**Supporting Online Material**

# Autofluorescence of atmospheric bioaerosols – the spectral fingerprint and taxonomic trends of pollen

Christopher Pöhlker[1*], J. Alex Huffman[2*], Jan-David Förster[1], and Ulrich Pöschl[1]

[1]*Max Planck Institute for Chemistry, Biogeochemistry Department and Multiphase Chemistry Department, P.O. Box 3060, D-55020 Mainz, Germany*

[2]*University of Denver, Department of Chemistry and Biochemistry, 2190 E. Illif, Denver, Colorado 80208, USA*

[*]*Corresponding authors: c.pohlker@mpic.de, a.huffman@du.edu*

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**Supporting Figures:**

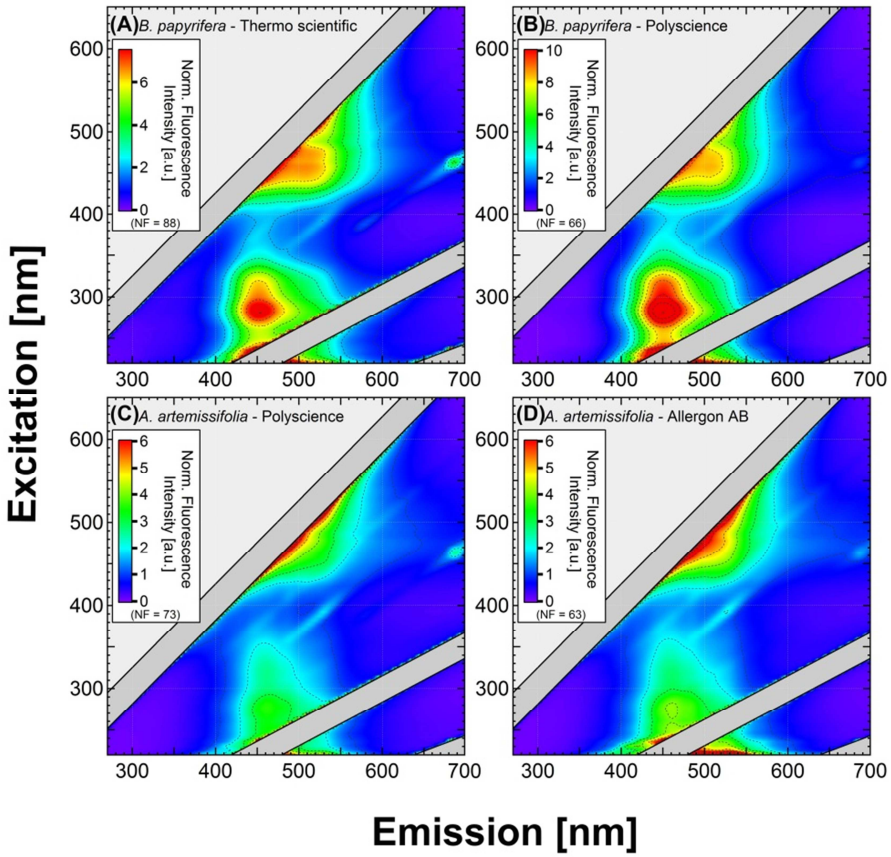


**Figure S1.** Comparison of excitation-emission-matrices (EEMs) of pollen purchased from different vendors: *Broussonetia papyrifera* purchased from Thermo Scientific (**A**) and Polyscience (**B**). *Ambrosia artimisiifolia* purchased from Polyscience (**C**) and Allergon AB (**D**). Fluorescence signatures in EEMs are shown to be identical, irrespective of commercial source.

