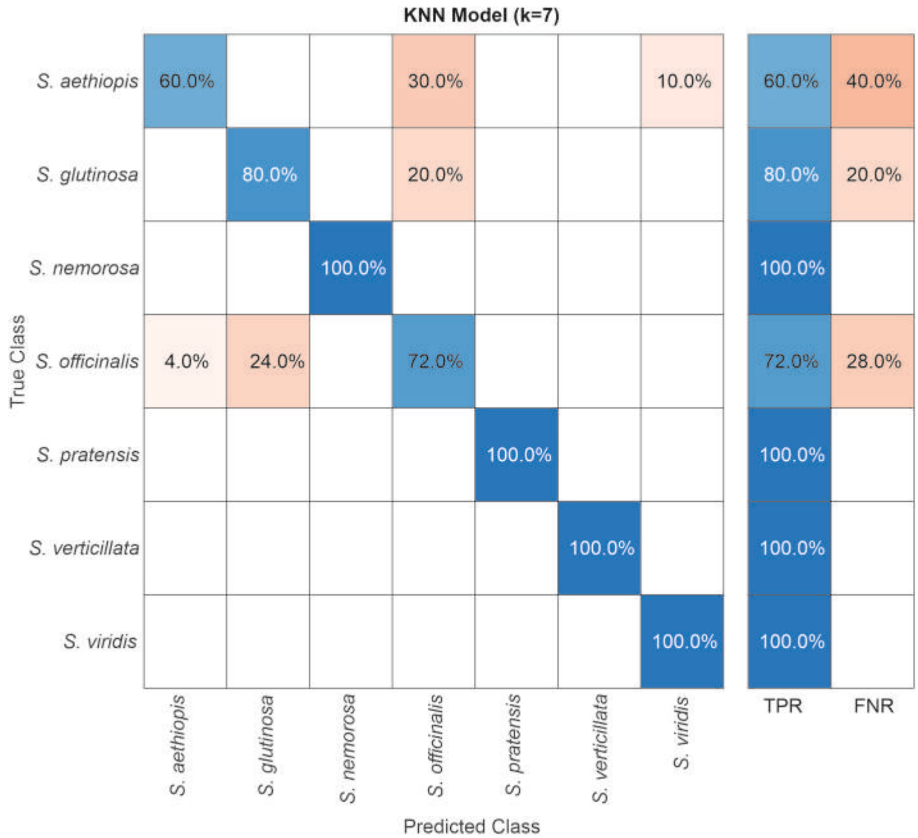

Fig. 8. KNN confusion matrix for the seven *Salvia* species found in Epirus and included in the training dataset. TPR: true positive rates; FNR: false negative rates.

paldat.org; Celenk et al., 2008; Özler et al., 2011; Azzazy, 2016).

3.4. Morphometric analysis

The pollen of Lamiaceae is stephanocolpate (from 6 colpus on) in most genera, while it is tricolpate only in a few species (Pozhidaev, 1992). The grain size is highly variable (15–100 μm) and the exine ornamentation under the LM is characterised by a variable-sized mesh network. A large number of subfossil grains of stephanocolpate Lamiaceae from sample US 29 were photographed with the LM at 1000X, with the aim of measuring the two main morphological parameters: the grain size and the mesh size of the reticulum. Specifically, equatorial diameter was measured as indicative of grain size because the stephanocolpate Lamiaceae often occur in polar view in pollen slides due to the opening of colpi.

The presence of such a large number of Lamiaceae grains in the US 29 sample allowed a further investigation under SEM, which led to a better definition of the pollen grain micromorphology. Morphometric analysis on SEM images was carried out on a total amount of 65 grains both from subfossil and selected modern species.

Most of the pollen grains of Lamiaceae have a bireticulate exine, a two-layered reticulum characterised by the presence of a suprareticulum (sr) supported by a microreticulate tectum (t) (Fig. 3). These features are only visible under SEM observation. A reticulum is a network-like pattern consisting of spaces (lumina) bordered by muri (Punt et al., 2007). Based on these characters, five lumina of the suprareticulum for each grain (modern and subfossil) were measured in two dimensions (length, width), the lumina of the reticulum were counted for each lumen of the suprareticulum and the equatorial diameter of each grain was measured. Measures were taken with ImageJ software (https://imagej.nih.gov/ij/) on SEM images. In particular, the analysis of the exine was carried out in equatorial position in order to have uniformity of the measuring point and a better visibility of the ornamentation. The adopted pollen terminology refers to Punt et al. (2007).

