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PII: S0166-5162(14)00148-7
DOI: doi: 10.1016/j.coal.2014.06.024
Reference: COGEL 2334

To appear in: *International Journal of Coal Geology*

Received date: 4 February 2014
Revised date: 18 June 2014
Accepted date: 26 June 2014

Please cite this article as: Urbanczyk, Justyna, Casado, Maria Angeles Fernandez, Díaz, Tomás E., Heras, Patxi, Infante, Marta, Borrego, Angeles G., Spectral fluorescence variation of pollen and spores from recent peat-forming plants, *International Journal of Coal Geology* (2014), doi: 10.1016/j.coal.2014.06.024

# Spectral fluorescence variation of pollen and spores from recent peat-forming plants

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## Abstract

The fluorescence properties of spores and pollen grains examined under ultraviolet incident light are used to assess the maturity of sedimentary organic matter and may have other applications in relation to recent sediments, in areas such as palaeoenvironmental research. In this study pollen grains and spores from 33 species common in peat ecosystems were mounted on a glass slide in accordance with standard palynologycal procedures for recent plants. The main objective of this work was to assess the variability of fluorescence spectra of pollens and spores within a single species or even within a single sample. A minimum of 10 spectra were recorded from each sample and were averaged to obtain a spectrum characteristic of each sample. Both the average scattering and the scattering in different spectral regions were calculated using the standard deviation (SD) and the coefficient of variation (CV). The effect of the preparation techniques was assessed on some samples of Ericaceae taxa. The results indicated similar spectra for alcohol-washed and distilled water-washed samples, whereas the application of an acetolysis solution caused an increase in intensity and a shift to longer wavelengths. The spectra corresponding to the S*phagnum* spores had the lowest intensity of all the families studied and displayed their maxima at the lowest registered wavelengths. They often showed a peak in the red region of the spectra, causing a larger scatter in fluorescence in this region. This peak is probably the result of wax or cytoplasmic material attached to the exospore. A significant number of Ericaceae taxa had two fluorescing pollen populations: a blue one of high intensity and

smaller size a yellow-orange one of low intensity and larger size. This difference could be related to different degrees of maturity of the pollen grains. In the case of pollen grains of herbaceous, tree and bush plants the largest scatter was found in the tails of the spectra towards the blue and red regions. The decreasing trend of fluorescence intensity with the shift of the spectra towards red was not observed in the pollen and spores of fresh plants. A good correlation was found between the spectral maxima ($\lambda_{max}$) and the red-green quotient ($Q_{R/G}$) regardless of the type of plant.

***Keywords:*** Fluorescence spectra, recent plants, reflected light microscopy, spectral fluorescence parameters

## 1. Introduction

Pollen grains and spores are ubiquitously found in sediments and have been used in numerous applications including palaeoenvironmental studies (determination of plant assemblage in a given place at a given moment –Traverse, 2007), basin formation and evolution (assessment of the distance to the coastline in a basin –Tyson, 1995), petroleum exploration (determination of the degree of maturity of the sediment based on its colour change –Teichmüller, 1986), etc. Their ubiquity is essentially due to the high resistance of the outer layer of the walls known as exine in pollen grains and exospore in spores, which is crucial for the fertilization process in the former and for the survival of the latter under adverse climatic conditions (Brooks and Shaw, 1972). This outer layer, often sophisticatedly ornamented and sculptured (Bedinger, 1992), is the most distinctive feature of each species, and shows strong resistance to chemical and biological decay. The inner layer essentially consists of cellulose and pectin and degrades rapidly during fossilization (Ivleva et al., 2005), whereas a significant part of the chemical composition of the outer layer consists of a complex and highly resistant biopolymer known as sporopollenin (Zetzsche and Kälin, 1931). A carotenoid nature has been suggested for sporopollenin (Brooks and Shaw, 1978), the composition of which varies for different species (Guilford et al., 1988). The use of degradative and pyrolysis techniques has revealed *n*-alkanes, *n*-alk-1-enes, α,ω-alkadienes, alkylphenols and benzaldehydes (Dungworth et al. 1971; Schenck et al.1981; Davis et al. 1985; van Bergen et al. 1995) to be the main structural moieties of sporopollenin in different

proportions. The composition of sporopollenin varies with the degree of fossilization which gives rise to different chemical and structural modifications (Yule et al. 2000). These modifications are reflected in the colour change of the spores and pollen from pale yellow through orange, reddish brown to finally black with increasing temperature due to the depth of burial. The systematic changes have been widely used as a thermal indicator through either qualitative (Staplin, 1969; Fisher et al., 1981; Pearson, 1982) or quantitative scales (Marshal, 1991; Yule et al., 1998, Ujiié, 2001). The colour transformation with increasing maturation corresponds to a decrease in O and H and an increase in the percentage of C, which is translated into a significant reduction of aliphatic groups and an increase in aromatic C=C bonds in the Fourier Transformed Infrared (FT-IR) spectra (Yule et al. 2000). The subtle changes in colour in the immature stage correspond to a reduction of carbonyl/carboxyl groups and a relative increase in aliphatic -$CH_2$- and -$CH_3$ groups. Since the early studies of van Gijzel (1963), it has become clear that the maturity transformations observable in spores and pollen under transmitted white light also have an effect on their fluorescence properties (van Gijzel, 1967a). The fluorescence phenomenon is due to the presence of "fluorophores" and "chromophores" (Lin and Davis, 1988) in a molecule. These possess electrons, which are susceptible to being excited by incident light to higher levels of energy, emitting fluorescence light when they return to their basal stage. FT-IR studies suggest that aliphatic unsaturated C-C double bonds and conjugated C-C double bonds adjacent to C-O groups are responsible for spore and pollen fluorescence properties (van Gijzel 1971; Yule et al., 2000). Although the first studies on spore and pollen fluorescence were performed using palynological slides (Berger, 1934), it was soon acknowledged that polished pellets and blocks could also be used and therefore the same preparations as for vitrinite reflectance measurements could be employed. Moreover a good correlation was established between the maturity of the source rock determined from the bulk chemical results or by the vitrinite reflectance measurements and the spectral fluorescence properties of sporinite (Jacob 1964; van Gijzel, 1967b; Ottenjann et al; 1982; Robert, 1979, Teichmüller, 1986). In general it has been found that the spectra decreased in intensity and shifted to a reddish colour with increasing maturity.

The most widely used parameters extracted from the fluorescence spectra of sporinite are the wavelength of the spectral maximum ($\lambda_{max}$) and the red/green quotient

($Q_{R/G}$). Other parameters such as the total emission flux (F), the quotient between the areas at higher and lower wavelengths than 530 nm (Pradier et al., 1988), the area corresponding to the different spectral colours (Crelling, 1983), or the chromaticity coordinates using the CIE (International Commission on Illumination) chart (Hagemann and Hollerbach, 1981) have been used in various attempts to differentiate numerically the spectroscopic curves. The differences between the fluorescence spectra of different pollen/spores species tend to decrease with increasing age of the sediment (van Gizel, 1967b). This, apart from the difficulty of distinguishing between the various species in polished blocks, might be the reason why the fluorescence spectra of sporinite are recorded all together in maturity studies. Nevertheless, the spectra of various species in sediments of the same age have been differentiated and significant differences have been found between *Sphagnum* spores and pollen grains of the same age in peats (van Gizel, 1967b). Other aspects, which affect the fluorescence spectra, are acid treatment during the preparation of the samples (van Gizel, 1967b; Mendonça Filho et al., 2010), environmental changes (Yeloff and Hunt, 2005) and differential alteration and corrosion of the grains (Havinga, 1967).

The aim of the present study is to record the natural variation of fluorescence spectra from pollen and spores of peat-forming plants as a first step to study their characteristics and variability in peat deposits. Fluorescence spectra can record the effects of alteration caused by prolonged dryness or microorganismal action providing additional and highly useful palaeoenvironmental information (Yeloff and Hunt, 2005).

## 2. Experimental

Strew mounts from pollen and spores (44) of 33 different species of peat forming plants, common in the peat bogs of northern Spain have been used in this study. Most of the *Sphagnum* samples were provided by the VIT Herbarium of the Natural Science Museum of Alava (Vitoria) while the other peat-forming plants were provided by the Faculty of Biology (BOS department) of the University of Oviedo. The samples were prepared in their respective storage locations. The *Sphagnum* spores from the VIT Herbarium were hand-picked from the capsule and spread on glass slides. The BOS samples were acetolysed using the method devised by Erdtman (1960) to remove the cytoplasmic and cellulosic material in order to improve the transparency of the specimens.

The samples for microscopic examination were fixed with Kaiser´s glycerol gelatin, after first making sure the fixer was free of fluorescence. The preparation technique used ensures that most of the grains are far enough apart inside the gel, to minimize the effects of different orientations or the presence of folded specimens in the fluorescence spectra.

An incident light optical microscope equipped with a LED light source, a combination of filters to provide ultraviolet illumination (excitation filter BP 360/40, dichroic mirror 400, suppression filter 470/40) and an oil immersion objective (50x) was used to record the spectra. Intensities were recorded in the 420-750 nm range at 5 nm intervals and were corrected for background noise. In order to be able to compare the intensities of the spectra a green 2941B007 standard with maximum intensity at 527 nm was employed. The maximum intensity of the standard was considered to be 1, and after measuring the standard through the optical system a factor was introduced to correct the spectral intensity of each spectrum. Fig. 1 shows the expected and recorded values of the standard. The spectra were only corrected for intensity. As the pollen and spore spectra cover a wider wavelength range than the certified values of the standard, no other functions for shape could be applied. Nevertheless comparison of the recorded and expected results for the standard indicated that a minor alteration of the spectra due to the transmittance of the optical system was to be expected, except for a small shoulder at 565 nm that was identified in the fluorescence spectra. The equipment also recorded images of the same specimen where the spectrum was taken. At least 10 spectra were recorded from each slide and they were averaged to produce a representative spectrum of the sample. The following spectral parameters– $\lambda_{max}$ (spectral maximum), $Q_{R/G}$ (red green quotient) calculated as the intensity at a wavelength of 650 nm divided by the intensity at 500 nm, Q+ (Intensity of the spectral maximum divided by the intensity at 500 nm) and $I_{max}$ (maximum intensity)– were used to compare the characteristics of the spectra. Additionally the standard deviation (SD) of the averaged intensity at each measured wavelength was calculated for each sample. As the intensities of the spectra were rather different and the SD is affected by the actual intensity value, the coefficients of variation (CV) were also calculated in order to compare the scatter of the different spectra within a given sample independently of the intensity. The average of the SDs and CVs through the measured spectral wavelengths (ASD and ACV, respectively)

provided an average value for these statistics, making it easier to assess the scatter of the spectra.

