



PRELIMINARY REVISION OF THE PALYNOLOGICAL COLLECTION OF PROFESSOR BLANKA PACLTOVÁ – A SIGNIFICANT COLLECTION OF CENOMANIAN MICROFLORA HOUSED AT THE NATIONAL MUSEUM, PRAGUE

This paper is dedicated to the memory of professor Blanka Pacltová.

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Abstract: A preliminary revision of the palynological collection of Professor Blanka Pacltová was carried out considering samples from the middle Cenomanian of the Peruc-Korycany Formation, the basal most member of the Bohemian Cretaceous Basin (the Czech Republic). This collection is mainly composed of slides with palynological residues for light microscope study, which were mounted in the 1960s and 1970s. This work presents an evaluation of the state of preservation of this collection, taking into account the presence of ancient angiosperm pollen types. High percentage of preparations is affected by degradation of glycerine jelly and their remounting is necessary. The present study additionally suggests a methodology for curation of this collection with the objective of long-term preservation.

Key words: palynological collection, Blanka Pacltová, curation, angiosperm pollen types, early Cenomanian, Bohemian Cretaceous Basin, the Czech Republic

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Introduction

Palynology is an important discipline straddling the interface between the geological and biological sciences (Wellman 2005). The term palynology was introduced to cover broader aspects, taking into account not only pollen, but other resistant microfossils, such as dinocysts, cryptogam spores, animal remains, etc. (Faegri and Iversen 1989). Concretely, palaeopalynology consists of the study of organic microfossils that are found in maceration preparations of sedimentary rocks (Traverse 2007).

The presence of palaeopalynological collections is not unusual in research institutions, universities and museums around the world, e. g. the Geological Survey of Canada, the British Geological Survey (UK), the Research Center of the Slovenia Academy of Sciences and Arts, the University of Sheffield (UK), Monash University (Melbourne, Australia), the National University of La Plata (Buenos Aires, Argentina), Harvard University (USA), the Naturhistoriska riskmuseet (Sweden), the Florida Museum (USA), the Natural History Museum (UK), the National Museum of Natural Sciences (Spain) and the National Museum (Prague, the Czech Republic); they generally house important collections (e.g.

Rushton 1979, Wellman 2005, Yañez et al. 2014). However, although these collections have great scientific and historical value, they usually go unnoticed in museums, because they are not attractive elements for their exhibitions.

The National Museum in Prague houses large palaeobotanical collections. One of the most important palaeopalynological collections of this museum was donated by Blanka Pacltová, Professor at the Faculty of Science of the Charles University in Prague (Svobodová 2008). Her collection comprises hundreds of slides of palynological material, mainly from the Late Cretaceous of the Bohemian Cretaceous Basin. These slides preserve Cenomanian to Coniacian microfloras and type specimens of early angiosperm pollen grains, published mainly in the 1960s and 1970s (e.g. Góczán et al. 1967, Pacltová 1968, 1971, 1977, 1979, 1981, Pacltová and Krutzsch 1970).

The interest in making a comparison between the Spanish mid-Cretaceous palynofloras linked to amber deposits (e.g. Barrón et al. 2015) and the Bohemian fossil sites led us to apply for a Synthesis Grant, in order to revise the Pacltová collection in the National Museum, Prague. The work was carried out during two weeks of the months of March and April, 2017. Reviewing a part of the collection allowed us to

develop some conservation guidelines for better preservation of palynological samples and preparations.

Therefore, the aims of the present paper are: (1) to publish initial data about the state of preservation of the Pacltová collection, (2) to make a brief description of some early Cenomanian angiosperm pollen preserved in the slides suitable for lectotype or neotype designation, and (3) to suggest ideas for restoration and long-term preservation of this collection.

Geographic and geological setting

The palynological material described in this paper was collected from the Bohemian Cretaceous Basin (BCB). It comes from the Peruc-Korycany Formation forming the basal-most lithostratigraphic unit of the BCB (Čech et al. 1980, Čech 2011). The age of the Peruc-Korycany Formation is bounded above by ammonites of the late Cenomanian *Calycoceras guerangeri* and *Metoicoceras geslinianum* zones in the Korycany and Pecínov members of the formation (Čech 2011). Palynological data indicates a late – middle Cenomanian age for these beds (Pacltová 1977, 1979), and sequence stratigraphic analyses by Uličný et al. (1997, 2009) indicate that they belong to the CEN 4 cycle of late – middle Cenomanian age.

Brief biography of Blanka Pacltová

Blanka Pacltová was born on October 18, 1928 in Nechanice near Hradec Králové. Later her parents moved to the nearby village of Kratonohy, where she spent most of her childhood. After finishing school, B. Pacltová went to study at a gymnasium in Prague, where she graduated in 1948. She was accepted to the Faculty of Science of Charles University in the fields of chemistry and biology, and after the creation of the geological faculty, she studied geology-palaeontology, specializing in palaeobotany. There she met Professor František Němejc, and during the last year of her study, she began working as an assistant in the Department of Geology and Palaeontology. She graduated in 1952, finished her PhD studies in 1959, and habilitated in 1963. Due to her political neutrality during the normalization after 1968, she was unable to get her professorship, but was finally able to get it in 1990, after the Velvet Revolution.

During the period 1955 to 1995, she taught numerous students. She trained several generations of Czech and Slovak palynologists – Magda Konzalová, Milada Vavrdová, Oldřich Fatka, Pavel Dufka, Jiří Bek, Marcela Svobodová, Nela Doláková, Renáta Pátová, Marie Boháčová, Eva Hubená, Eva Planderová, Paulina Snopková.

She was a supervisor of a number of foreign students: Urmila Ganguli (India), Miklós Juhász (Hungary), Gamal M. A. Lashin (Egypt), and a consultant for a number of world specialists, e.g. James A. Doyle, Geoffrey Norris (USA), Hans-Dieter Pflug, Wilfried Krutzsch (Germany), Norman F. Hughes (UK) and Henriette Méon (France).

Professor Blanka Pacltová is considered as a founder of palynology in former Czechoslovakia. She combined teaching her students with building the field of palaeopalynology, and was able to demonstrate its necessity not only for addressing

questions of development in organic life, but also the utility of palynology in practice. With assistance from Professor Odolen Kodym, she built a specialized palynological laboratory and established a library of scientific papers from all over the world, which was essential, but difficult to acquire during the totalitarian regime's rule of the country. She published more than 200 scientific papers, covering essentially all geological formations and microfossils of all plant groups. She tied her research on peat bogs to works by Karl Rudolph, took part in research on Southern Bohemian basins, worked on Tertiary coal-forming basins, and occupied herself with the evolution of angiosperms. Her work on the Normapolles complex is well known. In later years, she turned her attention to the oldest organisms of the pre-Cambrian, and micropalaeobotanical research on the origins of Barrandian stromatolites (Dašková 2008). For more details of life of Prof. Blanka Pacltová see Svobodová (2019).

