How to Describe and Illustrate Pollen Grains

Pollen from a Single Extant Taxon: Online Publication in PalDat – 86
Groups of Extant Pollen – 86
Fossil Pollen – 89
References – 95
For the description of a pollen grain, a number of features are used including size, polarity and shape, aperture condition, ornamentation, and pollen wall structure. Additional and often more specialized features depend on the group of plants under study. Gymnosperms (Cycadales, Ginkgoales, Pinales, Gnetales) vs. Angiosperms (magnoliids, monocots, commelinids, eudicots). These features can only be obtained by the application of a combined analysis with LM, SEM, and TEM (Fig. 1). In order to compare and categorize pollen, a common language and understanding of technical terms is necessary.

The description and illustration of a pollen grain depends on how the material is going to be presented and if one is describing a single fossil pollen grain, pollen of a particular extant species, pollen representing several species, a whole genus, several related genera, a complete family, or even a number of families. For future work it is important to provide both LM and SEM micrographs (even TEM), including incorporated scale bars, showing each taxon and close-ups of what are considered diagnostic features of pollen. When documenting the sculpture of pollen grains in SEM it has to be made sure that the magnification is high enough to distinguish the shape and outline of sculpture elements larger than 0.1 μm in diameter. LM- and SEM-diagnosis may be different from each other, due to the methods and techniques used. The methods used to prepare pollen grains for LM, SEM, and TEM must be mentioned along with the pollen descriptions, preferably in a material and method section.

**Pollen from a Single Extant Taxon: Online Publication in PalDat**

Pollen grains from single extant species have rarely been accepted by scientific journals. There is now a new online venue PalDat, for publishing pollen from a single species. PalDat is the world’s most comprehensive pollen database (www.paldat.org) and contains tools for pollen identification as well as global, free online submission and publication with review and editorial process (Weber and Ulrich 2017). PalDat already provides a large amount of pollen data on a variety of plant families. Each taxon entry (online publication) ideally includes a detailed description and micrographs (LM, SEM, and TEM) of the pollen, as well as images of the plant/inflorescence/flower and information on relevant literature (Fig. 2). PalDat is freely accessible and following a free registration it is open for contributions from all those willing to publish their pollen descriptions and micrographs online. Registered authors may also contribute as co-authors to existing publications by submitting new images and/or new data to pollen diagnosis (with review and editorial process). All changes are recorded in the database history as links to previous versions of the publication. Each contribution is citable and accessible for all users. Registered users can download publications in pdf form. The terminology used in PalDat follows this book.

**Groups of Extant Pollen**

Many of the classical papers on pollen morphology and ultrastructure, covering a large number of extant taxa, provide only a general description of pollen types with pollen of different species lumped together. Furthermore, micrographs are showing selected taxa and usually not the same taxon photographed in both LM and SEM. This makes the data unreliable and not very useful for among others paleopalynologists that want to compare their fossil pollen grains very precisely to particular extant taxa. The decision on particular potential modern analogues of the fossil pollen grain can have major effects on the paleo-vegetation reconstruction and paleoecological and paleoclimate interpretations of the fossil assemblage, as well as on the paleophytogeographic signal of the taxon. It is recommended, disregardless of the description, that all species be fully illustrated by LM and SEM (and TEM when possible) and their basic and diagnostic morphological features compiled in a table so they can be easily compared (Table 1). The example shown here are Winteraceae pollen tetrads. When portraying tetrads it is useful to show their basal-, lateral-, as well as apical view, in both LM (Fig. 3) and SEM (Fig. 4). Pollen grains should be portrayed in polar and equatorial view. Illustrating pollen from different taxa together on a plate/figure with the same magnification makes it easier to realize size differences. The SEM close-ups are then used to highlight the main sculpture features or the dissimilarities of the taxa. Ideally all close-ups showing sculpture elements should have the same magnification for an easy comparison (Fig. 5).
### Diagnosis worksheet

**PalDat worksheet with all pollen features obtained by a combined analysis using LM, SEM, and TEM. Blue dots indicate LM-, yellow dots SEM-, and red dots TEM-based analyses. PalDat pictures showing Plantago maritima**
Betula pendula

Taxonomy: Angiospermae, Fagales, Betulaceae, Betula

Published: 2016-03-25

Pollen Description

Shape, Size and Aperture

pollen unit: monad, dispersal unit and peculiarities: monad, size (pollen unit): small (10-25 μm), pollen class: porate, polarity: isopolar, shape: spheroidal, outline in polar view: circular, shape (dry pollen): irregular, outline in polar view (dry pollen): irregular, infoldings (dry pollen): irregularly infolded, interapertural area sunken, aperture number: 3, aperture type: porus, aperture condition: porate, triporate, aperture peculiarities: annulus, operculum, oncus

