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The role of pollen and NPP research in palaeoenvironmental reconstruction of the Neolithic archaeological site "Gorjani-Topole" (Slavonia region, eastern Croatia)

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Abstract

The palynological study of eight samples from the Gorjani-Topole archaeological site was conducted with the aim of palaeoenvironmental reconstruction. In total, 44 different palynomorphs were recorded: 12 pollen taxa, 23 fungal spores/remains, four algal cysts, one amoebae and four palynomorphs of unknown origin. Among the non-pollen palynomorphs, *Chomotriletes* (previously *Pseudoschizaea*) occurred with the greatest frequency and abundance. The low pollen richness accompanied by the presence of only a few sporopollenin-rich pollen types indicates unfavourable preservation conditions in the analysed core samples. The dominance of erosion/desiccation indicators supports the scenario of significant oscillations of the hydrological level in the sediments, with periodic drying of the substrate. Although the interpretation of changes in plant cover, due to the reduced pollen spectrum, is not feasible, it is still possible to indicate paleoecological trends and partially interpret them, considering the indicator value of the few preserved palynomorphs. The findings of cultivated grass pollen (Cerealia) and other anthropogenic indicators including weed pollen (*Convolvulus arvensis*) are of great importance. Moreover, *Riccia* moss spores or fungal *Epicoccum* spores could also indicate the anthropogenic impact on the study area.

1. INTRODUCTION

1.1. General background

Palynological analysis provides a window into the past landscape (TRAVERSE, 2007) and vegetation history reconstructed by high-resolution pollen analysis which is an important tool in the understanding of climatic change, ecological restoration and recording human impact on the environment (SHANG & LI, 2010). In *sensu stricto* meaning, palynology allows plant identification on local/extra-local or regional levels (considering plants' dispersal strategies) and reconstruction of plant cover (in the case of statistically significant proportions of palynomorphs in the substrate samples). Moreover, ecological indicator values of particular pollen species highlights temperature changes, hydrological or trophic substrate level in the particular study area and possible human impacts on the environment (COURT-PICON et al., 2006; ANDRIČ, 2007; FEURDEAN et al., 2013; KULKARNI et al., 2016; HRUŠEVAR et al., 2020). In *sensu lato* meaning, other microorganic proxies known as non-pollen palynomorphs (NPPs), including amoebae, fungal spores, algal cists, zooclast, etc., can be useful in explaining and confirming palaeoenvironmental changes (KARPIŃSKA-KOŁACZEK et al., 2014; DIETRE et al., 2016; DOYEN & ETIENNE, 2017; WOJEWÓDKA & HRUŠEVAR, 2020; WELC et al., 2021; DRUZHININA et al., 2023). Due to their restricted dispersal possibilities, NPPs often provide a local signal which can be directly related to anthropogenic activities (SHUMILOVSKIKH & VAN GEEL, 2020). In archaeological research and interpretation, pollen and NPP analysis becomes almost a standard tool for understanding the interaction between humans and nature (BAKELS, 2020; SHUMILOVSKIKH & VAN GEEL, 2020), although a multiproxy approach often depends on project finances and execution time (DRUZHININA et al., 2023). Charcoal particles, as indicators of fire history, are created by the burning of vegetation, often caused by human activity (MOONEY & TINNER, 2011). However, the distinction between naturally caused and artificial fires still remains problematic in interpretation. Since botanical data for the Gorjani-Topole (GT) site during the Neolithic are completely missing, the aim of this work was to use both pollen and non-pollen palynomorphs for palaeoenvironmental reconstruction. More specific objectives were: (i) to obtain additional information about the Neolithic flora of eastern Croatia, (ii) to gain information about the human economy of that area, and (iii) to enable a better understanding of the depositional processes in loess deposits.

1.2. Location, physical environment and vegetation

The Gorjani-Topole (GT) archaeological site is located in Slavonia, eastern Croatia (Fig. 1), approximately 10 km northwest of Đakovo town and 2 km east of Gorjani village. Administratively, the area belongs to the Osijek-Baranja county.

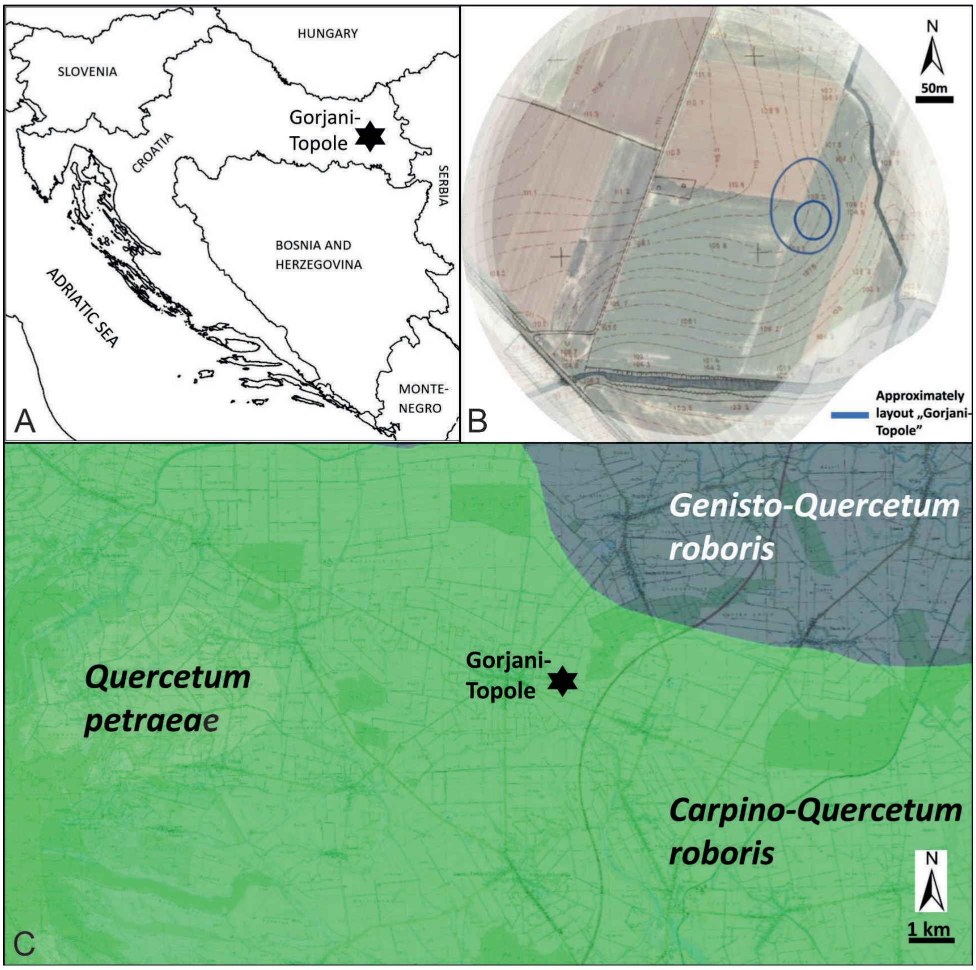

Figure 1. A) The study area: the position of Gorjani-Topole within the national/regional borders; B) Position of the archaeological site on a local level, recognised below intensively cultivated arable land; C) Potential vegetation (if the anthropogenic impact was absent) on the broader area.

The village was an important medieval centre, situated on the northern edge of the Đakovo loess plateau (NJEGAČ, 2002), built mainly from loess and loess-like Quaternary deposits (KOROLIJA & JAMIČIĆ, 1989 a,b; LEHMKUHL et al., 2021). Cores were acquired in locations where loess was deposited (Fig. 2a). The precise position of the study site is defined by the coordinates 45°23'N 18°23'E, at an altitude of ~ 110 m a.s.l. (ŠOŠIĆ KLINDŽIĆ et al., 2019).