Given the large variation observed between the spectra of some *Sphagnum* and Ericaceae taxa several tests were performed to rule out sample preparation procedures as being the main cause of the variability. Only alcohol-washed (al) and untreated (u) samples could be compared in the case of *Sphagnum* due to the scarcity of the material. In the case of Ericaceae species water-washed (w), alcohol-washed (al) and acetolysed samples using the Avetisjan procedure (av) could be prepared. The latter procedure (Avetisjan, 1950) was used in this case to assess the effect of acetolysis on the pollens (Pupuleku et al., 2010), because a much smaller amount of sample is required (only a few drops of solution spread directly over the slide together with the material to be mounted) than with the Erdtman method.

## 3. Results and discussion

### *3.1. Variation of the spectra within a single sample.*

The spectra from different pollen grains and spores within a single sample generally had a similar shape, though rather different intensities. In a few cases such as that illustrated in Fig. 2i for *Erica mackaiana* a single sample contained pollen grains with different fluorescence characteristics. Fig. 3 shows the fluorescence spectra recorded for three samples which can be considered as an example of the different phenomena observed when recording the fluorescence spectra:

Case i. All the spectra within the sample exhibited a similar shape and a similar $\lambda_{max}$ but different intensities as shown in Fig. 3a. This was observed in most of the samples including tree pollen (*Pinus pinaster, Quercus robur, Castanea sativa, Alnus glutinosa, Betula pubescens, Cupressus sempervirens, Eucalyptus globulus, Salix atrocinerea, Tilia cordata*), grass pollen (*Arnica montana, Taraxacum sp., Serratula tinctoria, Polygala serpyllifolia, Ranunculus repens, Scilla verna*), Ericaceae pollen (*Erica umbellata*) and some *Sphagnum* spores.

Case ii. The spectra within the sample exhibited two maxima and in the various grains the relative intensities of both maxima were different. This was particularly common in the *Sphagnum* samples that typically had a maximum centered in the blue region of the spectra (470-505 nm; Table 1) and a peak in the red region of the spectra

(invariably at 675 nm) attributed tentatively to pigmented wax or capsule debris attached to the wall (Fig. 3b). In some cases the intensity of the peak at 675 nm was higher than that reached in the 470-505 nm region, but the values recorded in Table 1 as $\lambda_{max}$ and $I_{max}$ reflect the maxima in the blue region. In the *Sphagnum* samples analysed the red peak was present in *S. papillosum, S. squarrosum, S. angustifollium, S. tenellum and S. auriculatum*, whereas the other examples of *S. tenellum and S. auriculatum*, and the spores from *S. capillifolium, S. fimbriatum, S. palustre* and *S. subnitens* only showed a blue maximum. The *Sphagnum* spectra in peat do not have a peak in the red region indicating that this material is rather labile and is easily destroyed even with a superficial humification degree. The presence of the reddish material in *Sphagnum* was not specific to some species because when two samples were available from the same species either, one (*S. tenellum and S. auriculatum)*, both (*S. papillosum*) or none (*S. fimbriatum*) showed a red peak. In addition within a single sample from the same plant only some grains exhibited a red peak (Fig. 3b). It was initially thought that the presence of material attached to the *Sphagnum* spores could be related to the preparation procedure, since spores had to be scratched from the capsule wall, because the discharge of *Sphagnum* spores is so violent that very few are left inside the capsules. In a few cases enough spores were present to attempt washing the sample with alcohol. However further treatment with acetolysis solution following Avetisjan procedure yielded sterile samples. Fig. 4 shows an example of the average spectrum of *S. angustifolium* spores spread over the slide without any treatment (untreated) and one after being washed with alcohol. It can be observed that the peak in the red region was still present. This also occurred in *S. tenellum* (a). The red peak was not observed in the spectra of any of the spores of *S. papillosum* and *S. squarrosum* after alcohol-washing, although it was present in the untreated samples (Table 2). In summary, the peak was only discernible in some of the spectra of a given slide, and several taxa of a single species had or not the red peak in their averaged spectra. The persistence of the red peak at 675 nm after alcohol washing suggests that this peak was not a result of the sample preparation procedure. The spectral parameters also indicated a drop in intensity and a slight shift to shorter wavelengths in the spectra of alcohol-washed samples. In addition a lower scatter could be observed in these samples, as reflected by the ACV (Table 2).

Case iii corresponds to samples with grains that showed rather different fluorescence spectra within a single sample. This was only observed in Ericaceae plants

in which *Daboecia cantabrica* as well as *Calluna vulgaris, E. australis, E. ciliaris, E. mackaiana, E.tetralix* and *E.vagans* showed some bluish pollens and some orange pollens (Fig. 3c), which were averaged separately.

**Variation in the fluorescence spectra of the spores.** The spores of the different *Sphagnum* species showed similar characteristics under incident ultraviolet light illumination exhibiting their characteristic trilete aperture (Fig. 2h). In the case of the *Sphagnum* spectra the largest SDs were observed in the region where the red peak (675 nm) is situated, corresponding to values higher than 2.3, whereas for the rest of the spectral range the SDs were significantly lower (Table 1). In the case of CVs, the ACV values ranged between 0.15 and 0.52 with values below the average up to a wavelength of 650 nm, maximum values being reached only in the red region of the spectra. The largest differences between minimum and maximum values for CV (Table 1) corresponded to samples that showed the largest variability in red peak intensity, and also a generally higher ACV (Table 2). Among the *Sphagnum* species studied, only *S. subnitens* systematically showed a higher CV throughout the whole spectral range, indicating a larger scattering of the spectra. Two samples for each species of *S. tenellum, S. papillosum, S. fimbriatum* and *S. auriculatum* were studied, whose spectra exhibited similar shapes for both samples. Nevertheless it can be seen from Table1 that the differences in intensity ($I_{max}$) and scattering (CV) within two samples of the same species were in the range of those observed between different *Sphagnum* species. The $I_{max}$ of the *Sphagnum* spectra ranged between 1.6 and 7.9 and were among the lowest values of all the spectra analysed. In general the maxima of *Sphagnum* spectra were in the blue region (470-495 nm), except for *S. palustre* which was shifted to higher wavelengths (505 nm) and exhibited a high fluorescence intensity ($I_{max}$ 7.9 see Fig. 5).

The spores of the fern *Pteridium aquilinum* also have a characteristic trilete morphology with a $\lambda_{max}$ in the blue region of the spectra (at 465 nm) and a fluorescence intensity higher (11.7) than that of the *Sphagnum* spores.

**Variation of fluorescence spectra of tree pollen grains.** The tree pollen represented in this study corresponds to *Alnus glutinosa, Betula pubescens, Castanea sativa, Cupressus sempervirens, Eucalyptus globulus, Pinus pinaster, Quercus robur,*

*Salix atrocinerea*, and *Tilia cordata*. From *Quercus*, *Pinus*, *Castanea* and *Eucalyptus* two taxa of each species were measured. The morphology of the main pollen types and their most distinctive characteristics (under incident fluorescence light) is shown in Fig. 2a, 2d, 2g, 2k, 2l, 2o and 2p. All these pollen types corresponded to Case i, where the spectra have a single maximum (Fig. 6). In general the spectral maxima ranged between 503 and 565 nm (Table 1) and were located at higher wavelengths than those of the *Sphagnum* spores except in the case of *Cupressus sempervirens* and *Salix atrocinerea* ($\lambda_{max}$=465 nm). The intensities ranged between 9.2 and 44.8 and were generally higher than those of the *Sphagnum* spores. Where there were two slides of a single species the differences in intensity varied to the same extent as for tree pollens of different species and families (Table 1). As can be seen in Fig. 2 the morphology of the tree pollens are very different and the examples with the highest intensities were *Pinus pinaster* (44.8), *Salix atrocinerea* (37.2) and *Castanea sativa* (35.1). In the case of the tree pollens the largest SD among the spectra were close to the spectral maxima, whereas the largest CVs were located in the tails of the spectra. The ACV values were smaller than those of the *Sphagnum* spores indicating a smaller variation between the fluorescence spectra of a single sample (Table 1).

**Variation of fluorescence spectra of Ericaceae pollen grains.** As mentioned above many samples of Ericaceae showed a relatively large variation of fluorescence properties (Fig. 2i). The Ericaceae pollen grains displayed the typical tetrahedral tetrads of 4 cells (each cell is a tricolporate grain), with the pore area of adjacent grains in contact with each other and a quasi-spherical outline (Fig. 2i and Fig 2f). The more bluish spectra in Fig. 3c, had spectral maxima at 500-515 nm, whereas the yellow-orange pollens had spectra with maxima in the region of 525-565 nm (Table 1). The bluish specimens generally showed higher fluorescence intensities (27.4-80.0) than the orange pollens (11.4-31.8) from the same taxon (Fig.7), whereas *Erica umbellate,* with a single population, had an intensity similar to those of orange pollens (19.7). The variability of pollen fluorescence properties within a single taxon might be due to the different degree of maturity or alteration of the pollen grains. Both maturity and alteration can cause a decrease in fluorescence intensity and a shift towards red in the spectra (Ottenjann et al., 1982; Khorasani and Michelsen, 1992). The ASDs of the bluish spectra were larger than those of the orange spectra for the same taxon due to

their higher fluorescence intensity, but the ACV did not show a systematic trend (Table 1). In both cases the maximum SD within a single spectrum was close to the spectral maximum and the CVs either remained more or less constant over the whole spectral range or had higher values towards shorter wavelengths.