Material and methods

A partial revision of the Pacltová collection was focused on levels from the middle Cenomanian of the Peruc Korycany Formation (of the Bohemian Cretaceous Basin). Concretely, 16 sedimentary layers from borehole VL-1 (samples VL-1/394 to VL-1/409) in Vrbno nad Lesy near Louny (south of the Ohře River area) and 23 horizons from the Ln-1 Louny borehole (samples Ln-1/280 to Ln-1/286 and Ln-1/290 to Ln-1/305) in western Bohemia were revised (Appendix). All these samples were prepared in the 1960s at Charles University in Prague. Thanks to a personal donation made by Prof. Blanka Pacltová, these palynological samples are now housed in the National Museum, Prague.

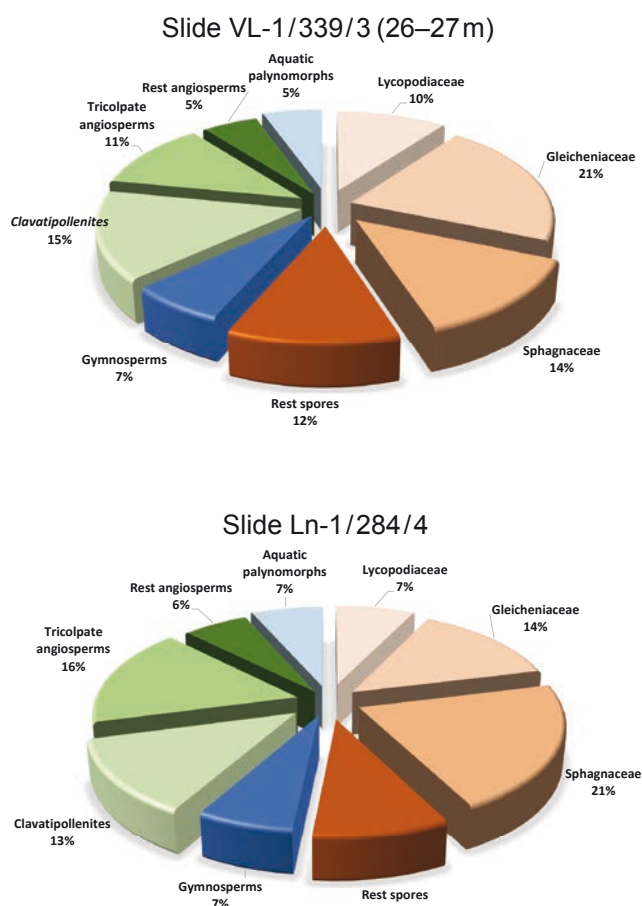
Samples were prepared using standard techniques based on acid treatment (hot HF, 10% HCl, HNO₃; see e.g. Phipps and Playford 1984). Resultant residues were mounted in glycerin jelly on glass slides (76 × 26 mm). After that, they were covered by transparent glass coverslips. Later, after the glycerin jelly solidified, the edges of the coverslips were sealed with Canada balsam (Pacltová 1971). Three slides per horizon were prepared from borehole VL-1, while five per horizon were made from borehole Ln-1 (Appendix). A laboratory label with the data of each level was affixed to each slide. Final laboratory residues have not been generally preserved. Additionally, a small number of type samples from the Cenomanian localities of Kněževes, Hostivice, Nové Strašecí, Zdětín, Praha-Barrandov, Brník and Roubanina were revised.

After light microscope study of the samples, and microphotographs taken with immersion objectives of the most relevant specimens, slides were placed in a vertical position in black standard microscope light boxes. In several cases, the boxes also contain a handwritten paper in pencil that indicates the borehole and the prepared levels, as well as the date of preparation.

The present microscope analysis of the slides was performed using an Olympus BX 50 light microscope equipped with an Olympus DP 74 camera at the Department of Palaeontology of the National Museum, Prague.

In order to know the importance of angiosperms in the flora of the Bohemian middle Cenomanian, a quantitative study

was carried out in five levels (one slide per level): VL-1/397/1, VL-1/402/1, VL-1/400/3, VL-1/399/3 and Ln-1/284/4. Due to the bad state of slide preservation, only two levels (VL-1/339/3 and Ln-1/284/4) were finally used, containing more than 100 palynomorphs. An assemblage quantitative analysis graphically presented in percentages was constructed using Microsoft Excel (Text-fig. 1).



Text-fig. 1. Quantitative results expressed as percentages of the main taxa palynological identified in level 339 of borehole VL-1, and in level 284 of borehole Ln-1 Louny.

Results

Preservation of slides

Most of the palynological slides of the Pacltová collection from the studied boreholes are poorly preserved, and in great part seriously degraded. As mentioned above, slides with palynological residues were mounted with glycerin jelly on glass slides, covered with glass coverslips and sealed with Canada balsam. Glycerin jelly is one of the preferred mounting mediums for fossil palynomorphs (Moore et al. 1991). It has excellent optical properties and can be handled easily (Batten and Morrison 1983). Its main disadvantage is that it absorbs water from the atmosphere, and this causes palynomorphs to swell when mounted in it (Faegri and Deuse 1960). Generally, palynomorphs remain in position

in slides mounted with glycerin jelly. It is possible to locate them from mechanical stage map references and return to them for further observation and comparison at a later date (Moore et al. 1991).

Glycerin jelly is composed of glycerol, gelatin and phenol or thymol (Hill 1983, Zander 1997). The two latter substances prevent any microbiological growth in the slides. However, they only work as an antibiotic for a few years after preparation, and if coverslips are poorly sealed, microorganisms quickly colonize and degrade the preparations.

After a careful microscope revision of Pacltová's slides of the previous mentioned levels, we observed that the most of them have the coverslips not well-sealed. Through time, the Canada balsam dried, cracked and became detached from coverslips in the most of the studied slides. Consequently, the glycerin jelly, which includes the palynomorphs, is exposed to environmental conditions, undergoing (i) loss of moisture and (ii) colonization by fungi. The former produces reduction of the volume of the glycerin jelly (Pl. 1, Fig. 1), formation of bubbles, and change of the orientation of the palynomorphs and their concentration in different places of the slides (Pl. 1, Fig. 9). The latter causes total destruction of both the glycerin jelly and the palynomorphs, since fungi metabolize the organic matter.

