

Article

Multiporate Pollen of Poaceae as Bioindicator of Environmental Stress: First Archaeobotanical Evidence from the Early–Middle Holocene Site of Takarkori in the Central Sahara

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Abstract: This paper reports on the most ancient unusual morphological trait of the apertures of Poaceae pollen found in archaeological layers. In Poaceae, high levels of hybridization, polyploidy, apomixis, and multiporate pollen are often related. Multiple genomes in polyploids are critical for the adaptation of plant species to stresses and could be revealed by anomalies in pollen development. Therefore, the paleoenvironmental research can gain great benefits from identifying polyploids in past contexts by observing anomalous pollen morphology during pollen counts. The occurrence of multiporate pollen in Poaceae has also been related to special features of the ecology of the species showing this anomaly, as well as to climatic and environmental stresses experienced by Poaceae living in a given region. Multiporate and bi- or tri-porate instead of monoporate pollen grains have been observed in samples taken from Takarkori rockshelter, an archaeological site in southwestern Libya (central Sahara) that has been occupied between ~10,200 and ~4650 cal BP. Multiporate pollen was found in organic sands and coprolites of ovicaprines. On the basis of archaeobotanical research, this work aims to investigate whether the presence of supernumerary pores in Poaceae pollen may be an effect of both climatic/hydrological changes and continued anthropogenic pressure on the wild grasses living in the region. The presence of multiporate pollen reveals that Poaceae that lived in central Sahara tackled several kinds of stress during the early and middle Holocene. The Takarkori pollen record suggests that climate change could have played a major role in the early Holocene, while human pressure became stronger during the middle Holocene. The change in environmental conditions determined adaptive responses of polyploid grasses even in the form of multiporate pollen.

Keywords: anomalous pollen; polyploidy; archaeobotany; wild cereals; desert; climate change; hunter-gatherer-fishers; pastoralists; coprolites; epigenetic



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1. Introduction

Plant reproductive biology and archaeology have found unprecedented interconnection through the discovery of anomalous pollen in archaeological contexts. Multiporate grass pollen was recorded with unusual frequency at the Holocene archaeological site of Takarkori, in central Sahara. The fact that this pollen belongs to the cereal family—Poaceae—suggests the availability of resources in an area long frequented by people who depended on it. This area has simultaneously undergone extensive and sometimes rapid environmental transformations over the last few thousand years.

1.1. Developmental Conditions of Multiporate Pollen

The Poaceae family is classically endowed with ana-monoporate pollen, i.e., a single “pore” aperture develops distally in each monad during microsporogenesis. Although this is the most common and widespread condition of the plant cycle, multiple pores have been reported in the pollen of several grass species. Poaceae include wild, cultivated,

and domesticated grain species distributed in almost all types of habitats: these perennial or annual plants occupy both wet and wooded places and dry and rural environments. Species that also produce multiporate pollen tend to be grassland plants, with herbaceous habitus and summer life cycle.

The first report of multiporate pollen of Poaceae concerns a hybrid generation between rye and wheat [1] (pp. 163–168). In recent years, the literature has begun to report the presence of anomalous pollen, with the description of multiporate monads among several tribes. There are examples among Andropogoneae: *Apluda mutica* L., *Bothriochloa ischaemum* (L.) Keng., *Heteropogon contortus* (L.) P. Beauv. ex Roem.&Schult.; Paspaleae: *Paspalum pauciciliatum* (Parodi) Herter; Phareae: *Pharus lappulaceus* Aubl.; Oryzeae: *Zizaniopsis bonariensis* (Balansa & Poitr.) Speg., and Paniceae: *Digitaria ciliaris* (Retz.) Koeler, *Echinochloa polystachya* (Kunth), *Panicum maximum* N.J. Jacquin and other millets including species of *Pennisetum* [2–4]. Species of Chloridoideae are occasionally multiaperturate [5]. In *B. ischaemum* a high frequency of multiporate pollen (up to 22%) was observed and correlated to apospory, that is the absence of meiosis in microsporogenesis with formation of diploid “pseudospores”; they produce a sporophyte by mitosis, without gamia [6].

Multiporate pollen has also been observed in hybrids of Poaceae, and in the amphiploids, that are hybrids where the chromosome number is the sum of the chromosome numbers of both parental species [7–9]. Due to chromosome doubling, amphiploids overcome the sterility limitation of hybrid pollen [10]. Amphiploidy can be spontaneous or artificially induced, resulting in polyploid species and, importantly, is the basis of the origin of domestic wheats. Up to ten pores were observed in *Tritileymus* M842-1 (*Triticum aestivum* x *Leymus mollis*) which is an amphiploid ($2n = 8x = 56$) obtained by crossing *Triticum aestivum* ($2n = 6x = 42$) and *Leymus mollis* (Trin.) Pilg. ($2n = 4x = 28$) [11]. Also in this hybrid, several abnormalities have been recorded during microsporogenesis such as the absence of tetrad formation, absence of callose deposition, irregular shape of some microspores, and irregularity in mitosis. Although multiporate pollen grains are less efficient for reproduction, due to germination from many pollen tubes and competition among them [12], hybrids between distinct species frequently show higher vigor and probability of survival. Interestingly, they also have greater adaptability and physiological homeostasis than diploid organisms [13]. In the amphiploid ($2n = 8x = 56$) obtained from *Avena barbata* ($2n = 4x = 28$) x *A. sativa* ssp. *nuda* ($2n = 6x = 42$), the development of multiporate pollen grains is a consequence of DNA demethylation [10]. This is an epigenetic mechanism that plays a major role in the expression of some genes. In parental genomes of many grass polyploids (cereals), there are changes of genome methylation patterns, rapid epigenetic gene silencing, and other genetic changes as activation of retrotransposons that seems to be tightly linked to molecular pathways activated by stress [14].

Amphiploids and polyploids have higher allelic diversity and heterozygosity often resulting in phenotype changes. Therefore, polyploidy is not only a fundamental component of plant evolution [15] but confers genomic characteristics that could provide more physiological and ecological flexibility to the plant. Polyploidy may be manifested as both an effect of environmental stress, as increased rates of polyploidization, and an adaptation to stress [16]. The numerous abnormalities observed in micro- and macrosporogenesis of these grasses may be the cause of apomixis, an asexual mode of reproduction during which the egg develops into seed without involvement of meiosis and fertilization, so the progeny will be genetically identical to the mother plant [17]. Apomixis in polyploids is probably caused by asynchronous expression of genes normally deputed for sexual reproduction [18]. Apomixis generates clonal seeds. The shift from normal to apomictic reproduction occurs in plant populations that show meiotic disorders, increased chromosome numbers, and defective pollen [13]. Poaceae include many apomictic species with different levels of ploidy. All species that have multiporate pollen are polyploid and many of them reproduce by apomixis [3]. Although the interplay between multiporate pollen development and apomictic reproduction requires further research to fully understand

what are cause and effect, the presence of multiporate pollen can be used for preliminary identification of apomixis [3,9].