3.5. KNN classification

K-nearest neighbour classification (KNN; Fix and Hodges Jr., 1951; Cover and Hart, 1967) was applied to validate the results obtained through a purely descriptive criterion. KNN is a machine learning method, belonging to supervised learning algorithms, which aims at classifying new objects into two or more destination classes, defined on a training dataset with given labels. In KNN, a set of k nearest neighbours ($k > 1$) is taken into account when making a decision regarding the classification. The algorithm can account for different distance measures and different k values. Moreover, a weight criterion for the distances can be introduced. Among the advantages of this method is that it does not require special assumptions regarding the distribution of the data, so it can fit well with data sets that are not large. Additional information about KNN can be found, among others, in Seidl (2009).

In our case, a classification model was first constructed within a training set represented by measurements of modern grains belonging to selected genera of the Lamiaceae family. The variables included in the modern dataset are: length (L) and width (W) of the lumina of the suprareticulum, the equatorial diameter (ED) of the pollen grain and the number of lumina of the reticulum (CellNB). These variables are characterised by values of different magnitude, i.e. CellNB and ED are characterised by values of an order of magnitude greater than L and W. In order to prevent some variables from having a greater weight in the classification, KNN was applied on standardised data (i.e. for each variable, values are centred around the mean and divided by the standard deviation). Moreover, with the aim of giving greater weight in classification to the closest observations, an inverse square was considered as distance weighting function. This choice implies a low sensitivity of the results, regardless of the k value adopted. We tested the KNN with different k values depending on the number of observations, which