Furthermore, the slides were stored in a vertical position into wood microscope slide boxes. These boxes have one row of racks, to easily store and remove the slides. Glycerin jelly used to mount the palynomorphs is not a solid, but a semi-fluid gel. Due to the vertical orientation of the slides in boxes, the palynomorphs and inorganic particles (e.g. cuticles and coal fragments, mineral remains, etc.) slowly moved from their original positions to the bottom of the preparation slide.

Finally, palynological slides have to be in airtight boxes, but the lids of the slide boxes of the Pacltová collection do not allow a hermetic seal. Environmental impurities, mainly dust, penetrated into the boxes over the years, depositing on their bottoms. This powder was the source of fungal spores that later germinated, colonizing and metabolizing the glycerin jelly of the preparations.

Pollen types

According to the hand written notes of Prof. B. Pacltová and her scientific references, slides from horizons: VL-1/399, VL-1/403, VL-1/405, VL-1/408, Ln-1/280, Ln-1/284, Ln-1/286, Ln-1/291, Ln-1/294, Ln-1/296, Ln-1/297 and Ln-1/303 contain angiosperm pollen types (see e.g. Pacltová 1968, 1971; Appendix). However, it is impossible to find them in the corresponding slides, even though the coordinates are given in the mentioned papers. In some of these levels, several species of angiosperm pollen grains such as *Krutzschipollis reticulatus* from VL-1/403, *Nemejipollis aquaticus* from Ln-1/297, *Tricolpites nemejcii* from VL-1/408, *Tricolporoidites bohemicus* from VL-1/405 and *T. subtilis* from Ln-1/296 were described for the first time. The degradation that the glycerin jelly of the preparations underwent has caused the loss of the type specimens of the mentioned species. Possibly also their change of position and orientation in the slide is what prevents their relation to

the figured specimens. Another problem is the publication of the types based on short descriptions was made exclusively from light-microscope studies, and sometimes the pollen grains were figured as poorly-focused pictures.

We tried to find these type specimens, but had no success. However, the light-microscope study carried out by us on the semi-destroyed pollen samples allowed us to recognize other pollen grains of early angiosperm taxa, which could be in certain circumstances designated as lectotypes/neotypes.

Systematic palaeobotany

Echinipollis cenomanensis PACLTOVÁ, 1968

Pl. 1, Figs 2, 3

1968 *Echinipollis cenomanensis* PACLTOVÁ, p. 104, pl. 2, figs 1–5.

1971 *Echinipollis cenomanensis* PACLTOVÁ; Pacltová, p. 119, pl. 16, figs 1, 2.

Original diagnosis. Pollen grain spherical, with four irregular apertures in the equatorial plane; exine thin, with echinulate sculpture; echinuli about 1 µm high; size of grain 17 µm in diameter.

Remarks. This species has been detected by us in horizon 122 of Praha-Barrandov, which corresponds with its type locality and stratum. It also occurs in slide 4 of horizon Ln-1/284. Only two specimens have been found and studied by us. Both present ambis more spherical than the holotype, which was figured in blurry pictures. In addition, a deep study of the irregular apertures indicated in the diagnosis may relate them to true colpi. A more accurate study may reveal a tetracolpate pattern. The botanical affinity of this species was considered unknown. However, the authors also provisionally consider that they may be produced by an aquatic plant. In examining the apertures, we found some similarities with the pollen of recent *Hippuris*.

Krutzschiperipollis cretaceus? PACLTOVÁ, 1968

Pl. 1, Figs 4, 5

1968 *Krutzschiperipollis cretaceus* PACLTOVÁ, p. 102, pl. 1, figs 6–9.

1971 *Krutzschiperipollis cretaceus* PACLTOVÁ; Pacltová, p. 121, pl. 15, figs 16–17.

Original diagnosis. Pollen grain spherical, rarely periporate, 5–6 circular pori about 1.5 µm; sculpture densely baculate; baculae about 0.5 µm high; endoexine thinner than ectoexine; size 19 µm in diameter.

Remarks. Perhaps this species should be re-described, because its diagnosis does not match characters displayed in figure of the holotype in Pacltová (1968: pl. x, figs 6–9, 1971: pl. 15, figs 16, 17). The pollen grain figured is reticulate and not baculate, as Pacltová writes in her diagnosis. The holotype does not show baculi, but clear reticulum, in which it is similar to the species *Krutzschiperipollis reticulatus*. The holotype of *K. cretaceus* belongs to slide 1/1 from Nové Strašecí. The studied specimen was found in horizon 122 from the Praha-Barrandov borehole. Botanical affinity to the family Chenopodiaceae is not clear.

Krutzschipollis reticulatus PACLTOVÁ, 1968

Pl. 1, Fig. 8

1968 *Krutzschipollis reticulatus* PACLTOVÁ, pp. 102, 104, pl. 1, figs 10–13.

1971 *Krutzschiperipollis reticulatus* PACLTOVÁ; Pacltová, p. 121, pl. 15, figs 7–9.

Original diagnosis. Pollen grain spherical, periporate, about 14 circular pori 1.8 µm in diameter; exine 1 µm thick, consisting of endexine and ectexine; ectexine reticulate; lumina of reticulum of irregular size and shape, partly arranged in rows; size of grain 15 µm.

Remarks. The holotype of this species occurred in slide 3 of horizon VL-1/403. Unfortunately, the slides from this horizon are destroyed. A well-preserved specimen of *K. reticulatus* has been found in slide VL-1/399/1 from the same borehole. It presents spheroidal amb, polyporate pattern with more than 12 pori, and reticulate ornamentation. The exine shows a clearly columellate pattern. According to our analysis, lumina of the reticulum is irregular in size and shape, but it is not arranged in rows. The botanical affinity of this species with the recent family Chenopodiaceae is unclear.

Tricolpites barrandei PACLTOVÁ, 1971

Pl. 1, Figs 6, 7

1971 *Tricolpites barrandei* PACLTOVÁ, p. 115, pl. 7, figs 1–9, 19–21.

Original diagnosis. Pollen grain tricolpate. Shape oblate. Polar axis 14(25)17 µm, equatorial axis 13(14)15 µm. Colpi reach nearly to poles. Apocolpium very small. Exine regularly reticulate, up to 2 µm thick. Nexine structureless (about 0.7 µm thin), always thinner than sexine. Sexine baculate, baculae robust, more than 1 µm high, forming microreticulum meshes about 1 µm.

Remarks. Although the holotype of *T. barrandei* was found in the Praha-Barrandov borehole, the studied specimen is in slide 1 of horizon VL-1/339. Possibly the holotype has been destroyed and a lectotype or neotype must be designated. This species can be easily characterized by its oblate, almost spherical, shape, thick columellate exine with conspicuous clavae, and reticulate (not microreticulate) ornamentation with lumina consisting of meshes with similar size (up to 1 µm) on the pollen body.