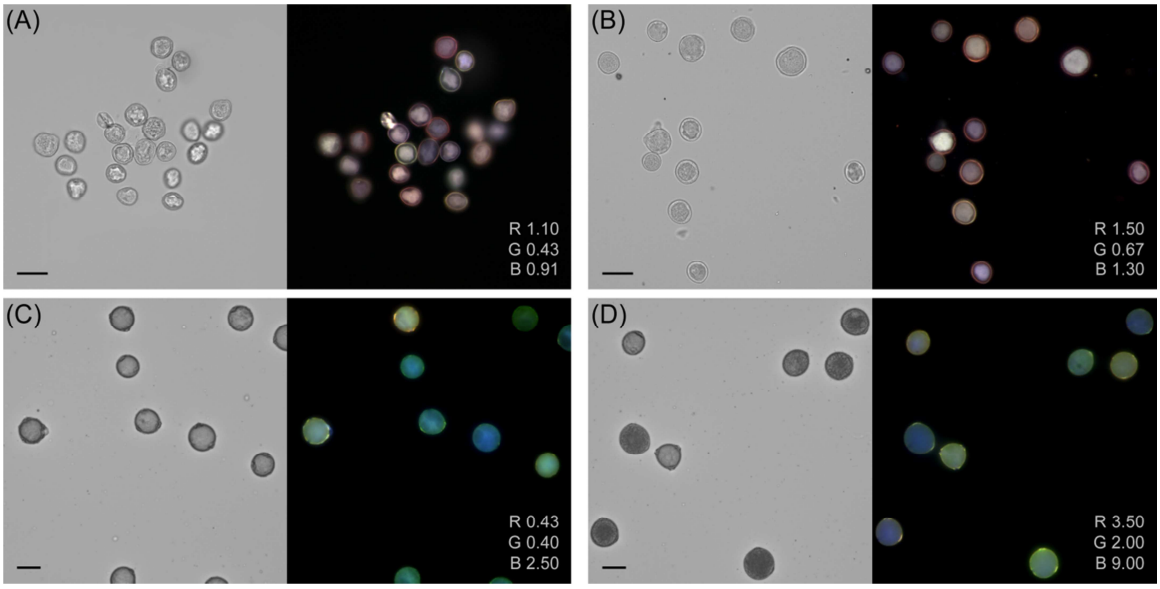


**Figure S2.** Microscopy images in bright field (left) and fluorescence mode (right) showing influence of age on fluorescence properties of two pollen species: **(A)** *Populus alba* harvested in March 2011 versus **(B)** *Populus alba* from March 2013; **(C)** *Fagus sylvatica* from April 2009 versus **(D)** *Fagus sylvatica* from May 2013. Regions with representative group of pollen grains are shown. White numbers show excitation exposure times for red (R), green (G), and blue (B) fluorescence channels. Shorter exposure times indicate higher fluorescence intensity. Analyzed samples suggest that fluorescence intensity of all grains increases with age - same effect is observed in corresponding EEMs (Fig. S3). No obvious differences in fluorescence microstructure between younger and older grains are observed. Scale bar = 30 µm.