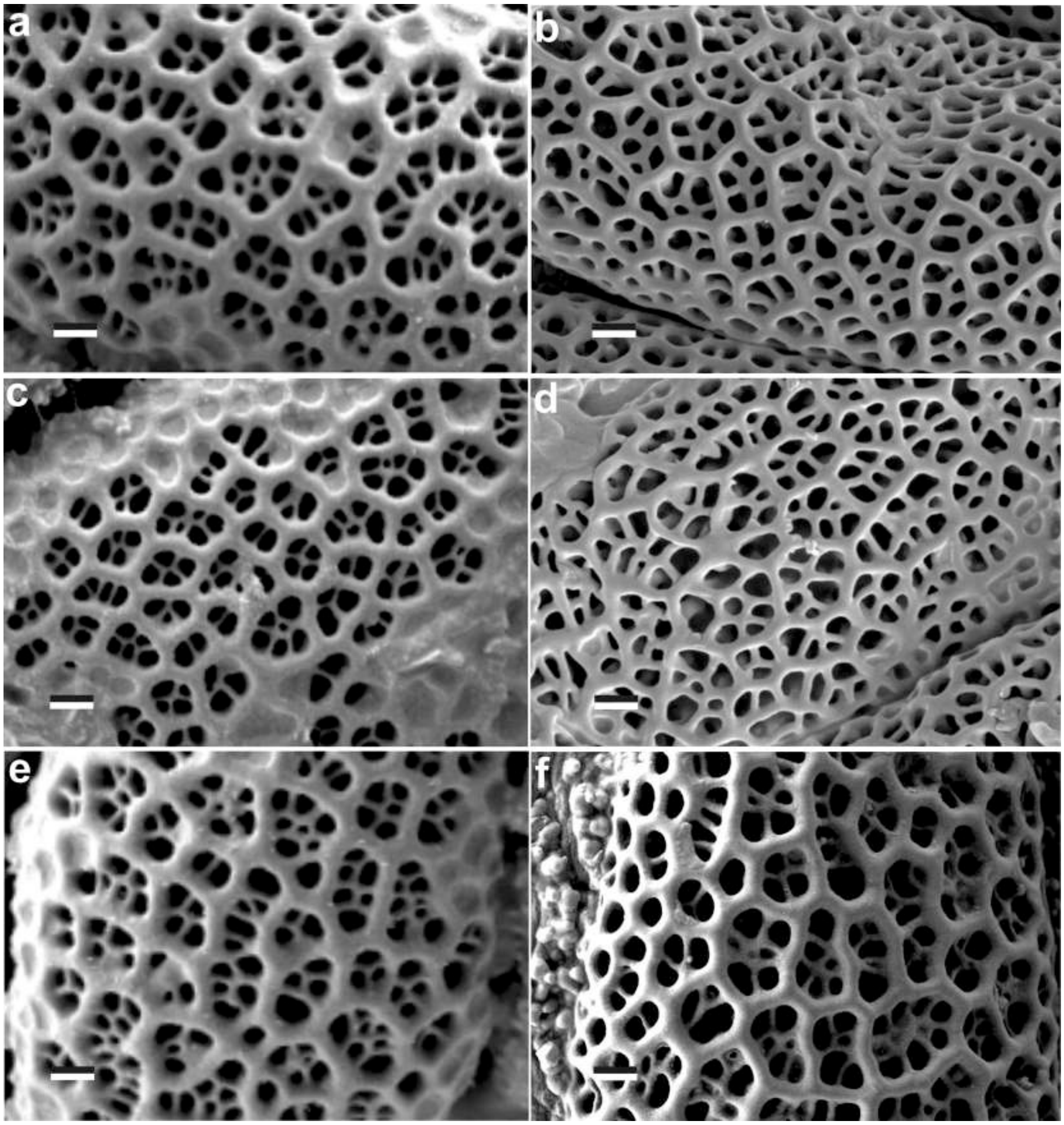

Fig. 9. Scanning electron microphotographs of *Salvia* pollen grain exine patterns; a) subfossil grain 1; b) *S. pratensis*; c) subfossil grain 3; d) *S. nemorosa*; e) subfossil grain 5; f) *S. glutinosa*. Scale bar = 1 μm.

varies in the different datasets analysed.

Confusion matrices were made to evaluate the classification power of the selected variables for the selected modern genera dataset. Afterwards, the model was exported and applied to subfossil grains in order to obtain their classification at a generic level. After subfossil pollen were classified within a certain genus, we also explored the possibility of classifying grains at a specific level using a KNN model. In doing that, only species which are present in the Epirus region were considered. The analysis was carried out by means of MATLAB classification tool.

4. Results

4.1. The pollen spectrum

A total of 387 grains were counted in sample US 29 and 27 taxa were recognised (Table 1); concentration is 12,900 grains/g. Woody taxa are mainly represented by *Hedera*, probably ivy plants growing on the nearby tower walls. Sparse amounts of trees belonging to several ecological groups are found in the sample. Mediterranean plants are only represented by few grains of *Quercus ilex* L. and *Cistus* while the deciduous forest is represented by deciduous *Quercus* and *Carpinus*. The wet environments around the site can be recognised in *Alnus*, *Ostrya*, *Corylus*, *Myriophyllum* and *Polypodium*. A certain number of NPPs (Non Pollen Palynomorph) were recovered in the sample, some of which classified as "algae" belonging to different families of groups (Fig. 4), but all testifying to the presence of paddles or water spots. Particularly relevant is the presence of tree crops, such as olive, vines, chestnut and walnut that witness the agricultural exploitation of the hill and the nearby alluvial plain (Fig. 5). Among the herb taxa, the most represented are Lamiaceae followed by Cichorieae, Asteroideae and Caryophyllaceae. The presence of Asteraceae coupled with coprophilous fungal spores (Fig. 5), mainly belonging to the Sordariales order and the genus *Sporomiella* (Fig. 4), suggests that the area was exploited as pastureland. The most peculiar feature of this sample is the anomalous abundance of Lamiaceae that might have an anthropogenic significance. Microcharcoals would attest both slash and burn practices to renew pastures, and the use of fires for domestic activities.

