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Systematic Palaeontology (Palaeobotany)

# The need for the SEM in palaeopalynology

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**Abstract**

One of the commonly quoted weaknesses of pollen analysis is its poor taxonomic resolution, which can be achieved with the Light Microscope (LM). This prevents detailed palaeoecological interpretations from being made. Although the Scanning Electron Microscope (SEM) has been widely available for almost 40 years, it is rarely used in routine palaeopalynological research. The usual reason given is that single-grain techniques are too time-consuming. However, this need not be the case. By combining LM and SEM, fossil pollen grains can be identified more accurately. Moreover, it is possible to distinguish between similar, but botanically distinct, taxa. In this way, palaeopalynology can supply phylogeneticists and palaeoclimatologists with a plethora of useful data. ***To cite this article: D.K. Ferguson et al., C. R. Palevol 6 (2007).***


**Résumé**

**De la nécessité du MEB en paléopalynologie.** Une des faiblesses de l'étude des pollens la plus fréquemment citées est la faible résolution taxonomique obtenue par microscopie à lumière transmise (MLT). Cette difficulté empêche l'établissement d'une interprétation paléoécologique détaillée. Le MEB (microscope électronique à balayage) est un outil à la disposition des scientifiques depuis presque 40 ans, mais il est malgré tout rarement utilisé en routine pour les recherches en paléopalynologie. La principale raison invoquée en est que la technique du grain par grain consomme beaucoup de temps, ce qui n'est pas forcément le cas. En combinant les techniques MLT et MEB, les grains de pollens fossiles peuvent être identifiés avec plus d'exactitude, et il devient possible, en outre, de distinguer des taxons similaires, mais botaniquement distincts. Dans ce sens, la paléopalynologie peut fournir aux phylogénéticiens et paléoclimatologistes une pléthore de données utiles. ***Pour citer cet article : D.K. Ferguson et al., C. R. Palevol 6 (2007).***


*Keywords:* LM; SEM; Micromorphology; Ornamentation; Single-grain technique

*Mots clés :* Microscope à lumière transmise (MLT) ; MEB ; Micromorphologie ; Ornementation ; Technique grain à grain

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## 1. Introduction

All of us have experienced at some time the problem of describing people in such a way that they can be immediately identified by a third person. The result of our exertions is mostly a list of useless generalizations. Only in exceptional cases does the person we are attempting to describe have some striking feature, like a mole or an extraordinarily long nose, which singles them out. Why then do we rarely have problems recognizing our acquaintances? The answer is simple: they all have a unique *combination of characters*. The facial features are particularly diagnostic, a fact used by the police in tracing criminals. If only a limited number of features were available, confusion can arise. How often have you thought you knew a person in front of you and called out to them, only to realize that you had made a mistake?

Organisms – like people – are recognized by a combination of characters. This explains why certain plants can be identified at a distance, far too far away for the botanist to see the diagnostic characters. Therefore, good illustrations of the whole plant remain an essential part of systematic botany. Although the practitioners of phenetics gave characters equal weight, it is clear that not all characters can play an equal role in establishing the plant's identikit. In angiosperm taxonomy, the floral features are of paramount importance. This explains why mistakes can be made when only vegetative material is available.

Unfortunately, for the palaeobotanist, plant parts (leaves, pollen, and seeds) get dispersed and are rarely found in organic connection. In this way only part of the identikit remains. In order to rectify this situation, more characters have to be added as compensation for those that are missing, in order to create a new identikit. This is the reason why palaeobotanists studying leaf compressions usually attempt to prepare cuticles in order to bolster the macroscopic features with details of the leaf anatomy. In much the same way, we make it our policy to examine not only the general features of fossil pollen grains (shape, apertures, exine stratification) with the light microscope (LM), but complement this with a detailed examination of the ornamentation using a scanning electron microscope (SEM).