Tricolpites cf. nemejcii PACLTOVÁ, 1971

Pl. 2, Figs 1–3, 7

1971 *Tricolpites nemejcii* PACLTOVÁ, pp. 113–114, pl. 4, figs 1–5, pl. 5, figs 1–12, pl. 6, figs 1–12.

Original diagnosis. Pollen grain tricolpate. Shape oblate to subprolate. Polar axis 23(25)28 µm, equatorial axis 16(18)23 µm long. Colpi long, mostly narrow, occasionally open, reaching nearly to poles. Apocolpia very small in relation to rest of grain. Nexine thinner (less than 1 µm) than sexine. Exine thin, reticulate, densely covered by clavae, about 1 µm high. Clavae form regular, perfect microreticulum. Meshes of microreticulum about 0.4–0.7 µm in diameter, almost rounded polygonal in shape.

Remarks. The three specimens studied by us were found in levels from borehole VL-1 Vrbno near Louny

(western Bohemia), which is the type locality of *T. nemejcii*. Specifically, they occurred in horizons VL-1/397 and VL-1/399. Pollen grains of slide 3 from horizon VL-1/408, where the holotype was described, are now destroyed. This species is easily characterized by its prolate shape with long colpi, reaching the polar area but never fusing. In many cases, it has a polar axis around 30 µm long. Considering the exine of this species, the sexine is always at least two times thicker than the nexine, which is clearly columellate. The sexine is also densely clavate, and shows a reticulum with lumina of meshes, always lower than 1 µm. In our opinion, the specimens studied by us do not exhibit all the typical morphological features of *T. nemejcii*. For example, the specimen from slide 1 of LV-1/399 (Pl. 2, Fig. 1) shows colpi wider than normal in this species. On the other hand, one of the specimens from slide 1 of horizon LV-1/397 (Pl. 2, Figs 3, 7), which is in polar view, does not show a clear differentiation between nexine and sexine. In the last specimen (Pl. 2, Fig. 2), the nexine's thickness is approximately of the same thickness as the sexine. These are the reasons why we attribute the studied specimens to *T. nemejcii*, but with uncertainty.

***Tricolporoidites bohemicus* PACLTOVÁ, 1971**

Pl. 2, Figs 4–6

1971 *Tricolporoidites bohemicus* PACLTOVÁ, p. 116, pl. 10, figs 1–13, pl. 11, figs 5–19, pl. 12, figs 11–14.

Original diagnosis. Pollen grain tricolporoid, small (less than 20 µm in diameter). Shape spheroidal-oblate to subprolate. Polar axis 14(16)18 µm, equatorial axis 13(15)17 µm in diameter. Colpi short, not reaching poles, interrupted by small visible longitudinal apertures on equator. Exine thick, consisting of thin bacula and thicker capita, umbrelloid in shape. Capita touch each other. Meshes of microreticulum about 0.4–1 µm in diameter. Meshes irregular polygonal in shape.

Remarks. Four specimens from slide 4 of Ln-1/284 and slides 1 of VL-1/397 and LV-1/399 were studied. The holotype was designated by Pacltová (1971) in slide 3 of VL-1/405, which is now unfortunately destroyed. *T. bohemicus* is typically tricolporoid, but is similar in shape and size to the tricolpate *Tricolpites barrandei*. The former species also exhibits a thick columellate exine with conspicuous clavae, presenting a reticulate ornamentation with smaller meshes than *T. barrandei*.

***Tricolporoidites* Type C**

Pl. 2, Fig. 10

1971 *Tricolporoidites* Type C; Pacltová, pp. 115–116, pl. 9, figs 1–4.

Description. Pollen grain tricolporoid. Shape subprolate. Polar axis 21 µm, equatorial axis 19 µm in diameter. Colpi long, reaching poles, slightly protruding at equator, interrupted by narrow poroid, 4 µm long. Colpi seems built by cavernae. Exine thin, smooth to chagrenate.

Remarks. Pacltová (1971) indicated that this species is rare in the Peruc-Korycany Formation. It is possible that she studied a single specimen that was found in slide Ln-1/293/2. Surely, the scarcity of specimens prevented

B. Pacltová from description of a new species. We have detected this species in slide VL-1/399/1. In our opinion, the apertures of this pollen type are not tricolporoidate, but clearly tricolporate, since the specimen figured by Pacltová (1971) and the specimen figured by us found in VL-1 (Pl. 2, Fig. 10) show clear compound colpate apertures, with alongate to rounded pori in the equatorial area.

Quantitative study

A quantitative analysis of two horizons (VL-1/399 [26–27 m] and Ln-1/284), containing 259 and 148 specimens, respectively, allowed us to infer two similar assemblages (Text-fig. 1), characterized by trilete spores of the families Sphagnaceae, Lycopodiaceae and Gleicheniaceae, and angiosperm pollen grains related to the family Chloranthaceae (*Clavatipollenites*) and the eudicots (tricolpate and tricolporoidate pollen grains). The best-represented spores belong to the species *Stereisporites antiquasporites* (Pl. 2, Fig. 15), *Camarozonosporites ambigens*, *Hamulatisporis* sp. (Pl. 2, Fig. 17), *Clavifera triplex*, *Gleicheniidites senonicus* (Pl. 1, Fig. 9), *G. latifolius* and *G. carinatus* (Pl. 2, Fig. 8). The chloranthaceous monosulcate and reticulate/clavate *Clavatipollenites* is abundant and well-represented by several species. We have found several forms with thick exines, which remain to be described in the future (Pl. 2 Fig. 11). Very rarely, monosulcate grains of the genera *Retimonocolpites*, *Transitoripollis* (Pl. 2, Fig. 14) and *Tucanopollis* also appear. Tricolpate pollen grains of eudicots are common and diversified. The most abundant pollen can be related to several species of *Tricolpites*, such as *T. vulgaris*, *T. minutus*, *T. barrandei* (Pl. 1, Figs 6, 7), etc. In addition, grains of *Cupuliferoidaepollenites* (Pl. 2, Fig. 13), *Nyssaepollenites* (Pl. 2, Fig. 9) and *Tricolporoidites* also occur. Gymnosperm pollen grains are very scarce, being represented by *Classopollis* cf. *revrei* (Pl. 2, Fig. 16), *Cycadopites follicularis*, *Inaperturopollenites dubius*, *Monosulcites* sp., *Gnetaceapollenites* sp. (Pl. 2, Fig. 12) and undetermined bisaccate pollen grains. Aquatic palynomorphs are represented by small acanthomorph acritarchs and phycomas of prasinophycean algae.