4.2. Morphometry of Lamiaceae subfossil grains (LM and SEM)

The analysed subfossil pollen grains are stephanocolpate (6 colpus). Measurements of the grain size (equatorial diameter) and of the

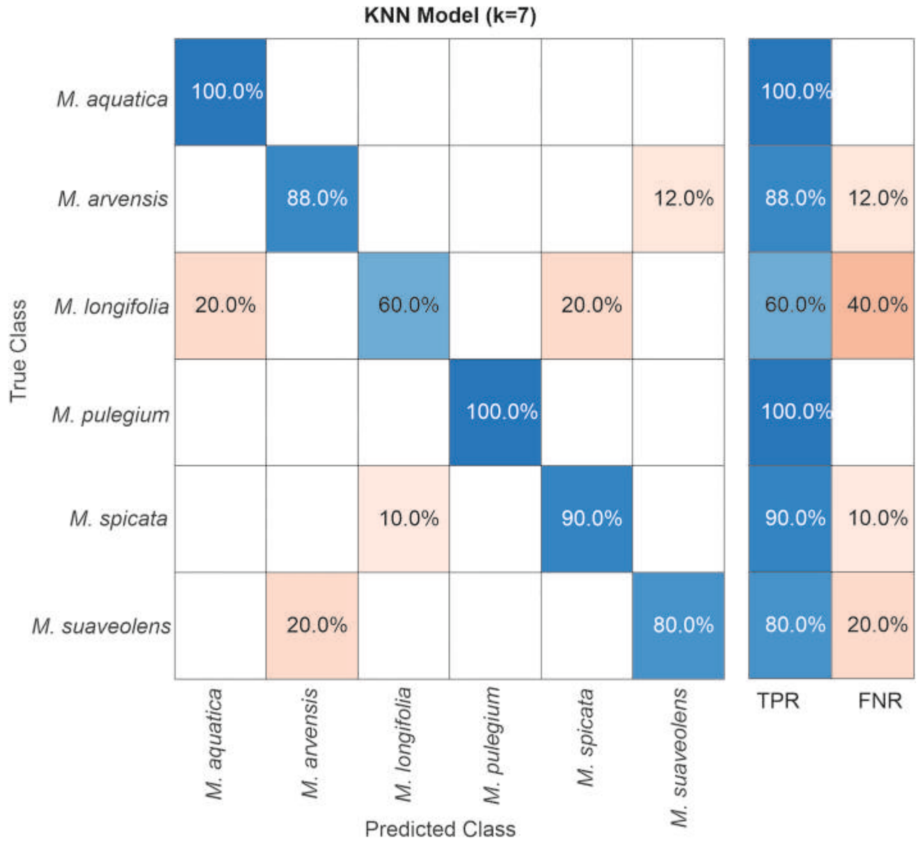

Fig.10. KNN confusion matrix for the six *Mentha* species found in Epirus and included in the training dataset. TPR: true positive rates; FPR: false negative rates.