A close observation of the grains with different fluorescence colors revealed that the orange grains had larger and better developed walls than the blue ones. Therefore a systematic study was carried out to record the size of the blue and orange pollens separately. For each type of pollen 25 measurements were performed, the results of which are presented in Table 3. In general the pollen exhibiting orange fluorescence (labelled II) tended to be larger than those with blue-yellowish colors (labelled I) for each sample. The size of the orange grains ranged between 37.1 µm (*E. vagans*) and 49.5 (*E. mackaiana*), while the bluish grains ranged between 28.1 µm (*E. vagans*) and 36.1 µm (*E. mackaiana*). The differences in colour for *C. vulgaris* (b) were not clear enough for the eye to split them into two populations. The pollen sizes ranged from 26.4 to 37.4 µm (average value of 33.3 µm) indicating predominance of the "smaller" size population. The possibility that the acetolysis preparation procedure based on the Erdtman method (er) caused the pollen to swell (Reitsma, 1969) and an additional shift of the fluorescence spectra to longer wavelengths in some pollen grains was investigated by preparing some samples with three different solutions (distilled water, alcohol and Avetisjan acetolysis). As the Erdtman procedure requires a large amount of grains, the contents of various pollen sacs are generally used in the preparation. This particular sample was subjected exclusively to Erdtman acetolysis, which is the standard procedure for fresh plant palynological preparations in the laboratory, and it was not used for the milder procedures applied to observe the variability in size and fluorescence colors of the Ericaceae specimens.

Samples of *Calluna vulgaris, E. australis, E. tetralix and E. vagans* were washed with distilled water (w) alcohol (al) and acetolytic reactants using the Avetisian procedure (av). For some of the species, various samples from different specimens were prepared. The morphology of *E. australis* and *E. vagans* pollens after the different treatments is shown in Fig. 8. Both the fluorescence spectra (Table 4) and the size of the specimens (Table 3) were recorded for these samples. In most of the cases, as can be seen in Table 4, the spectra corresponded to only one population, except for water-washed *E. australis* (d) and Avetisjan-acetolysed *E. tetralix* (c) which corresponded to

two populations. In both cases minor differences in the values of $I_{max}$ (16.5-13.3 and 14.1-19.2, respectively) and $\lambda_{max}$ (460-470 and 500-510, respectively) could be observed between the two populations (Table 4), which were not large enough to justify splitting them into two groups of pollen sizes (Table 3). These differences were much smaller than those observed between the bluish and orange populations shown in Table 1. The sizes measured indicate that there were no systematic differences observed in the sizes of the pollen as consequence of the cleaning solution and also that the grain sizes tended to correspond to the blue-yellowish population observed in the Erdtman-acetolysed samples (Table 3). The data in Table 4 also show that the $\lambda_{max}$ values generally corresponded to the blue region of the visible spectrum, except for the av-samples, which were more yellowish in color (Fig. 8). The spectra of *E. vagans* (c) after the three preparation procedures are presented in Fig. 9, where the similarity between the water-washed and alcohol-washed spectra can be appreciated whereas the Avetisjan-treated sample had a higher intensity spectrum that is shifted to higher wavelengths as can be seen in the images of Fig. 8.

The effect of the preparation procedure on the fluorescence spectral parameters can be better appreciated in the plots of Fig. 10, where the pairs of values corresponding to alcohol-water and alcohol-Avetisjan are plotted for the different parameters. In all the cases the values of the spectral parameters were spread out along the mediatrix line without any systematic trend. The scatter was low in the case of $\lambda_{max}$ and the characteristic quotients ($Q_{R/G}$ and Q+) and slightly larger for the $I_{max}$ values (Fig. 10). A different trend was observed when the alcohol-washed and the Avetisjan-treated samples were compared. In this case the acetolysis solution tended to cause an increase in $I_{max}$, a significant shift to higher wavelengths of the spectra (increase in $\lambda_{max}$ and $Q_{R/G}$), and a decrease in Q+ (Fig. 10). The results of this study showed that alcohol-washing hardly modified the characteristics of the spectra compared to washing with distilled water and that the acetolytic reactants caused a shift to higher wavelengths. This shift was of a similar magnitude for the pollens of homogeneous size and with similar fluorescence characteristics. The Avetisjan-treatment did not affect the size of the grains in a systematic way (Table 3), nor did it increase the variation of the spectral fluorescence parameters (Table 4) and therefore the acetolytic solution cannot be considered responsible for the great variation of pollen fluorescence observed in the Erdtman-treated samples. Although no direct evidence of a shift to orange was observed

in the Avetisjan-acetolysed samples, the morphology of the orange grains in the Erdtman treated samples suggests that they were more mature than their yellow counterparts. The existence of two populations of different size and spectral properties in these samples could be related to differences in the maturity of the pollens. The acetolysis treatment may have caused the larger and more mature pollens to experience a greater shift in spectral properties than the less mature pollen, accentuating the differences in their fluorescence properties. However all the samples prepared to test this hypothesis contained only the smaller and probably less mature populations.

**Variation of the fluorescence spectra of herbaceous plant pollen grains.** The examples of herbaceous plant pollen included in this study are shown in Fig. 11. They were *Arnica montana* (Fig. 2n), *Serratula tinctoria* (Fig. 2c) and *Taraxacum* sp. (Fig. 2q) from Asteraceae. They had a relatively thick wall with an irregular arrangement of spines and very different fluorescence characteristics. The other herbaceous plants include *Scilla verna* (Fig. 2b) that had elongated pollen with yellow fluorescence colours, *Polygala serpyllifolia* (Fig. 2e) that had a characteristic psilate ornamented surface and yellow-orange fluorescence colours and *Ranunculus repens* (Fig. 2j) with a round and perforated shape and blue fluorescence colours. As can be seen in Fig. 11, the *Serratula tinctoria* spectrum showed the highest intensity (74.5). Only a few herbaceous species with rather different pollen morphologies have been considered for this study making it difficult to depict general observations about their fluorescence spectra. The highest intensity was recorded for *Serratula tinctoria* (Fig. 2c), whose pollens were among the most intensely fluorescing of the set analysed. The range of intensities was similar to those of the Ericaceae pollen, and was close to the tree pollen spectra with the highest intensities (Table 1). The scattering within the spectral range tended to be higher in the blue region of the spectra.

### *3.2. Variability of the spectral parameters*

Although the differences between the spectra of the various species considered in this study have already been discussed, this section will focus on some spectral parameters that could also be used to follow the changes that occurred during the formation of the peat or sediments from which the pollen and spores were taken in order

to determine the degree of alteration undergone. Fig 12 shows the variation in intensity and $Q_{R/G}$ versus the spectral maxima. The spores from the ferns (Hypolepydaceae) and mosses (Sphagnaceae) have been plotted as a single group and pollens have been grouped into bushes (Ericaceae), trees (Pinaceae, Fagaceae, Betulaceae, Cupressaceae, Myrtaceae/Myrtoideae, Salicaceae, Malvaceae/Tilioidea) and herbaceous plants (Asteraceae, Polygalaceae, Ranunculaceae/Ranunculoideae, Asparagaceae/Scilloideae). Despite the scattering in the values of Fig. 12a, it can be seen that the lowest intensities corresponded to the spores (values below 10), whereas most of the pollen species showed values between 10 and 50. In addition the spores had typically lower intensities than most of the pollen grains with the few exceptions already mentioned. The typical trend of the fluorescence intensity to decrease with the shift of the spectra towards red (Teichmüller, 1986), which is observed in organic sediments of increasing maturity was not observed in the pollen and spores of the fresh plants considered in this study (Fig. 12a). This is evidenced by the low intensity of the blue spectra of the spores. It should be noted that the intensity of the pollen spectra may have been enhanced due to acetolysis, since the non-acetolysed samples had lower $I_{max}$ values. Nevertheless the non-acetolysed Ericaceae had notably higher $I_{max}$ values than most of the non-acetolysed *Sphagnum* (Table 1 and Table 4).

There is a good correlation between the spectral maxima ($\lambda_{max}$) and the $Q_{R/G}$ regardless of the type of plant, which did not follow a linear trend (Fig. 12b). The spores typically had a lower $Q_{R/G,}$ than most of the pollen grains. Minor differences were observed in the case of Q+ (not shown), which measures the distance between $\lambda_{max}$ and the 500 nm wavelength in each spectrum. It can be noticed that the Q+ reached values close to 1 when the $\lambda_{max}$ was close to 500 nm. The spores and the more yellowish Ericaceae pollen had values close to one, whereas the values of Q+ were greater in the case of the tree pollen and the more orange Ericaceae grains. It can be seen from these data that The Q+ parameter appears to have little discriminating ability.

The $\lambda_{max}$ and intensity values were grouped into various classes in order to construct a histogram that would make it possible to determine the most common intervals for each type of plant. In the case of $\lambda_{max}$ the class width selected was 25, whereas for $I_{max}$ it was 10. Fig 13 shows the distribution of the spectra among various classes. A clear shift to lower wavelengths in the case of the spores compared to the pollens was observed (Fig. 13a). Both the tree and bush pollens belonged to the 500 nm

modal class. The specimens collected from the herbaceous plants were too few to draw firm conclusions about the modal values of these plants. In the case of intensity, the modal values for spore intensity corresponded to class 0 comprising values below 10 (Fig. 13b), while the Ericaceae pollens tended to have higher intensities (30) than the tree pollens (10). The herbaceous plants showed intermediate intensities (20).

## 4. Conclusions

The fluorescence spectra of pollens and spores of fresh peat-forming plants showed a certain variation within a single species and even within a single taxon, which may be relevant to the application of fluorescence property variation in palaeoenvironmental studies. The sample preparation procedure based on spreading spores or pollen grains on a glass plate, which is typically employed in palynofacies studies, has shown to be appropriate for obtaining fluorescence spectra that generally exhibited a similar shape but a different intensity in each taxon. The presence of two spectral types within a single taxon was common in the case of Ericaceae, indicating that this family was particularly sensitive to variation in fluorescence properties. The variation of fluorescence properties in each taxon was related to size suggesting that the spectra of more mature and larger grains were shifted to longer wavelengths.

The preparation procedure (water-washing versus alcohol-washing) had little effect on the intensity and shape of the fluorescence spectra and the differences did not reflect any systematic trend, whereas the acetolytic solution used in the Avetisjan procedure generally caused an increase in spectral fluorescence intensity and a shift to longer wavelengths.

