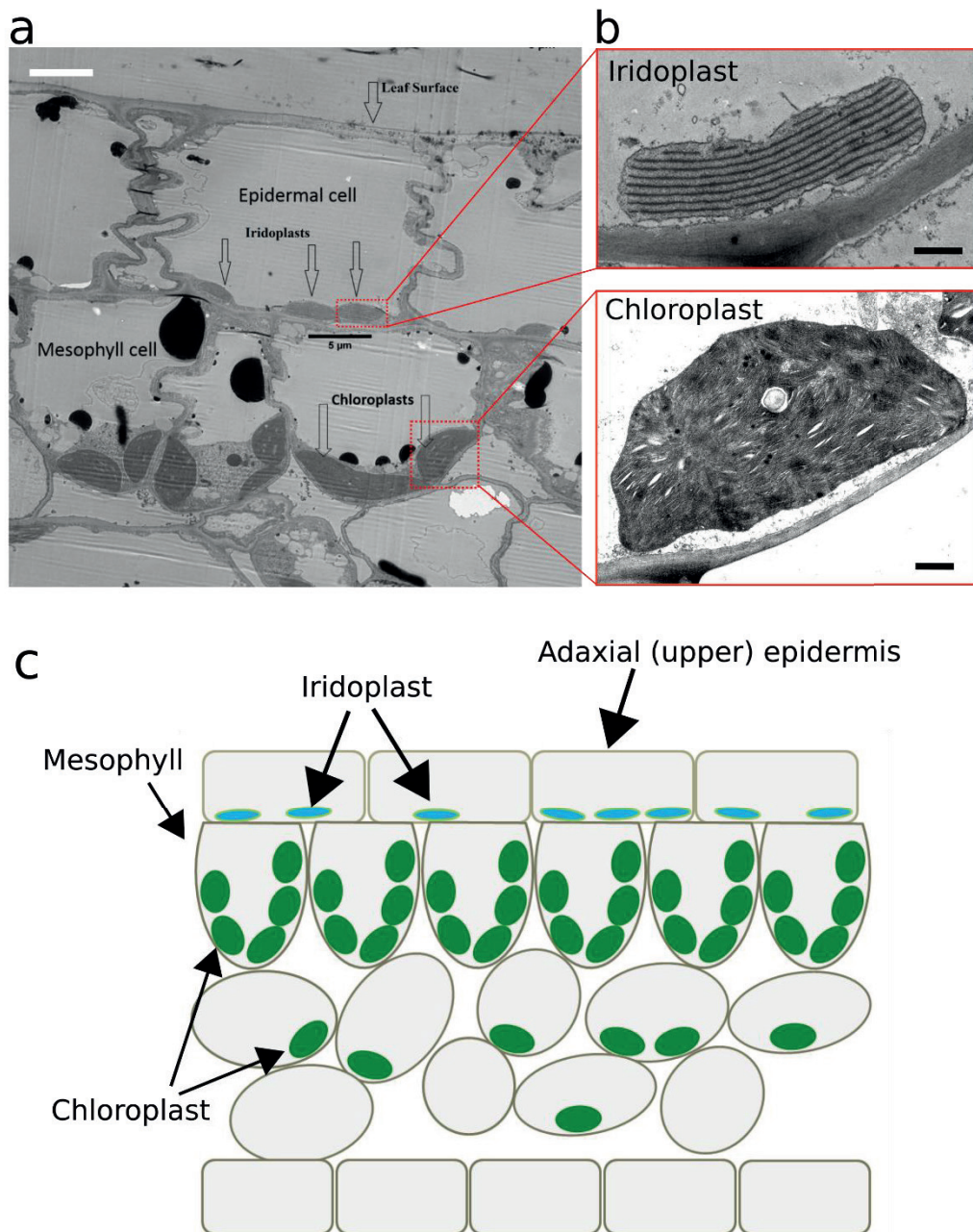
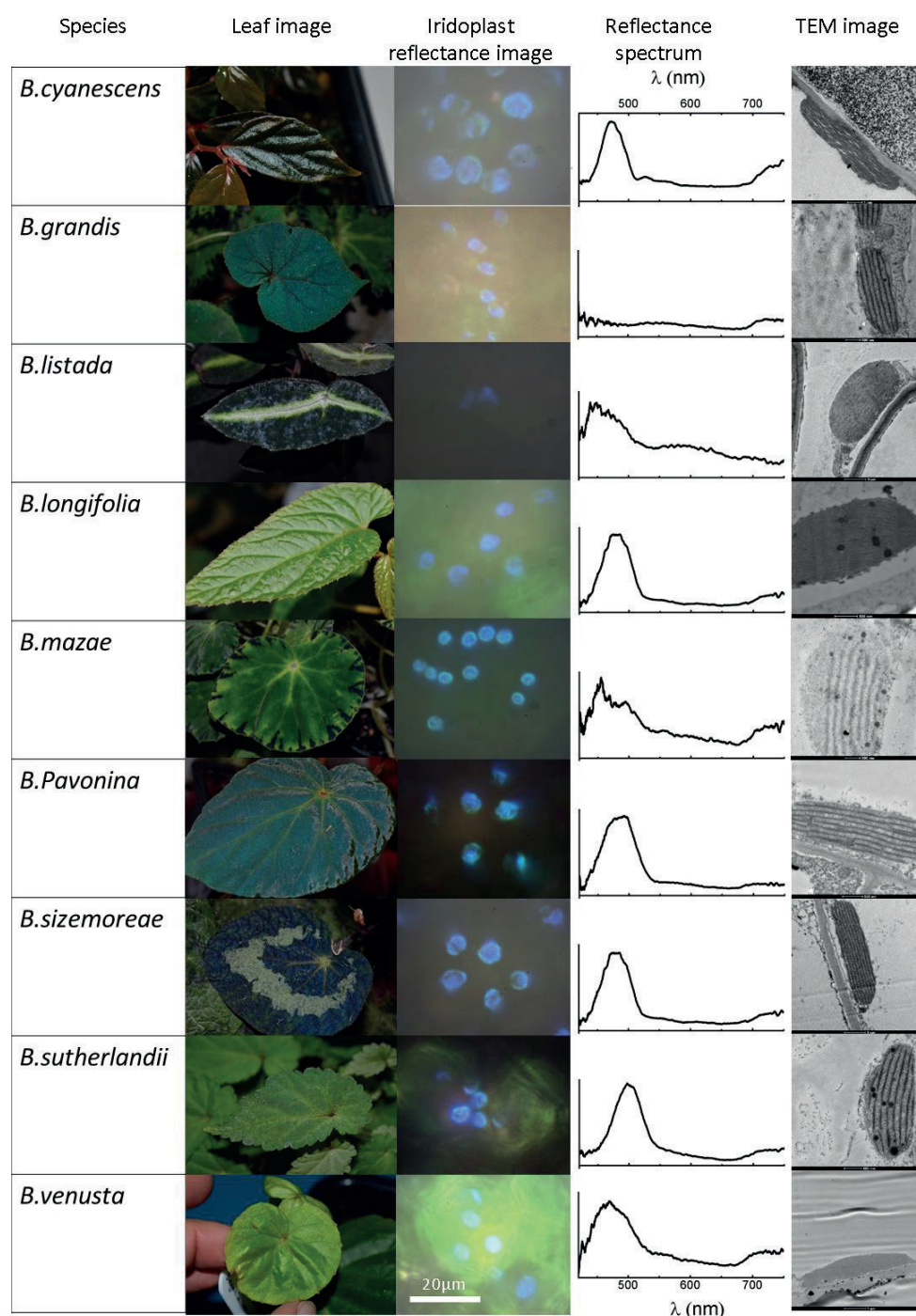