According to Köppen's climatic classification, the studied site belongs to the Cfb climate type; it has a moderately hot, humid climate with warm summers with a mean temperature of the hottest month below 22 °C. In a recent decade (from 2009 to 2018 inclusive), the average annual temperature slightly exceeded 12 °C, and the average annual precipitation varied around 700 mm (KLEPO, 2020), of which about 390.2 mm fell during the growing season (LEKO, 2016). The common northwest wind blows throughout the year (KLEPO, 2020).

Today the Đakovo plateau is under high human pressure and intensively cultivated arable land prevails, therefore flood-plain oak forests and mixed oak-hornbeam forests have been strongly reduced. Only small patches of alder-oak riparian floodplain forests on nutrient-rich alluvial soils of the temperate regions of the Balkan Peninsula (alliance *Alno-Quercion roboris* Horvat 1950) and oak-hornbeam forests on the deep

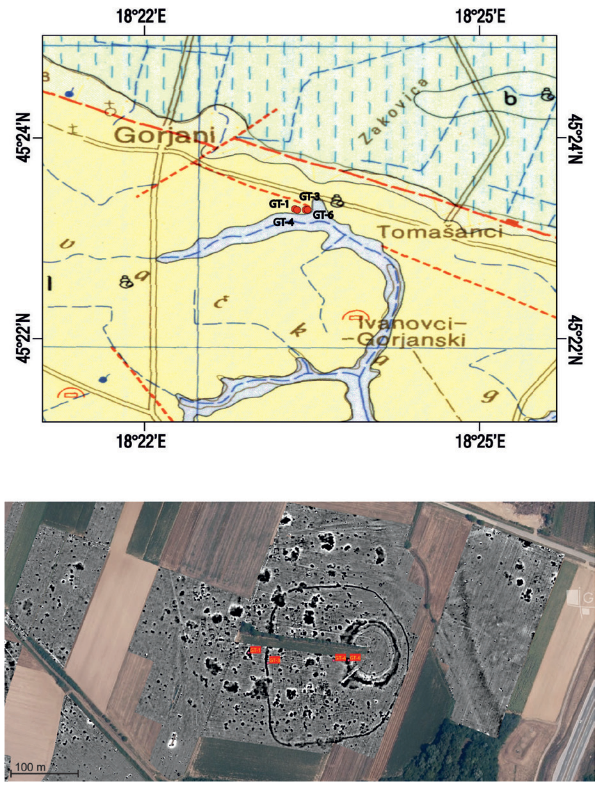

Figure 2. Position of cores 1, 3, 4 and 6 on the Basic Geological Map (KOROLIJA & JAMIČIĆ, 1989a) and a magnetogram of the site of Gorjani Topole.

nutrient-rich soils of the Balkans and Northern Italy (alliance *Erythronio-Carpinion* (Horvat 1958) Marinček) remain (BIOPORTAL, 2023). The forest coverage of the Osijek-Baranja county is 28.93 %, which is significantly below the 44 % which is the national average (ANONYMOUS, 2017). Potential vegetation of the Gorjani area within a 10 km radius includes forests dominated by the common oak (*Quercus robur* L.), sessile oak (*Q. petraea*) and common hornbeam (*Carpinus betulus*) within *Carpino-Quercetum roboris*, *Genisto-Quercetum roboris* s. lat. and *Quercetum petraeae* s. lat. (NIKOLIĆ, 2015). Beech trees of *Luzulo albidae-Fagetum* s. lat. dominated the slopes of the nearby higher hills of Dilj Mt. According to TRINAJSTIĆ (1998) vegetation of that area belongs to the European planar vegetation belt of the Euro-Siberian – North American region.

1.3. Previous archaeological research

The location of the archaeological site Gorjani-Topole (GT) was confirmed by geophysical research and archaeological excavation a few years ago (ŠOŠIĆ KLINDŽIĆ et al., 2021), although it was discovered much earlier, in 2007 from Google Earth satellite imagery (ŠOŠIĆ KLINDŽIĆ et al., 2019). Systematic excavations began in 2020, but were latter interrupted by the COVID 19 pandemic and continued in 2021 (ŠOŠIĆ KLINDŽIĆ et al., 2024). The study site, located at an altitude of 110 m a.s.l. consists of three ditches and a circular settlement, with the outer enclosure occupying 1.15 ha. The Middle enclosure covers 0.82 ha, and the inner enclosure occupied 0.82 ha. A magnetic survey of the area also showed the expansion of the settlement outside the enclosed areas. Excavation took place on part of the outer ditch, inner ditch and palisade as well as the remains of a house. A comprehensive description of the site is presented in ŠOŠIĆ KLINDŽIĆ et al. (2019, 2024).

Pottery fragments discovered on the GT site belong to the Sopot culture (ŠOŠIĆ KLINDŽIĆ et al., 2019), with a calibrated date range from 12 samples from 4900 to 4300 cal years BC (ŠOŠIĆ KLINDŽIĆ et al., 2024). South of the enclosure, some fragments of pottery belonging to the Starčevo culture were also found (ŠOŠIĆ KLINDŽIĆ et al., 2019). Thanks to the long tradition of Sopot culture research, with regular excavations from the 1970s, many archaeological sites dated ~5000 BC have been identified (ŠOŠIĆ KLINDŽIĆ et al., 2021) leading to a hypothesis that the GT study site was part of the network of settlements separated by distances of between 3 and a maximum of 6 km (KALAFATIĆ et al., 2021). Although archaeological excavations are numerous, and knowledge about the Sopot culture is accumulating, relatively little is known about animal husbandry in eastern Croatia during the Neolithic. According to TOMAC (2022), food economy in the Late Neolithic of eastern Croatia corresponds well to what is generally known from that period on a regional scale. Although the archaeozoological remains from GT were insufficient for any extensive conclusion, the nearby archaeological site of Gorjani-Kremanjača, situated less than one kilometre from the GT study site, indicates that cattle were, without any doubt, the most important animal species for the inhabitants of the broader Gorjani area, followed by pigs and caprines (sheep and goats collectively), (TOMAC, 2022).

2. MATERIALS AND METHODS

2.1. Fieldwork

Archaeological field research was conducted during the spring of 2021. Sites were cored using a gasoline powered percussion hammer Vibrokorer Eijkel kamp Cobra TT RD32 equipped with percussion gouges, used to take reasonably undisturbed samples from depths to about 7 metres, without the use of a drilling liquid (so samples were suitable for chemical analysis). Samples were extracted from the piston corer in the field and stored in a cold store at 4°C. Ten samples were prepared for grain-size analysis and eight samples for palynological analysis from four different cores (Fig. 3).

2.2. Laboratory work

The acid-alkaline treatment of the sediment was done at the Croatian Geological Survey. Sediments were passed through the 250 μm and 10 μm mesh sieves, so the analysed organic fraction was between these specified sizes. In order to enable mutual comparison of the size differentiated samples analysed, *Lycopodium* tablets (STOCKMARR, 1971) of known spore concentration (Batch no. 280821291; 13761 spores/tablet) were added to each sample. The preparation procedure included treatment with cold HCl for carbonate removal and HF for silicate removal, while sodium pyrophosphate ($Na_4P_2O_7$) enables the dispersion of clay particles and heavy liquid ($ZnCl_2$) was applied to separate the organic matter from the undissolved inorganic fraction. The above mentioned procedure is in accordance with FAEGRI & IVERSEN (1989) and MOORE et al. (1991). Palynomorphs were stored in silicate oil.