The spectra of the *Sphagnum* spores had maxima at the lowest wavelengths and showed the lowest intensity of all the families analysed. They often exhibited a shoulder in the red region of the spectra, which was responsible for the larger scattering of fluorescence in this region attributed to wax or cytoplasmic material attached to the exospore. In the case of the pollen grains of herbaceous, tree and bush plants the largest scattering was found in the tails of the spectra in the blue and red regions. The trend for the fluorescence intensity to decrease with the spectral shift towards red typically observed in spores and pollen of different age was not observed in the pollen and spores

of fresh plants. A good correlation was found between the spectral maxima ($\lambda_{max}$) and the red-green quotient ($Q_{R/G}$) regardless of the type of plant.

***Acknowledgement:*** Financial support from MICINN (CGL2009-13990-C02-01/02) MINECO (CGL2013-46458-C2-1-R) and a FPU fellowship from MEC for J. Urbanczyk are gratefully acknowledged.

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**Figure Captions**

Fig.1 Certified values and obtained values of the fluorescence standard 2941B007 used in this study.

Fig. 2. Appearance of the most characteristic pollen and spores studied under incident fluorescence light illumination. Oil immersion objective: a) *Eucalyptus globulus*; b) *Scilla verna*; c) *Serratula tinctoria*; d) *Tilia cordata*; e) *Polygala serpyllifolia*; f) *Calluna vulgaris*; g) *Castanea sativa*; h) *Sphagnum angustifolium*; i) *Erica mackaiana*; j) *Ranunculus repens*; k) *Betula pubescens*; l) *Quercus robur*; m) *Salix atrocinerea*; n) *Arnica montana;* o) *Alnus glutinosa*; p) *Pinus pinaster*; q) *Taraxacum* sp.

Fig. 3. Variation of fluorescence spectra within a single taxon. Examples from the three case studies observed.

Fig. 4. Fluorescence spectra of *Sphagnum angustifolium* spores prepared by 2 different methods.

Fig. 5. Average fluorescence spectra of the studied spores.

Fig. 6. Average fluorescence spectra of the studied tree pollens.

Fig. 7. Average fluorescence spectra of Ericaceae pollens.

Fig. 8. Appearance of Ericaceae grains after 3 different preparation procedures: a and d) water-washed; b and e) alcohol-washed; c and f) Avetisjan-acetolysed.

Fig. 9. Examples of fluorescence spectra of water-washed (w), alcohol-washed (al) and Avetisjan-acetolysed (av) *Erica vagans* (c) sample.

Fig. 10. Variation in spectral fluorescence parameters of alcohol-washed versus water-washed and Avetisjan-acetolysed Ericaceae taxa: a) $\lambda_{max}$, b) $I_{max}$, c) $Q_{R/G}$, d) Q+.

Fig. 11. Average fluorescence spectra of Herbaceous pollen.

Fig. 12. Relationships between $\lambda_{max}$ and $I_{max}$ (a) and $\lambda_{max}$ and Q+ (b) for the taxa studied.

Fig. 13. Histogram showing the distribution of $\lambda_{max}$ (a) and $I_{max}$ (b) values from fluorescence spectra in the various types of plants analysed

Figure1

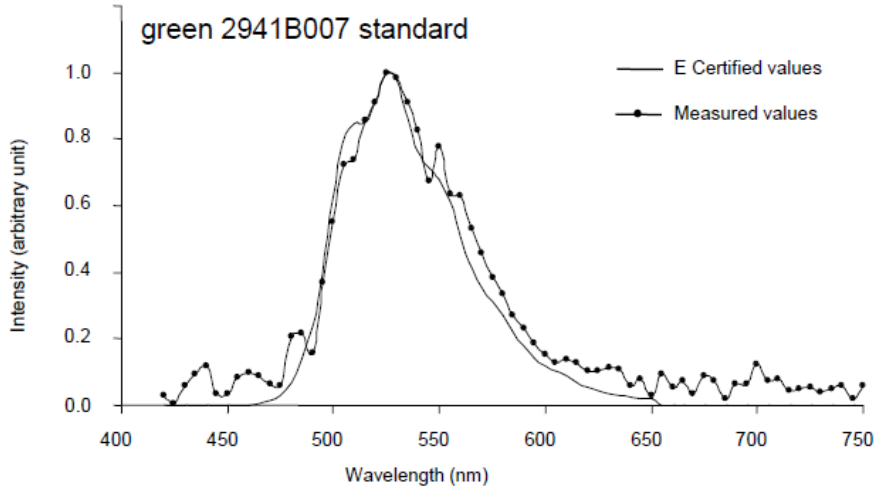


Figure2

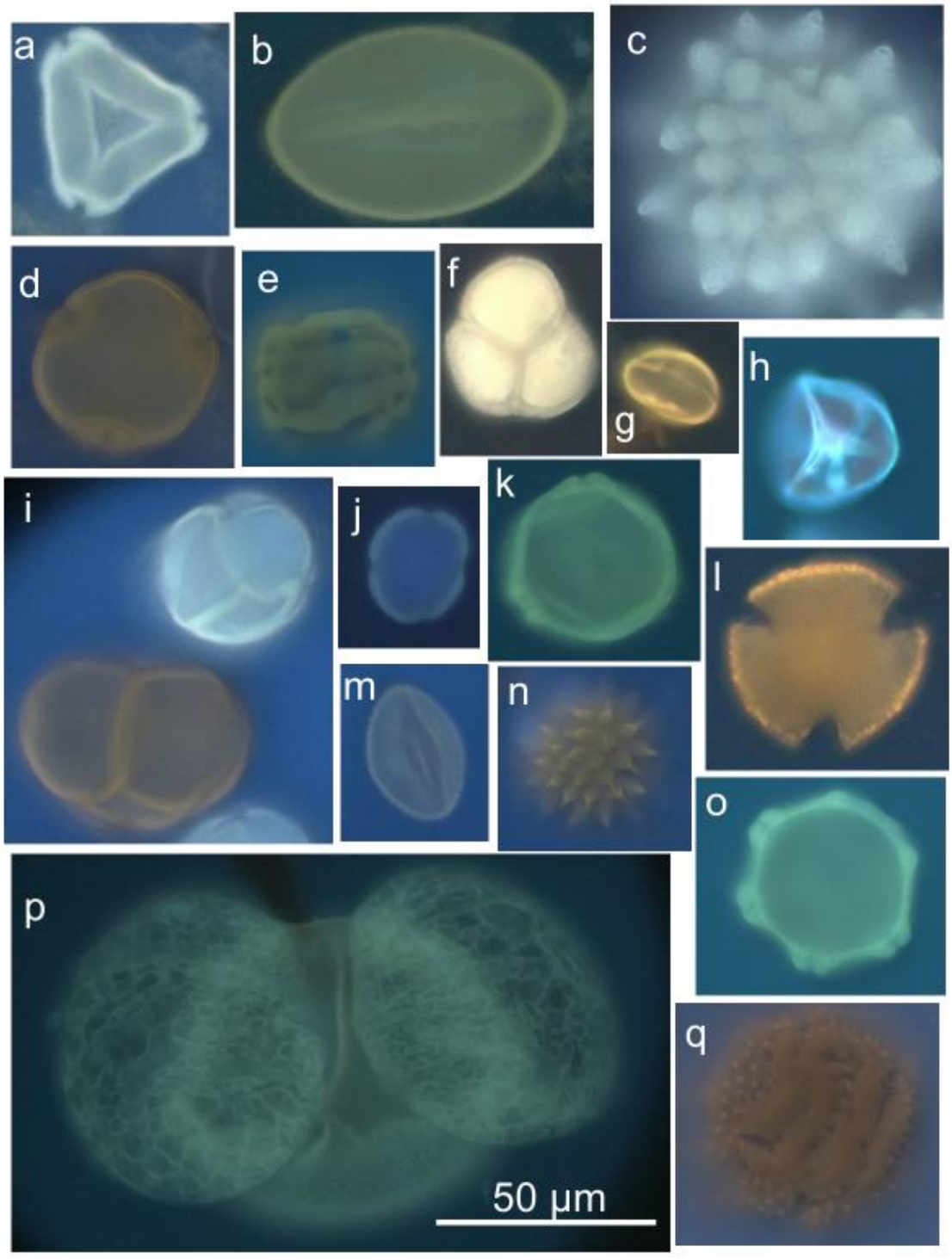
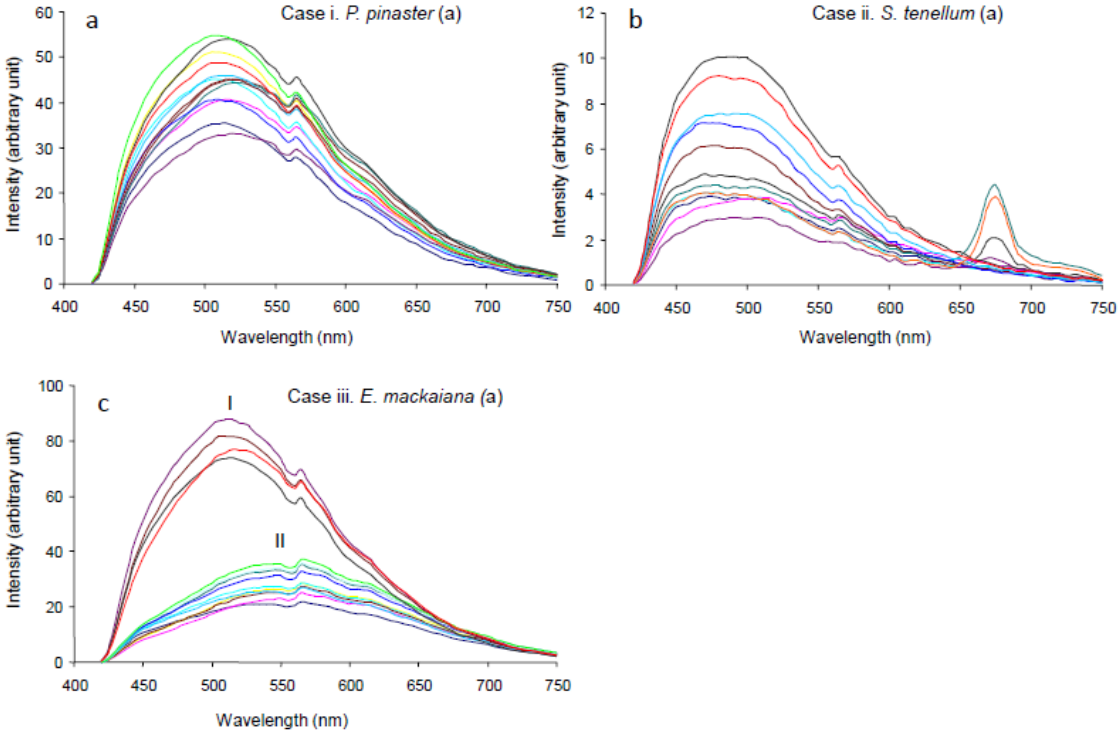