To sum up, Poaceae multiporate pollen is an effect of reproductive cycle anomalies. It is often related to high levels of hybridization, polyploidy and amphiploidy, and apomixis [12]. The occurrence of this pollen anomaly in Poaceae has been connected to special properties of the plant species, as well as to the ability to reply to environmental stresses experienced by individuals living in a given region.

1.2. Aim of the Paper and the Archaeobotanical Evidence

This paper reports on multiporate pollen observed in an archaeological context with the aim of testing whether pollen with this anomaly could be a new type of environmental marker.

The Takarkori rockshelter, located in the Tadrart Acacus mountains in southwestern Libya (central Sahara), has been the subject of interdisciplinary research that has shed new light on the complexity of the relationships between humans and plants. Interdisciplinary studies have yielded a wealth of information about climate, vegetation, and people that lived in the region (e.g., [19,20]). Different cultures, with different degrees of social complexity, inhabited this desert area while challenging environmental transformations for millennia. The preservation of organic matter in this sheltered site is exceptional and allowed the preservation of molecular residues, lipids, and ancient DNA. They revealed, together with archaeology and archaeobotany, hitherto unknown details of plants consumption and uses [21,22]. The cp-DNA of some wild cereals preserved by dehydration was extracted, amplified, and sequenced, confirming the morphological identification of the African minor cereals *Echinochloa colona* (L.) Link., *Panicum laetum* Kunth., and *Sorghum bicolor* (L.) Moench [23]. Further evidence of the extraordinary state of preservation of the plant remains was in the findings of weavings, likely used for the construction of baskets to collect or store grains [24] and of wood with the colour still preserved from the pigments used for rock art [25]. Well preserved pollen grains were extracted by sediments accumulated into the site [20]. Among them, the multiporate pollen of Poaceae is an interesting anomaly that has never been reported in the Holocene Sahara. Our hypothesis is that this particular pollen is linked to a combination of environmental/anthropogenic stresses that occurred at the site and in the region at the time of occupation.

2. Materials and Methods

The site of Takarkori (N 24.53714, E 10.51969; 1100 m asl) has been excavated and studied in the framework of the Archaeological Mission in the Sahara (former Joint Italian-Libyan Archaeological Mission in Acacus and Messak) of the Department of Antiquities, Tripoli, and Sapienza University of Rome. In this area, the archaeological research identified a first exploitation by hunter-gatherer-fishers, which was followed by a long occupation of pastoral communities. These were herders of cattle, and of sheep and goats.

Archaeological chronology is supported by ceramics, lithics and 43 radiocarbon dates on heaths, floors, organic sand and burials (see Table 3 in [20]), besides 30 radiocarbon dates on seed accumulations (see Table 1 in [23]). The chronological phases of occupation of the rockshelter, based on radiocarbon dates, archaeological materials, geoarchaeology and stratigraphy, can be resumed in the four main phases reported below, from the oldest to the most recent (e.g., [26]):

- Late Acacus (LA) = hunter-gatherer-fishers, ~10,170–~8180 cal BP
- Early Pastoral (EP) = pastoralists, ~8300–~6890 cal BP
- Middle Pastoral (MP) = pastoralists, ~7160–~5610 cal BP
- Late Pastoral (LP) = pastoralists, ~5700–~4650 cal BP

The excavation campaigns of Takarkori lasted from 2003 to 2006. The rockshelter showed a particularly well-preserved sedimentary sequence and was excavated in four sectors over 143 sqm of total surface (Figure 1). The stratigraphy of the site is reported in the interdisciplinary paper by Cremaschi et al. [20] who described its deposit as made by sediments rich in organic matter, charcoal and dried plant parts, human and faunal

remains. Archaeobotanical analyses including pollen and macroremains have been carried out on sediment samples collected from every stratigraphical unit unearthed during the excavation.

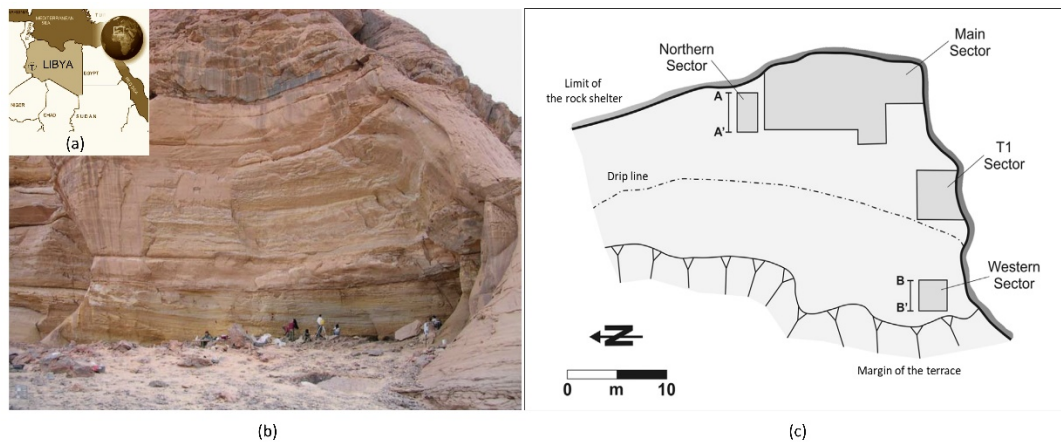


Figure 1. Takarkori rockshelter, Libya: (a) Location of the site in Libya (T = Takarkori); (b) The site during the 2004 field season; (c) The four sectors of excavation (Cremaschi et al. [20] modified). The trench with the pollen sequence reviewed here was in the Northern Sector. The coprolites were taken from different layers in the Main sector.