## 2. Methodology

We continue to apply the methodology developed by Zetter [12] and described in detail by Zetter and Ferguson [13]. It is important at the outset to scrape the surface of the samples clean, to prevent any possible contamination by recent pollen grains. The sediment is then ground in a mortar and gently boiled in HF in a copper pan to remove any silicates. This solution is transferred to a large polythene bucket to which 3–4 l of water is added and left to stand until the solids have settled out. The liquid is then decanted, and the residue boiled in concentrated HCl for 5 min to prevent the formation of calcium fluoride. After decanting the HCl, the samples are washed in distilled water and centrifuged 3–4 times before undergoing acetolysis (chlorination plus acetylation). The samples are now transferred to a test tube and about 1.5 cm glacial acetic acid added, followed by ca. 3 cm of a freshly prepared solution of saturated sodium chlorate. Then 3–4 drops of concentrated HCl are added and the mixture stirred with a glass rod. The test tubes are placed in a bath of boiling water for 3 min. The samples are then centrifuged at 2000 rpm for 20 s and the liquid fraction decanted. To eliminate any remaining chemicals, the samples are washed and centrifuged at least three times. In order to remove the water, the samples are then washed in concentrated acetic acid or acetic anhydride. A mixture of nine parts acetic anhydride and one part concentrated $H_2SO_4$ are now added and the test tubes placed in a warm water bath for 3–4 min [1]. After the mixture is centrifuged and the liquid fraction decanted, the residue is washed once

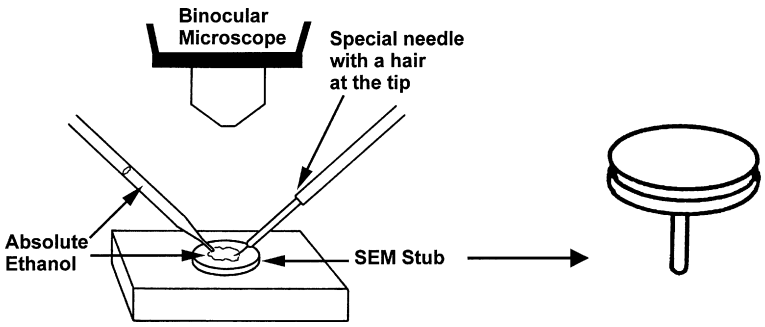
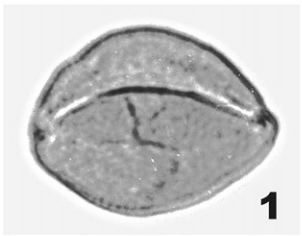