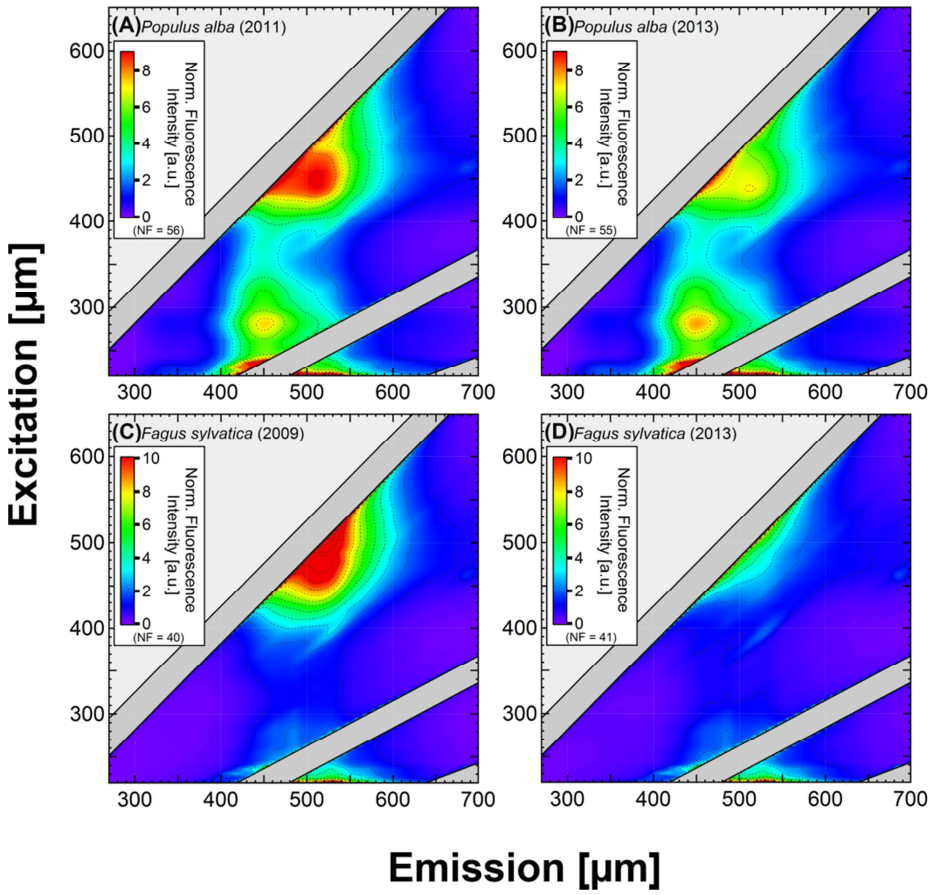


**Figure S3.** Excitation-emission-matrices (EEMs) showing influence of age on fluorescence properties by means of two pollen species: **(A)** *Populus alba* harvested in March 2011 versus **(B)** *Populus alba* from March 2013; **(C)** *Fagus sylvatica* from April 2009 versus **(D)** *Fagus sylvatica* from May 2013. Spectra show that fluorescence intensity of pollen bulk samples tends to increase with age, however, spectral signature in EEMs is conserved. *P. alba* shows slight intensity increase with age (Fig. S3A versus Fig. S3B), corresponding with comparably slight intensity increase in fluorescence microscopy images (Fig. S2A versus Fig. S2B). In contrast, *F. sylvatica* shows strong intensity increase with age (Fig. S3C versus Fig. S3D), corresponding with similarly strong intensity increase in fluorescence microscopy images (Fig. S2C versus Fig. S2D).