2.3. Palynological analysis

All analysed proxies: pollen, NPPs and charcoal were counted on the same slide using an Olympus BH-2 transmitted light microscope at x400, x600 and x1000 (oil immersion), magnifications combined with the interference contrast. Photomicrographs were taken with an AmScopeTM camera adapter connected to AmScope v.3.7 camera software. Palynomorph concentration was calculated by the formula: Palynomorphs conc. = ((number of *Lycopodium* spores added) x (number of particles counted)) / ((number of *Lycopodium* spores counted) x (weight of dry sediment processed in grams)), following MAHER (1981). The pollen and plant spore identification was based on pollen keys: MOORE et al. (1991), BEUG (2015) and the reference collection of the Department of Biology, Faculty of Science, University of Zagreb. Palynological residues and slides are stored in the collection of the Croatian Geological Survey. The identification of non-pollen palynomorphs was based on the following publications: VAN GEEL et al. (1983), VAN GEEL et al. (1989), SCOTT (1992), PANTALEÓN-CANO et al. (1996), KUHRY (1997), CARRIÓN & VAN GEEL (1999), CARRIÓN & NAVARRO (2002), REVELLES et al. (2016), REVELLES & VAN GEEL (2016). The NPP names were adjusted to those of MIOLA (2012). Newly described palynomorphs are followed by the abbreviation ZAG, already used for palynomorph description in collaboration with the University of Zagreb, Faculty of Science and Croatian Geological Survey (eg. DRUZHININA

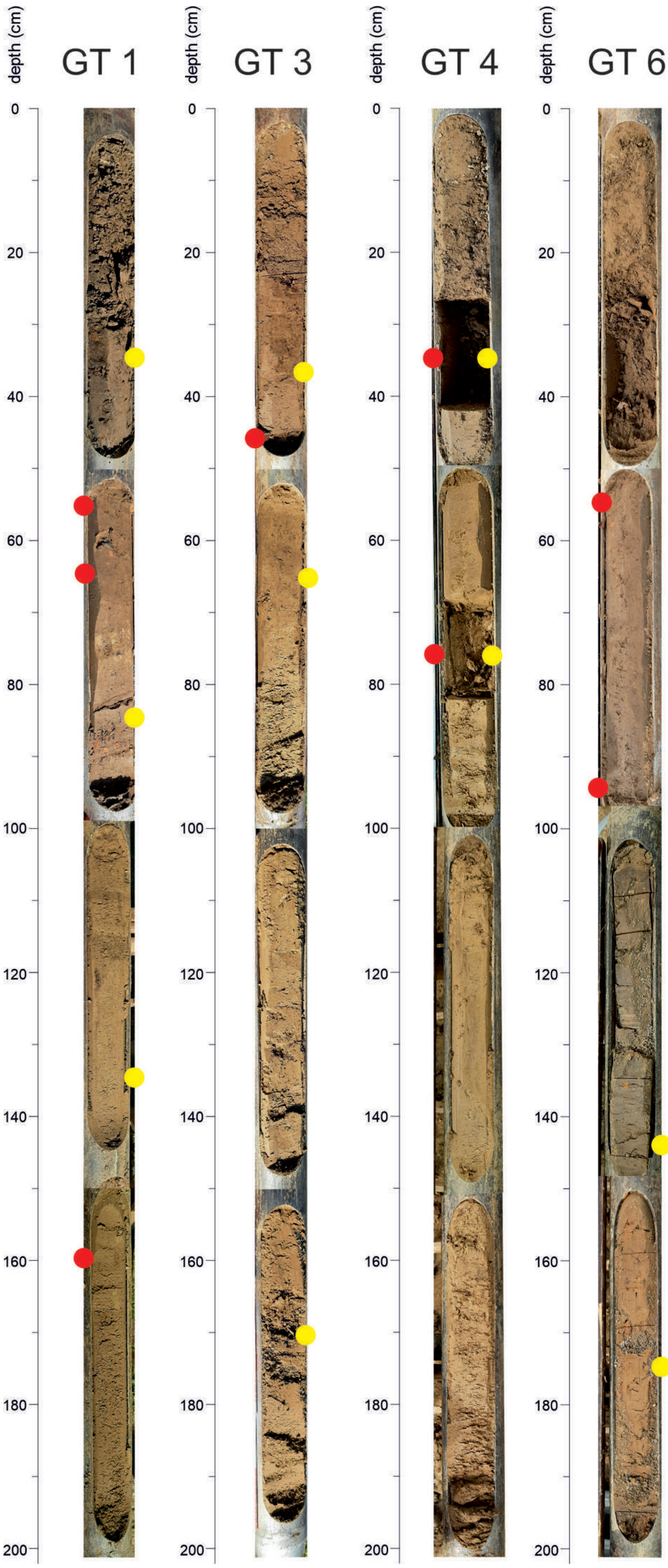

Figure 3. Positions of the samples; red dots for palynology, and yelow dots for grain-size analysis.

et al., 2023). On the same palynological slides pollen, NPPs and charcoal were observed and counted.

3. RESULTS

The multiproxy study includes eight samples, isolated from the four cores: GT-1, GT-3, GT-4, GT-6 that were conducted with the aim of palaeoenvironmental reconstruction.

Grain-size analysis identifies silt as the dominant grain-size fraction in all the studied samples. In addition, the samples contain small amounts of clay-sized (13-21 %) and sand-sized (1-8 %) minerals (Suppl. 1).

3.1. Palynomorphs and charcoal

In total, 44 different polymorphs were recorded: 12 pollen taxa, 23 fungal spores/remains, four algal cysts, one amoebae and four palynomorphs of unknown origin (Suppl. 2). Within non-pollen palynomorphs, 22 previously undescribed morphotypes were recorded here based on morphological features (Pl. 1, Table 1). Palynomorph concentrations range from $5x10^3$ to $2x10^5$ palynomorphs per gram (Suppl. 3). For reconstruction of possible palaeoecological trends, each palynomorph was assigned to one of four different ecological categories: i) indicators of wetness, ii) indicators of erosion/dessication, iii) indicators of anthropogenic influence and iv) unknown (Fig. 3).

The GT-1 (158-168 cm) sample contains 5,504 pollen grains/g, 33,026 NPPs/g and 517,414 charcoal particles/g (Suppl. 3). Within arboreal pollen (AP), only *Corylus* was observed, and non-arboreal pollen (NAP) is completely missing. NPPs were dominated by HdV-351 and *Nigrospora*, and only one *Chomotriletes* was counted (Suppl. 2). Charcoal particles were rare (Suppl. 2). Indicators of anthropogenic influence prevailed, and indicators of erosion/dessication were sparsely presented (Fig. 4).

The GT-1 (60-70 cm) sample contains 8,257 pollen grains/g, 123,849 NPPs/g and 1.629,302 charcoal particles/g (Suppl. 3). Within AP, only *Quercus* was observed, and NAP is completely missing. NPPs were dominated by *Chomotriletes*, succeeded by HdV-38 and HdV-200. Charcoal particles were numerous (Suppl. 2, Suppl. 3). Only indicators of erosion/dessication were represented (Fig. 4).

Sample GT-1 (50-60 cm) contains 5,504 pollen grains/g, 126,601 NPPs/g and 11.504,196 charcoal particles/g (Suppl. 3). Within AP, only *Alnus* was observed, and NAP is completely missing. NPPs were dominated by *Chomotriletes* and *Glomus*. Moreover, in this sample *Chomotriletes* and charcoal particles reach the highest

Table 1. Descriptions of newly observed NPP types.