Pollen analyses were carried out on sediments and coprolites taken from all the sectors [20,27,28]. The analyses on Poaceae pollen reported here derive from new observations of the slides already studied for palaeoenvironmental and ethnographic purposes. These detailed morphological analyses refer to 30 samples taken from the wall of TK-NS, a 4 × 2 m trench. Seven Units, including loose, organic or laminated sand, form the stratigraphy (Table 1). Moreover, 55 coprolites from the Main sector were also reconsidered: 45 specimens belong to Late Acacus layers, and 10 specimens belong to Middle Pastoral layers. The list of samples, with descriptions of the stratigraphical units, cultural phases, and chronology, is reported in Table 1.

Table 1. Takarkori rockshelter, Libya: List of pollen samples in the TK-NS sequence, with descriptions of stratigraphical units, cultural phases, chronology, and coprolites analysed in different phases.

TK-NS Pollen Sequence (Cremaschi et al., 2014: [20])							Main Sector
Sample No.	Depth (cm)	Unit	Description	Cultural Phase	Chronology (cal BP)	Coprolites (Number, Layer, and Square)	
1	6.0	I	Organic loose sand, lamination; common plant remains, charcoal and coprolites; large stones from vault collapse	MP2—Middle Pastoral 2	6300–5750	10 specimens from one layer (L25 V24)	
2	11.0						
3	18.5						
4	21.5						
5	26.5						
6	32.5						
7	35.0						
8	42.5	II	Organic sand; frequent charred and uncharred plant remains; lenses of white ash	MP1—Middle Pastoral 1	6950–6300		
9	49.0						
10	51.5						
11	53.5	III	Loose thin laminated sand; frequent uncharred plant remains and coprolites	EP1—Early Pastoral 1	8250–7800		
12	58.5						
13	61.5						
14	66.0						

Table 1. Cont.

TK-NS Pollen Sequence (Cremaschi et al., 2014: [20])						Main Sector
Sample No.	Depth (cm)	Unit	Description	Cultural Phase	Chronology (cal BP)	Coprolites (Number, Layer, and Square)
15	71.5	IV	Organic sand, from loose to moderately hard; frequent charred and uncharred plant remains. Lenses of white ash and black charred material	LA3—Late Acacus 3	8950–8450	25 specimens from three layers (L103 S32; L69 T22; L147 V22-23)
16	75.0					
17	81.0					
18	84.0					
19	87.5					
20	91.5	V	Organic loose sand including two thin layers of laminated sand cemented by organic matter	LA2—Late Acacus 2	9450–8950	20 specimens from one layer (L275 R23)
21	97.0					
22	101.0	VI	Loose sand; scarce plant fragments, often charred			
23	105.0					
24	110.0					
25	115.0					
26	120.0	VII	Loose organic sand; rare plant remains distributed in thin layers; rare weathered sandstone fragments	LA1—Late Acacus 1	9950–9450	
27	121.0					
28	122.5					
29	130.0					
30	132.0					

Pollen extraction from sediments was made with a method including sieving and heavy liquid separation [20], while coprolites were boiled in 10% NaOH solution [28]. All treatments included acetolysis. *Lycopodium* tablets were added to calculate pollen concentration. Residues were included in glycerol to mount permanent slides. For each sample, one or two whole slides were observed, counting the monoporate and multiporate pollen grains of Poaceae. In previous studies [20], pollen spectra have been calculated on a pollen sum that included all pollen. Large pollen grains are the grass records with a maximum diameter $\geq 40 \mu\text{m}$ that is often considered as “Cerealia type”. Nomenclature largely follows the African Pollen Database, and Chenopodiaceae is preferred to Amaranthaceae because it was used in previous papers.

Data were analyzed by Pearson’s correlation coefficients and Student’s *t*-test ($p < 0.05$), performed to examine the relationship between the presence of multiporate Poaceae pollen and environmental/anthropogenic stresses (variables considered: $>40 \mu\text{m}$ Poaceae pollen—Asteraceae, Chenopodiaceae, Cyperaceae, *Typha*, all other Poaceae). Pollen data [20] together with multiporate Poaceae pollen were ordinated by Principal Component Analysis (using XLSTAT) to obtain further information on differences between samples in terms of plant composition.

3. Results

Poaceae pollen was present in high quantity and in a good state of preservation in all samples from the sequence (67%, on average; LA: 64%; Pastoral: 71%) and in 49 coprolites (LA: 78%, MP: 50% on average). Pollen was completely absent from 6 coprolites (5 from L147, and 1 from L275). Multiporate pollen grains have been found both in the sequence and in coprolites. Coprolites show rare multiporate pollen in three layers:

- LA2, L275, 3 specimens with 3.2%, 0.5%, 0.5%
- LA3, L69, 1 specimen with 0.9%
- MP2, L25, 2 specimens with 0.9%, 0.8%.

In TK-NS, multiporate pollen was in 53% of the samples representing 0.1% of the total grasses (Table 2). Diporate and triporate were the most common with pore sizes which varied significantly from each other. In general, the exine and size of the different records

were quite variable suggesting intraspecific variability and the possible presence of several species sharing the multiporate pollen anomaly (Figure 2).

Table 2. Takarkori rockshelter, Libya: Multiporate pollen observed in the TK-NS sequence (last column) and the number of Poaceae observed; the table also reports on the percentage of Poaceae in pollen spectra, their concentration (p/g), and the presence of large pollen of grasses (X = > 40 μm are > 1% in the spectrum).

Sample No.	TK-NS Pollen Sequence (Cremaschi et al., 2014: [20])		Large Poaceae (X = > 40 μm ; § = > 60 μm)	Poaceae Pollen (This Paper)		
	Poaceae Sum			Monoporate	Multiporate	
	% On Total Pollen	p/g		Count	Count	%
1	50.2	55,613	X	1866	0	
2	50.6	67,752	X	179	2	1.12
3	59.5	25,922	X; §	1026	2	0.19
4	61.3	103,526	X	935	2	0.21
5	74.7	192,008	X	406	0	
6	76.3	430,656	X	454	1	0.22
7	73.3	140,453	X	610	0	
8	87.2	145,972	X; §	188	0	
9	76.9	222,823	X	1612	1	0.06
10	75.3	77,963	-	3691	2	0.05
11	72.5	77,145	-	103	0	
12	73.8	78,635	X	362	0	
13	80.6	340,566	X	193	1	0.52
14	82.6	191,471	X	367	2	0.54
15	96.4 *	40,956	X	29	0	
16	86.2	5373	X	57	0	
17	77.6	52,360	X	176	1	0.57
18	73.5	116,702	X; §	189	0	
19	63.4	35,476	X	208	0	
20	49.3	44,790	X	441	0	
21	37.8	20,713	X	376	3	0.80
22	52.0	13270	X	724	4	0.55
23	44.8	21,426	X	143	1	0.70
24	41.1	8877	X	384	1	0.26
25	71.6	5654	X	1587	0	
26	44.0	5103	X	1504	1	0.07
27	79.9	7901	X; §	902	1	0.11
28	75.0	22,123	X	1415	2	0.14
29	55.5	640	X	2108	0	
30	74.9	14,439	X	2066	0	

* This value is misleading because the sample was a fireplace from which only a few pollen grains, almost all Poaceae, were extracted.