Fig. 1. Transferring pollen grains from a glass slide onto a SEM stub prior to sputter coating.
Fig. 1. Transfert d'un grain de pollen d'une lame de verre sur un support pour MEB avant son revêtement métallique.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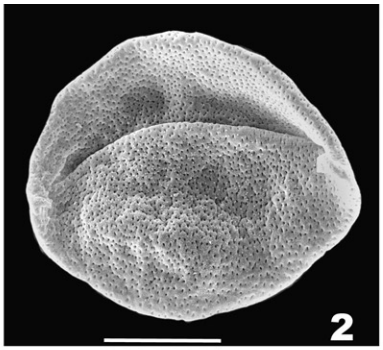
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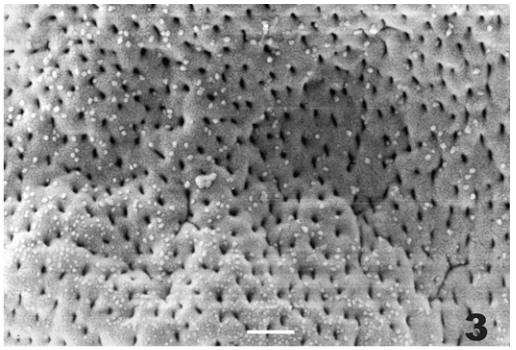
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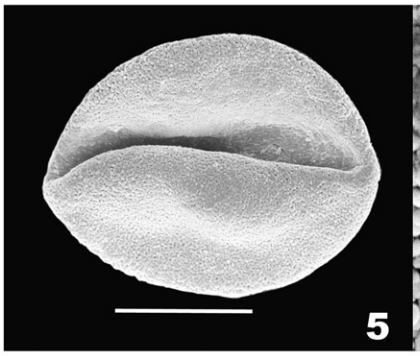
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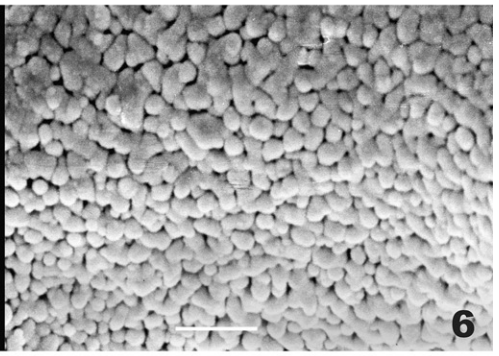
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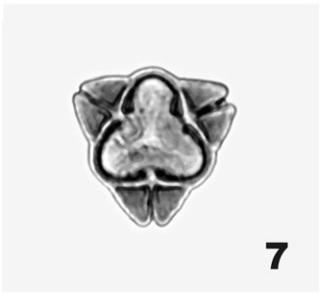
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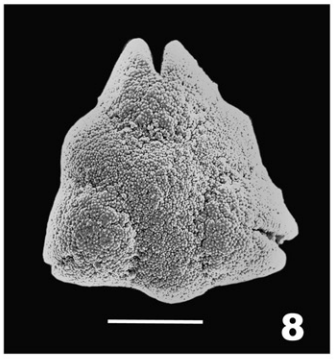
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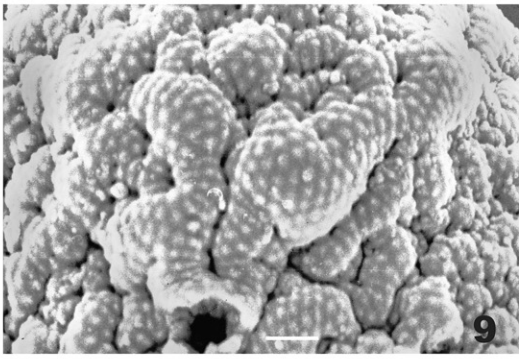
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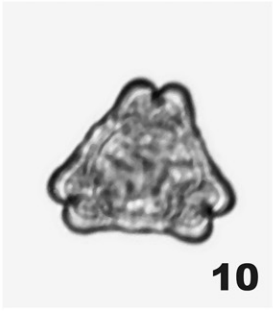
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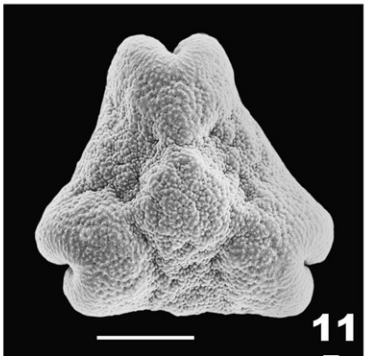
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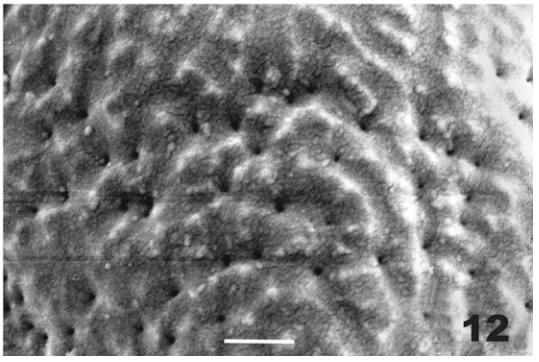
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in acetic acid and at least three times in water. In a few cases, it is necessary to separate the organic fraction from the inorganic material with a solution of zinc bromide.