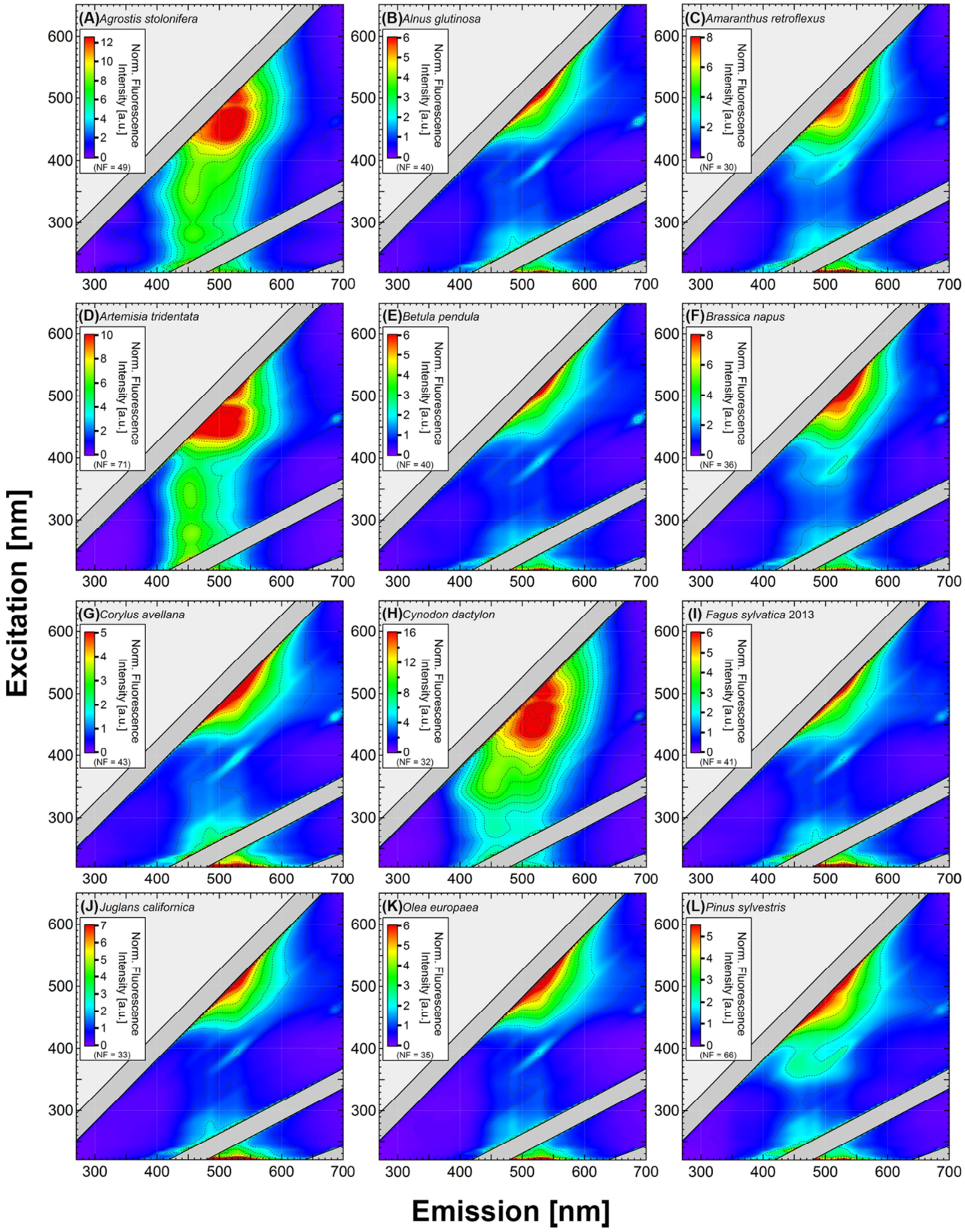
(A) Agrostis stolonifera
(B) Alnus glutinosa
(C) Amaranthus retroflexus
(D) Artemisia tridentata
(E) Betula pendula
(F) Brassica napus
(G) Corylus avellana
(H) Cynodon dactylon
(I) Fagus sylvatica 2013
(J) Juglans californica
(K) Olea europaea
(L) Pinus sylvestris
Norm. Fluorescence Intensity [a.u.]
(NF = 49)
(NF = 40)
(NF = 30)
(NF = 71)
(NF = 40)
(NF = 36)
(NF = 43)
(NF = 32)
(NF = 41)
(NF = 33)
(NF = 35)
(NF = 66)
Excitation [nm]
Emission [nm]