Data elaboration shows that there is not linear relationship between multiporate pollen and many other records [>40 μm Poaceae pollen (Pearson correlation coefficient $r = -0.11$), *Typha* ($r = -0.19$), Cyperaceae ($r = 0.04$) and Chenopodiaceae ($r = 0.06$)], while a negative correlation resulted between the multiporate and all other Poaceae ($r = -0.38$), and a positive correlation exists with Asteraceae ($r = 0.44$). Although from the Student's *t*-test the difference is not statistically significant, the value of *r* suggests that multiporate pollen tends to decrease when all other grasses increase, while tends to increase when shrubs and herbs of the daisy family, common in the arid environments of the desert [29], increase.

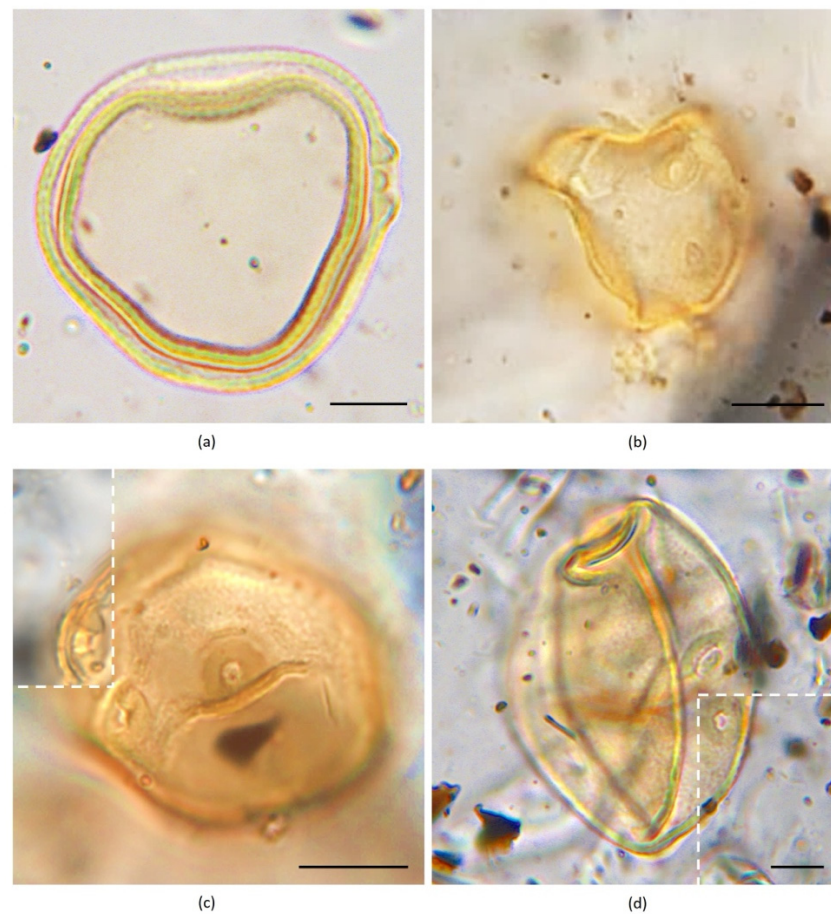


Figure 2. Takarkori rockshelter, Libya: Poaceae pollen: (a) Monoporate pollen from a coprolite of ovicaprine taken from the Main sector (pollen diameter = 51 μm ; porus+annulus = 13 μm); (b) Diporate pollen from sample 4 (pollen diameter = 28.2 μm ; porus+annulus = 5.5 μm and 6 μm); (c) Triporate pollen from sample 9 (pollen diameter = 34.8 μm ; porus+annulus = 7.9 μm , 7.8 μm , and 7.7 μm); (d) diporate pollen from sample 28 (pollen diameter = 63.7 μm ; porus+annulus = 10.6 μm and 9.4 μm). Figures (c,d) have been arranged for visualization of two different focuses. The scale bar is 10 μm .

4. Discussion

Since the occurrence of multiporate pollen in normally monoporate species is related to hybridization causing changes in reproductive biology [3,9], we argue that most Takarkori's Poaceae were polyploids probably also equipped with apomixis. Actually, the tribes Andropogoneae and Paniceae, largely in the form of spikelets and caryopses in the archaeobotanical record of this rockshelter [23] and abundant in other sites of the area (e.g., [30,31]), are known to include polyploids and also some obligately apomictic species (e.g., [32,33]).

In Takarkori, anomalous pollen was common in the samples. Multiporate pollen was found in the pollen sequence and in coprolites dated to ~9500–5700 cal BP [20,23,26]. They seem to have been distributed in layers of different ages, mainly concentrated in the Late Acacus and late Middle Pastoral (see Table 2). The presence of multiporate pollen reveals that Poaceae that lived in central Sahara have tackled several environmental stresses, under climate or anthropogenic change pressures, during the early and middle Holocene (Table 3).

Table 3. Takarkori rockshelter, Libya: Pollen samples from TK-NS pollen sequence with main results on pollen zones [20], and plant macroremains (spot and mix accumulations: [23]; data on palaeoenvironment are from the palynological study of Wadi Teshuinat [31].