Glycerine is added to the organic residue to form a suspension. With a pipette a drop of this liquid is transferred to a glass slide. Using a dissecting needle to which a nasal hair has been affixed, those grains which are of particular interest are brushed to the edge of the glycerine, where they can be located and transferred to another glass slide with a fresh drop of glycerine for photography under a LM. Because no cover slip is used, it is possible to photograph the same grain in various orientations. After this, the pollen is transferred to an aluminium SEM stub to which a drop of absolute ethanol has been added (Fig. 1). The ethanol removes all traces of the glycerine from the surface of the pollen grains, so that these can be examined in great detail under the SEM. The stubs are sputtered with gold in a BIORAD Sputter Coater for 4 min before being examined in a SEM at 10 kV. The advantage of this single-grain technique is that the very same grain is examined under both LM and SEM, thereby establishing detailed identikits for the various morphotaxa.

## 3. Discussion

It should be clear from the above methodology that we are not suggesting that light microscopy be replaced by SEM, a practice that is followed by a number of palynologists [5,11]. Rather, by submitting the pollen grains, which have already been examined under a LM, to a closer scrutiny (10,000–20,000 ×) with a SEM, we hope to obtain additional – micromorphological – characters with which to expand the pollen's identikit. This combined LM/SEM investigation can be used to:

- achieve more accurate identifications than is possible simply with LM or SEM;
- reveal mistaken identities and distinguish LM doubles.

In this way, palaeopalynology can play a greater role in:

- establishing the origin of clades and in the timing of molecular clocks;
- extracting palaeoclimatic and palaeoecological parameters from pollen assemblages;
- ascertaining biogeographical patterns in the past.

A few examples should suffice to emphasize these points.

### 3.1. Phylogenetics

Many of the basal angiosperms, which are to be found in the Cretaceous, produced minute pollen grains [2,3,4,10]. In pollen grains less than 12 μm in size, the resolution obtained with a LM is generally insufficient to establish the diagnostic features. As a result, there is a real danger that different clades may prove to be indistinguishable, and will be lumped into botanically meaningless morphotaxa (Plate 1, **1–6**). This is clearly an unfortunate situation during a phase of cladogenesis. It is therefore essential that a SEM be used to establish any differential trends (Plate 1, **7–12**). Details of the ornamentation can also be used to establish cases of convergence (Plate 3, **1–6**).

Because anemophilous and amphiphilous flowers produce pollen in much larger quantities than other plant parts, and are therefore more likely to be represented in the fossil record, they should be better sources of information on the first and last occurrences of a given taxon. Thus, while unequivocal fruits and seeds of the Saururaceae are first known from the Upper Eocene [8], we have recently found representatives of this family

Plate 1. (**1–6**) *Monocolpopollenites* spp. from the Santonian of Gmünd, Austria. Under the LM, these grains are very similar and could be easily confused. Even low-power SEM is not sufficient to distinguish them unequivocally. However, under high magnification, it is clear that *Monocolpopollenites* sp. 1 (**1–3**) has perforate ornamentation with minute granules scattered over the entire tectum. In *Monocolpopollenites* sp. 2 (**4–6**) the ornamentation consists of short rugulae which can be fused, especially around the sulcus. (**7–12**) Examples of Normapolles from the Santonian of Gmünd, Austria, displaying different botanical affinities. (**7–9**) *Oculopollis* sp. The rugulate to verrucate sculpturing with a suprasculpture of regularly spaced microechinae suggests an affinity with the Juglandaceae. (**10–12**) *Trudopollis* sp. The perforate, microechinate sculpture resembles that found in the Myricaceae and Betulaceae. Bar in SEM overview 10 μm, in SEM close-up 1 μm.

Planche 1Transfert d'un grain de pollen d'une lame de verre sur un support pour MEB avant son revêtement métallique. (**1–6**) *Monocolpopollenites* spp. du Santonien de Gmünd, Autriche. En MLT, ces grains sont similaires et peuvent être facilement confondus. Le MEB de faible puissance n'est pas suffisant pour les distinguer de façon univoque. Cependant, à plus fort grossissement, il est clair que *Monocolpopollenites* sp. 1 (**1–3**) a une ornementation perforée, avec de menus granules dispersés sur tout le tectum. L'ornementation de *Monocolpopollenites* sp. 2 (**4–6**) consiste en de courtes rugules qui peuvent fusionner, notamment près du sulcus. (**7–12**) Exemples de Normapolles du Santonien de Gmünd, Autriche, montrant différentes affinités botaniques. (**7–9**) *Oculopollis* sp. : la sculpture de l'exine rugulée à verruquée avec une suprasculpture de microépines régulièrement espacées suggère une affinité avec les Juglandaceae. (**10–12**) *Trudopollis* sp. : la sculpture perforée à microépines ressemble à celle observée chez les Myricaceae et les Betulaceae. La barre dans les photos MEB est de 10 μm pour la vue d'ensemble et de 1 μm pour le détail.