NPP TYPES	DESCRIPTIONS
ZAG-4	Fungal spores, claster of three cells, each 18 x 14 µm; monosepate, slightly constricted at septum, dark redish-brown colour.
ZAG-5	Multicellular fungal palynomorph with eight cells, irregular in shape (subspherical to cuneat) and dimension (shortest diameter 10 µm, longest diameter to 30 µm), monoporate, surface of cells psilate to scabrate, dark redish-brown colour.
ZAG-6	Multicellular fungal palynomorph with seven cells, iregular in shape (subspherical to cuneat) and dimension (shortest diameter 7 µm, longest diameter to 12 µm), aporate, surface smooth, translucent- brown colour.
ZAG-7	Multicellular fungal palynomorph, probably conidia; total lengh 70 µm; cells irregular in shape (subspherical to rectangular), with diameter < 10 µm; brown colour.
ZAG-8	Multicellular fungal palynomorph with seven cells; total lengh 40 x 30 µm; cells irregular in shape (mostly rectangular), approx. 10 to 15 µm in lenght; surface smooth; brown colour.
ZAG-9	Probably fungal palynomorph; multicelular; total lengh 40 x 25 µm; cells irregular in shape (mostly rectangular), approx. 5 to 15 µm in lenght; surface smooth; reddish.
ZAG-10	Multicellular fungal palynomorph, ascospores or conidia; total lengh 70 x 30 µm; cells irregular in shape (mostly rectangular to cuneat), approx. 5 to 15 µm in lenght; surface smooth, translucent-brown colour.
ZAG-11	Multicellular fungal palynomorph with 16 cells, tapering to a point at one end of axi, triserial, linear; each cell irregular in shape (subspherical to rectangular) and dimension (shortest diameter 8 µm, longest diameter 26 µm) with total length of filament 90 µm; surface smooth to psilate; dark brown colour.
ZAG-12	Multicellular fungal palynomorph with 14 cells, tapering to a point at one end of axi, curved; cells mostly rectangular in shape with dimension 5-7.5 x 7.5-10, triangular to a point end of axi; reddish.
ZAG-13	Multicellular fungal palynomorph with 20 cells, very similar to ZAG-12 but always found without end of axi; cells mostly rectangular in shape and slightly larger than ZAG-12 with dimension 7.5-10 x 12-15 µm, triangular at the bend of the structure; reddish.
ZAG-14	Fungal spore measuring 32 x 25 µm; cylindrical-subfusiform in shape; heteropolar, truncate at the basal side and apical at the end, with two narrow arched pores; brown.
ZAG-15	Fungal spore from class Sordariomycetes, measuring 45 × 35 µm; cylindrical in shape; heteropolar, truncate at the basal side and apical at the end, with no visible pore; brown.
ZAG-16	Multicellular fungal conidia; dimension 70 x 55 µm; cells irregular in shape, mostly rectangular; each cell approx. 12 x 10 µm, inaperturate; surface smooth, translucent-brown colour.
ZAG-17	Multicellular fungal palynomorph, always incomplete, probably remains of conidia; cells irregular in shape, mostly rectangular, trapezoidal or pentagonal; each cell approx. 15-20 x 10-12 µm, inaperturate; surface smooth, translucent-brown colour.
ZAG-18	Unbranched hyphae, long-septate.
ZAG-19	Branched hyphae, short-septate.
ZAG-20	Microfossil hyaline, globose, 25 µm in diameter, ornamented with numerous, densely arranged, anastomosing processes; similar to HdV-989 (CARRIÓN & VAN GEEL, 1999) but smaller.
ZAG-21	Globose spores of unknown origin; dimension 25 µm in diameter, protruding spines 7,5-10 µm long; reddish; similar to HdV-182 (VAN GEEL et al., 1983; CARRIÓN & NAVARRO, 2002) but slightly bigger, with less visible reticulum.
ZAG-22	Globose spores of unknown origin; dimension 20 µm in diameter, protruding spines 5-7,5 µm long; brown; similar to HdV-182 (VAN GEEL et al., 1983; CARRIÓN & NAVARRO, 2002) but with less visible reticulum.
ZAG-23	Probably resting egg of unknown origin, dimension 90 x 55 µm; muri made irregular surface pattern; yellow colour.

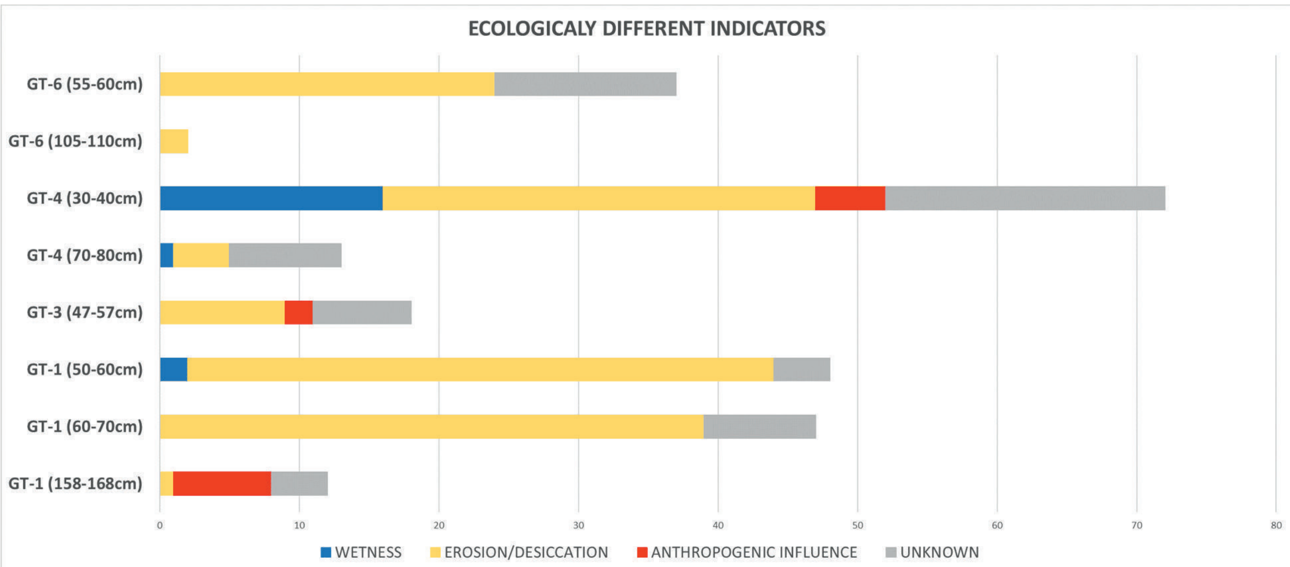

Figure 4. The proportion of various ecological indicators based on counted palynomorphs within 100 *Lycopodium* spores in each analysed sample: indicators of wetness (*Alnus*, trilete, Antocerotidae, *Riccia*, *Assulina*, HdV-225, HdV-984, HdV-989, ZAG-20), erosion/desiccation indicators (*Glomus*, HdV-200, UAB-7, UAB-48, *Chomotriletes*), anthropogenic indicators (*Senecio, Xanthium, Convolvulus arvensis*, Cerealia, HdV-351), unknown (almost all newly described palynomorphs and other pollen and NPPs not included in the any abovementioned categories).

abundance (Suppl. 2) and concentration values (Suppl 3). Indicators of erosion/dessication prevailed, and indicators of wetness were rare (Fig. 4).