Figure3



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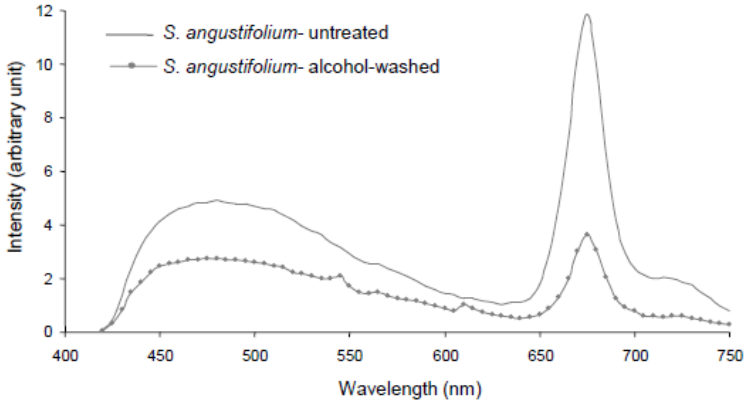
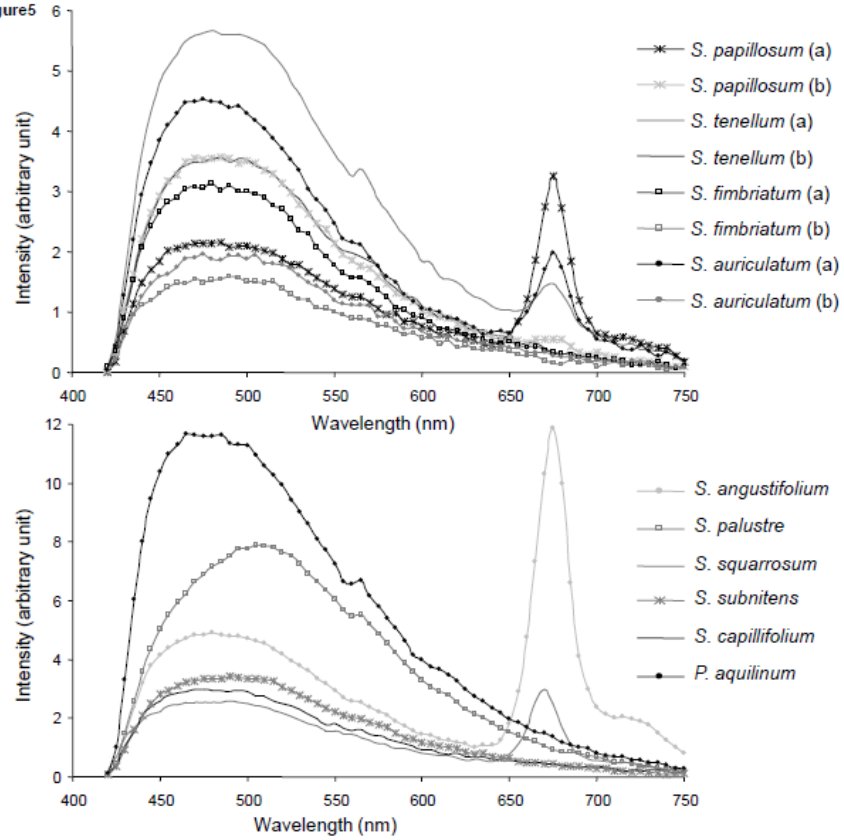


Figure5



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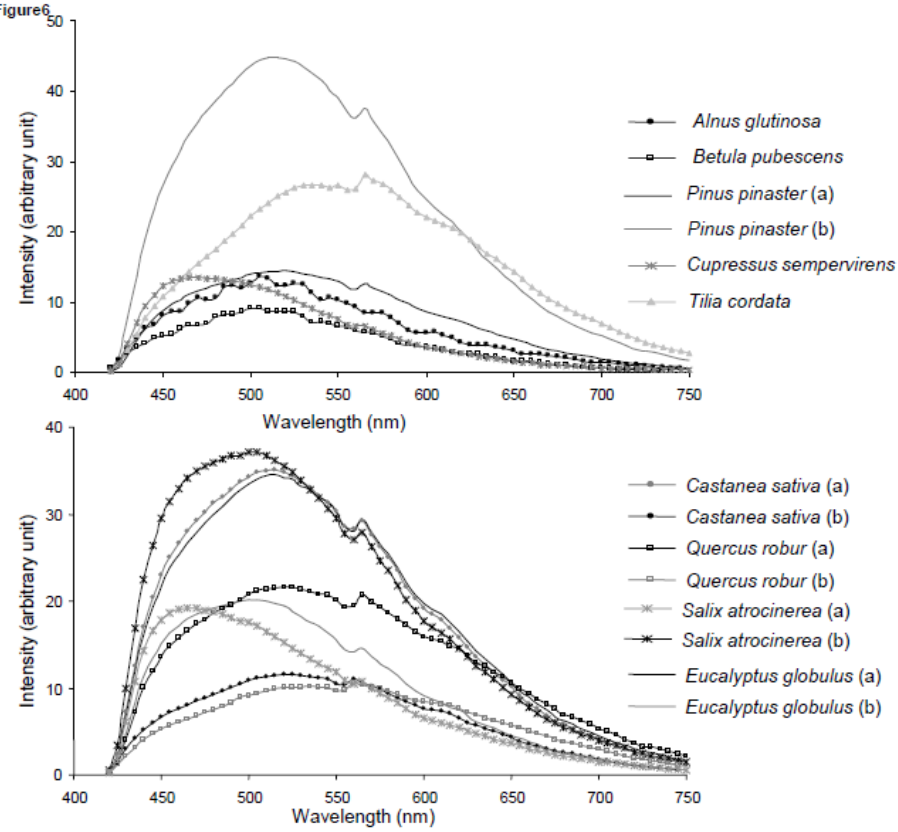