Photonic multilayer structure of *Begonia* chloroplasts enhances photosynthetic efficiency

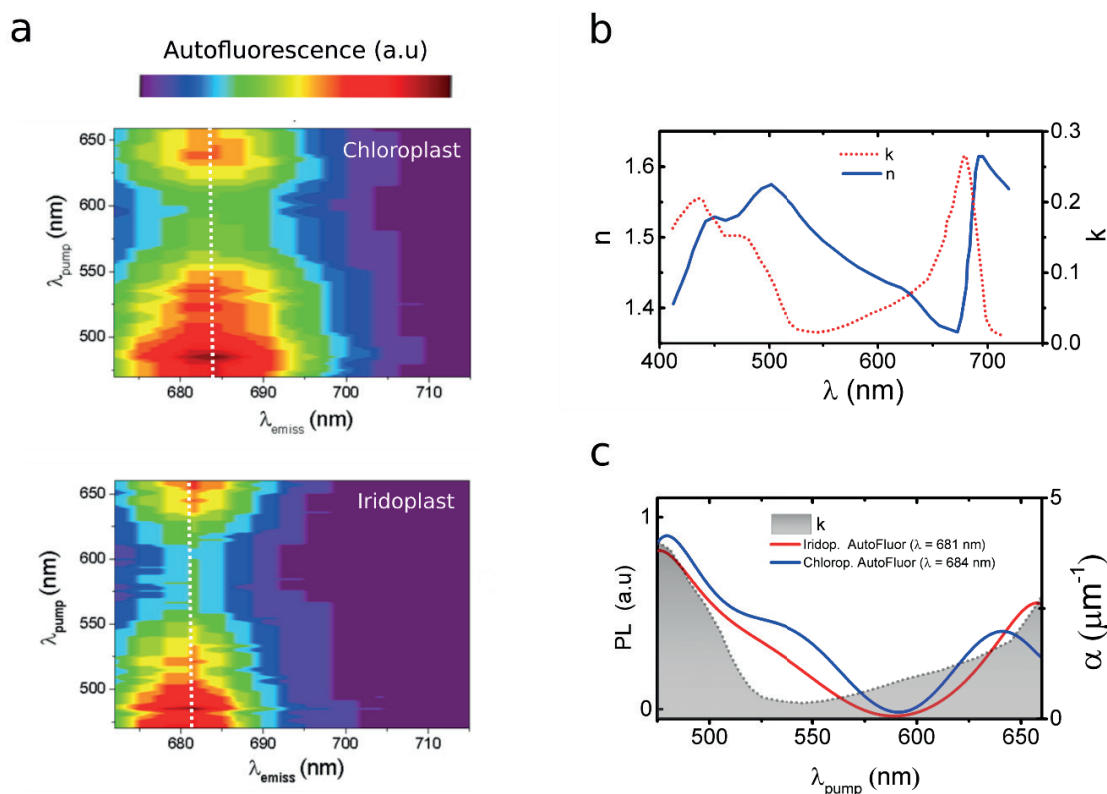


Supplementary Figure. S1 | Location and context of iridoplasts and chloroplasts in *Begonia*.