The GT-3 (47-57 cm) sample contains 5,504 pollen grains/g, 44,035 NPPs/g and 4.161,326 charcoal particles/g (Suppl. 3). Within NAP only *Xanthium* t. was observed while AP is completely missing. The most abundat NPP is *Glomus*, reaching here the highest value in comparison with others samples (Suppl. 2). Charcoal particles were numerous (Suppl. 3). Indicators of erosion/dessication prevailed, and indicators of anthropogenic influence were sparsely presented (Fig. 4).

Sample GT-4 (70-80 cm) contains 8,257 pollen grains/g, 33,026 NPPs/g and 404,573 charcoal particles/g (Suppl. 3). Within AP, only *Pinus* and *Tilia* were observed, NAP is missing and local vegetation is presented by Antocerotidae. NPPs were not abundant, albeit with the relative domination of *Chomotriletes,* fungal tissue and UAB-7 (Suppl. 2). Charcoal particles were rare (Suppl. 3). Palynomorphs of unknown ecological value prevailed, succeeded by indicators of erosion/dessication and wetness (Fig. 3).

The GT-4 (30-40 cm) sample contains 38,531 pollen grains/g, 165,132 NPPs/g and 1.857,735 charcoal particles/g (Suppl. 3). Within AP, only *Pinus* was observed. The NAP spectrum includes *Senecio* t., *Convolvulus arvensis* and Cerealia, while the local vegetation includes undifferentiated trilete spores, Antocerotidae and *Riccia* spores. Within NPPs, *Chomotriletes* is the most abundant, succeeded by fungal tissue, HdV-200 and HdV-984. Additionaly, two *Epicoccum* spores were counted. The amoebae *Assulina* was only observed in this sample (Suppl. 2). Charcoal particles were numerous (Suppl. 3). Only this sample contains all four ecological categories. Indicators of erosion/dessication are relatively dominant, succeeded by palynomorphs of unknown ecological value. Indicators of wetness were represented in a greater proportion than anthropogenic ones (Fig. 4).

Sample GT-6 (105-110 cm) lacks any pollen grains. NPPs concentration is 5,504 per gram, and chracoal concentrations is 1.588,019 particles per gram (Suppl. 2). Within the palynomorphs only *Chomotriletes* was observed (Suppl. 2). Charcoal particles were numerous (Suppl. 3). Due to the lack of other proxies, palaeoecological trends were unclear.

The GT-6 (55-60 cm) sample contains 5,504 pollen grains/g, 101,831 NPPs/g and 4.981,482 charcoal particles/g (Suppl. 3). Within AP, only *Corylus* was observed. *Chomotriletes* was the most abundant NPP (Suppl. 2). Charcoal particles were very numerous (Suppl. 3). Indicators of erosion/dessication were relatively dominant (Fig. 4).

4. DISCUSSION

4.1. Core-specific interpretation of the palaeoenvironment

GT-1 core

The lower part of the core (from 158-168 cm) is characterized by the absence of erosion/dessication indicators, with only one *Chomotriletes* observed. VAN DE SCHOOTBRUGGE et al. (2024) concluded that *Chomotriletes* is the valid senior synonym of a variety of taxa, including *Pseudoschizaea* and *Concentricystes*. They considered it a freshwater organism cyst, dominant in floodplain soils and ephemeral freshwater lakes. The appearance of hazel pollen (*Corylus*) coincides with the fungus HdV-35, which is usually associated with an anthropogenic presence in a particular area (VAN GEEL et al., 1981). However, the number of charcoal particles is relatively low. Some authors (PYNE et al., 1996) have shown that in fires with low burning intensity (under conditions of increased humidity), more charcoal particles are released. The presence of hazel may indicate slightly drier conditions in the lower part of the core.

The topmost layers of the core (50-60 cm and 60-70 cm depths) contain individual pollen grains of oak (*Quercus robur-pubescens* t.) and alder (*Alnus*), pollen taxa common in the plain area of central Croatia (HRUŠEVAR et. al., 2020, 2023) within flooding soil and high groundwater levels. The presence of the *Glomus* fungus and the high frequency of the non-pollen palynomorph *Chomotriletes* (previously *Pseudoschizaea*) indicate pronounced erosion processes (SCOTT, 1992; CARRIÓN et al., 2018; BRISSET et al., 2020), possibly caused by increased fire activity. Namely, within all of the analysed samples, this one is characterized by the highest charcoal concentration. The fungus HdV-200, which is common in the sediment formed by the decomposition of helophytes at the bottom of water pools during dry periods (VAN GEEL et al., 1989), further supports the indication that conditions of alternating humidity were pronounced.

GT-3 core

Xanthium pollen was observed in the sample, a species that may indicate livestock breeding/grazing (TONKOV et al., 2011). However, the finding of this secondary anthropogenic indicator is not further strengthened by non-pollen palynomorphs that would additionally confirm human influence. Charcoal particles were abundant.

GT-4 core

Pollen indicators of anthropogenic activity are absent from the lower sample (70-80 cm), and indicators of wetness are scarce, although an Antocerotidae moss spore was observed. Within arboreal pollen, pine (*Pinus*) and lime (*Tilia*) are represented. Both taxa have a high proportion of sporopollenin in the exine which makes them resistant to non-wrapping conditions of preservation (TRAVERSE, 2007), hence the reason they don't reflect the prevalent vegetation. The higher abundance of fungal hyphae and tissues, accompanied by *Epicoccum* spores probably reflects more drier conditions.

The upper sample (30-40 cm) contains the most species rich pollen spectrum within the analysed samples. Observed palynomorphs indicate the existence of a temporary oligotrophic water body, accompanied by indicators of erosion/desiccation (*Chomotriletes*). Water mosses *Riccia* and Antocerotidae, which grows on wet habitats, were represented by their spores, accompanied by HdV-225, HdV-984 and HdV-989, probably of algae origin. At the same time, it is the only sample in which an amoeba, more specifically *Assulina muscorum*, was observed. The aforementioned amoeba is an indicator of oligotrophic habitats with a lower pH substrate (VAN GEEL et al., 1989; FIŁOC & KUPRYJANOWICZ, 2015). All this indicates that conditions for preservation were somewhat more favourable than in other samples. Within plants, pollen of the field bindweed (*Convolvulus arvensis*) and one pollen grain of cereals (Cerealia) were observed. Only grass pollen > 37 µm were considered as cultivated grass (eg. DÖRFLER, 2013). Considering that some of the largest wild grasses pollen, such as *Glyceria* t., *Bromus hordeaceus* t. (WALLER et al., 2017) and *Arrhenatherum* t. (TWEDDLE et al., 2005) can easily be mistaken for cereals, the annulus diameter assessment was also taken into account.

Both taxa indicate the presence of humans and anthropogenic pressure on the studied area. Moreover, *Riccia* moss is often found in water bodies exposed to grazing (CARRIÓN & NAVARRO, 2002), similar to some taxa of *Anthoceros* which could be linked with anthropogenic pressure (KOZÁKOVÁ et al., 2015). Among the fungi, *Epicoccum* spores were observed. This is a common invasive taxa on crops (FATIMA et al., 2016) although they can be found on different materials, even in marine organisms (FATIMA et al., 2016; PIECUCH et al., 2020).

GT-6 core

The sample at a depth of 105-110 cm cannot be interpreted, even indirectly, due to the poor preservation of the palynomorphs.

The near-surface sample of this core (depth section 55-60 cm) contains individual pollen grains of hazel (*Corylus*), a shrub species that appears as a pioneer due to fire and can indicate the presence of people in an area (MITHEN et al., 2001; GROß et al., 2018). A very high concentration of charcoal particles can support anthropogenic activity, and perhaps fire is the cause of increased erosion, which is reflected in the high number of *Chomotriletes* indicators.