Discussion

The curation of palynological collections is not easy. These usually include a large number of preparations, which include light-microscope slides, laboratory residues, sediment samples and, in some cases, SEM stubs, TEM grids and photographic negatives (Chapman 1985, Collinson 1987, 1995). As we previously explained, the Pacltová collection is basically light-microscope slides, which were mounted with glycerin jelly in the 1960s. This substance has non-durability during storage (Collinson 1995). Concretely, the unsealed glycerin jelly slides have a lifespan of less than 20 years (Chapman 1985). More recently, Traverse (2007) indicated that his slides had a maximum life of 40 years, much less in many cases.

Chapman (1985) indicated that an inspection of the Couper collection, which is stored in the Sedgwick Museum (Cambridge CB2 3EQ, England), showed in a great part

(35–45 %) signs of degradation. After that inspection, the collection was moved to an unheated dark room at 60% relative humidity, and the edges of the coverslips treated by the museum staff with polyurethane varnish in an attempt to prevent any further deterioration.

At this moment, the Pacltová collection has degraded most of its slides. In order to recuperate the collection, it would be necessary to make a study to determine which palynomorph slides could be recovered. According to Collinson (1995), treatment will involve removal of the coverslips, removal of the original mounting medium and remounting of a new medium. Rescue of glycerin jelly slides has been discussed by Hill (1983) and Sincock (1984). The former author removes the coverslips with the aid of 5% HCl in warm (45 °C). The latter suggests an enzymatic hydrolysis, including 10% trypsin solution. The Pacltová materials could be remounted easily, with glycerin jelly or with other mounting medium such as the plastic resin “Elvacite” (Rushton 1979). According to Chapman (1985), life expectancy of glycerin jelly mounts can be increased by sealing them immediately with a broad band (approx. 3–4 mm), adding a water-excluding substance under the coverslip (paraffin wax or Depex), and handling them carefully. Our study indicates that the mounts of the Pacltová collection with Cretaceous palynological assemblages that should be recovered are: slide 1 of VL-1/397 (23.5–25 m) horizon, slide 1 of VL-1/399 (26–27 m) horizon, slide 3 of VL-1/399 (26–27 m) horizon, slide 3 of VL-1/400 (27–29 m) horizon, slide 1 of VL-1/402 (31–32 m) horizon, slide 4 of Ln-1/284 horizon, slide 6B4 35/A1859, slide Barrandov Cenoman and slide Pecák 40003.

Glycerin jelly slides that are well prepared may last indefinitely, provided that they are stored flat, in a room that is maintained at a fairly even, cool temperature (Batten 1999). More specifically, the slides must be labelled and stored horizontally, coverslip uppermost, and preferably in trays within a purpose built cabinet with stops to prevent drawers from being pulled out in one movement (Collinson 1987, 1995, Moore et al. 1991). If the slides are stored in a slide boxes, they also have to be horizontally arranged, coverslip uppermost, and these boxes airtight and opaque.

Prof. B. Pacltová described and figured for the first time quite a number of Cretaceous angiosperm pollen taxa from the Bohemian Cretaceous Basin. She described 6 genera, 15 species, 7 types and made 2 new combinations (Pacltová 1968, 1971). In addition, together with Góczán, Groot and Krutzsch, she described 8 genera, 7 species and 1 type of Normapolles (Góczán et al. 1967). As we mentioned, we can find no types. Possibly, they changed their place and/or orientation during the dehydration of the glycerin jelly, and they may have been degraded by the action of the fungal infection. However, if it is possible to restore the Pacltová slides, holotypes must be preferentially located and re-described. If they are unfortunately lost, it will be necessary to designate lectotypes or neotypes (Kvaček 2008) in the recovery slides. Several of the specimens of *Echinipollis cenomanensis*, *Krutzschipollis reticulatus* and *Tricolpites barrandei* identified in the studied samples may be designated as lectotypes or neotypes. However, better specimens of *Tricolpites nemejcii* than those figured in Pl. 2 as “cf.” should be used to typify this species.

This collection should also be integrated by slides prepared with a sufficient volume of residues to allow a quantitative study not only of palynomorphs, but also of other elements, to make palynofacial studies. Hayek and Buzas (1997) recommended the identification of around 200 to 500 specimens per horizon to obtain a good ecological estimation, whereas Feist-Burkhardt and Götz (2002) indicated the study of 300–500 particles to make a palynofacial analysis. To obtain this number of elements in the slides, it is necessary to mount the slides with around 0.5 ml volume of laboratory residues. B. Pacltová did not indicate the volume of laboratory residues used per slide. The slides revised by us, although badly preserved, generally show a low number of palynomorphs, which does not exceed one hundred per slide. Such low numbers may be due to B. Pacltová not using a micropipette, and instead picking up several drops of residue with a standard pipette.

A quantitative study would be very interesting, to reconstruct the vegetation that inhabited margins of the Bohemian Cretaceous Basin during the Cenomanian. As we pointed out above, quantitative study of horizons VL-1/339 (26–27 m) and Ln-1/284 allowed us to recognize two similar assemblages in palynological composition (Text-fig. 1). The calculated percentages indicate that the Ln-1/284 assemblage reflects more humid conditions, by the relative higher amounts of Sphagnaceae and aquatic palynomorphs. The assemblage from borehole VL-1 exhibits higher percentages of Gleicheniaceae, Lycopodiaceae and *Clavatipollenites*, which may indicate an increase of relevance of pioneer plants that colonize degraded places, such as riverbanks or ravines with sunny exposure and poor soils.

It would be necessary to include as part of the Pacltová collection the laboratory residues of each sample studied by her. If these residues still exist, their recuperation would allow mounting new slides where, with high probability, a number of the described species will be present. From the 1960s to the present, these residues underwent dehydration. According to Chapman (1985), residues from which palynomorphs have been prepared should be fluid stored in plastic clip-on topped tubes in glycerol, to which a few drops of thymol or phenol are added to prevent fungal growth. If necessary, preservation of the residues will also allow staining of the samples for better study of the palynomorphs, and the use of isolation techniques (e.g. Zetter 1989) with types, for carrying out a complete study of them.

The preservation of residues will also allow mounting on scanning electron microscope (SEM) stubs. Nowadays, to describe a palynological species, it is recommended to combine a light microscope, SEM and sometimes TEM techniques. For example, the taxonomical study of Jurassic circumsulcate pollen grains of Cheirolepidiaceae from Central Spain (Circumpolles gen. et sp. indet.; see Peyrot et al. 2007) with the aid of both light microscope and confocal laser scanning did not allow description of a new species, nor comparison with others. At least, it would be necessary to use a SEM as e.g. the case of the Cretaceous spore genus *Costatoperforosporites* (see Mendes et al. 2017).