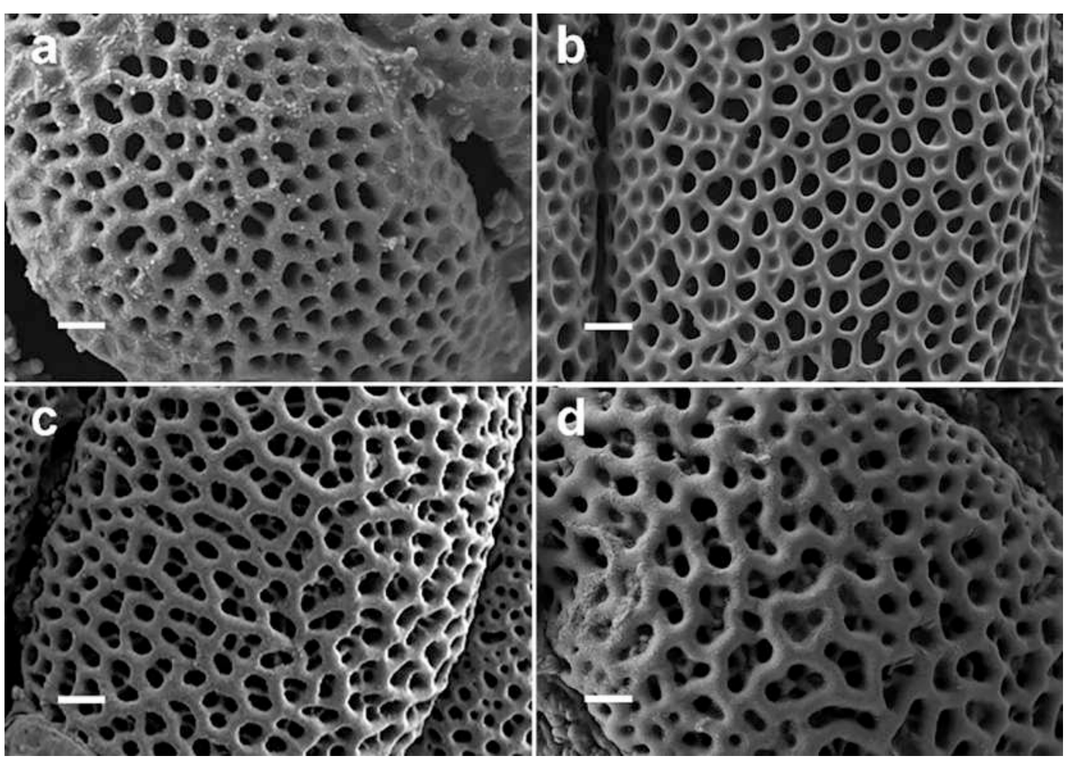

Fig. 11. Scanning electron microphotographs of exine patterns for *Mentha* (a-subfossil; b-*M. arvensis*) and *Thymus* (c-subfossil; d-*T. praecox*). Scale bar = 1 μm.

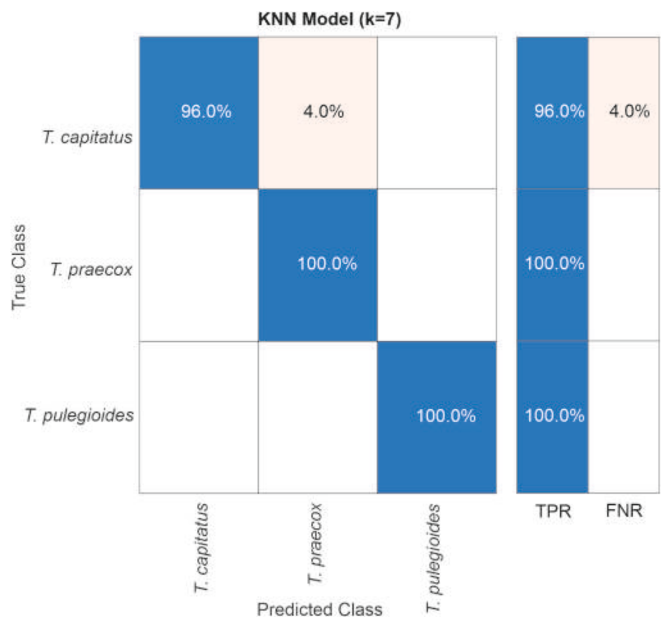

Fig. 12. KNN confusion matrix for the three *Thymus* species found in Epirus and included in the training dataset. TPR: true positive rates; FPR: false negative rates.

suprareticulum mesh (length and width), carried out on the 114 subfossil grains of Lamiaceae by means of LM, allowed three distinct types to be defined. Pollen type 1 includes 25–40 μm-sized grains with a suprareticulum mesh >1 μm; 27 grains of this type were identified (Fig. 6a). Pollen type 2 includes 15–20 μm-sized grains with a suprareticulum mesh <1 μm, hard to be defined under the LM; 21 grains of this type were identified (Fig. 6b). Pollen type 3 includes 20–35 μm-sized grains with a suprareticulum with clearly distinguishable meshes ≤1 μm; 63 grains of this type were identified (Fig. 6c). The remaining 3 pollen grains were borderline between the second and the third type so they were excluded from the count.