4.2. Palaeoecological history

In the continental biogeographical region of Croatia, reconstruction of vegetation with an emphasis on changes in plant cover, was possible only for the area of central Croatia where peatlands occur (HRUŠEVAR et al., 2020, 2023), while in eastern Croatia such data are mostly missing. For this reason, archaeological excavations provide a great possibility for understanding plant cover and changes in the vegetation of the Slavonia region (BAKRAČ et al., 2015), although they usually refer to a very local area or short time period.

However, all the analysed samples collected from four cores (GT-1, GT-3, GT-4, GT-6) were poor in palynomorphs, (Suppl. 2) and none of them reach statistical significance of approximately 300 to 1,000 grains in order to ensure the statistical robustness of the pollen percentages (KNELLER, 2009). In this example, even the counts below 150 pollen grains, proposed by some other authors (LYTLE & WAHL, 2005; KEEN et al., 2014; DJAMALI & CILLEROS, 2020) were not achieved and without the statistically significant pollen sum, the plant cover of the studied site and vegetation changes cannot be meaningfully interpreted. As the pollen spectrum is reduced in terms of both the qualitative (different pollen types observed) and quantitative (the number of palynomorphs counted) analysis, it is only possible to indicate palaeoecological trends and partially interpret them (Suppl. 2, Fig. 4), considering the indicator value of the few preserved palynomorphs. Within AP the presence of *Tilia*, *Quercus* and *Corylus* was expected in mixed oak deciduous forests, where oaks, elms, limes, ash and hazel were predominant at low to mid elevations during most of the Holocene (FEURDEAN et al., 2011; TANȚĂU et al., 2006). The presence of *Alnus* and *Corylus* (TINNER et al., 2000) or *Quercus* and *Corylus* (JAMRICHOVÁ et al., 2017) could be favoured by fire. This fits well with our samples, where charcoal particles are common. Hazel was also used as food resources by Mesolithic (KUNEŠ et al., 2008; REGNELL, 2012) and Neolithic communities (MARINOVA et al., 2013) frequently colonizing humid lowlands previously used for crops and pasture (PÈLACHS et al., 2009). There is evidence of its promotion by the Mesolithic people. They removed shade-tolerant trees (MITHEN et al., 2001) or expanded hazel by selective pruning for greater nut yield production (GROß et al., 2018). Moreover, cattle grazing could increase the ratio of light demanding hazel (KOLÁŘ et al., 2018) and promote this taxon at the expense of *Tilia* because its leaves are less palatable (HAEGGSTRÖM, 1990). All NAP types belong to anthropogenic indicators. The finding of Cerealia pollen, a primary anthropogenic indicator (BEHRE, 1981, FEURDEAN et al., 2013; MERCURI et al., 2013) and *Convolvulus arvensis,* a weedy taxa (HULINA, 1998) in summer cereals and root crops and/or fallow land (BEHRE, 1990) is of great significance. Both taxa indicate agricultural activity within the research area. Within Asteraceae *Xanthium* t. (TONKOV et al., 2011) and *Senecio* t. could be considered as anthropogenic indicators. The latter is also frequently found as an indicator of wet communities (HÁJKOVÁ et al., 2013), as it includes a great number of different species and many genera of the subfamily Asteroideae (BEUG, 2015). Despite the fact that *Senecio* t. also appears in natural communities or shows no significant relationship between its occurrence and archaeological sites in more recent publications (ABRAHAM et al., 2023), we consider it here as a synanthropogenic taxon, in the sense of LATAŁOWA (1992), KUNEŠ et al. (2015) and SERVERA-VIVES et al. (2023). Although the received AP and NAP pollen spectra indicate possible anthropogenic pressure, these taxa are also part of the natural vegetation in the studied area, and their findings perhaps explain taphonomic processes better than providing evidence of human influence.

Due to the lack of pollen in the analysed samples, NPPs can be of great importance for palaeoenvironmental interpretation (eg. SHUMILOVSKIKH et al., 2016; ENEVOLD et al., 2019.; DRUZHININA et al., 2023). Qualitative analysis of NPPs indicates the greatest diversity of fungal spores in our samples, which was partly expected due to their better preservation in soil compared to some other microfossils (SHUMILOVSKIKH & VAN GEEL, 2020). Numerous new types were observed (ZAG-4 to ZAG-23) albeit their indicator values are unknown. Quantitative analysis indicates the largest number of the palynomorph *Chomotriletes*, considered to be an alga (PÈLACHS et al., 2009). Also, *Chomotriletes* is an indicator of erosion and periodic drying of the substrate (SCOTT, 1992; CARRIÓN et al. 2018; BRISSET et al., 2020). Along with this palynomorph, *Glomus*, HdV-200, UAB-7, UAB-48 could indicate erosion/dessication processes

(REVELLES et al., 2016; BRISSET et al., 2020). Palynomorphs of probable algal origin including HdV-225 (VAN GEEL et al., 1989; KUHRY, 1997), HdV-984 (CARRIÓN & VAN GEEL, 1999), which likely belongs to the genus *Euastrum*, and to the latter very similar HdV-989 (CARRIÓN & VAN GEEL, 1999) are used here as indicators of wetness. The occurrence of HdV-351 during human habitation has been recorded by VAN GEEL et al. (1981) and MIOLA et al. (2010). As NPPs represent a large group of the resistant remains of a taxonomically wide variety of organisms, (SHUMILOVSKIKH & VAN GEEL, 2020) their determination is often difficult. Even though they are providing a very local palaeoenvironmental signal, and are considered as very useful indicators, their individual findings, in our study site, must be taken with caution. ENEVOLD et al. (2019) highlighted that many of the NPP types are rare with random occurrences so that only the more frequent types may be useful and informative.

All charcoal particles were insufficiently large (< 250 μm) to be considered indicators of local fires (OLSSON et al., 2010; BURROWS et al., 2014), and can be considered as evidence of burning in the broader area of sediment sampling (FEURDEAN et al., 2015; ADOLF et al., 2018). Although the increased number of fire indicators is related to anthropogenic pressure, no signs of human activity were observed in the sample with the highest concentration of charcoal particles eg. GT-1 (50-60 cm). Additionaly, in the two samples with a high concentration of charcoal particles, eg. GT-6 (55-60 cm) and GT -3 (47-57 cm), the former is completely free of anthropogenic indicators, and in the latter, only the clotbur pollen (*Xanthium*), a secondary anthropogenic indicator (TONKOV et al., 2011), was observed.

4.3. Taphonomic processes

A low number of palynomorphs or their absence is generally a consequence of sedimentological processes and the existing geochemistry and/or climate that does not support the preservation of micro- and/or macro-plant remains, and such conditions in the sediment are often the result of the interaction of landscape and climate (GASTALDO & DEMKO, 2011). Peatlands and lake sediments are considered the most favourable habitats for palaeoenvironmental reconstructions (GODWIN, 1981; MOORE et al., 1991), while wet/swampy habitats, exposed to periodic drying, are considered unfavourable. Namely, the wetting and drying of the substrate leads to changes in oxic and anoxic conditions, which can result in an increased rate of decomposition of organic matter (carbon recycling) and a drop in the pH value of the sediment, which is why wetland habitats, although favourable in principle, often prove to be "challenging" for palynological research (GASTALDO et al., 1989; GASTALDO & DEMKO, 2011).