**Figure S4.**

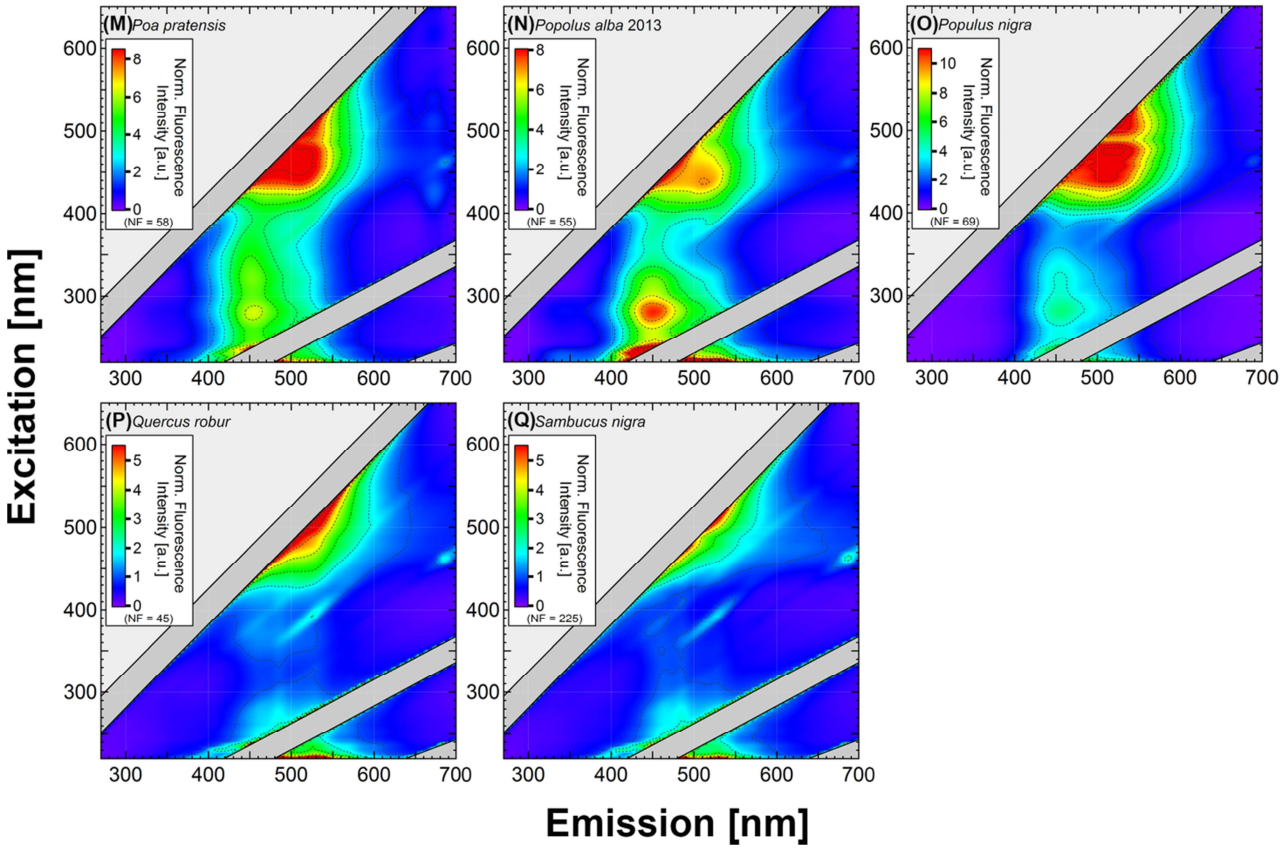


**Figure S4 cont.** Excitation-emission-matrices (EEMs) of pollen in dry state. Intensity color code has been adjusted to fluorescence intensity of individual samples. All EEMs are normalized and a normalization factor (NF) is reported in each panels (Sect. 2.3). *Agrostis stolonifera* (A) pollen was treated with acetone for dewaxing after harvest. However, the corresponding EEM does not show substantial alterations.