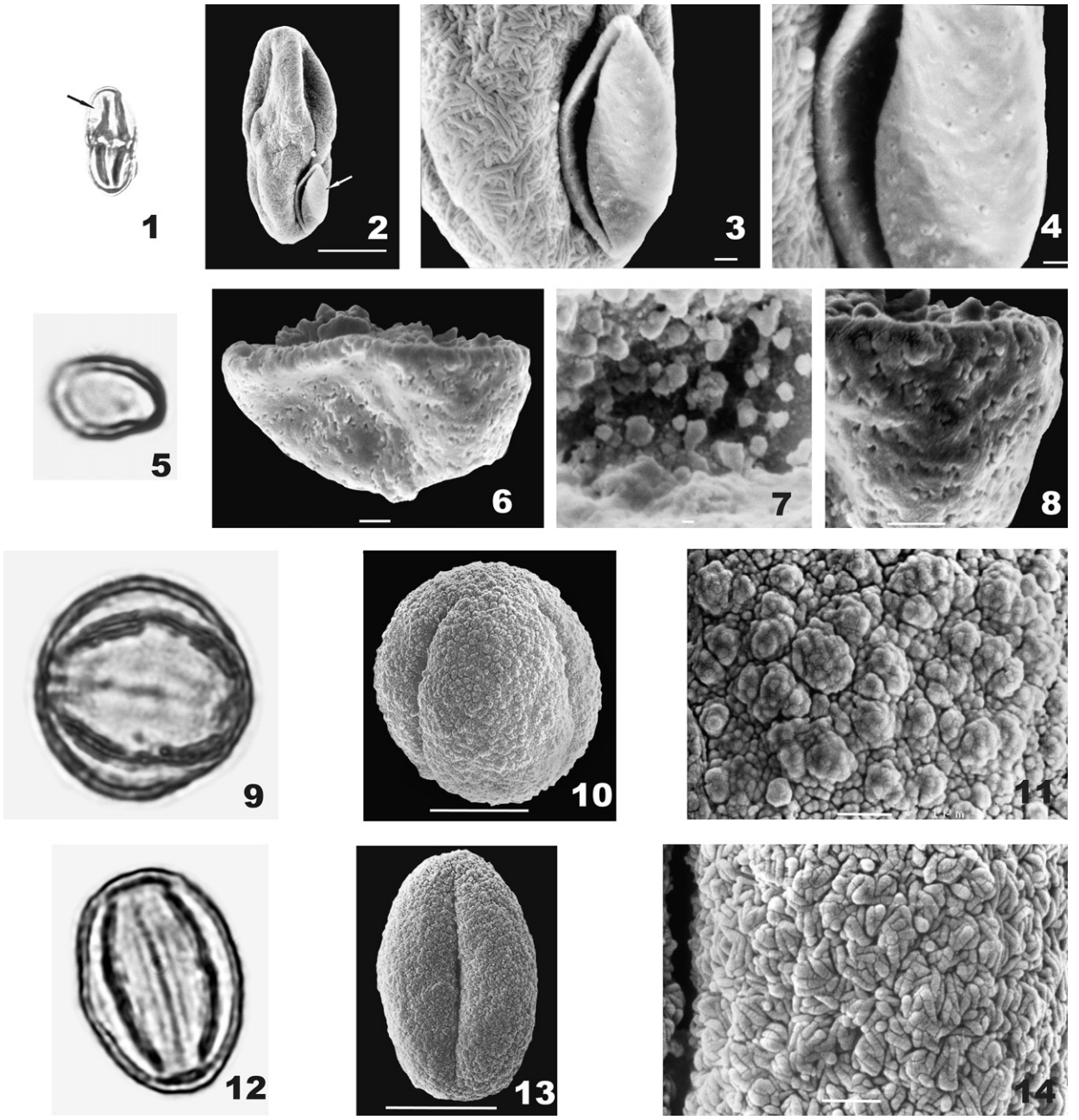



Plate 2. (**1–4**) *Saururipollis* gen. nov. from the Upper Miocene of Stoob, Austria, attached to a tricolporate Apiaceae pollen grain. The pollen is so small (long axis 8–10 μm) that it could easily be missed with the LM. Under the LM these pollen grains can be seen to be oblate, elliptical in polar view, and monosulcate with the sulcus having pointed extremities. The surface appears to be psilate. Under the SEM the tectum is clearly perforate, with each perforation surrounded by a ring-like structure. (**5–8**) 'Anemopsipollis' from the Middle Eocene Princeton Chert, British Columbia. This apparently inaperturate, psilate pollen grain under LM (Fig. 5) is clearly sulcate when viewed under the SEM. Moreover, the sulcus membrane has a microechinate ornamentation. Under the SEM, the tectum can be seen to be perforate, with irregularly shaped perforations, which are sometimes united to produce small foveolae. Such pollen grains are produced by *Anemopsis* (Saururaceae), a monotypic genus from California and Mexico. (**9–14**) *Quercus* spp. from the Lower Sarmatian of Lavanttal, Austria. Apart from a slightly different shape, these two species are indistinguishable under the LM. However, it is clear from the different ornamentation that two species are involved. *Quercus* sp. 1 (**9–11**) is verrucate with microechinae as suprasculpture and between the verrucae. *Quercus* sp. 2 (**12–14**), on the other hand, has somewhat clustered microrugulae. Bar in SEM overview 10 μm, in SEM close-up 1 μm.

in the Middle Eocene (Plate 2 , **5**–**8**). Without the SEM, it would have proved impossible to identify these tiny pollen grains (Plate 2, **1**–**4**).