According to Collinson (1987), SEM stubs and TEM grids have a yet-unknown shelf life, and photographs may represent the best or even only source of information if they deteriorate. Previously, Chapman (1985) had indicated that

stubs and grids must be stored in hermetic clear plastic boxes filled with an inert gas. However, he was in doubt about the durability of these materials.

A new and complete palynological study of the boreholes and outcrops of the Peruc-Korycany Formation should be carried out. This new study would supplement the results carried out by Prof. B. Pacltová during her professional career. It would be necessary to collect and prepare new samples in the levels already studied as well as in new locations, in order to obtain a comprehensive knowledge of the Bohemian Cenomanian. In addition, the creation of a new palynological collection will have as a consequence the need of storage of light-microscope slides for qualitative and quantitative studies, of sediment samples and macerated residues as well as SEM stubs and TEM grids for taxonomical and ultrastructural studies. The application of curation methods from the first phases of the new collection will allow correct storage of it, and the possibility of extended durability of the collection.

Suggestion for restoration and long term preservation of the Pacltová palynological collection

A preliminary revision of the Pacltová palynological collection, which was mainly mounted in the 1960s and recently transferred to the National Museum, Prague allowed us to verify that most of the collections underwent more than forty years of unfortunate poor curation. Due to content degradation of most of the slides, a large number of angiosperm pollen grain type specimens from the Cenomanian Peruc-Korycany Formation (Bohemian Cretaceous Basin, the Czech Republic) is lost forever.

In order to stop the collection degradation and recuperate most of the slides, we suggest the following steps:

1. Make a complete inventory of slides and laboratory residues and sediments of the Pacltová collection, with focus on the material from the Peruc-Korycany Formation.
2. Select the best preserved slides in order (i) to detect spore and pollen types and (ii) to value the state of preservation of their glycerin jelly.
3. Recuperate each slide, removing the coverslip and using a new medium of mounting.
4. If the mounting medium will be glycerin jelly again, the jelly should be dehydrated by applying gentle heat before sealing the slides.
5. Seal the slides with paraffin wax, or a time-resistant varnish.
6. Label and inventory the new slides.
7. Study the slides with light microscope, in order to detect type specimens.
8. Store the slides horizontally, coverslip uppermost, into airtight slides boxes. The boxes should put into a room with cool temperature.
9. Add glycerol and a few drops of thymol or phenol to each tube with laboratory residue. It is absolutely necessary to label these tubes with references which relate them to the slides.

10. Mount more slides from the stored residues to study each level from qualitative and quantitative points of view.
11. Isolate specimens for their study with SEM and/or TEM.
12. Store the SEM stubs and TEM grids into airtight plastic boxes filled with an inert gas.
13. Prepare in the laboratory new samples of Pacltová's studied levels and of new levels, to have a complete palynological overview of the Bohemian Cenomanian.

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Explanations to the plates

PLATE 1

Present state of the Pacltová collection

1. Palynological slide of the Pacltová collection (slide 5 of Ln-1/297) with destruction of glycerin jelly, mainly due to lose of moisture, which produced a progressive reduction of volume of the mentioned substance. Scale bar: 50 µm.