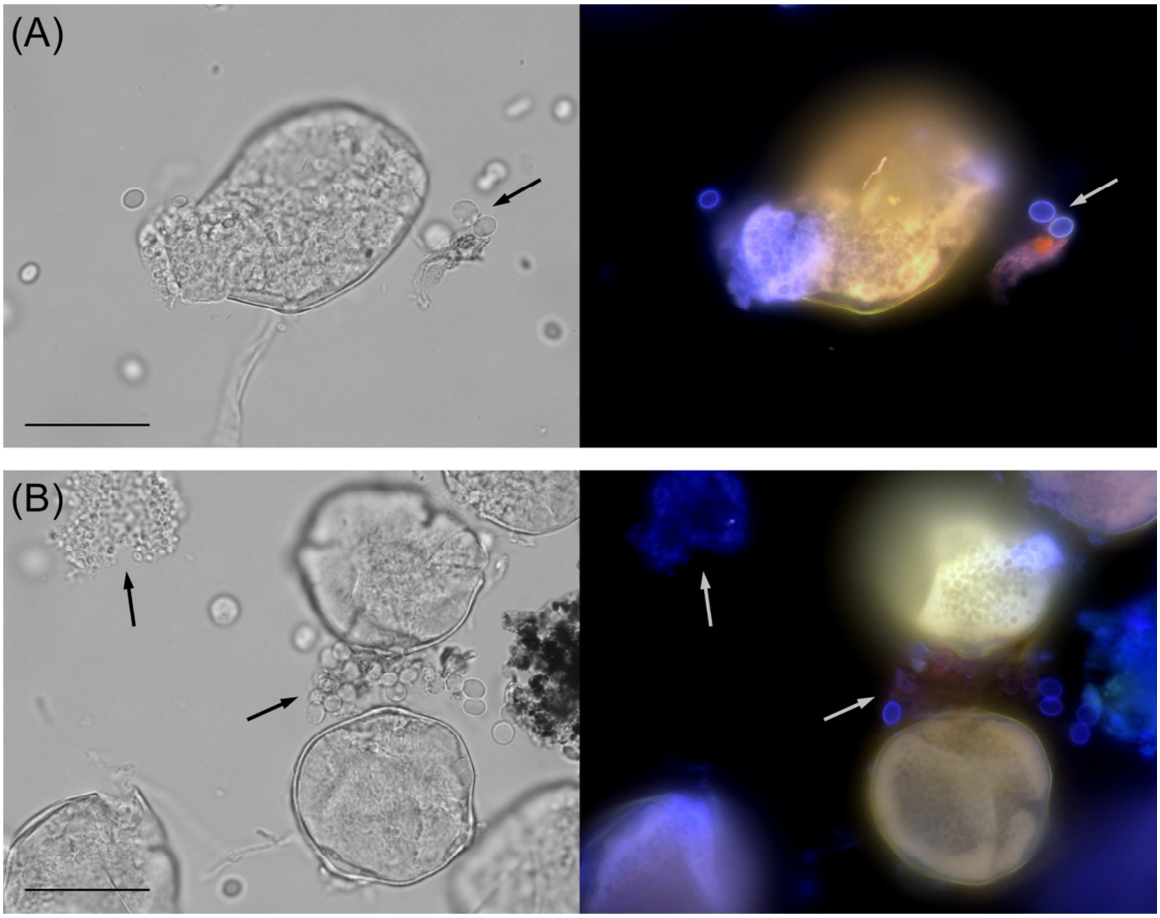


**Figure S5.** Microscopy images in bright field (left) and fluorescence mode (right) showing the "contaminating" small particles in the *Carpinus betulus* sample (in (**B**), right arrows) in comparison with cytosolic starch granules (in (**B**), left arrows). Ruptured pollen grains were not observed in the commercially obtained *C. betulus* sample. Pollen rupture as shown in (**A**) and (**B**) has been caused by mechanical stress. Comparison of the "contaminating" particles (4-6 µm) and cytosolic starch granules (~ 1 µm) shows substantial differences in particles size. Further, (**A**) illustrates the clear and blue cell wall-like fluorescence of the small particles (arrows). Scale bar = 30 µm.