### 3.2. Palaeoclimatology

In the Cenozoic of the northern hemisphere, the Fagaceae constituted an important family. While it is generally possible to distinguish the genus *Quercus* with a LM, it proves difficult to allocate the pollen to a group of species. It may even be impossible to decide whether the pollen came from an evergreen form or a deciduous taxon. From a palaeoclimatic point of view, this is a most unfortunate state of affairs. However, the ornamentation is often very characteristic (Plate 2, **11**, **14**). As a result, it is usually possible to decide whether an evergreen or deciduous taxon is involved [7].

### 3.3. Palaeobiogeography

As it is impossible to see the diagnostic characteristics under the LM, some pollen grains have been referred to the wrong genus or even family. Some bisaccate pollen referred to *Pinus* (Pinaceae) has turned out to be *Cathaya* when the surface of the grains has been examined under the SEM (Liu et al. [6]). *Cathaya* is a monotypic genus, which is confined to a few localities in China at the present day. Because of its unique microechinate ornamentation, *Cathaya* pollen grains can be recognized as such, even if the corpus is underdeveloped (Plate 3, **7**–**12**). Such aberrant pollen grains with relatively large sacci are usually referred to as *Podocarpidites* or *Podocarpus* (Podocarpaceae) in LM studies. However, the surface of authentic *Podocarpus* pollen grains is entirely smooth. When the SEM results are taken into account, a completely different palaeogeographic pattern emerges. *Podocarpus* can now be shown to have had a very restricted distribution in the Northern Hemisphere, while *Cathaya*, was once widespread in the Holarctic region [6].