TK-NS Pollen Sequence						
Sample No.	Pollen Zones		Plant Accumulations of Caryopses and Spikelets		Regional Palaeoenvironment and Context	Climate
	Label	Vegetation	Spot	Mix		
1	Tk2c	Spread of dry savanna and xeric vegetation, reduction of grassland with significant change toward environmentally dry conditions; wet habitats became smaller	1 spot of <i>Echinochloa</i> 65% and <i>Panicum</i> 18%	5 mix of Paniceae chaff: <i>Echinochloa</i> (79%, 65%) and <i>Panicum</i> (34%, 15%)	Rapid regression of humid environments giving way to savanna-desert: gradual establishment of drier climate. Spread of xerophilous desert savanna and psammophilous vegetation. Pastoralists try to reorganize by abandoning cattle ranching and intensifying the raising of goats and sheep, with lower water requirements; this is to cope with the early stages of the arid crisis that transformed the Sahara into a desert	Warm/Dry
2						
3						
4						
5						
6	Tk2b	A mosaic of xeric and freshwater habitats was present during this phase; Poaceae included a high quantity of different large pollen grasses due to increased natural availability and selection of ‘new’ wild cereals	10 spots of <i>Echinochloa</i> (59%, 52%, 50%), of <i>Urochloa</i> (82%, 75%, 65%), of <i>Panicum</i> (32%, 20%)		Wooded savanna with plant landscape beginning to be exploited by pastoralists from with cattle herds and small livestock that took over from hunter-gatherers; drying up the environment with oscillations. The results of palynological and macroremains analyses conducted at the Uan Muhuggiag rockshelter and Mathendush Cave suggest the spread of a Sahelian-type savanna in the area during the middle part of the middle Holocene	
7						
8						
9						
10						
11						
12						
13	Tk2a	Spread of grassland, wet environments and reduction of dry savanna vegetation; intensive plant accumulation	5 spots of Paniceae and Andropogoneae: <i>Pennisetum</i> 15%; <i>Sorghum</i> 72%, 15%; <i>Urochloa</i> 87%, 75%, 63%; 1 spot of <i>Polygonum</i> 87%	1 mix of Paniceae and Andropogoneae with chaff: <i>Brachiaria/Urochloa</i> 32%; <i>Pennisetum</i> 25%	The results of palynological and macroremains analyses conducted at the Uan Muhuggiag rockshelter and Mathendush Cave suggest the spread of a Sahelian-type savanna in the area during the middle part of the middle Holocene	Warm/From dry to wet
14						
15						
16						
17	Tk1c	Environmental instability, increased seasonality and changes in plant exploitation are evident; further reduction of permanent water bodies is visible at the end of the LA3 phase; notable increase of large pollen types, belonging to some <i>Panicum</i> , <i>Echinochloa</i> and <i>Setaria</i>				
18						
19						

Table 3. Cont.

TK-NS Pollen Sequence						
Sample No.	Pollen Zones		Plant Accumulations of Caryopses and Spikelets		Regional Palaeoenvironment and Context	Climate
	Label	Vegetation	Spot	Mix		
20				4 mix of Paniceae and Andropogoneae with chaff:		
21			1 spot of <i>Setaria</i> 71%	<i>Brachiaria</i> 26%, 19%;		
22	Tk1b	Widening of shallow-water marginal zones or general lowering of the water level; wooded savanna is present but xerophilous plants began to expand while grassland reduces		<i>Brachiaria/Urochloa</i> 17%; <i>Urochloa</i> 45%; <i>Pennisetum</i> 14%, 11%		
23						Transformation of plant cover in relation to an arid climate. There is a concomitant erosion phase with a dry interval, demonstrated, for example, by the disruption of the sedimentary sequence in Uan Afuda Cave and the ingression of windblown sand in the Ti-n-Hanakaten sequence
24						
25						
26						
27						
28	Tk1a	Grassland and sparsely wooded savanna; local permanent freshwater habitats with <i>Potamogeton</i> , and wet environments with reeds, cattails				Cold/Dry
29						
30						

4.1. Polyploidy as Effect and Adaptation to Environmental or Anthropogenic Stresses

Thanks to the conservation of exine in fossil pollen, polyploids can be identified in past contexts, giving paleoenvironmental research the possibility of obtaining hitherto unknown information on plant genetics of some archaeological records. Multiple genomes, which can be revealed by anomalies in pollen development, are in fact critical for the adaptation of plant species to external stresses.

To the best of our knowledge, only two cases of the discovery of these anomalies in archaeological deposits have been described in the literature. Radaeski et al. [4], studying fossil pollen from the Late Holocene Águas Claras site in Brazil, advanced the hypothesis that multiporate pollen in monoporate populations had some ecological significance. They observed diporate pollen of Poaceae in the late Holocene (~3420 cal BP); then, this pollen increased from ~1340 cal BP, when a vegetation change with expansion of forest occurred at the expense of grassland. The presence of diporate pollen was interpreted as the evidence that grassland was scattered even where forest vegetation dominated pollen spectra; in that context, their increase was linked to the stress determined by an increase in atmospheric humidity. In contrast, the presence of pollen from large Poaceae with many germination pores has been correlated with anthropogenic stress by Fall [34] who pointed out the appearance of multiporate pollen following the arrival of Polynesian settlers on the island of Tonga. In the swamps of this island, Poaceae percentage was low (1–2%) before the arrival of settlers (~2850 cal BP); then, grass pollen increases abruptly (up to 85%) with the arrival of settlers who introduced the practice of controlled burning of the swamps, as indicated by the presence of microcharcoals. Concurrently with this increase, multiporate pollen appeared, and their large size suggested that these were plants cultivated and so, in this case, the appearance of abnormal pollen would be related to selective hybridization and agriculture.

In the Takarkori rockshelter context, the Principal Component Analysis proved effective in interpreting the data as the components obtained have a clear ecological-environmental connotation (Figure 3). The first PC explains 10.39% (F1) of the total variance, the second PC explains 9.72% (F2). The first PC has low loadings with similar value for many pollen taxa and describes a high biodiversity in a context characterized by plants with a synanthropic value (e.g., *Tamarix*, *Artemisia*, and *Poaceae* > 40 μm), separating pollen spectra in “more anthropic” and “less anthropic”. The second PC has high loadings for pollen taxa typical of arid or semi-arid environments (e.g., Cichorieae), while pollen taxa related to wet conditions have low loadings (e.g., *Typha* and *Lemna*). The second component separates pollen spectra along a humidity gradient, from “prevalently wet” to “prevalently warm/dry” conditions. The position of multiporate pollen in the Quadrant I suggests that drier climate conditions might have triggered an adaptive reply (the anomalous pollen) in *Poaceae* of Takarkori, regardless of human’s presence. Many samples have relatively low scores for the second PC. Overall, the arrangement of samples in the PCA space seems to be influenced more by anthropogenic action and by seasonal wet phases, as is visible during the transition between the Late Acacus and the Early Pastoral phase (mainly Quadrant II and III). Later, in the Middle Pastoral phase, heterogeneity is observed among the samples with different ecological conditions (Quadrant I and IV).