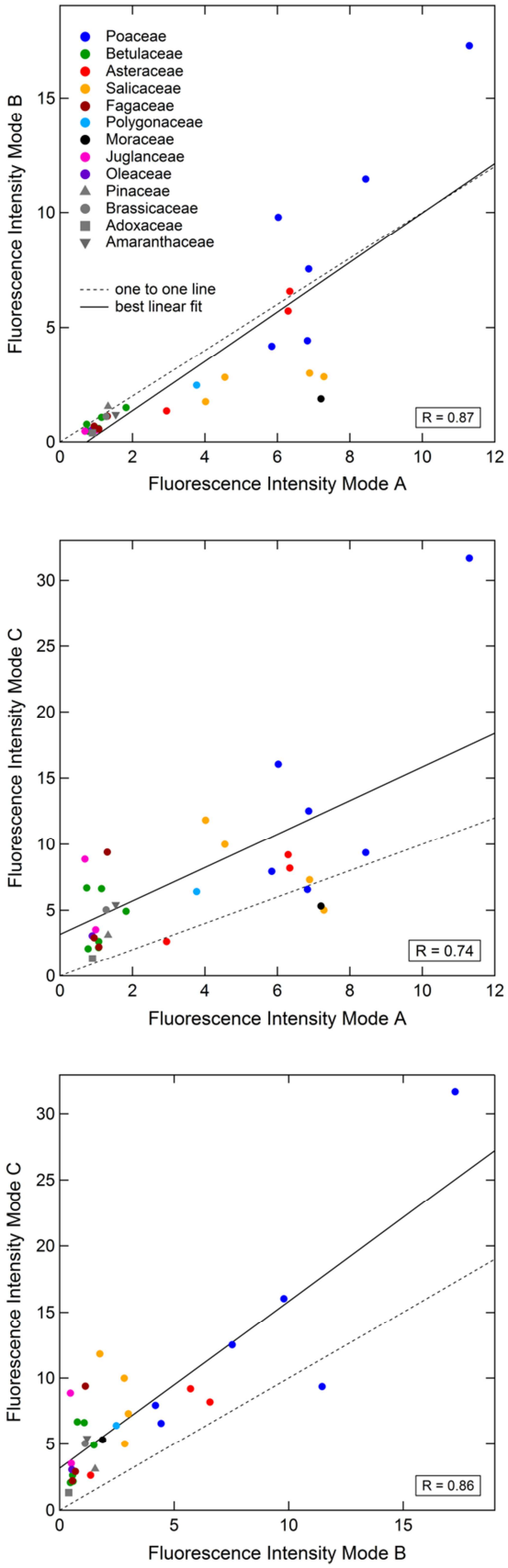


**Figure S6.** Scatter plots showing positive correlation between fluorescence intensities of main fluorescence modes A, B, and C (Fig. 5). Color code represents pollen families.

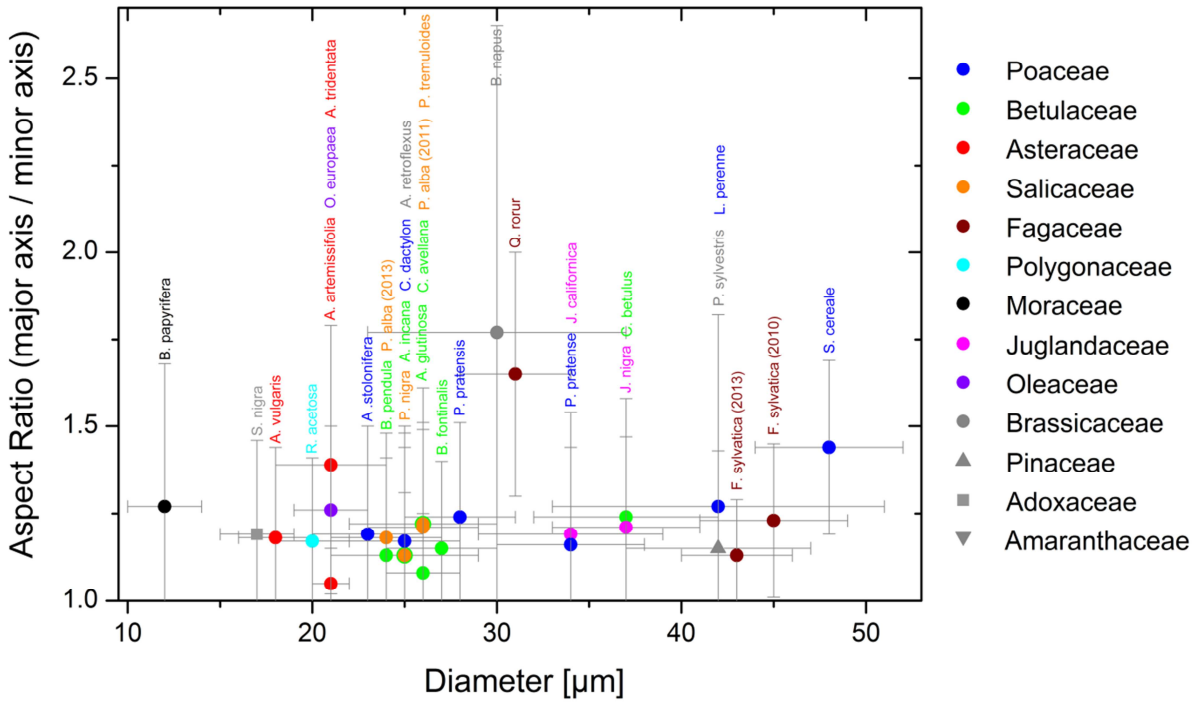


**Figure S7.** Overview of pollen grain size and shape (represented as aspect ratio) in dry state for 29 species analyzed in this study. Aspect ratio of ~ 1 approximates spherical grains, whereas aspect ratio >1 represents elongated grains. Typical aspect ratio for most pollen species in this study is 1.1 – 1.2. Many pollen species show grain size ~ 25 µm. No clear separation of families observed, based on size and shape. Error bars represent one standard deviation. Color code represents pollen families.