Although loess sediments could be good archives of palynomorphs used for palaeoenvironment reconstruction (SHANG & LI, 2010) loess-palaeosol sequences deposited under an oxidizing environment are mostly unfavourable for the reconstruction and interpretation of vegetational changes (MOORE et al., 1991; ZHANG et al., 2017), due to oxidation, microbial activity, possible high pH and deterioration of pollen grains (ZELIKSON, 1995; ZHANG et al., 2017). In this sense, it can be concluded that all the analysed (sub)samples were exposed to unfavourable processes of biostratinomy and/or diagenesis, which is why only palynomorphs with a higher proportion of sporopollenin, such as the pollen of pine (*Pinus*), lime (*Tilia*) and the aster family (*Senecio*, *Xanthium*) or trilete plant spores were the only types preserved (JACOBSON & BRADSHAW, 1981; TRAVERSE, 2007) in sediment, truncating the pollen spectrum. This is especially expected in loess and loess-like sediments, where Asteraceae pollen (ZELIKSON, 1995) including *Artemisia*, *Aster*, *Taraxacum* (ZHANG et al., 2017), Chenopodiaceae (ZELIKSON, 1995; ZHANG et al., 2017) and *Pinus* (ZHANG et al., 2017) were mostly overrepresented in the pollen spectra. High sporopollenin content in the exines of *Pinus*, *Tilia*, *Corylus*, *Alnus*, Cichoriaceae and Asteraceae (HAVINGA, 1964) makes the finding of this pollen type expected in the study site.

In contrast, grazing areas with animal breeding practices are often characterised by Asteraceae (including Asteroideae and Cichorioideae) and Chenopodiaceae NAP types, (MAZIER et al., 2006; FEURDEAN et al., 2013; FLORENZANO et al., 2013; RATTIGHIERI et al., 2013; KOZÁKOVÁ et al., 2015), which highlight difficulties in interpretation (anthropogenic vs. taphonomic influence) when pollen spectra are low.

Although NPPs can be found in any type of sediment (SHUMILOVSKIKH & VAN GEEL, 2020) the comparative impact of taphonomic processes on their preservation has not been investigated in detail. Still, microfossils composed of chitin (fungal palynomorphs, microforaminifers and scolecodonts) may be very well preserved in sediments, recording past sedimentation conditions (MEDEANIC et al., 2011; SHUMILOVSKIKH & VAN GEEL, 2020). According to SHUMILOVSKIKH & VAN GEEL (2020) Helminths eggs, insect fragments, fungi, fabers and unknown NPPs are most frequent in analysed soil samples on a global level. This is in accordance with our findings that NPPs in loess samples presented with a higher concentration and diversity (both qualitatively and quantitatively) in comparison with pollen palynomorphs, while the preservation potential of charred particles (charcoals) is even higher (BRYANT & HOLLOWAY, 2009).

5. CONCLUSIONS

The low number of different pollen taxa and their individual appearance prevents plant cover reconstruction or interpretation of vegetational change during the Neolithic Sopot cultural layer in eastern Croatia. However, all pollen taxa belong to mixed oak forests which is in agreement with the expected planar vegetation of the continental biogeographical region during the Middle Holocene. The appearance of cereal pollen, as a primary anthropogenic indicator, highlighted the human impact in the studied site, even though the rest of the observed weedy taxa probably reflect the poor preservation conditions in the soil samples (only taxa with higher sporopollenin contents were preserved). The analysed loess and loess-like sediments were better archives of NPPs than of pollen, showing a versatile abundance of algae, fungi and unknown NPPs. For this reason, interpretation of the palaeoenvironmental trends in the studied archaeological site were based more on the indicative ecological value of NPPs than pollen taxa, making possible a distinction between wetter, dynamic (erosion/dessication) or anthropogenic paleoenvironments. Charcoal particles probably reflects the regional fire history.

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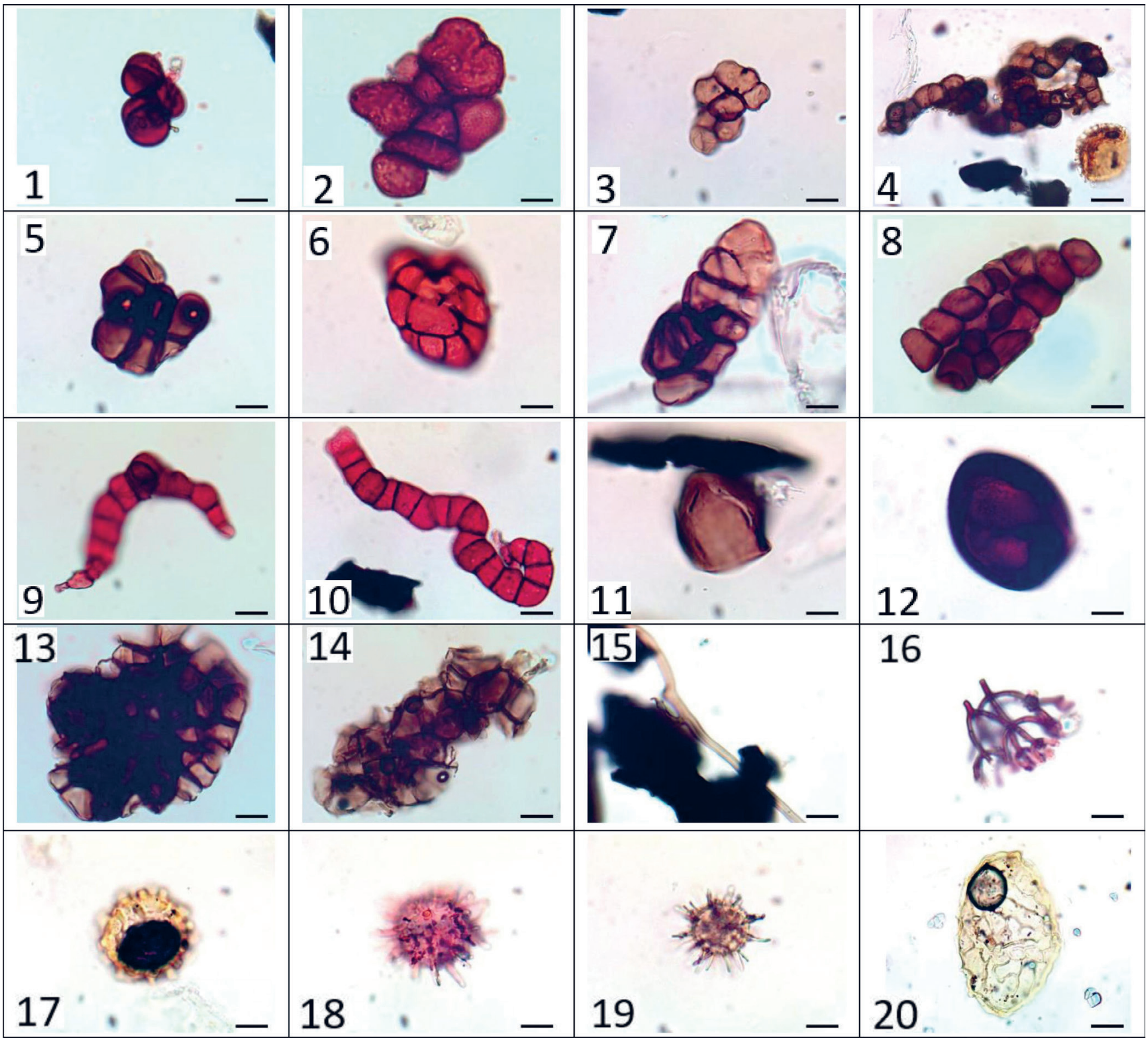

Plate 1. Description of the newly observed palynomorphs. FUNGI: ZAG-4 (1), ZAG-5 (2), ZAG-6 (3), ZAG-7 (4), ZAG-8 (5), ZAG-9 (6), ZAG-10 (7), ZAG-11 (8), ZAG-12 (9), ZAG-13 (10), ZAG-14 (11), ZAG-15 (12), ZAG-16 (13), ZAG-17 (14), ZAG-18 (15), ZAG-19 (16); ALGAE: ZAG-20 (17); UNKNOWN ORIGIN: ZAG-21 (18), ZAG-22 (19), ZAG-23 (20). Scale bar = 10 μm (photos 1-19), scale bar = 20 μm (photo 20).