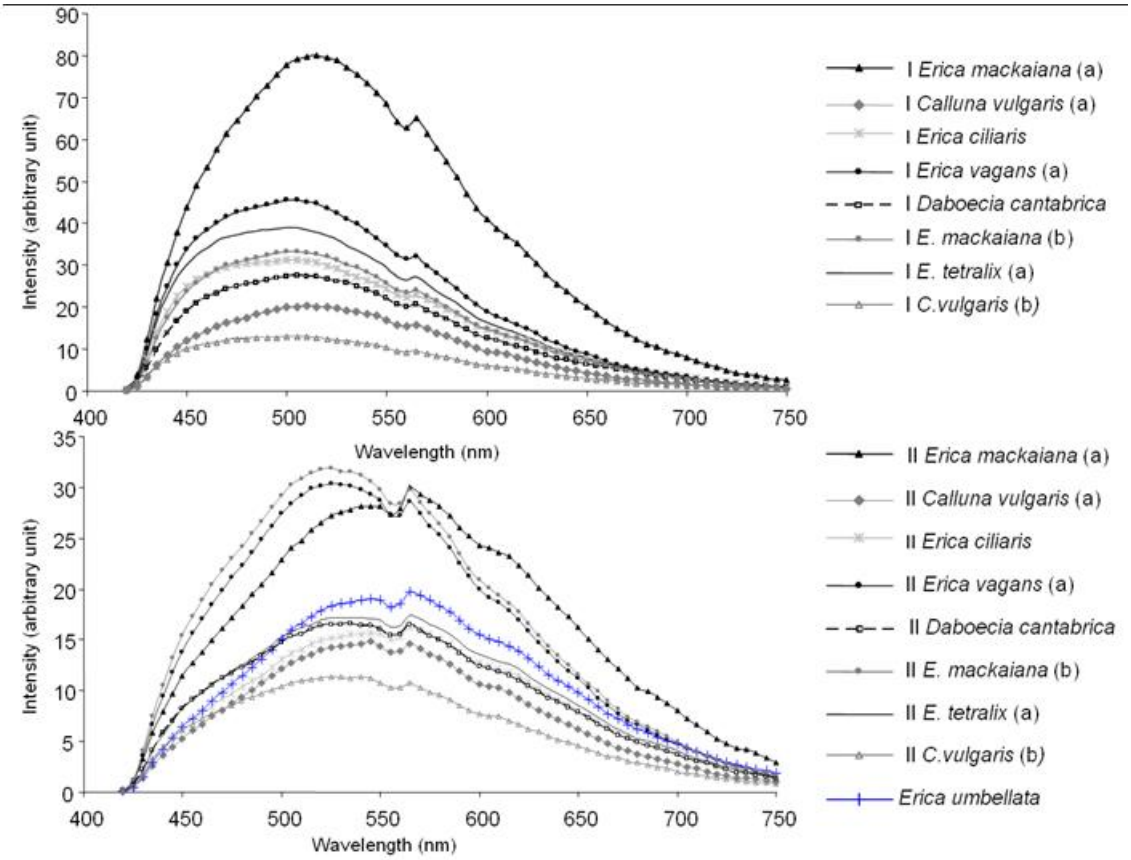


Figure 7

Figure8

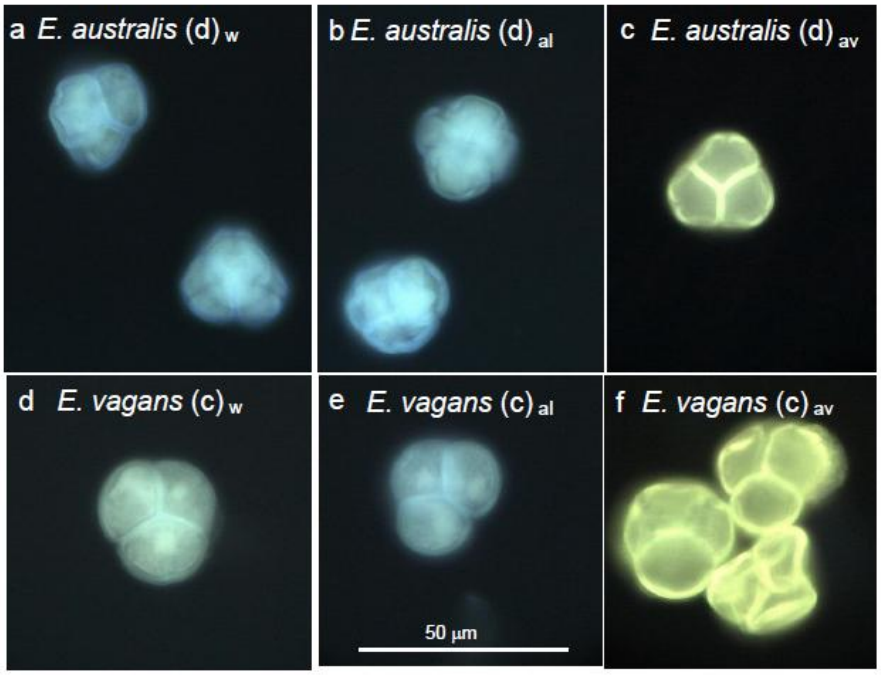

Figure9

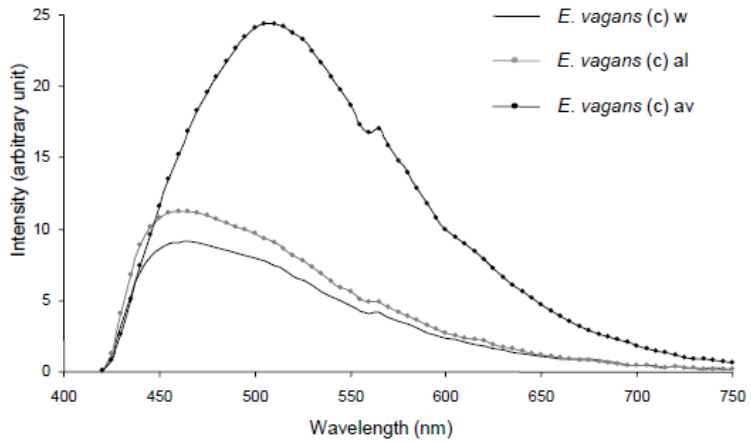

Figure10

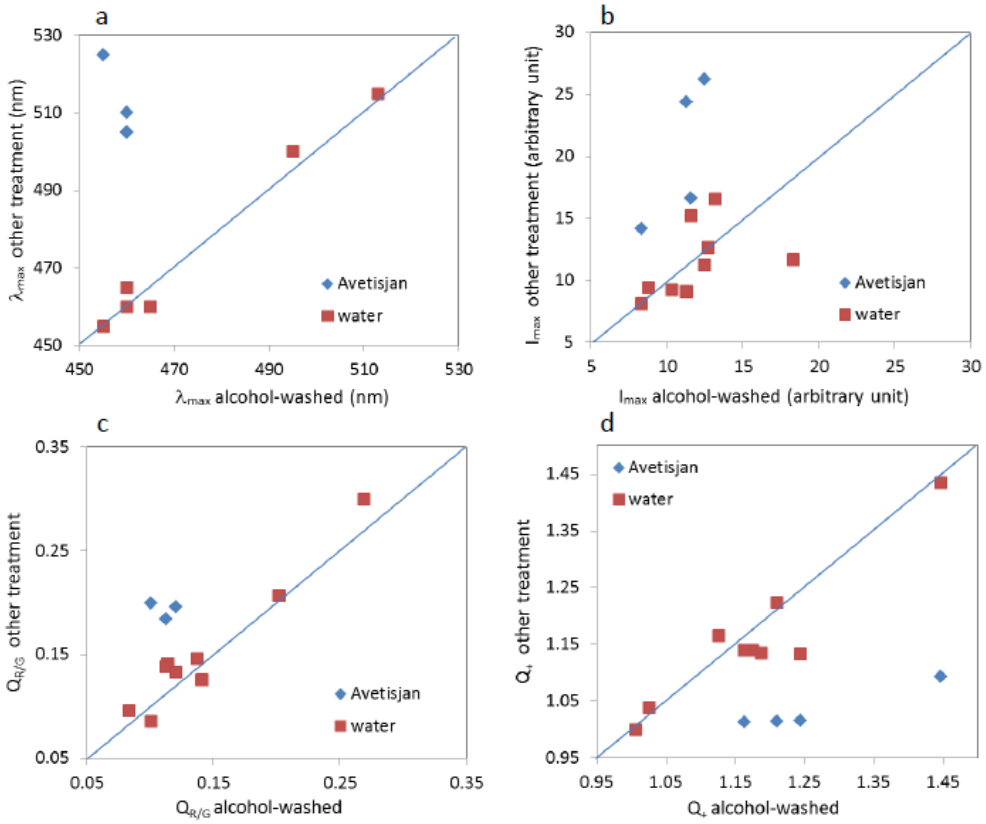

Figure11

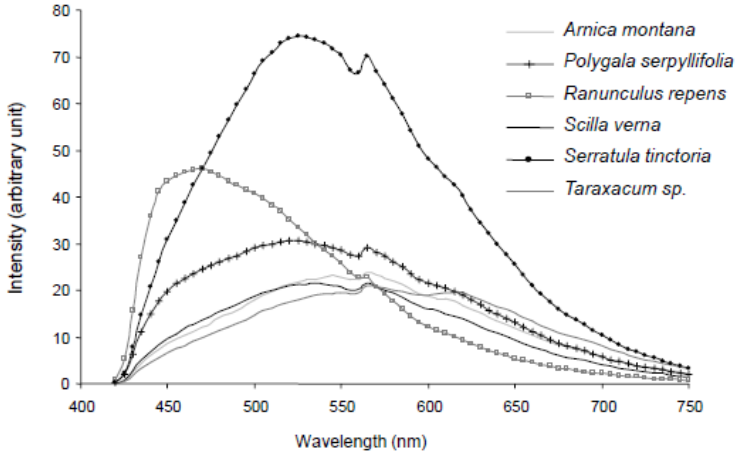

Figure12

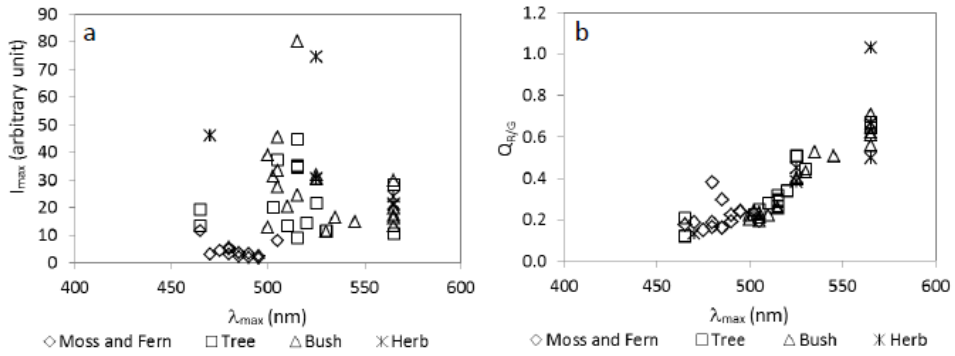

Figure13

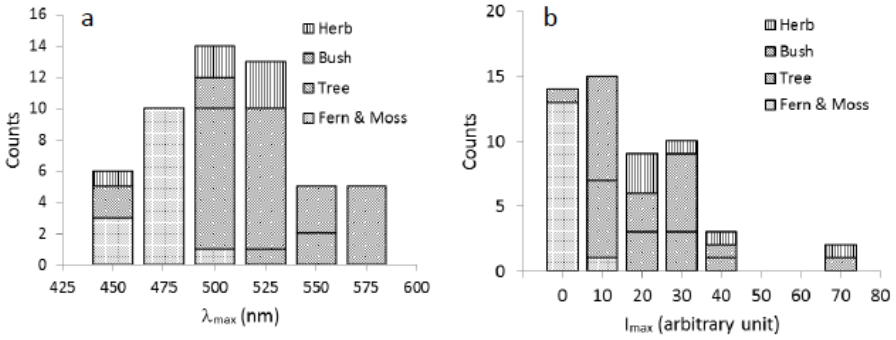

Table 1. Relevant spectral parameters of the samples studied: Spectral maximum ($\lambda_{max}$), Intensity of the spectral maximum ($I_{max}$), Red/Green quotient ($Q_{R/G}$) and Q+. An estimation of the scattering of spectral intensity is provided by the minimum (min) and maximum (max) values of the standard deviation (SD) and the coefficient of variation (CV) through the spectral range and their respective average values (ASD and ACV).