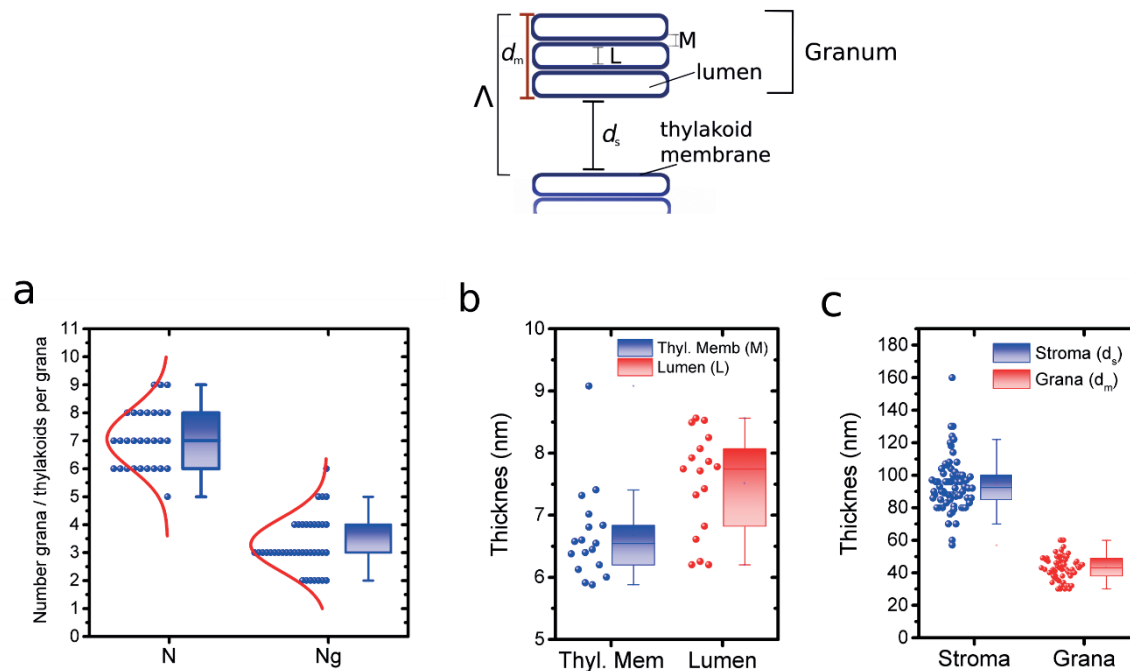
a, TEM image of mesophyll and epidermal cells in a cross section of a leaf of *Begonia pavonina*, showing the position of both iridoplasts and chloroplasts. Scale bar (white line) is 5 μm . **b**, representative TEM images of single organelles. Note the highly periodic granum stacks in the iridoplast compared with randomly packed grana in the chloroplast. Scale bars are 1 μm . **c**, diagram showing location of iridoplasts within a cross-section of *Begonia* leaf tissue. Iridoplasts are found in the adaxial epidermis, i.e. the layer of cells which form the upper surface of the leaf.