Ornamentation and Structure

LM ornamentation LM: pellate, nexine: -, exine: -, SEM ornamentation SEM: rugulate, microechinate, TEM tectum: eutectate, infratectum: columnellate, foot layer: continuous, endexine: absent, intine: monolayered, wall peculiarities: -

Miscellaneous

pollen coatings: absent, reserves in cytoplasm: starch, cell number: 2-celled, Ubisch bodies: present

Annotations

tectum very mighty

Author(s) of diagnosis: Halbritter, Heidemarie; Diethart, Bernadette

Pictures

1. flower(s)  2. flower(s)  3. flower(s)
4. pollen grain with generative cell  5. upper focus  6. optical section  7. lower focus
8. polar view  9. equatorial view  10. aperture  11. exine surface  12. dry pollen grains  13. dry pollen grain in polar view

Fig. 2 Online publication in PalDat. Screenshot showing part of the online publication of Betula pendula (Halbritter and Diethart 2016)
Table 1 Winteraceae pollen tetrads

|                                    | Takhtajania perrieri | Exospermum stipitatum | Tasmannia insipida |
|------------------------------------|----------------------|------------------------|---------------------|
| Tetrad diameter (LM; μm)           | 58–65                | 32–38                  | 28–33               |
| Apertures surrounded by an annulus-like rim (width; μm) | Yes, 2.5–6           | No                     | No                 |
| Width of aperture region (μm, longest axis) | 12–17               | 5–6                    | 7–11               |
| Exine thickness (LM; μm)*           | Max. 5.5             | Max. 3                 | Max. 3.2           |
| Nexine thickness (LM; μm)*          | Max. 0.9             | Max. 0.7               | Max. 0.9           |
| Sexine thickness (LM; μm)*          | Max. 4.2             | Max. 1.7               | Max. 2.3           |
| Sculpture (SEM)                     | Reticulate           | Perforate to nanoreticulate | Reticulate |
| Muri                               | Broad and rounded    | (Broad and rounded)    | Narrow and crested |
| Diameter of (largest) lumina (μm; longest axis) | 7–11                 | ≤1                     | 5–6                |
| Number of lumina/perforations (one grain in lateral view) | c. 15/20             | c. 120                 | c. 15–20           |
| Height ratio columellae vs. muri    | ~1–1.5:1             | ?                      | ~1:1               |
| Columellae per μm                   | 2 per 5 μm           | 2–3                    | 1–2                |
| Free-standing columellae            | Frequent, mostly ≤1 μm; gemmae, bacula, and clavae | Absent | Rare, mostly ≤0.5 μm; verrucae, gemmae, and clavae |
| Ulcus membrane (SEM)                | Granulate to microverrucate | Granulate to nanoverrucate | Granulate, nano- to microclavate |

Main features of three different Winteraceae pollen tetrads

Annotation: Measurements like exine, nexine, and sexine thickness provided in Table 1 (asterisks) are commonly used in (paleo) palynological literature. Scientists should be aware that such measurements (e.g., 0.7 or 0.9 μm) vary highly, up to 30%, depending on the methods and tools used. Therefore, the measurements should not be overrated or used for taxonomic discrimination.

Fossil Pollen

From the birth of paleopalynology this branch of science has been plagued by the lack of taxonomic foundation when interpreting paleoenvironments. It is very unfortunate that numerous new “scientific” publications dealing with the subjects of paleoecology, paleovegetation, paleoclimate and various aspects of paleophytogeography still present only a list of taxa observed in LM. Some publications include LM micrographs of the most “common” taxa, but only in exceptional cases the LM micrographs are accompanied by SEM micrographs. The absence of illustrations makes it impossible for any reader to verify, or later revise, the taxonomic background and to conclude if the modern living relative or potential modern analogue of the fossil taxon is justified. Every proper scientific journal should make it a mandatory request that all pollen types are represented by at least one LM micrograph. Furthermore, all taxa that suggest some sort of different, abnormal or exceptional paleo-parameters, in an otherwise “homogeneous” assemblage, or taxa that are used to set any sort of boundaries (temperature, precipitation, biozone, time, etc.), should be illustrated using both LM and SEM (in some cases even TEM). These contrasting taxa might include a dry element in an otherwise humid assemblage, a tropical element in an otherwise temperate assemblage, or an African element in an otherwise North American-Eurasian assemblage. Even though the journal would not allow these illustrations in the printed version most of them now offer the possibility to archive online supplementary files where the pollen can be illustrated.