| Species | $\lambda_{max}$ | $I_{max}$ | $Q_{R/G}$ | Q+ | ASD | $SD_{min}$ | $SD_{max}$ | ACV | $CV_{min}$ | $CV_{max}$ | Family | Type |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Pteridium aquilinum* | 465 | 11.7 | 0.18 | 1.04 | 2.75 | 0.07 | 6.40 | 0.52 | 0.40 | 0.66 | Hypolepydaceae | Fern |
| *S. papillosum* (a) | 485 | 2.2 | 0.30 | 1.03 | 0.55 | 0.03 | 3.73 | 0.50 | 0.12 | 3.99 | Sphagnaceae | Moss |
| *S. papillosum* (b) | 485 | 3.6 | 0.16 | 1.01 | 0.25 | 0.03 | 0.81 | 0.31 | 0.09 | 1.47 | Sphagnaceae | Moss |
| *S. squarrosum* | 495 | 2.6 | 0.24 | 1.00 | 0.62 | 0.08 | 4.25 | 0.52 | 0.21 | 1.48 | Sphagnaceae | Moss |
| *S.angustifollium* | 480 | 4.9 | 0.38 | 1.05 | 1.58 | 0.04 | 9.54 | 0.45 | 0.19 | 0.81 | Sphagnaceae | Moss |
| *S.auriculatum* (a) | 475 | 4.5 | 0.15 | 1.06 | 0.68 | 0.02 | 0.44 | 0.43 | 0.16 | 0.56 | Sphagnaceae | Moss |
| *S.auriculatum* (b) | 490 | 2.0 | 0.22 | 1.03 | 0.19 | 0.04 | 2.30 | 0.23 | 0.18 | 1.15 | Sphagnaceae | Moss |
| *S.capillifolium* | 470 | 3.0 | 0.19 | 1.03 | 0.18 | 0.03 | 0.41 | 0.15 | 0.11 | 0.32 | Sphagnaceae | Moss |
| *S.fimbriatum* (a) | 480 | 3.1 | 0.17 | 1.04 | 0.63 | 0.03 | 1.72 | 0.39 | 0.20 | 0.60 | Sphagnaceae | Moss |
| *S.fimbriatum* (b) | 495 | 1.6 | 0.24 | 1.02 | 0.21 | 0.04 | 0.43 | 0.33 | 0.22 | 0.89 | Sphagnaceae | Moss |
| *S.palustre* | 505 | 7.9 | 0.20 | 1.02 | 1.02 | 0.04 | 2.21 | 0.28 | 0.21 | 0.61 | Sphagnaceae | Moss |
| *S.subnitens* | 490 | 3.4 | 0.19 | 1.02 | 0.82 | 0.04 | 1.95 | 0.49 | 0.32 | 0.66 | Sphagnaceae | Moss |
| *S.tenellum* (a) | 480 | 5.7 | 0.19 | 1.02 | 1.04 | 0.05 | 2.36 | 0.42 | 0.21 | 0.91 | Sphagnaceae | Moss |
| *S.tenellum* (b) | 485 | 3.6 | 0.16 | 1.01 | 0.38 | 0.02 | 0.95 | 0.30 | 0.16 | 3.97 | Sphagnaceae | Moss |
| *Pinus pinaster* (a) | 520 | 14.4 | 0.34 | 1.03 | 1.60 | 0.05 | 3.52 | 0.20 | 0.20 | 1.33 | Pinaceae | Tree |
| *Pinus pinaster* (b) | 515 | 44.8 | 0.29 | 1.03 | 3.44 | 0.10 | 6.60 | 0.17 | 0.13 | 0.46 | Pinaceae | Tree |
| *Quercus robur* (a) | 525 | 21.6 | 0.51 | 1.04 | 1.11 | 0.05 | 2.26 | 0.08 | 0.04 | 0.20 | Fagaceae | Tree |
| *Quercus robur* (b) | 565 | 10.6 | 0.67 | 1.18 | 1.30 | 0.09 | 2.47 | 0.19 | 0.12 | 0.24 | Fagaceae | Tree |
| *Castanea sativa* (a) | 515 | 35.1 | 0.30 | 1.02 | 1.80 | 0.06 | 3.89 | 0.10 | 0.06 | 0.26 | Fagaceae | Tree |
| *Castanea sativa* (b) | 530 | 11.6 | 0.45 | 1.10 | 0.57 | 0.09 | 1.07 | 0.10 | 0.14 | 0.78 | Fagaceae | Tree |
| *Alnus glutinosa* | 510 | 13.6 | 0.28 | 1.14 | 0.61 | 0.15 | 1.19 | 0.13 | 0.07 | 0.48 | Betulaceae | Tree |
| *Betula pubescens* | 515 | 9.2 | 0.27 | 1.12 | 0.55 | 0.13 | 1.24 | 0.39 | 0.07 | 9.09 | Betulaceae | Tree |
| *Cupressus sempervirens* | 465 | 13.5 | 0.12 | 1.08 | 1.25 | 0.06 | 2.87 | 0.24 | 0.08 | 0.55 | Cupressaceae | Tree |
| *Eucalyptus globulus* (a) | 515 | 34.6 | 0.32 | 1.03 | 2.87 | 0.08 | 5.67 | 0.16 | 0.14 | 0.28 | Myrtaceae/Myrtoideae | Tree |
| *Eucalyptus globulus* (b) | 503 | 20.1 | 0.23 | 1.00 | 0.64 | 0.04 | 1.27 | 0.08 | 0.06 | 0.28 | Myrtaceae/Myrtoideae | Tree |
| *Salix atrocinerea* (a) | 465 | 19.2 | 0.21 | 1.10 | 0.96 | 0.05 | 2.52 | 0.13 | 0.08 | 0.20 | Salicaceae | Tree |
| *Salix atrocinerea* (b) | 505 | 37.2 | 0.25 | 1.00 | 2.75 | 0.11 | 6.51 | 0.14 | 0.10 | 0.22 | Salicaceae | Tree |
| *Tilia cordata* | 565 | 28.2 | 0.64 | 1.26 | 2.43 | 0.09 | 4.36 | 0.17 | 0.14 | 0.38 | Malvaceae/Tilioideae | Tree |
| *I Calluna vulgaris* (a) | 510 | 20.3 | 0.22 | 1.02 | 1.38 | 0.03 | 2.94 | 0.14 | 0.09 | 0.20 | Ericaceae | Bush |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *I Calluna vulgaris* (b) | 500 | 12.9 | 0.22 | 1.00 | 1.71 | 0.03 | 3.40 | 0.29 | 0.23 | 0.41 | Ericaceae | Bush |
| *I Daboecia cantábrica* | 505 | 27.4 | 0.23 | 1.00 | 3.58 | 0.04 | 7.19 | 0.29 | 0.19 | 0.29 | Ericaceae | Bush |
| *I Erica australis* (a) | 515 | 24.2 | 0.26 | 1.04 | 1.69 | 0.05 | 4.24 | 0.13 | 0.06 | 0.35 | Ericaceae | Bush |
| *I E. ciliaris* | 503 | 31.3 | 0.24 | 1.00 | 5.13 | 0.12 | 11.33 | 0.30 | 0.22 | 0.44 | Ericaceae | Bush |
| *I E.mackaiana* (a) | 515 | 80.0 | 0.26 | 1.03 | 2.71 | 0.03 | 5.08 | 0.09 | 0.11 | 0.23 | Ericaceae | Bush |
| *I E.mackaiana* (b) | 505 | 33.4 | 0.21 | 1.00 | 5.06 | 0.07 | 11.01 | 0.31 | 0.10 | 0.56 | Ericaceae | Bush |
| *I E.tetralix* (a) | 500 | 39.0 | 0.20 | 1.00 | 2.82 | 0.04 | 6.17 | 0.15 | 0.19 | 0.41 | Ericaceae | Bush |
| *I E.vagans* (a) | 505 | 45.5 | 0.19 | 1.00 | 2.79 | 0.07 | 5.50 | 0.16 | 0.09 | 0.28 | Ericaceae | Bush |
| *II Calluna vulgaris* (a) | 545 | 14.8 | 0.51 | 1.22 | 2.21 | 0.03 | 4.20 | 0.29 | 0.28 | 0.55 | Ericaceae | Bush |
| *II Calluna vulgaris* (b) | 530 | 11.4 | 0.43 | 1.09 | 0.30 | 0.01 | 0.55 | 0.06 | 0.02 | 0.13 | Ericaceae | Bush |
| *II Daboecia cantábrica* | 535 | 16.5 | 0.53 | 1.12 | 1.82 | 0.05 | 3.68 | 0.18 | 0.22 | 0.40 | Ericaceae | Bush |
| *II Erica australis* (a) | 565 | 13.5 | 0.61 | 1.30 | 1.49 | 0.02 | 3.07 | 0.20 | 0.12 | 0.54 | Ericaceae | Bush |
| *II E.ciliaris* | 550 | 16.2 | 0.62 | 1.24 | 1.90 | 0.05 | 3.46 | 0.21 | 0.14 | 0.72 | Ericaceae | Bush |
| *II E.mackaiana* (a) | 545 | 30.0 | 0.71 | 1.31 | 3.11 | 0.10 | 6.53 | 0.16 | 0.06 | 0.23 | Ericaceae | Bush |
| *II E.mackaiana* (b) | 525 | 31.8 | 0.40 | 1.09 | 3.95 | 0.03 | 7.76 | 0.24 | 0.27 | 0.43 | Ericaceae | Bush |
| *II E.tetralix (a)* | 550 | 17.4 | 0.56 | 1.14 | 2.43 | 0.04 | 4.57 | 0.25 | 0.13 | 0.19 | Ericaceae | Bush |
| *II E.vagans* (a) | 525 | 30.4 | 0.41 | 1.11 | 2.34 | 0.05 | 4.79 | 0.15 | 0.11 | 0.38 | Ericaceae | Bush |
| *Erica umbellata* | 565 | 19.7 | 0.65 | 1.31 | 4.27 | 0.06 | 8.34 | 0.38 | 0.18 | 0.65 | Ericaceae | Bush |
| *Arnica montana* | 545 | 23.8 | 0.67 | 1.32 | 2.98 | 0.11 | 5.40 | 0.27 | 0.14 | 2.50 | Asteraceae | Herb |
| *Polygala serpyllifolia* | 525 | 30.6 | 0.45 | 1.05 | 2.69 | 0.06 | 5.19 | 0.15 | 0.10 | 0.24 | Polygalaceae | Herb |
| *Ranunculus repens* | 470 | 46.1 | 0.13 | 1.13 | 5.09 | 0.18 | 12.91 | 0.28 | 0.23 | 0.34 | Ranunculaeae | Herb |
| *Scilla verna* | 535 | 21.5 | 0.50 | 1.15 | 1.66 | 0.07 | 3.25 | 0.15 | 0.10 | 1.18 | Asparagaceae/Scilloideae | Herb |
| *Serratula tinctoria* | 525 | 74.5 | 0.38 | 1.12 | 2.96 | 0.08 | 10.68 | 0.07 | 0.03 | 0.29 | Asteraceae | Herb |
| *Taraxacum* sp. | 550 | 21.0 | 1.03 | 1.42 | 2.29 | 0.09 | 4.18 | 0.18 | 0.10 | 0.60 | Asteraceae/Cichorioideae | Herb |

Table 2. Spectral parameters of the *Sphagnum* samples prepared without any treatment (u) and washed with alcohol (al). The spectral fluorescence parameters are: Spectral maximum ($\lambda_{max}$), Intensity of the spectral maximum ($I_{max}$), Red/Green quotient ($Q_{R/G}$) and Q+. ASD and ACV represent the average values of the standard deviation and the coefficient of variation, respectively through the spectral range. Y=yes, N=no indicates the presence/absence of a red peak at 675 nm frequently observed in *Sphagnum* spores.