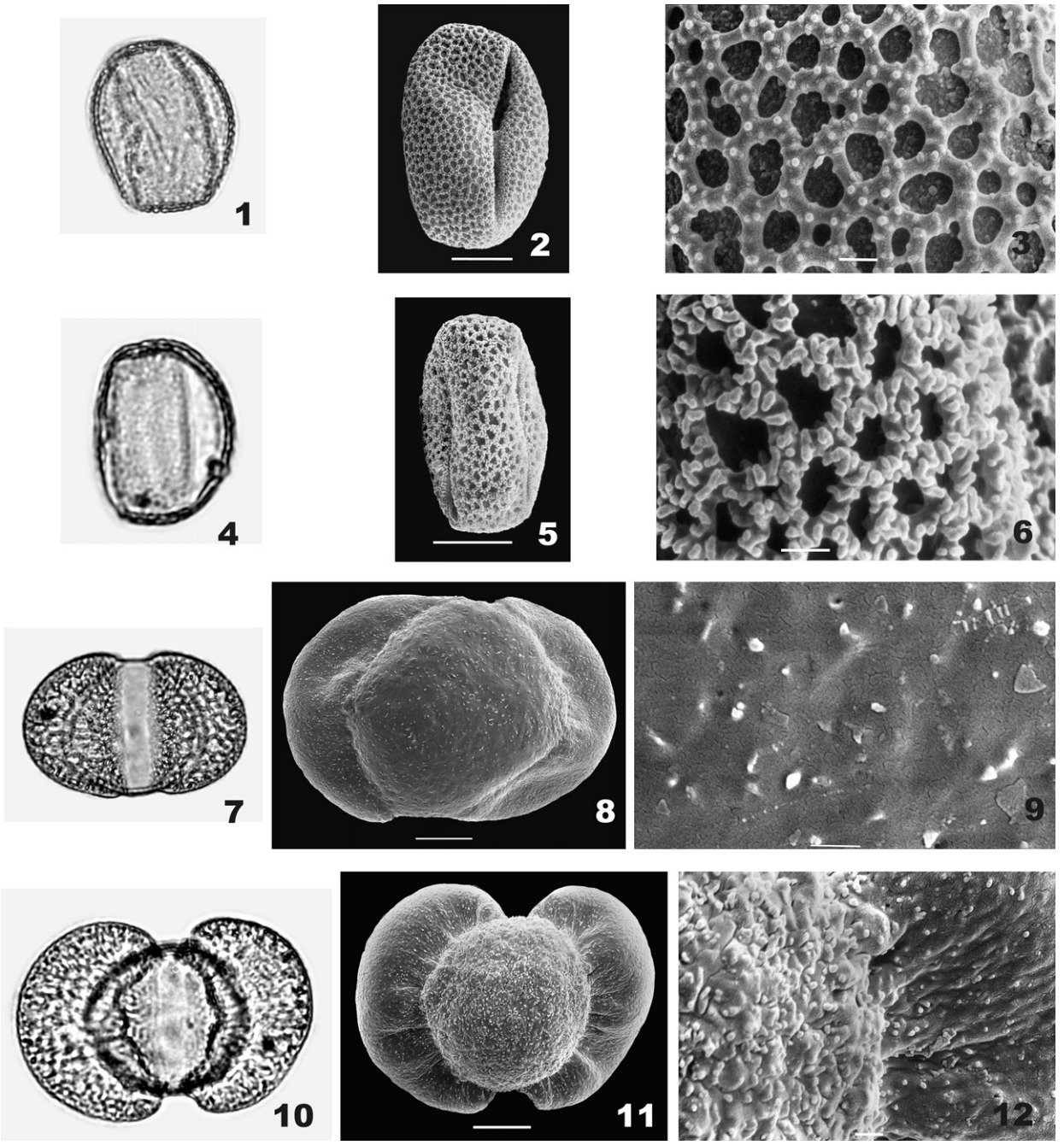
## 4. Conclusions

Considering all the advantages which the SEM has to offer, it comes as a surprise to note how rarely this facility is employed by palaeopalynologists as a routine technique. However, if progress in palaeopalynology is to be made, it is essential to take full advantage of all the available techniques. We hope that our contribution will finally persuade those colleagues,