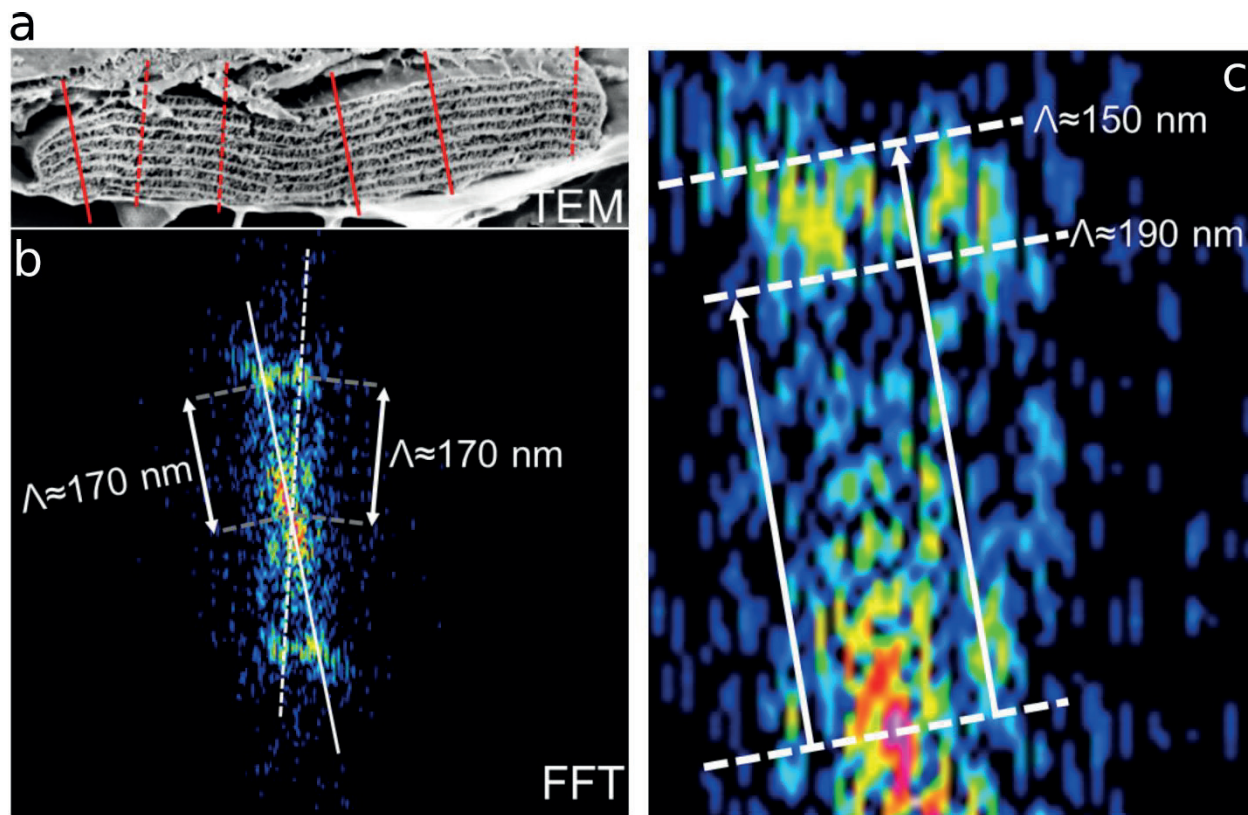
Supplementary Figure. S2| Extended table of *Begonia* species showing reflected light microscopy and spectra and TEM images of iridoplasts. Note *B. grandis* iridoplasts did not show a reflectance peak. This is due to the relatively small size of iridoplasts in this species ($\approx 2 \mu\text{m}$) making their contribution in the total reflected spectra negligible (considering the measurement spot for reflectance is $\approx 9 \mu\text{m}$ in our case). However, microscope images confirm the presence of iridoplasts in the epidermal cells.



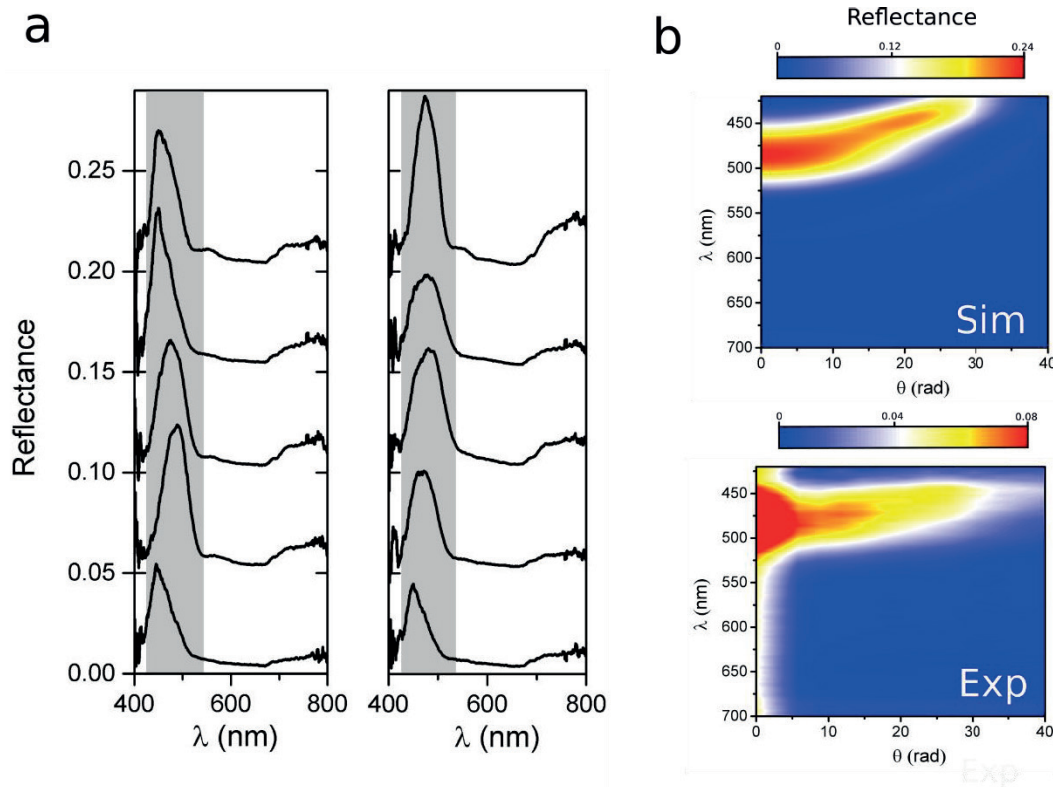
Supplementary Figure. S3 | Material properties of iridoplast. **a**, confocal PLE autofluorescence measurements for both chloroplasts and iridoplasts. We measured fluorescence in the emission range $660 < \lambda < 720$ nm from single organelles under single wavelength excitation. Each panel was produced from the average of $n = 7$ iridoplasts and $n = 10$ chloroplasts in the same leaf. The excitation wavelength was varied between $470 < \lambda < 660$ nm to obtain the 2D contour plots shown in the image. The observed pattern is associated with autofluorescence of chlorophyll in both cases although some differences are apparent, probably due to differences in pigment composition. The white dashed line shows the emission wavelength at which the maximum of autofluorescence takes place. Note that autofluorescence for excitation in the green ($\lambda \approx 550$ nm) is low in both cases, which indicates a low absorbance in that spectral region. **b**, refractive index of thylakoid membrane ($\bar{n}_t = n + ik$) as extracted from reference¹ and used in this work to calculate the optical properties of the iridoplast. Real (n , blue solid line) and imaginary (k , red dotted line) terms of the refractive index are plotted. Note that the absorbance will be determined by k . As expected, material dispersion at the thylakoid membrane is determined by chlorophyll absorbance. **c**, solid lines show autofluorescence/photoluminescence (PL) as a function of excitation wavelength for iridoplast (red line) and chloroplast (blue line) at maximum emission wavelength $\lambda = 681$ and $\lambda = 684$ nm respectively, corresponding to the white dashed lines in **a**. For comparison we have also plotted the absorption coefficient ($\alpha = 4\pi k/\lambda$) spectrum of thylakoid membranes as calculated from the imaginary term (k) of the refractive index shown in **b**. It is observed that the variation of PLE and α shown very similar spectral features, therefore linking absorbance and autofluorescence. These results demonstrate that the thylakoids in iridoplast and chloroplast present similar pigmentation, mostly chlorophyll.