| Species | $\lambda_{max}$ | $I_{max}$ | $Q_{R/G}$ | Q+ | ASD | ACV | Red peak |
|---|---|---|---|---|---|---|---|
| *S. papillosum* (a) $_{u}$ | 485 | 2.2 | 0.30 | 1.03 | 0.55 | 0.50 | Y |
| *S. papillosum* (a) $_{al}$ | 480 | 1.8 | 0.23 | 1.01 | 0.22 | 0.26 | N |
| *S. angustifolium* $_{u}$ | 480 | 4.9 | 0.38 | 1.05 | 1.58 | 0.45 | Y |
| *S. angustifolium* $_{al}$ | 475 | 2.8 | 0.25 | 1.07 | 0.45 | 0.33 | Y |
| *S. auriculatum* (a) $_{u}$ | 475 | 4.5 | 0.15 | 1.06 | 0.68 | 0.43 | N |
| *S. auriculatum* (a) $_{al}$ | 465 | 2.6 | 0.15 | 1.09 | 0.16 | 0.21 | N |
| *S. tenellum* (a) $_{u}$ | 480 | 5.7 | 0.19 | 1.02 | 1.04 | 0.42 | Y |
| *S. tenellum* (a) $_{al}$ | 470 | 4.4 | 0.20 | 1.05 | 0.79 | 0.46 | Y |
| *S. squarrosum* $_{u}$ | 495 | 2.6 | 0.24 | 1.00 | 0.62 | 0.52 | Y |
| *S. squarrosum* $_{al}$ | 470 | 1.6 | 0.24 | 1.12 | 0.12 | 0.16 | N |

Table 3. Values of the statistics used to describe the size distribution of Ericaceae pollen grains in the studied samples (Av=average, Max=maximum, Min=minimum, SD=standard deviation, CV=coefficient of variation). The corresponding fluorescence spectral values are shown in Table 1 for Erdtman-treated samples (er) and in Table 4 for distilled water-washed (w), alcohol-washed (al) and Avetisjan-treated (av) samples.

| Component | Av (µm) | Max (µm) | Min (µm) | SD (µm) | CV |
|---|---|---|---|---|---|
| I *C. vulgaris* (a) $_{er}$ | 35.8 | 44.0 | 30.8 | 3.27 | 0.09 |
| II *C. vulgaris* (a) $_{er}$ | 47.0 | 57.2 | 39.6 | 5.50 | 0.12 |
| *C. vulgaris* (b) $_{er}$ | 33.3 | 37.4 | 26.4 | 3.93 | 0.12 |
| I E. australis (a) $_{er}$ | 39.7 | 46.2 | 35.2 | 3.21 | 0.08 |
| II E. australis (a) $_{er}$ | 49.2 | 57.2 | 41.8 | 3.68 | 0.07 |
| I E. mackaiana (a) $_{er}$ | 36.1 | 39.6 | 30.8 | 2.26 | 0.06 |
| II E. mackaiana (a) $_{er}$ | 49.5 | 57.2 | 44.0 | 2.71 | 0.06 |
| I E. vagans (a) $_{er}$ | 28.1 | 30.8 | 26.4 | 1.7 | 0.06 |
| II E. vagans (a) $_{er}$ | 37.1 | 44.0 | 33.0 | 3.29 | 0.09 |
| C. vulgaris (c) $_{w}$ | 33.1 | 37.4 | 28.6 | 2.50 | 0.08 |
| C. vulgaris (c) $_{al}$ | 31.9 | 35.2 | 28.6 | 2.02 | 0.06 |
| C. vulgaris (d) $_{w}$ | 30.2 | 35.2 | 26.4 | 2.16 | 0.07 |
| C. vulgaris (d) $_{al}$ | 31.2 | 35.2 | 26.4 | 2.35 | 0.08 |
| C. vulgaris (d) $_{av}$ | 29.7 | 33.0 | 26.4 | 1.92 | 0.06 |
| E. australis (b) $_{w}$ | 38.8 | 44.0 | 35.2 | 2.00 | 0.05 |
| E. australis (b) $_{al}$ | 40.3 | 44.0 | 37.4 | 2.30 | 0.06 |
| E. australis (c) $_{w}$ | 26.3 | 28.6 | 24.2 | 1.52 | 0.06 |
| E. australis (c) $_{al}$ | 26.3 | 28.6 | 24.2 | 1.50 | 0.06 |
| E. australis (d) $_{w}$ | 24.7 | 26.4 | 22.0 | 1.15 | 0.05 |
| E. australis (d) $_{al}$ | 25.2 | 28.6 | 22.0 | 1.57 | 0.06 |
| E. australis (d) $_{av}$ | 25.2 | 28.6 | 22.0 | 1.69 | 0.07 |
| E. tetralix (b) $_{w}$ | 31.8 | 35.2 | 26.4 | 2.43 | 0.08 |
| E. tetralix (b) $_{al}$ | 33.6 | 37.4 | 28.6 | 2.06 | 0.06 |
| E. tetralix (c) $_{w}$ | 30.6 | 35.2 | 26.4 | 2.61 | 0.09 |
| E. tetralix (c) $_{al}$ | 30.4 | 35.2 | 26.4 | 2.20 | 0.07 |
| E. tetralix (c) $_{av}$ | 31.2 | 35.2 | 26.4 | 2.31 | 0.07 |
| E. vagans (b) $_{w}$ | 31.8 | 35.2 | 26.4 | 2.20 | 0.07 |
| E. vagans (b) $_{al}$ | 31.5 | 35.2 | 26.4 | 2.95 | 0.09 |
| E. vagans (c) $_{w}$ | 27.4 | 30.8 | 24.2 | 1.97 | 0.07 |
| E. vagans (c) $_{al}$ | 26.6 | 30.8 | 24.2 | 1.90 | 0.07 |
| E. vagans (c) $_{av}$ | 27.3 | 33.0 | 24.2 | 2.38 | 0.09 |

Table 4. Spectral parameters of the Ericaceae samples using different sample preparation procedures: distilled water-washed (w), alcohol-washed (al) and Avetisjan-treated (av) samples. The spectral fluorescence parameters are: Spectral maximum ($\lambda_{max}$), Intensity of the spectral maximum ($I_{max}$), Red/Green quotient ($Q_{R/G}$) and Q+. ASD and ACV represent the average values of the standard deviation and the coefficient of variation, respectively through the spectral range.

| Species | $\lambda_{max}$ | $I_{max}$ | $Q_{R/G}$ | Q+ | ASD | ACV |
|---|---|---|---|---|---|---|
| *C. vulgaris* (c) $_{w}$ | 515 | 9.3 | 0.30 | 1.04 | 0.46 | 0.11 |
| *C. vulgaris* (c) $_{al}$ | 513 | 10.3 | 0.27 | 1.02 | 0.64 | 0.22 |
| *C. vulgaris* (d) $_{w}$ | 455 | 8.1 | 0.10 | 1.44 | 0.41 | 0.15 |
| *C. vulgaris* (d) $_{al}$ | 455 | 8.3 | 0.08 | 1.45 | 0.53 | 0.22 |
| *C. vulgaris* (d) $_{av}$ | 525 | 14.2 | 0.46 | 1.09 | 1.08 | 0.16 |
| *E. australis* (b) $_{w}$ | 500 | 9.4 | 0.21 | 1.00 | 1.48 | 0.36 |
| *E. australis* (b) $_{al}$ | 495 | 8.8 | 0.20 | 1.01 | 0.51 | 0.14 |
| *E. australis* (c) $_{w}$ | 460 | 16.5 | 0.13 | 1.17 | 1.07 | 0.18 |
| *E. australis* (c) $_{al}$ | 465 | 13.2 | 0.14 | 1.13 | 1.18 | 0.22 |
| I *E. australis* (d) $_{w}$ | 460 | 13.7 | 0.12 | 1.23 | 0.95 | 0.19 |
| II *E. australis* (d) $_{w}$ | 470 | 8.7 | 0.16 | 1.04 | 0.28 | 0.09 |
| *E. australis* (d) $_{al}$ | 460 | 12.5 | 0.11 | 1.24 | 0.92 | 0.23 |
| *E. australis* (d) $_{av}$ | 510 | 26.2 | 0.18 | 1.02 | 3.29 | 0.27 |
| *E. tetralix* (b) $_{w}$ | 465 | 11.7 | 0.15 | 1.14 | 1.80 | 0.38 |
| *E. tetralix* (b) $_{al}$ | 460 | 18.3 | 0.14 | 1.17 | 1.08 | 0.17 |
| *E. tetralix* (c) $_{w}$ | 460 | 15.2 | 0.09 | 1.22 | 1.57 | 0.27 |
| *E. tetralix* (c) $_{al}$ | 460 | 11.6 | 0.10 | 1.21 | 0.79 | 0.18 |
| I *E. tetralix* (c) $_{av}$ | 500 | 14.1 | 0.17 | 1.00 | 1.11 | 0.19 |
| II *E. tetralix* (c) $_{av}$ | 510 | 19.2 | 0.23 | 1.03 | 2.67 | 0.34 |
| *E. vagans* (b) $_{w}$ | 460 | 12.7 | 0.11 | 1.19 | 1.12 | 0.29 |
| *E. vagans* (b) $_{al}$ | 465 | 12.6 | 0.14 | 1.13 | 1.21 | 0.28 |
| *E. vagans* (c) $_{w}$ | 465 | 9.1 | 0.13 | 1.14 | 1.01 | 0.29 |
| *E. vagans* (c) $_{al}$ | 460 | 11.3 | 0.12 | 1.16 | 1.25 | 0.32 |
| *E. vagans* (d) $_{av}$ | 505 | 24.4 | 0.20 | 1.01 | 3.01 | 0.26 |

**Highlights**

- Fluorescence spectra of spores and pollen of fresh peat-forming plants are studied
- The spectra differ in intensity but have similar shape for each taxon
- Effect of preparation techniques in the fluorescence is studied
- Spores show more bluish and less intense spectra compared to pollen grains
- A good correlation of red/green quotient and spectral maxima is observed