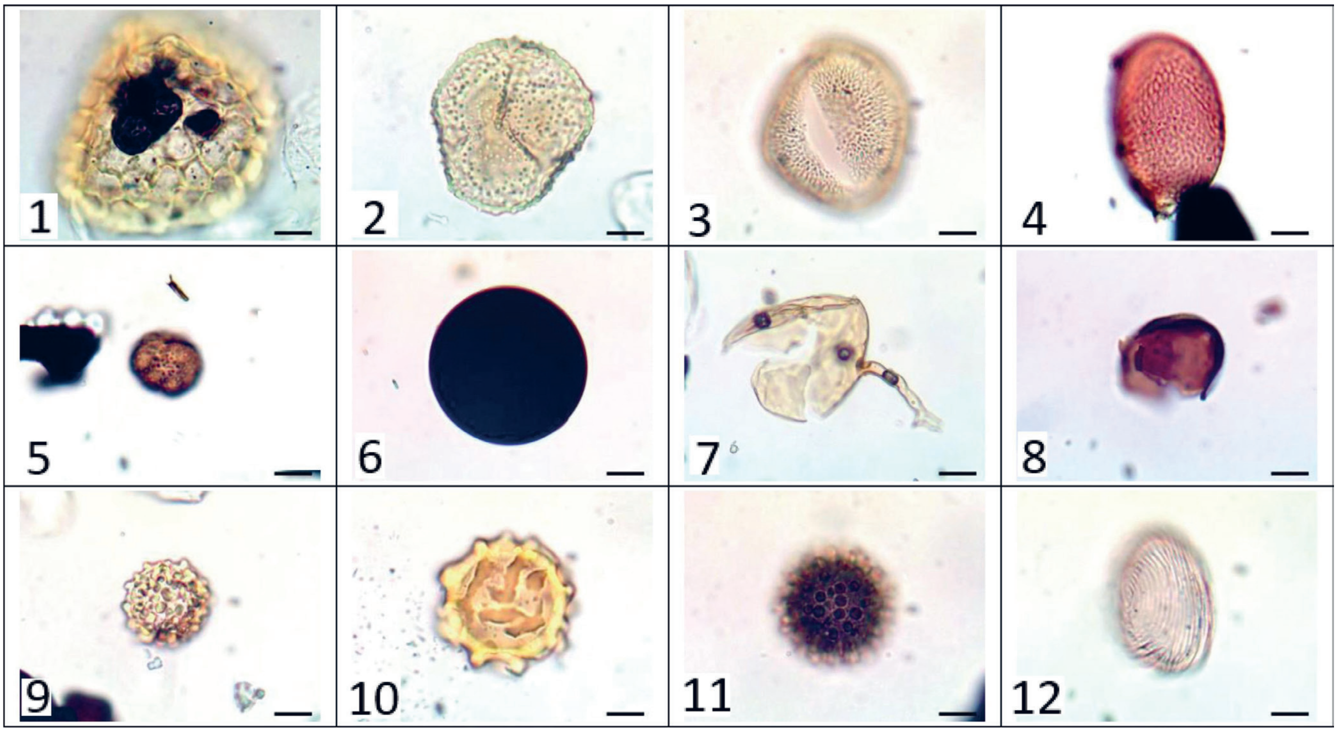

Plate 2. Some selected palynomorphs. POLLEN AND SPORES: *Riccia* (1), Antocerotidae (2), *Convolvulus arvensis* (3); AMOEBAE: *Assulina muscorum* (4); FUNGI: *Epicoccum* (5), *Nigrospora* (6), *Glomus* (7), HdV-351, family Sordariaceae (8); ALGAE: HdV-225 (9), HdV-989 (10), HdV-984 (11), *Chomotriletes* (12). Scale bar = 10 µm.

Supplement 1. Grain-size analysis.

Sample	Grain-size – Gorjani Topole		
	Sand	Silt	Clay
GT -1			
34-41	2	80	18
80-90	4	77	19
130-140	5	82	13
GT-3			
37-47	1	82	17
67-77	1	80	19
165-175	8	78	14
GT-4			
30-40	2	77	21
70-80	3	78	19
GT-6			
145-150	3	79	18
175-180	2	79	19

Supplement 2. The qualitative and quantitative content (in units) of palynomorphs.

		DEPTH/ TAXA	GT-1 (50-60 cm)	GT-1 (60-70 cm)	GT-1 (158-168 cm)	GT-3 (47-57 cm)	GT-4 (30-40 cm)	GT-4 (70-80 cm)	GT-6 (55-60 cm)	GT-6 (105-110 cm)
POLLEN	ARBOREAL POLLEN	*Pinus*					2	1		
		Tilia						1		
		Quercus robur-pubescens t.		3						
		Alnus	2							
		Corylus			2				2	
	NON-ARBOREAL POLLEN	*Senecio*					2			
		Xanthium				2				
		Convolvulus arvensis					1			
		Cerealia					1			
	LOCAL SPORES	Trilete undiff.					4			
		Antocerotidae					3	1		
		Riccia					1			
NON-POLLEN PALYNOMORPHS (NPPs)	AMOEBAE	*Assulina*					1			
	KNOWN FUNGI	*Glomus*	4			6				
		Epicoccum					2	1		
		Nigrospora			3		1			
		HdV-38		2						
		HdV-200		2			3			
		HdV-351			7		1			
		UAB-7						2		
		UAB-48						2		
	NEWLY DESCRIBED FUNGAL PALYNOMORPHS	ZAG-4	1							
		ZAG-5	1	2						
		ZAG-6			1		2		7	
		ZAG-7				4	2			
		ZAG-8					2	1		
		ZAG-9						1		
		ZAG-10						1		
		ZAG-11							1	
		ZAG-12							1	
		ZAG-13							2	
		ZAG-14					1			
		ZAG-15					1			
		ZAG-16						1		
		ZAG-17					4			
		ZAG-18					1			
		ZAG-19	2	2		3	2	2	2	
	PROBABLY ALGAE	HdV-225					1			
		HdV-984					3			
		HdV-989					2			
		ZAG-20					1			
		Pseudoschizaea	38	35	1	3	28	2	24	2
	UNKNOWN	ZAG-21		2						
		ZAG-22					1	1		
		ZAG-23					1			
EXOTIC MARKER		*Lycopodium*	100	100	100	100	100	100	100	100

Supplement 3. Basic categories and their concentration values.

Depth	POLLEN	NPPs	CHARCOAL	POLLEN conc.	NPPs conc.	CHARCOAL conc.
GT-1 (50-60 cm)	2	46	4180	5,504	126,601	11.504,196
GT-1 (60-70 cm)	3	45	592	8,256	123,849	1.629,302
GT-1 (158-168 cm)	2	12	188	5,504	33,026	517,413
GT-3 (47-57 cm)	2	16	1512	5,504	44,035	4.161,326
GT-4 (30-40 cm)	14	60	675	38,530	165,132	1.857,735
GT-4 (70-80 cm)	3	12	147	8,257	33,026	404,573
GT-6 (55-60 cm)	2	37	1810	5,504	101,831	4.981,482
GT-6 (105-110 cm)	0	2	577	0	5,504	1.588,019