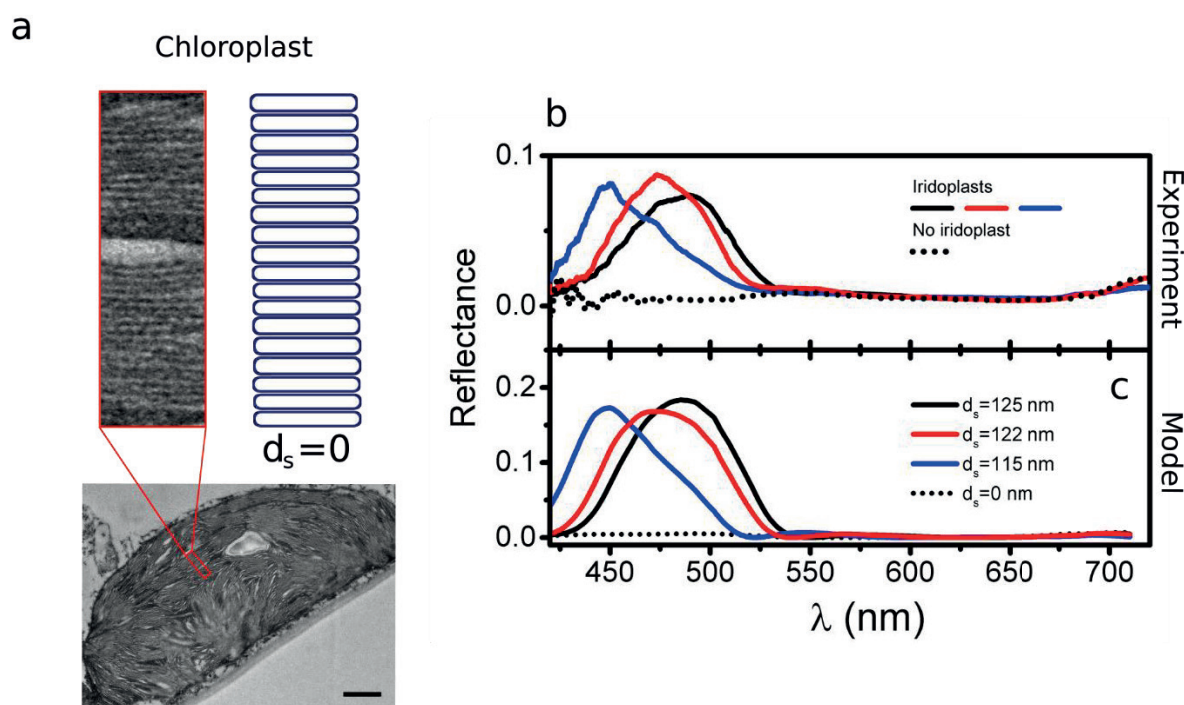
Supplementary Figure. S4| Statistical study of thickness of iridoplast membranes. a, Study of the number of grana stacks per iridoplast, N , and the number of thylakoids per grana, N_m . Measurements performed on $n = 29$ TEM images of iridoplasts. Mean values of $N = 7 \pm 1$ and $N_m = 3 \pm 1$ are the most common in the leaves analyzed in this study. **b,** Thickness of thylakoid membrane ($M/2$) and lumen compartment (L) obtained from a sample size $n = 17$ collected from 6 different TEM images. Mean values are $M = 6.7 \pm 0.8$ nm and $L = 7.5 \pm 0.8$ nm. Although these numbers were obtained from high quality TEM images the error is high due to the difficulty in obtaining a good contrast between lumen and thylakoid membrane. This is a well-known problem for researchers studying thylakoid dynamic properties². **c,** Thickness for stroma and grana showing average values of $d_s = 94 \pm 16$ and $d_m = 43.4 \pm 7.6$ nm obtained from a sample size of $n = 70$ extracted from a set of 7 images. These values lead to a period of the photonic structure of $\Lambda = 137 \pm 24$ nm. Note that TEM images show often under-evaluated distance values compared to those obtained by more morphologically robust techniques such as cryo-SEM. However, cryo-SEM does not provide the contrast and resolution to distinguish thylakoid membrane from lumen.