The study of the subfossil grains with SEM allowed a more accurate observation of the exine characters, not clearly distinguishable under the LM. As a first step, the three pollen types, already identified under the LM, were distinguished under SEM and their distinctive features measured (Table 2). In particular, the reticulum of the subfossil grains belonging to pollen type 1, which was distinguishable by its large meshes under the LM (>1 μm), showed a bireticulate structure characterised by the coexistence of a wider suprareticulum and a very dense reticulum (Fig. 6a). Most grains belonging to pollen type 2 appeared cloaked by pollenkitt, a substance with a protective and adhesive function useful to interact with pollinating animals (Pacini and Hesse, 2005). The pollenkitt occluded the exine and prevented evaluating the mesh size under both LM and SEM. Following the acetolysis treatment, it was possible to remove the pollenkitt (see Supplementary Material, Fig. 1SM) and examine the ornamentation of the exine, which under SEM observation revealed the presence of a suprareticulum and a reticulum characterised by small meshes seldom rated as perforated (Punt et al., 2007). As the other types, also subfossil grains belonging to pollen type 3 showed a bireticulate structure characterised by the coexistence of a suprareticulum and a reticulum (Fig. 6c).

4.3. Selection of modern species for comparison

As a first step for the selection of current species to compare with subfossil pollen grains, only genera found in the Greek flora (ipni.org and portal.cybertaxonomy.org/flora-greece/intro) were examined. Among the 35 Lamiaceae genera found in Greece (A, Table 3), the 19 having 6 colpi were considered for subsequent screening (B, Table 3). The number of colpi was checked on grains observed under LM and SEM, using images on the available works on the Lamiaceae species (*PalDat – a palynological database,* 2000 onwards, www.paldat.org; Celenk et al., 2008; Özler et al., 2011; Azzazy, 2016) and pollen atlases (Reille, 1992, 1995; Beug, 2015).

The subsequent exclusion criteria were based on morphological and morphometric similarity of modern grains to subfossil pollen (see Table 2), and in particular they examined: the average dimension of the equatorial diameter with respect to fossil pollen (C, Table 3), the occurrence of bireticulate or perforate-bireticulate exine, as observed in fossil pollen (D, Table 3), the number of lumina in each suprareticulum lumen in the range of fossil pollen (E, Table 3), the length of the suprareticulum lumina in the range of fossil pollen (F, Table 3) and, finally, the width of the suprareticulum lumina in the range of fossil pollen (G, Table 3; for details on measures see Supplementary Material).

In the end, only 7 genera passed all selection criteria (G, Table 3) and were analysed by the KNN classification method.

4.4. KNN classification

In general, KNN results do not show high sensitivity with respect to adopted k. We tested different k values, obtaining a greater accuracy for k values ranging from 6 to 12. The results shown here were obtained with k = 10 and the overall accuracy is 80.3%. The classification model performs well for the genera *Mentha, Micromeria, Salvia* and *Satureja* (Fig. 7), showing true positive rates over 85%. For *Salvia*, a residual percentage of cases can be incorrectly predicted as *Thymus*. Concerning *Origanum* and *Thymus*, however, higher chances of misclassification result. In particular, *Origanum* can be misclassified as *Satureja* in the 23% of cases, while *Thymus*, correctly classified in 65% of cases, can be incorrectly predicted as all the other genera included in the modern dataset (Fig. 7). Poor results were obtained for *Calamintha*, which is misclassified in most cases.

Application of the classification model to subfossil grains provided the results shown in Table 4 (for scores see Supplementary Material). For pollen type 1, four out of five grains are attributed to *Salvia*, and one (grain 3) to *Thymus*. Regarding the latter, the scores related to classification (see Supplementary Material) are divided between *Salvia* and *Thymus*. The fact that a percentage of cases where *Salvia* can be predicted as *Thymus* is included in the classification model of modern genera (Fig. 7), makes us quite confident in attributing fossil type 1 to *Salvia*. All grains of pollen type 2 are attributed to *Mentha* and the five grains of pollen type 3 are attributed to *Thymus*.

After having assigned a genus to each pollen type, the KNN model was used to try to assign a species to each genus. The first step, as in the case of genus classification, was to apply the method to the modern species in order to understand their predictability. In this case, we adopted k = 7 in relation to a lower number of observations. The species used for the classification are those found in the Epirus region (for details on measurements see Supplementary Material).