Pollen grains from the original preparations of the Pacltová collection

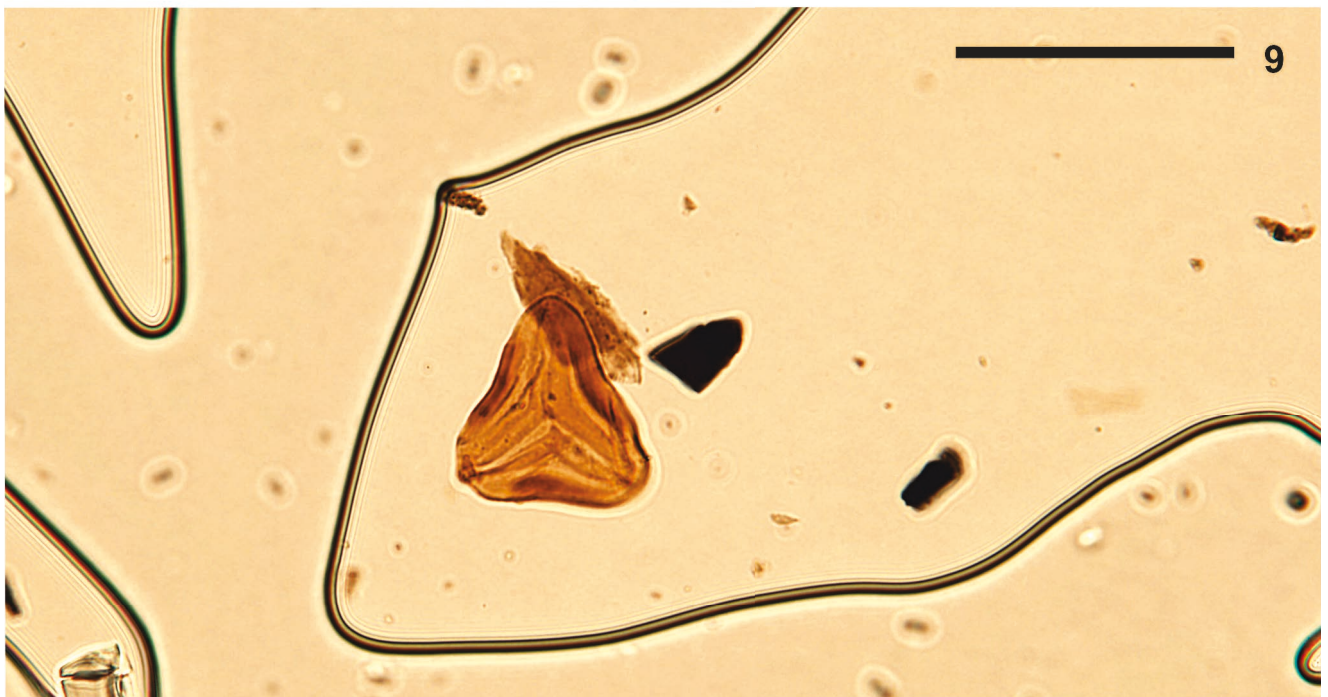
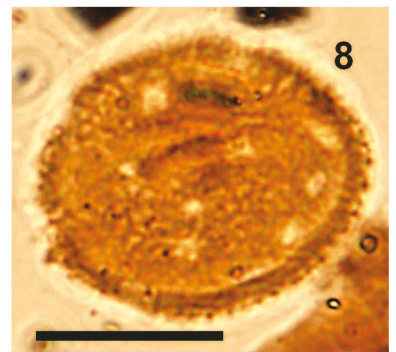
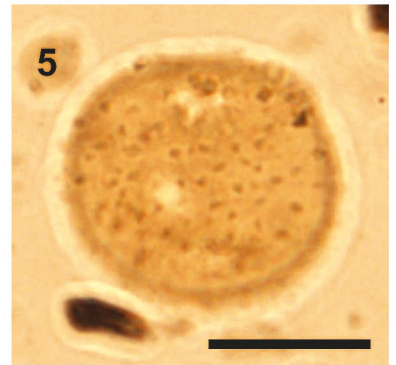
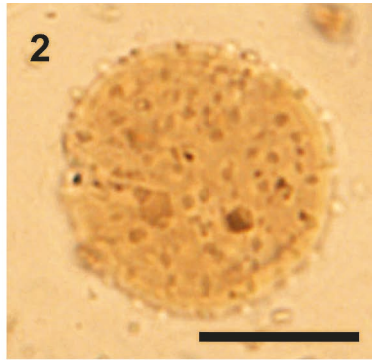
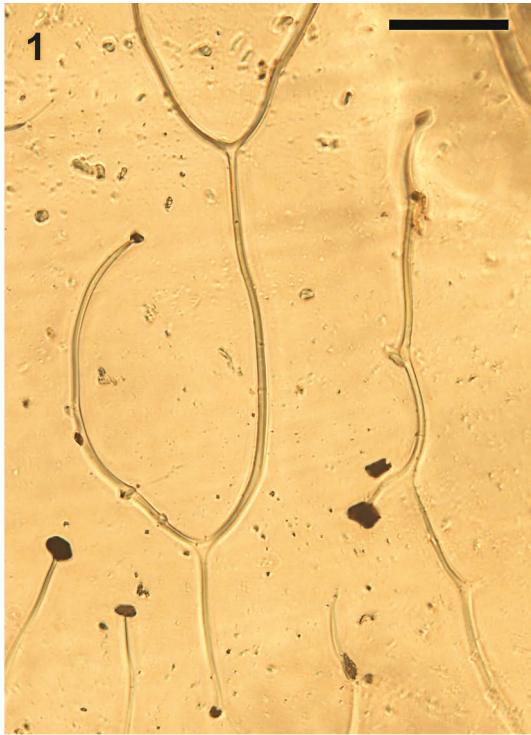
2. *Echinipollis cenomanensis* PACLTOVÁ: specimen from Praha-Barrandov. Focus shows spherical amb and echinulate exine. Scale bar: 10 µm.
3. *Echinipollis cenomanensis* PACLTOVÁ: the same specimen showing a true colpus. Scale bar: 10 µm.
4. *Krutzschiperipollis cretaceus?* PACLTOVÁ: specimen from Praha-Barrandov. Focus shows spherical amb and baculate exine. Scale bar: 10 µm.
5. *Krutzschiperipollis cretaceus?* PACLTOVÁ: the same specimen exhibiting several pori and baculate surface. Scale bar: 10 µm.
6. *Tricolpites barrandei* PACLTOVÁ: specimen from slide 1 of VL-1/399. Polar view showing tree colpi and a thick columellate and clavate exine. Scale bar: 10 µm.
7. *Tricolpites barrandei* PACLTOVÁ: the same specimen showing a clearly reticulate ornamentation. Scale bar: 10 µm.
8. *Krutzschiperipollis reticulatus* PACLTOVÁ: specimen from slide 1 of VL-1/399. Spherical, periporate and reticulate pollen grain. Scale bar: 10 µm.
9. *Gleicheniidites senonicus* N.-E.ROSS, specimen from slide 3 of Brník V, horizon 113. The reduction of the glycerin jelly provokes the change of position of the palynomorphs, and their migration along the slide. Finally, they are accumulated in some parts of the preparation where the glycerin jelly is not degraded yet. Scale bar: 30 µm.