Supplementary Figure. S5| Experimental determination through Fast Fourier Transform (FFT) image of the period of the multilayer stack (Λ) in iridoplast. **a, Freeze fracture Cryo-SEM image of an iridoplast. Red solid and dotted lines show the stacking directions at different positions of the iridoplast. **b**, FFT of the image in (**a**) demonstrating a period of $\Lambda \approx 170$ nm. The two main directions in which the grana are stacked are also highlighted by dotted and solid white lines, matching the directions shown in (**a**). We obtain a variability between $\Lambda = 150$ nm and $\Lambda = 190$ nm for the stacking within the same iridoplast (**c**, zoom in **b**). Note that these values present a very good agreement with those parameters obtained from the optical model while still falling within the error of the ultrastructural analysis in Supplementary Fig. S4 ($\Lambda = 137 \pm 24$ nm). In addition we performed FFT analysis on $n = 7$ TEM images giving an average value of 133.8 ± 9.1 nm as expected from the previous discussion.**

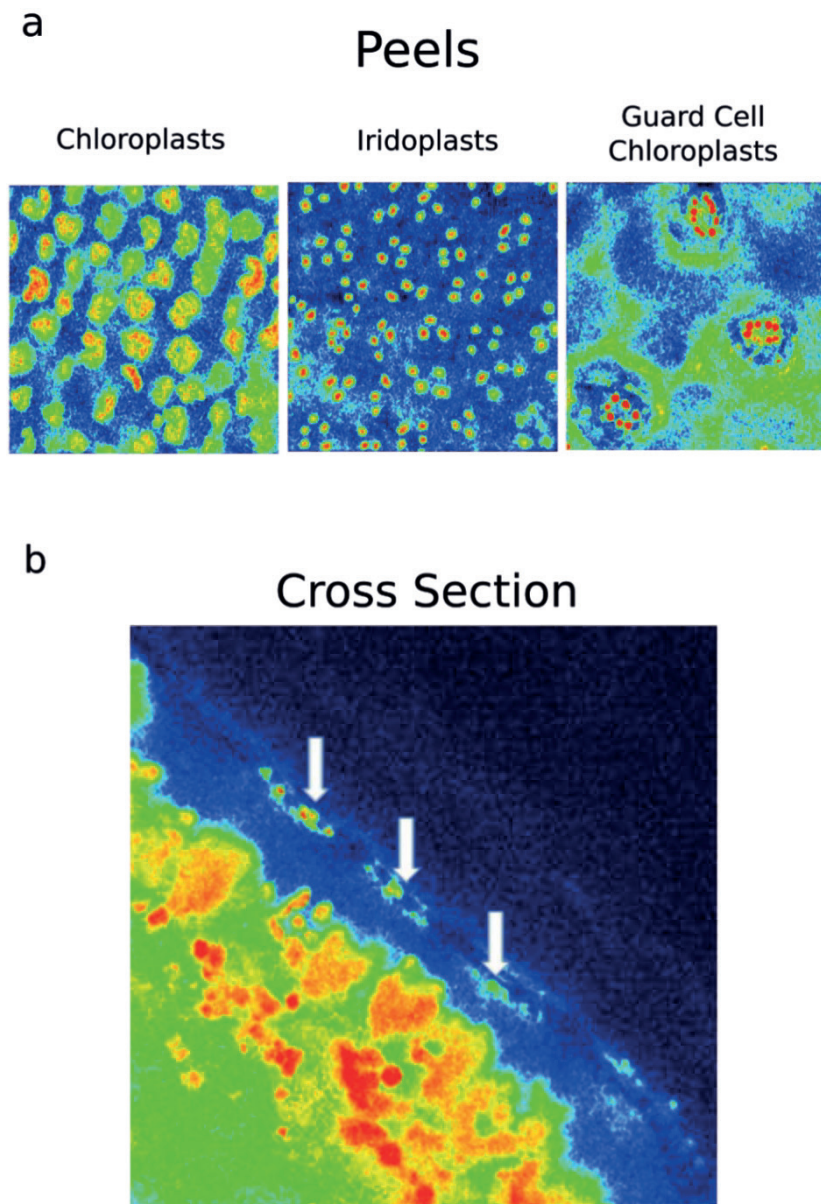


Supplementary Figure. S6 | We performed several series of measurements on different leaves and on more than 20 iridoplasts from the same dark-adapted leaf. Reflectance peaks have similar central wavelengths ($\lambda_c \approx 470$ nm) and are consistent in shape and bandwidth. The slight variations are demonstrated to fit within our model depending on the period of the iridoplast. The statistical data suggest all iridoplasts would present similar values for Λ when under the same light conditions. **a**, Reflectance spectra of ten different iridoplasts from the same leaf under similar conditions of light adaptation and measurement. The shadowed area highlights the expected spectral band for the iridoplast reflectance. **b**, for a full characterization of the optics of iridoplasts we have measured the angular dependence of the reflectance of single iridoplasts. An example of that measurement (bottom) is shown in **(b)** together with a simulation (top) for the angular reflectance of an iridoplast with the properties shown in Fig. 2 of the main text and $d_s = 125$ nm.



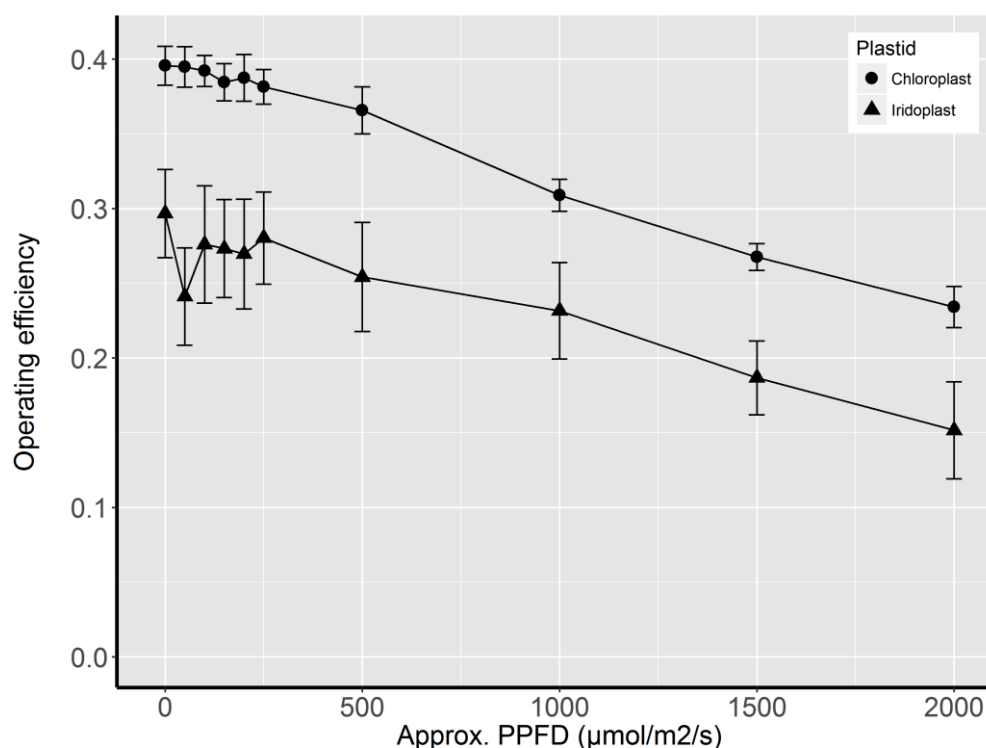