The results of the application of the KNN model for the classification of *Salvia* at a specific level (Fig. 8) show an overall 84.8% of accuracy and indicate a good probability of correctly classifying *Salvia* pollen grains at the specific level based on the morphometric variables used. However, the classification of pollen type 1 subfossil grains at the specific level is less straightforward. Pollen grain 1 is predicted as *S. pratensis* L., pollen grains 2 and 3 as *S. nemorosa* L. and grains 4 and 5 as *S. glutinosa* L.. These three species show similar exine micromorphology, as shown in Fig. 9 that compares pollen grains from pollen type 1 (grain 1; 3; 5) with the three identified species of *Salvia* (*S. pratensis; S. nemorosa; S. glutinosa*).

The results of the application of the KNN model for the classification of *Mentha* at a specific level show an overall accuracy of 88.9% (Fig. 10). Within the six species, a lower than 80% of true positive rates was only found for *M. longifolia* L., which can be predicted as either *M. aquatic* L. or *M. spicata* L. The application of the model to pollen type 2 subfossil grains allowed them to be attributed with good confidence to *M. arvensis* L. (Fig. 11a, b).

Finally, the results of the application of the KNN model for the classification of *Thymus* at a specific level show an overall 98.3% accuracy (Fig. 12). On these bases, grains 1, 2 and 3 of the subfossil type 3 can be classified as *T. praecox* Opiz (Fig. 11c, d). Grain 4 can be attributed to *T. pulegioides* L., however, the classification scores indicate a borderline situation, with a values around 0.5 for both *T. praecox* and *T. pulegioides.*

5. Discussion

5.1. Pollen grain morphometry as a method for species determination

The results presented in this work demonstrate that deep morphometric analysis of pollen grains can lead to species determination. In the case of Lamiaceae, it is clear that the diagnostic feature that allows species distinction is the morphology of the two-layered exine, which is visible only through SEM observation. This means that the routine LM analysis, which is peculiar to palynological investigation of sediment samples, may not be sufficient to reach the species level in this family but may guide in the genus determination. In fact, a simpler morphometric analysis at LM, based on the measurement of grain size and relative mesh size of the suprareticulum, allowed distinguishing three different pollen types in the US 29 sample. The subsequent comparison with pollen atlases and reference material allowed narrowing the number of genera with which to compare subfossil material. In the case of sample US 29, it was possible to analyse subfossil pollen with SEM due to the large number of Lamiaceae grains and thus their easy recovery on the small stubs used for observations.

Genus and species determinations were realised by means of comparison with selected modern species through the application of the KNN classification method. The criteria adopted for selection proved fit for purpose and included morphometric similarity and geographic location. On this basis, the presence in the subfossil sample of pollen grains of *Salvia (nemorosa/glutinosa/pratensis)*, *Mentha arvensis* and *Thymus praecox* can be asserted.

Attribution to three different sage species for subfossil grains was unexpected since KNN classification on modern species had shown a good probability of correctly classify *Salvia* grains at the specific level despite the great similarity in the exine micromorphology.

5.2. The origin of Lamiaceae in US 29

The anomalous large pollen quantities of Lamiaceae species in US 29 cannot be easily linked to a single interpretive hypothesis. In fact, these plants could have been present in natural associations growing near the site or rather being connected to human activities. The archaeological interpretation of the setting where the sample was collected indicates that it was used as a shelter for shepherds during late-post medieval times, and in particular from the second half of 15th up to the16th century AD. During this phase, due to the gradient of the floor, the existing older latrine had become a waste and drainage point for the shepherds who occupied the tower structures as shelter for themselves and their flocks.

The identified Lamiaceae species develop in different ecosystems, and in particular *Salvia nemorosa* in temperate and submediterranean grasslands, ruins and arid uncultivated lands; *Salvia glutinosa* in woodlands and scrub; *Mentha arvensis* in damp meadows; *Thymus praecox* in mountain vegetation and dry meadows (Tutin et al., 1972; Pignatti, 1982; portal.cybertaxonomy.org/flora-greece/intro).