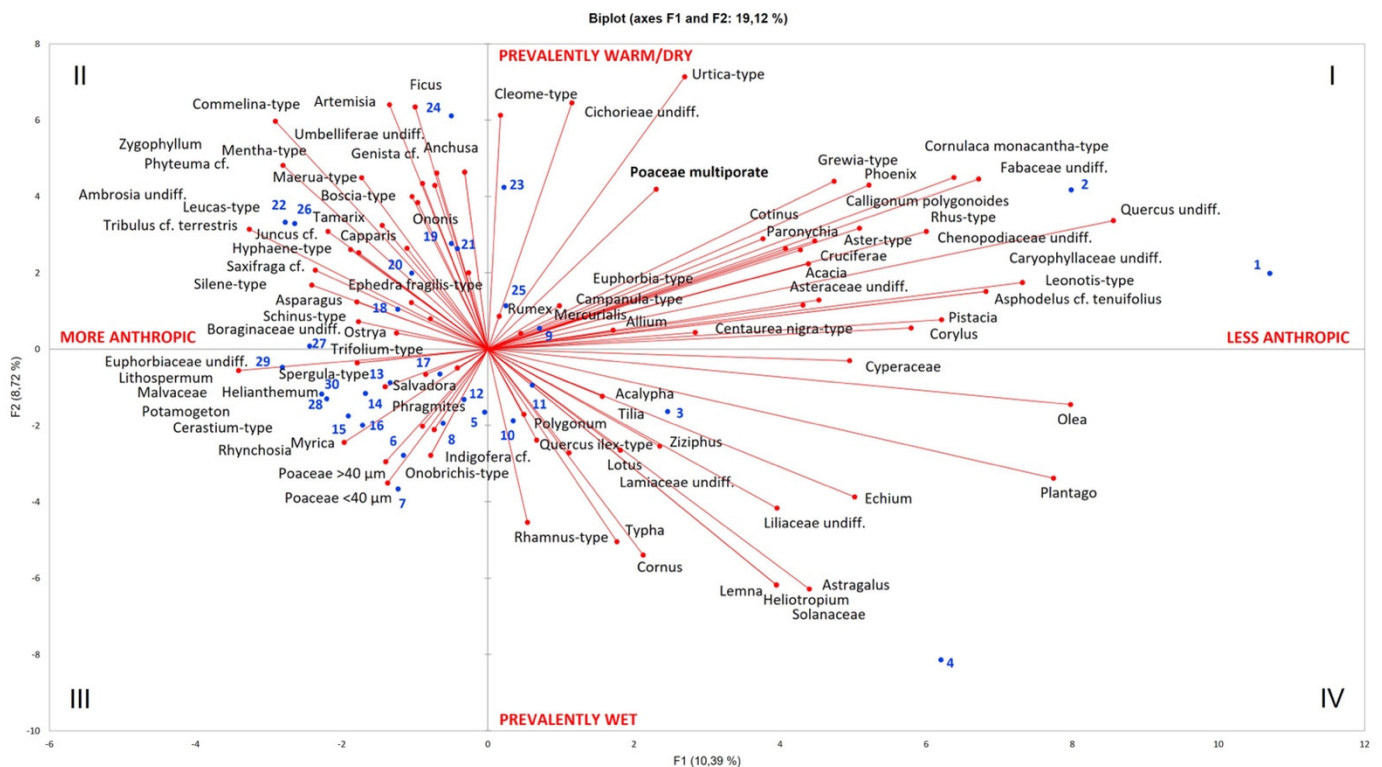


Figure 3. Principal Component Analysis (PCA) of pollen data from the TK-NS sequence [20] together with multiporate *Poaceae* pollen. The graph shows the sample points from dataset projected on the max variance plane given by PCA. The first axis separates pollen spectra along an anthropic pressure gradient (from “more anthropic” to “less anthropic”), and the second one along a climate gradient from prevalently wet to prevalently warm/dry conditions.

In the archaeobotanical record of Takarkori probably the concomitant stresses of climate instability and human selection have acted on suitable grasses that responded with polyploidy and multiporate pollen. Below the two determinants are discussed to emphasize their role in the development of anomalous pollen in the plant populations living in the Sahara at early and middle Holocene.

4.1.1. Multiporate Poaceae and the Climate Stress

In the TK-NS sequence, the highest values of multiporate pollen have been observed as concomitant with high values of Asteraceae (Figure 4: bottom), that are dated approximately from the early Holocene to the transition to the middle Holocene (~9500–8400 cal BP: point a in Figure 4) and in the second part of the middle Holocene (~5700 cal BP: point b in Figure 4).