Supplementary Figure. S7 | Model and measurement comparison of absolute reflectance spectra. **a**, TEM images and sketch of thylakoid stacking in chloroplasts of the mesophyll cells of *B. Grandis x Pavonina*. They show large granum stacks with more than 20 thylakoids stacked together (typical of shade-adapted plants). Therefore, for the sake of modelling the photonic properties the distribution of thylakoids within a chloroplast may be considered as a particular case of an iridoplast with $d_s = 0$ (see Fig 2a of the main text for comparison with ultrastructure of the iridoplasts). **b**, Measurements from several iridoplasts from the same leaf at normal incidence. We present reflectance measurements of three iridoplast of the same leaf after dark adaptation. The three of them present a strong reflectance in the same spectral range with slight differences depending on the organelle inspected. Note that the absolute values of reflectance (as compared to a silver mirror) reach values close to 8% in the three cases. For the sake of completeness we performed a measurement in the epidermal cells and using the same illumination spot but this time over a region where no iridoplast was present (dotted line). For this last case, reflectance is negligible in the blue-green spectrum although the small reflectance peak for $\lambda > 700$ nm is present with and without iridoplast. This small increase in reflectance for longer wavelengths is likely due to backscattering on pigmented mesophyll cells. **c**, Optical modelling of the data in (b). In order to compare the absolute values of reflectance measured with the optical model we performed a fit to the three spectra presented here. The parameters used for this fitting are the same shown in Fig. 2b of the main text: $N = 8$, $N_m = 3$, $M = 9$ nm, $L = 7.5$ nm, $n_s = 1.35$, $n_L = 1.35$ and n_t with values shown in Supplementary Fig S3. In the four calculated spectra the distance between grana (d_s) was varied to fit to the spectral shape (see Fig 2 in the main text of this work). Note that individual iridoplasts will vary in value of d_s . We also calculated the reflectance for the case $d_s = 0$, that is, with no partition gap between them, hence resembling the distribution of thylakoids in a normal chloroplast as sketch in (a). Not surprisingly, absolute values of measured reflectance are consistently lower than the calculated ones despite the very good fit of the spectral shape. More importantly however, the spectral blueshift,