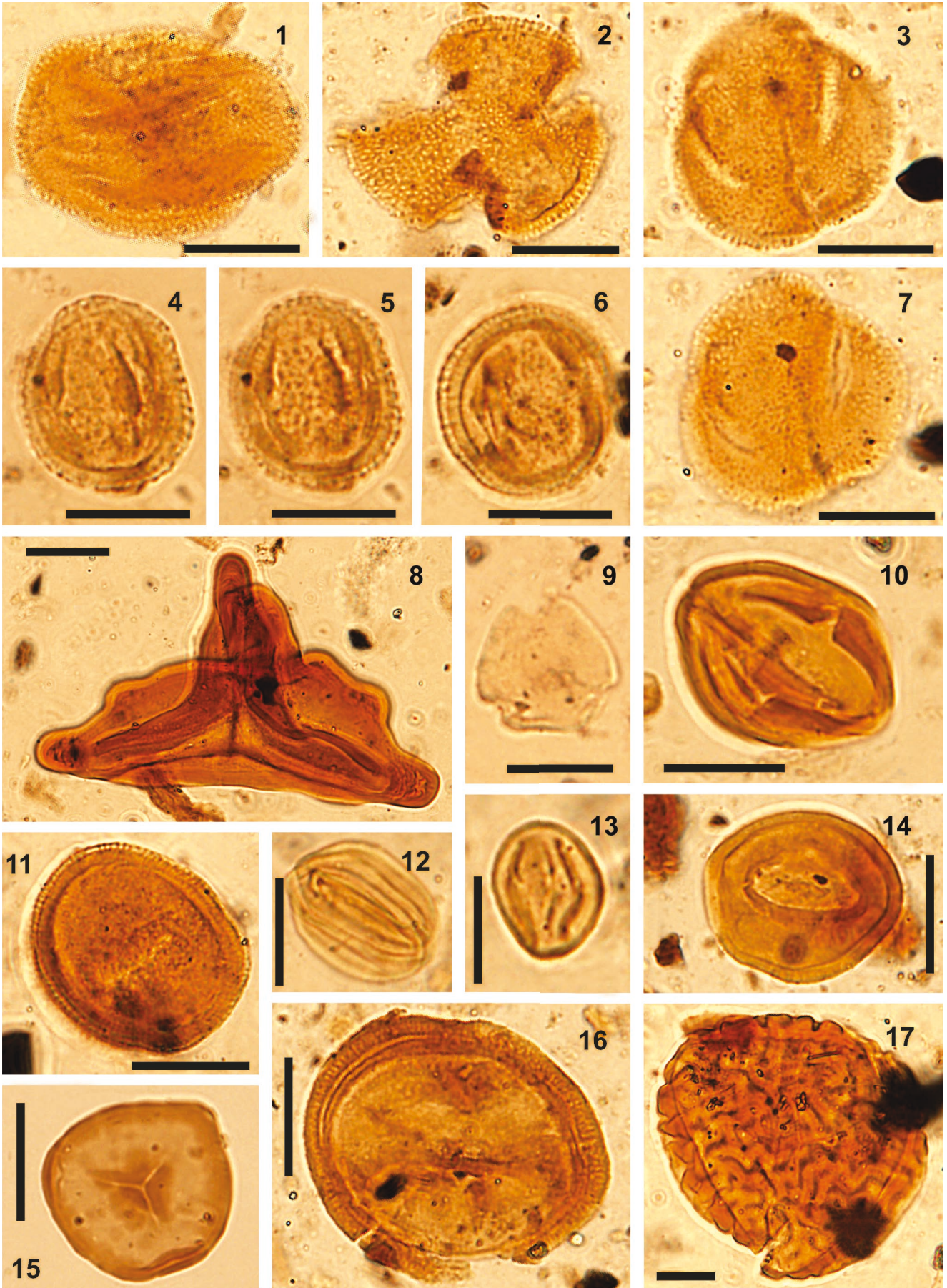
PLATE 2

Pollen grains from the original preparations of the Pacltová collection

1. *Tricolpites* cf. *nemejci* PACLTOVÁ: specimen from slide 1 of VL-1/399. Equatorial view showing colpi wider than the ordinary in this species. Scale bar: 10 µm.
2. *Tricolpites* cf. *nemejci* PACLTOVÁ: specimen from slide 1 of VL-1/397. Polar view with columellate and clavate exine, and reticulate surface. Scale bar: 10 µm.
3. *Tricolpites* cf. *nemejci* PACLTOVÁ: specimen from slide 1 of VL-1/397. Polar view exhibiting its three colpi, reticulate surface and undifferentiated nexine and sexine. Scale bar: 10 µm.
4. *Tricolporoidites bohemicus* PACLTOVÁ: specimen from slide 4 of Ln-1/284. Specimen showing the colpiroids and thick exine. Scale bar: 10 µm.
5. *Tricolporoidites bohemicus* PACLTOVÁ: the same specimen exhibiting reticulate surface. Scale bar: 10 µm.
6. *Tricolporoidites bohemicus* PACLTOVÁ: specimen from slide 4 of Ln-1/284. Spheroidal grain with thick clavate exine. Scale bar: 10 µm.
7. *Tricolpites* cf. *nemejci* PACLTOVÁ: the same specimen of Fig. 3 with different focus, which reveals different sizes in meshes of reticulum. Scale bar: 10 µm.
8. *Gleicheniidites carinatus* (BOLCH.) BOLCH.: specimen from slide 3 of VL-1/399. Trilete spore with trapezoid-shaped interradsial crassitudes. Scale bar: 10 µm.
9. *Nyssaepollenites* sp.: specimen from slide 4 of Ln-1/284. Tricolpate and psilate pollen grain. Scale bar: 10 µm.
10. *Tricolporoidites* Type C: specimen from slide 2 of VL-1/399. Tricolpate pollen grain with psilate exine. Scale bar: 10 µm.
11. *Clavatipollenites* sp.: specimen from slide 1 of VL-1/399. Monosulcate pollen grain with thick exine. Scale bar: 10 µm.
12. *Gnetaceaepollenites* sp.: specimen from slide 1 of VL-1/399. Polyplcate pollen grain with psilate exine. Scale bar: 10 µm.
13. *Cupuliferoidaepollenites* sp.: specimen from slide 1 of VL-1/399. Tricolporoidate and psilate pollen grain.
14. *Transitoripollis* sp.: specimen from slide 1 of VL-1/397. Monosulcate pollen grain with thick exine. Scale bar: 10 µm.
15. *Stereisporites antiquasporites* (L.R. WILSON et R.M. WEBSTER) DETTMANN: specimen from slide 3 of Brník V, horizon 113. Psilate trilete spore. Scale bar: 10 µm.
16. *Classopollis* cf. *revrei* LAING ex SVOBODOVÁ-PĚKNÁ: specimen from slide 1 of VL-1/397. Circumsulcate pollen grain with thick exine. Scale bar: 10 µm.
17. *Hamulatisporis* sp.: specimen from slide 1 of VL-1/399. Trilete spore regulate and strongly convoluted. Scale bar: 10 µm.

PLATE 1





Appendix

List of studied slides per horizon of VL-1 and Ln-1 boreholes (see Material and methods for details). Grey colour indicates slides where Prof. B. Pacltová (1968, 1971) found and described new angiosperm pollen species. The disappearance, absence or destruction of particular slides is indicated with the term “absent”.

VL-1 borehole					
horizon number	slide number				
VL-1/394	1	2	3		
VL-1/395	1	2	3		
VL-1/396	1	2	3		
VL-1/397	1	2	3		
VL-1/398	1	2	3		
VL-1/399	1	2	3		
VL-1/400	1	2	3		
VL-1/401	1	absent	3		
VL-1/402	1	2	3		
VL-1/403	1	2	3		
VL-1/404	1	2	3		
VL-1/405	1	2	3		
VL-1/406	1	2	3		
VL-1/407	1	2	3		
VL-1/408	1	2	3		
VL-1/409	1	2	3		
Ln-1 Louny borehole					
horizon number	slide number				
Ln-1/280	1	absent	3	absent	absent
Ln-1/281	1	absent	3	absent	5
Ln-1/282	absent	absent	absent	4	absent
Ln-1/283	absent	absent	3	absent	absent
Ln-1/284	absent	absent	absent	4	absent
Ln-1/285	absent	absent	3	absent	absent
Ln-1/286	absent	absent	absent	4	5
Ln-1/290	1	2	3	4	5
Ln-1/291	1	absent	absent	absent	absent
Ln-1/292	1	absent	3	4	absent
Ln-1/293	1	absent	absent	4	5
Ln-1/294	1	2	absent	absent	absent
Ln-1/295	1 and 1/2	2	absent	4	5
Ln-1/296	1	2	3	4	5
Ln-1/297	absent	2	3	4	5
Ln-1/298	1	absent	3	4	absent
Ln-1/299	1	2	absent	4	absent
Ln-1/300	1	2	3	4	5
Ln-1/301	1	2	3	4	5
Ln-1/302	1	2	3	4	5
Ln-1/303	1	2	3	absent	5
Ln-1/304	1	2	3	4	absent
Ln-1/305	1	2	3	4	5