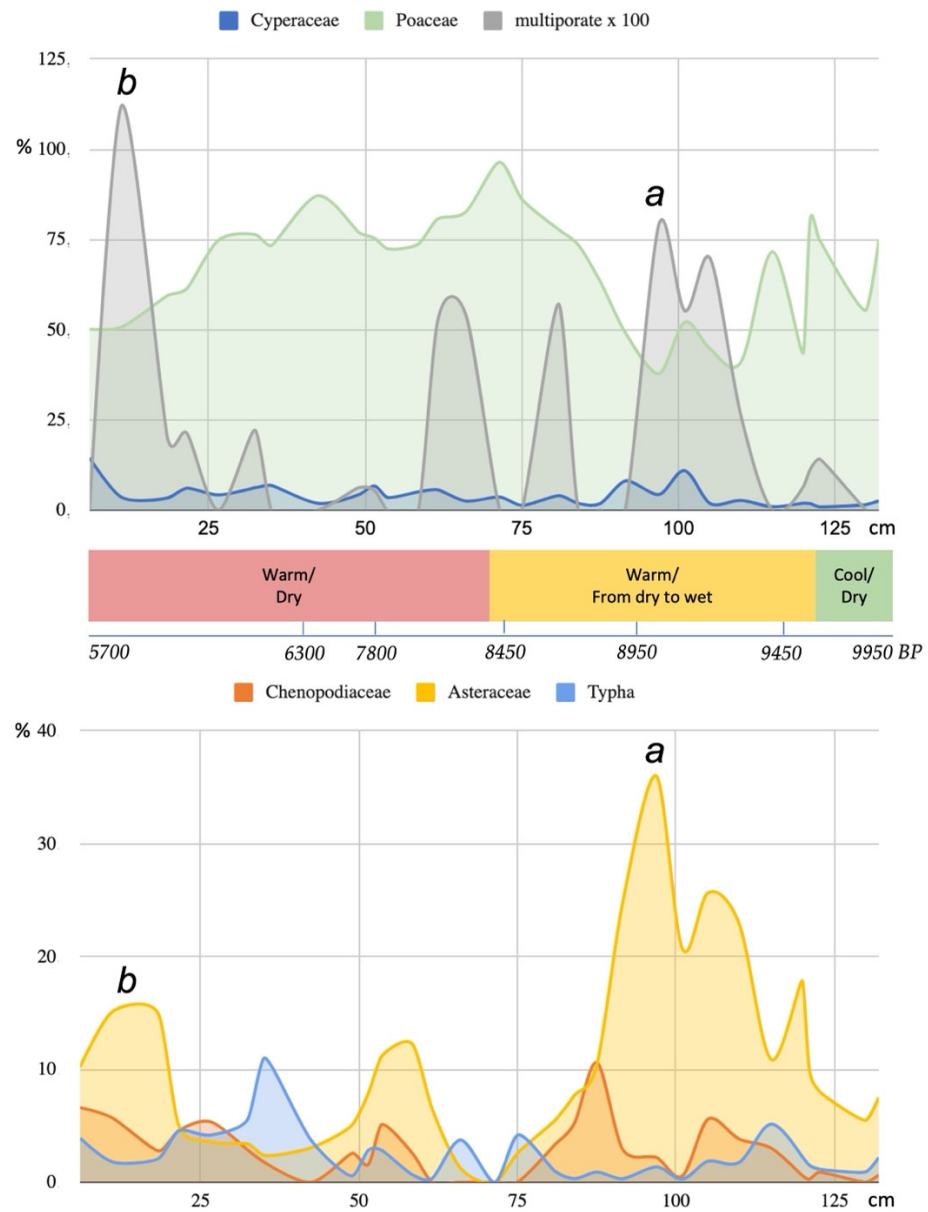


Figure 4. Takarkori rockshelter, Libya: TK-NS pollen sequence: main curves indicating wet (Poaceae and Cyperaceae, top) and dry (Asteraceae and Chenopodiaceae, bottom) climate/environmental conditions at the site. Climate phases in the region (Table 3) and radiocarbon dates along the sequence (Table 1) are reported. The presence of *Typha*, marking local availability of fresh-water places, is reported in the bottom diagram. Points “a” and “b” mark the occurrence of multiporate pollen in samples characterised by high values of Asteraceae, including many shrubby and herb species adapted to steppic and dry savannah in the Sahara.

From geoarchaeological, archaeobotanical and zooarchaeological data, several climatic phases have been identified, with alternating cool and warm, arid and humid oscillations in the region (Table 3). Between ~9500 and 8100 cal BP, most of the calcareous tufa deposits

reflecting a prolonged period of humid climate due to the northward expansion of the SW African monsoon system, were observed in Tadrart Acacus [35]. This massif, like other Saharan mountains especially sensitive to climate change, hosted plant migrations as a refugium and was covered by grasslands [31] (and see [36] for Tibesti and Hoggar). During this African Humid Period (AHP), tropical plant taxa migrated some 500 km northwards compared to their present-day distribution [37], and Mediterranean taxa descended to the central Sahara mountains as shown by remnants in the Tadrart Acacus and in Tassili N'Agger [31,38]. Similar patterns have been recognized in the analysis of faunal, especially aquatic, remains [39].

During the early to middle Holocene, the transition between the Late Acacus and the Early Pastoral phase corresponds to the transition from a cool and dry to a warm oscillation moving from dry to wet conditions locally. Contrary to the situation described by Radaeski et al. [4], the transition to a drier climate might have triggered an adaptive reply in the wild cereals of Takarkori. It would seem, therefore, that it was not the presence of a humid or arid climate but the change in environmental conditions that determined adaptive responses of polyploid grasses. Then, in the TK-NS sequence, relatively significant multiporate pollen were found during periods of environmental instability.

In the Pastoral phases, the AHP gave way to a progressive drying of the climate. In the Central Sahara, this phase is known to have begun from ~6000 cal BP and led to an expansion of grassland and reduction of forested and tree savanna vegetation. At the end of the AHP, the Sahara experienced a major environmental crisis with the southward displacement of its desert margin [40], rapid decrease of precipitation at 5800–4800 cal BP [41], decline of wetlands [42,43], and related loss in biodiversity [37,44]. Pollen data from Takarkori shows that large stands of grasses moved from grasslands to grow closer to the contracted wet environments: their genotypes were largely equipped with high levels of genetic variability and plasticity for climate-responsive traits [23]. This alone may have been able to give them the ability to survive dramatic environmental transformations by enduring water shortages, at least in the early stages of transformation processes.

4.1.2. Multiporate Poaceae and the Anthropogenic Stress

Wild grains that have been accumulated in the Takarkori rockshelter include genera whose multiporate pollen has already been documented in the literature, such as *Panicum* [3] and *Echinochloa* [4]. These grasses may have pollen with large size, between 40 and >60 μm , and may often be included in the Cerealia type [31]. In fact, wild cereals are often marked by large pollen as well as the domesticated species. In the TK-NS sequence, large pollen of Poaceae was found in almost all samples (Table 2) but there is not a linear correlation between multiporate and large pollen of Poaceae in the sequence.

In order to answer the question of whether human action may have been a stress agent for the production of multiporate pollen in wild polyploids, it may be useful to consider data from coprolites and from plant macroremains. The highest amount of multiporate Poaceae pollen in coprolites was found in Layer 275 (LA2, ~9500–8600 cal BP): the morphometric analysis of the coprolites in this sample demonstrated that young Barbary sheep, together with very few adults, were kept in a stone-based enclosure [27]. Pollen analysis proved that the enclosure was likely made of reeds and other wetland plants like *Phragmites* and *Typha*, reinforced with stones at the base. Grasses were a relatively abundant plant resource easily available. The high accumulation of stems and other plant remains in the infilling of the enclosure suggests that grass fodder was largely transported to the penning area. To do this, humans repeatedly selected grasses. They made a pressure on wild cereals, thus triggering the production of multiporate pollen. The combination of pollen and archaeological evidence suggested to Rotunno et al. [27] that the fodder collected to feed the young animals was selected from high stands of weeds and wild cereals, mostly growing close to wet environments, known to the gatherers and repeatedly visited to collect wild cereals for food [23].