change in shape and evolution of the absolute reflectance between iridoplasts can be described as a change in d_s with our model.

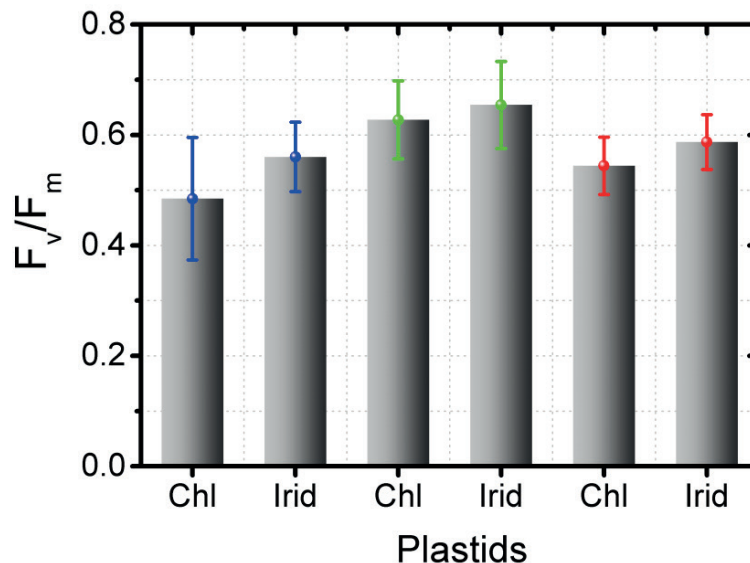


Supplementary Figure. S8 Photosynthetic properties of chloroplasts and iridoplasts images separately with fluorescence microscopy. This technique has been applied successfully before to investigate photosynthesis in guard cell chloroplasts, both in intact leaves and in epidermal peels^{3,4}. We imaged chloroplasts and iridoplasts by peeling off the top two layers of cells (i.e. adaxial epidermis and mesophyll) simultaneously. This allowed clear imaging and measurement of mesophyll chloroplasts and iridoplasts. **a**, For comparison, we also imaged guard cell chloroplasts in the abaxial epidermis, which showed reduced maximum quantum efficiency (Fig 4) and operating efficiency (data not shown) consistent with previous

studies^{3,4}. **b**, leaf cross section showing both iridoplasts in the epidermis (white arrows) and mesophyll chloroplasts below. Peels and cross sections gave similar results.



Supplementary Figure. S9| Response of operating efficiency (F_q'/F_m') to increasing light intensity (Photosynthetic photon flux density, PPFD) in mesophyll chloroplasts and iridoplasts. Consistent with previous observations of other epidermal plastids, iridoplasts show reduced operating efficiency compared to chloroplasts and efficiency reduces with increasing PPFD for both chloroplast types ⁴. Operating efficiency is a measure of the efficiency with which light absorbed by PSII is used for photochemistry, when chloroplasts are in a light-adapted state. In this sense, it differs from maximum quantum efficiency (F_v/F_m), which measures PSII efficiency in a dark-adapted state. Thus operating efficiency reports both on the first step of PSII photochemistry but also gives information on the efficiency of subsequent steps of electron transport. For the data presented here, this suggests that a high rate of photosynthesis in iridoplasts is limited by downstream processes, but this is unlikely to present a disadvantage in low-light conditions, where the higher F_v/F_m may improve overall efficiency relative to the mesophyll chloroplasts. Data are the means of at least 6 replicates $\pm$ standard error.



Supplementary Figure. S10| Comparison of quantum yield measurements of chloroplasts and iridoplasts under excitation of three different wavelengths: $\lambda = 468 \pm 10\text{nm}$ (blue dots), $\lambda = 532 \pm 20\text{nm}$ (green dots) and $\lambda = 633 \pm 10\text{nm}$ (red dots). A similar increase in iridoplast quantum yield over chloroplast quantum yield is observed for all wavelengths, indicating that this effect is not an artefact of a specific measurement wavelength. The difference in absolute values with different measuring light is an established observation⁵. Error bars indicate standard deviation with $n=5$ (Blue), $n=4$ (Green), $n=13$ (Red).

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