This would mean: 1) either that flocks had eaten the plants during their wondering through different pastures, 2) or the shepherds had collected the plants for their food supply and/or as forage supplements for flocks.

The first hypothesis could be supported by the fact that goats have a great ability to both grab the most hidden and hard-to-reach grasslands and to draw nutrients even when these are scarce, feeding themselves with potentially less palatable food (Cannas and Pulina, 2008; Sepe and Argüello, 2019). Concerning the second hypothesis, although the use of Lamiaceae in human consumption is still of great importance in this area, as in the whole Mediterranean (Malamas and Marselos, 1992), the origin of these plants in the sample may be more likely to be related to the presence of flocks, and flock faeces. The co-occurrence of grazing indicators (pastoral indicators and coprophilous fungi; Figs. 4, 5) would support this hypothesis as the still persistent use of aromatic plants to improve physiological functions of flocks, such as nutrient digestibility, immune system functioning and endocrine and metabolic system stimulation (Pieroni et al., 2006; Franz et al., 2010; Vishal, 2013; Odhaib et al., 2021). Moreover, it seems to be still usual in Greece to border pastures with aromatic plants such as thyme, rosemary and mint (Alfredo Carannante,[2] personal communication). Many studies have focused on analysing the quality of fodder for feeding goats, and it has been shown that the use of natural antioxidants and flavonoids, extracted in particular from aromatic plants, is an interesting option for meat and milk quality improvement (Boutoial et al., 2012). For example, the feeding of rosemary to goats increases the protein value of milk while the addition of thyme increases lactose and decreases coagulation time without affecting smell and taste (Boutoial et al., 2012). Likewise, the introduction of sage seeds increases the fat in the milk, which is useful for cheese ripening and storage (Schettino et al., 2017). Many experiments have been conducted in Greek areas with long pastoral tradition: the practice of adding fresh or distilled leaves of Mediterranean plants, such as oregano (*Origanum vulgare* L.), mint (*Mentha spicata* L.), sage (*Salvia officinalis* L.) and rosemary (*Rosmarinus officinalis* L.) to the goat fodder has proven to have a number of benefits. The goats showed better health condition and the milk and meat better nutritional qualities (Boutoial et al., 2013; Odhaib et al., 2021).

6. Conclusions

The abundant presence of Lamiaceae pollen in US 29 provided the exceptional opportunity to analyse the morphology of the grains in detail. The results of this in-depth study have shown that morphometry is diagnostic for genus and even species determination within this family. The KNN classification applied to morphometric data of both subfossil and modern pollen grains of selected species has proven to be a valuable method for discriminating Lamiaceae species. However, this type of analysis requires observation under SEM, which is not always applicable in sediment samples, unless you have a large concentration of grains belonging to this family.

On the other hand, the interpretation of this anomalous presence in the sample is not so straightforward. The identification of large amounts of at least three species of Lamiaceae (*Salvia nemorosa/glutinosa/pratensis*, *Mentha arvensis* and *Thymus praecox*) seems to indicate that, these aromatic plants were specially harvested by shepherds around the site and used as fodder supplements for flocks. This would allow these plants, generally included among the ruderals, to be listed among the Local Pastoral Pollen Indicators. The main anthropogenic activity at the site of Kastrì in the 15th–16th century was therefore related to flock husbandry and pastoralism, confirming the archaeological interpretation of the sampled structure as a shelter for shepherds along the transhumance routes, still practised today.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

[2] IRIAE, International Research Institute for Archaeology and Ethnology, Naples, Italy.

Elda Russo Ermolli reports equipment, drugs, or supplies was provided by University of Naples Federico II. Elda Russo Ermolli reports a relationship with University of Naples Federico II that includes: board membership. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

We would like to thank the Herbarium Neapolitanum NAP, Annalisa Santangelo and Roberta Vallariello from Department of Biology, Federico II University, for providing the modern pollen samples used in this study. We thank the anonymous reviewers for their careful reading of our manuscript and their many insightful comments and suggestions.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.revpalbo.2024.105091.

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