Planche 2. (**1**–**4**) *Saururipollis* gen. nov. du Miocène supérieur de Stoob, Autriche, attaché à un grain de pollen Apiaceae tricolporé. Le pollen est si petit (axe longitudinal de 8 à 10 μm) qu'il peut facilement échapper à l'observation en MLT. En MLT, ces grains de pollen peuvent apparaître oblats, elliptiques en vue polaire et monosulcés, avec le sulcus montrant des extrémités pointues. La surface semble être psilée. Avec le MEB, le tectum apparaît clairement perforé, avec chaque perforation entourée par une structure de type anneau. (5–8) « Anemopsipollis » du chert de Princeton, Colombie britannique (Éocène moyen). Le grain de pollen apparemment inaperturé et psilé sous MLT (Fig. 5) présente clairement, vu au MEB, une membrane de sulcus, avec une ornementation à microépines. On observe au MEB que le tectum peut être perforé, avec des perforations irrégulières qui parfois se réunissent pour produire de petites fovéoles. De tels grains de pollen sont produits par *Anemopsis* (Saururaceae), un genre monotypique de la Californie et du Mexique. (**9**–**14**) *Quercus* spp. du Sarmatien inférieur de Lavanttal, Autriche. Excepté une forme légèrement différente, ces deux espèces ne sont pas distinguables en MLT. Avec le MEB, les différentes ornementations révèlent clairement que nous sommes en présence de deux espèces. *Quercus* sp. 1 (**9**–**11**) est verruqué, avec des microépines à la fois comme supra sculpture et entre les verrues. *Quercus* sp. 2 (**12**–**14**) présente, par opposition, des microrugules agglomérées. La barre dans les photos MEB est de 10 μm pour la vue d'ensemble et de 1 μm pour le détail.

Plate 3. (**1**–**6**) Two fossil representatives of the Hamamelidaceae. Under the LM, Hamamelidaceae gen. et sp. indet. (**4**–**6**) from the Middle Eocene Princeton Chert, British Columbia, looks so similar to *Parrotia* sp. (**1**–**3**) from the Lower Sarmatian of Lavanttal in Austria, that it could be considered to be conspecific. However, while *Parrotia* has muri with regularly spaced microechinae, the American representative has muri with densely packed microrugulae. (**7**–**12**) Variation in *Cathaya* pollen grains from the Lower Sarmatian of Lavanttal, Austria. Under the LM normally developed pollen grains (**7**–**9**) are often confused with those of *Pinus* Subgenus Haploxylon. However, under the SEM *Cathaya* displays irregularly scattered microechinae, a feature unique to this genus. The presence of microechinae can be used to distinguish between aberrant pollen grains of *Cathaya* (**10**–**12**) and those of *Podocarpus* sensu lato, which lack this feature. Bar in SEM overview 10 μm, in SEM close-up 1 μm.

Planche 3. (**1**–**6**) Deux fossiles représentatifs des Hamamelidaceae. Sous MLT, les Hamamelidaceae gen. et sp. indet. (**4**–**6**) provenant du chert de Princeton, Colombie Britannique (Éocène moyen) semblent similaires au *Parrotia* sp. (**1**–**3**) du Sarmatien inférieur de Lavanttal, Autriche, et pourraient être considérés comme conspécifiques. Cependant, alors que *Parrotia* a des muri avec des microépines régulièrement espacées, le représentant américain a des muri avec des microrugules densément regroupées. (**7**–**12**) Variations dans les grains de pollen *Cathaya* du Sarmatien inférieur de Lavanttal, Autriche. Sous MLT, les grains de pollen normalement développés (**7**–**9**) sont souvent confondus avec ceux du *Pinus* sous-genre Haploxylon. Cependant, au MEB, *Cathaya* montre des microépines irrégulièrement dispersées, une caractéristique typique de ce genre. La présence de microépines peut être utilisée pour distinguer les grains de pollens aberrants de *Cathaya* (**10**–**12**) de ceux de *Podocarpus* senso lato, qui ne montrent pas cette particularité. La barre dans les photos MEB est de 10 μm pour la vue d'ensemble et de 1 μm pour le détail.




who have remained sceptical, to rise to the challenge [9].

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