The abundance of wild cereal caryopses and spikelets at the site also documents an undoubted predilection and selection ability of certain nutrient-rich wild cereal species by Takarkori's inhabitants. Human exploitation of wild grasses, their accumulation and cultivation can be regarded as forms of anthropogenic stress. Accumulations of wild grains used as food and, likely, as fodder and litter, related to the Late Acacus and Middle Pastoral periods were common in the area. In Takarkori, the concentration of seeds was visible as accumulations of different species during the Late Acacus and Pastoral phases.

In the four millennia that wild grains have been exploited by the people of the Central Sahara, there is evidence that systematic harvesting and processing of these grains evolved more than once into cultivation. As it seems that wild species have never acquired all the characteristics proper to domestication, a cultivation without domestication was suggested by Mercuri et al. [23]. Nevertheless, anthropogenic pressure on the grains themselves caused noticeable changes in the morphology of the seeds of *Panicum laetum*, which became rounder, and *Echinochloa colona*, which became more elongated [23]. Jungle rice-*E. colona* was probably brought to the Takarkori area by pastoralists. The harvested plants were also used as fodder for animals, a kind of pressure that reasonably increased during the Pastoral period [20,23]. At the beginning of the Middle Pastoral period, the plant coexisted with a very similar species, liverseed grass-*Urochloa panicoides*; later, an increasing expansion of jungle rice at the expense of liverseed grass was favoured by domesticated sheep and goats overgrazing that facilitated *Echinochloa*, a more competitive, opportunistic, and invasive species, and by human selection [23]. Interestingly, intermediate forms and hybrids are currently known for *Urochloa* and *Brachiaria* [45], which are so similar that ambiguous morphologies of caryopses are common in the archaeobotanical records of the Tadrart Acacus area (e.g., [30,46]). The presence of multiporate pollen of Poaceae in the Middle Pastoral period could therefore also be explained in the context of continuous and increasing anthropic pressure by pastoralists, an action that took place during a long phase of climatic instability.

4.2. Multiporate Pollen as Palaeoecological Marker

Takarkori's multiporate pollen of Poaceae is an important pollen marker that for the first time could offer the possibility of obtaining palaeoecological information from an anomaly rather than from the normal morphology of the identified pollen.

Recently, a similar consideration was made on gymnosperms found in a 320 million years old deposit. There, a 3% limit of anomalous pollen was taken as an indicator of increased environmental stress. Based on field observations, one sac or three sacs malformation rate < 3% is normal for conifers under low stress; however, the exposure to UV-B radiation induced trees to produce 3-sac pollen. In the paleo-deposit, pollen with 3-sacs instead of 2-sacs was common, but a spike in 3-sacs pollen was interpreted as an indicator of ozone weakening events [47]. As in the case study of Takarkori, this research showed that certain types of malformations may be useful to indicate what kind of stress those plants were experiencing. It is likely that these anomalies have brought, and still can bring, benefits to the species that are capable of them. Variability in pollen tetrad morphology and the size of *Classopollis*, another extinct gymnosperm pollen type, revealed evidence for unreduced $2n$ pollen: the polyploid hybrids, which were more stress tolerant, may have reduced the ancient extinction risk of these plants during the environmental crisis at the Triassic–Jurassic transition, 200 million years ago [48].

In general, doubling of the genome may be accompanied by diversification in gene expression and epigenetic changes, which are important for controlling phenotypic plasticity and promoting adaptation to environmental stress conditions, such as rapid climate change [4]. Accordingly, the benefits of polyploidy could promote the adaptation of species to changing environments and their continuous transformation [49]. In the particular case of multiporate instead of monoporate pollen, studies on the mechanical model of pollen grain shape and exine show that the pores deform significantly more than the exine as the pollen swells. Therefore 'the presence of the pores relieves the stress on the exine and

reduces it below the values which it would attain if the pores were not present' [50]. This can be of strategic importance to maintain pollen viability during rapid environmental changes or continuous stresses.

5. Conclusions

This article shows for the first time the use of anomalous pollen as an environmental marker (bioindicator) applied to Holocene archaeological contexts. The coexistence of plant species with phenotypic plasticity and some kind of stress is indisputable in the Takarkori chrono-cultural-sequence. Many grass species were involved, as evidenced on the one hand by the abundance of archaeobotanical records, especially Paniceae and Andropogoneae, and on the other hand by the morphological variety of the multiporate pollen grains.

However, it is not easy to determine whether climate change or anthropogenic pressures were the cause of the stress. This could have been in agreement with phases of natural environmental changes that involved major transformations in wadi and lake water availability and vegetation cover in the Sahara during the Holocene. Stress could also have been caused by the continuous selection of wild cereals harvested for food or fodder. The Takarkori pollen record suggests that climate change could have played a major role in the early Holocene. The occurrence of multiporate pollen and the increase of shrubby vegetation during dry periods can be considered primarily as consequences of the climatic changes during that period. During the periods of grass reduction and dryness expansion, polyploids were more common and humans did not hesitate to collect and transport to the site these plants that ensured consistent and predictable characteristics. The continuative anthropogenic pressure on Poaceae in the region brought about by their exploitation, cultivation, and the spread of pastoralism, maintained polyploid populations assuring food and fodder throughout environmental crises. Human pressure became relatively stronger even on polyploids during the late middle Holocene, eventually resulting in overexploitation that irreversibly reduced these resources [31].

Polyploids and amphiploids, which are one of the determinants of multiporate pollen formation, largely reproduce by apomixis. This mode of reproduction naturally occurs in about 35 families of angiosperms, and today is chosen in agriculture because it ensures the perpetuation of clonal genotypes through self-reproduction of seeds (agamospermy) and without the loss of vigor of the polyploid hybrid [51]. In the present period of profound and rapid climate change, affecting both wild and cultivated plant species, with significant repercussions on all human populations, the Poaceae family continues to have the same enormous attractiveness for humans, considering that it includes the grains that form the basis of human nutrition in most cultures. Takarkori has once again made it possible to gain a deeper insight into the history of African wild grains. In its archaeobotanical record, it could be possible to observe the development of multiporate pollen under climatic and human stress conditions over millennia. Understanding the significance of Poaceae multiporate pollen grains may allow a better knowledge of how plants have responded and are responding to exogenous stresses and what the consequences are for the ecology of some of them, including wild cereals.

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