For those who want to produce a taxonomically valid study based on fossil material are advised to use the single-grain method when investigating fossil pollen and make sure not to sieve the sample
Fig. 3 LM micrographs of Winteraceae pollen tetrads. Tetrads shown in basal-(left), lateral-(middle), and apical (right) view at high focus (upper three rows) and in optical cross section (lower three rows). *Takhtajania perrieri* (first and fourth row), *Exospermum stipitatum* (second and fifth row), *Tasmannia insipida* (third and sixth row).
Fig. 4 SEM micrographs of Winteraceae pollen tetrads. Tetrads shown in basal view (upper row), lateral view (middle row) and apical view (lower row). Takhtajania perrieri (left), Exospermum stipitatum (middle), Tasmannia insipida (right)
Fig. 5 Details of Winteraceae pollen tetrads. SEM close-ups of Takhtajania perrieri (A-B), Exospermum stipitatum (C-D) and Tasmannia insipida (E-F), showing sculpture on distal face of pollen (A, C, E) and the aperture region and ulcus membrane (B, D, F).

during preparation (see “Methods in Palynology”). This allows the researcher to study all elements occurring within a sample using both LM and SEM and to investigate even very small and/or rare pollen grains. The small and/or rare pollen (Fig. 6) would otherwise be overlooked during the old-fashion routine LM observation, where the researcher usually counts 300–600 grains. When illustrating fossil pollen it is important to show the grain in both LM and SEM. Close-ups taken with the SEM should have magnification high enough so all sculpture elements larger than 0.1 μm become distinguishable. Sculpture and suprasculpture elements smaller than 1 μm are not observed or hard to distinguish using LM only, but will be revealed using high magnification SEM (Fig. 7). Many pollen grains that look similar or the same in LM can be distinguished using SEM. In some cases it is beneficial to turn the pollen grain once it has been photographed in SEM, re-sputter and photograph again. This applies especially to heteropolar pollen grains (Fig. 8) as well as pollen dispersed in permanent tetrads. When single pollen
Fig. 6 Small and rare pollen, Paleocene, Western Greenland. A. small fossil grains (≤10 μm in diameter) usually absent in samples after sieving. LM micrographs (left) in equatorial (upper) and polar view (lower). B. pollen in equatorial view, SEM. C. striate sculpture not seen under low magnification LM.

Fig. 7 Ornamentation LM vs. SEM, fossil, Middle Eocene, Western Greenland. A-C. Eucommia sp. A. Pollen psilate in LM. B. Pollen in SEM, equatorial view, note sculpture. C. Ornamentation nanoechinate (≤0.5 μm) and granulate. D-F. Ilex sp., E. LM and SEM overviews show the typical clavate sculpture known for this genus. F. Microrugulate suprasculpture present on the distal part of the clavae, only observed using high magnification SEM.
Fig. 8 Fossil heteropolar pollen grain, Paleocene, Western Greenland. A. LM micrographs showing proximal (left) and distal (middle) poles of pollen grain and equatorial view (right). B. SEM overviews showing both poles of the pollen grain and the different aperture arrangements. C-D. SEM close-ups of proximal (C) and distal poles (D) show that the muri are much broader on the proximal pole.

grains or tetrads are studied using SEM, changes in sculpture over the pollen surface are often observed, for example polar vs. equatorial region, mesocolpium vs. aperture region vs. aperture membrane (Fig. 9). Some pollen or tetrads also have Ubisch bodies or viscin threads (Hesse et al. 2000). These differences in the sculpture of fossil pollen need to be documented and it is therefore often necessary to show more than a single close-up taken with the SEM.
Fig. 9 Fossil tetrad, *Rhododendron* sp., Miocene, North-east China. A, D. Tetrad, overviews in LM vs. SEM. B-C, close-ups at same, magnification show difference in sculpture at polar region of pollen grain (B) vs. interapertural area (C). E. exine surface with viscin thread, SEM

**References**

Halbritter H, Diethart B (2016) *Betula pendula*. In: PalDat – a palynological database. Published on the Internet https://www.paldat.org/pub/Betula_pendula/300732 [accessed 2017-04-28]

Hesse M, Vogel S, Halbritter H (2000) Thread-forming structures in angiosperm anthers: their diverse role in pollination ecology. Plant Syst Evol 222: 281–292

**Open Access** This chapter is licensed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license and indicate if changes were made.

The images or other third party material in this chapter are included in the chapter’s Creative Commons license, unless indicated otherwise in a credit line to the material. If material is not included in the chapter’s Creative Commons license and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder.