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Photosynthetic and CO_2 diffusion capacities in relation to phyllidia anatomy in Briophytes

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Abstract

Bryophytes were the first plants to conquere terrestrial habitats and represent a particular group among land plants as they mainly occur under gametophytal stage, were no stomata are found. Although mosses are the main representatives of Bryophytes, their physiological characterization has been sparsely studied as well as the one of *Selaginella denticulata* (Desv. ex Poir.) Spring., a Lycophyte. Aiming to discern how much lower is photosynthesis in mosses and *S. denticulata* as compared to that of ferns and Spermatophytes, the latter and six moss species were grown under optimal conditions. On average, photosynthesis in mosses was three times lower than in *S. denticulata* and both tested groups had significantly lower rates than ferns and Spermatophytes. These differences were not due to differences in the electron transport rate (*ETR*) or in the maximum velocity for carboxylation ($V_{c,max}$), but rather to differences in mesophyll conductance (g_m), the latter being four times higher in *S. denticulata*. g_m was smaller in these two groups as compared with ferns and Spermatophytes. Finally, an anatomical characterization of studied species was performed to assess which foliar traits were those more responsible for establishing limited g_m and maximum photosynthesis. In agreement with previous studies in other plant groups, g_m was significantly related to variations of the cell wall thickness and the chloroplasts surface area exposed to the mesophyll per leaf area.

Key words: leaf anatomy, mesophyll conductance, mosses, photosynthesis, *Selaginella denticulata.*

Resum

“Capacitats fotosintètica i difusiva del CO_2 en relació a l'anatomia dels fil·lidis dels Briòfits”

Els Briòfits foren les primeres plantes que conqueriren hàbitats terrestres i representen un grup singular entre les plantes terrestres ja que el seu cicle vital està dominat per l'estadi gametofídic, on no es troben estomes. Malgrat que les molses són el grup amb més exemplars d'entre els briòfits, la seva caracterització fisiològica ha estat poc estudiada, tal com ocorre amb el licòfit *Selaginella denticulata* (Desv. ex Poir.) Spring. Amb l'objectiu de determinar quant menor és la fotosíntesi de molses i *S. denticulata* en comparació a la de falgueres i espermatòfits, aquest licòfit i sis molses es van créixer en condicions òptimes. La fotosíntesi de les molses va ser tres vegades menor que la de *S. denticulata* i, ambdós grups, presentaren valors significativament menors que els observats a falgueres i espermatòfits específicament. Aquestes diferències no foren degudes a diferències en les taxes de transport d'electrons (*ETR*) ni en la velocitat màxima de carboxilació ($V_{c,max}$), sinó a diferències en la conductància del mesòfil (g_m), la qual fou quatre vegades major a *S. denticulata*. g_m fou també menor en aquests dos grups que les descrites a falgueres i espermatòfits. Finalment, es va realitzar una caracterització anatòmica de les espècies estudiades per a establir quins trets foliars eren els més implicats en la limitació de la g_m i la màxima fotosíntesi. En concordança amb estudis previs en altres gups de plantes, g_m es relacionà significativament amb les variacions en la gruixa de la paret cel·lular i en la superfície de cloroplasts exposada al mesòfil per àrea foliar.

Paraules clau: anatomia foliar, conductància del mesòfil, fotosíntesi, molses, *Selaginella denticulata.*

Introduction

According to fossil data, first land plants appeared in the mid-Paleozoic era, between 480

and 360 million years ago. Transition from an aqueous to a gaseous medium exposed plants to new conditions that required the development of physiological and morphological changes (Kenrick *et al.*, 1997). With an estimated number of 13000 species, Bryophytes (liverworts, hornworts and mosses) are a group of land plants whose diversity is second to that of the angiosperms. They were among the first-green plants to successfully conquer terrestrial niches and are unique among all land plants because their life cycle is dominated by a gametophytal or haploid phase (Hedderson *et al.*, 1996; Goffinet *et al.,* 2001; Shaw *et al.*, 2004). Moreover, they have contributed to atmospheric and edaphic changes and, therefore, in subsequent evolution of all forms of land plants (Shaw *et al.,* 2004). Specifically, mosses are the main representatives of Bryophytes, consisting of 7000-8000 species. They have a crucial ecological importance in the habitats where they grow. Their physical structure and physiological characteristics allow them to develop in almost every world's ecosystem, so can be found in many aquatic habitats and in almost all terrestrial conditions. Indeed with liverworts and lichens, they confer a significant biodiversity to many environments where vascular plants are sparse or absent (Newton *et al.*, 2000; Hyvönen *et al.*, 2004).

After first land plants establishment, evolution continued and first vascular ones appeared, i.e. the Lycophytes. They date to the late Silurian – early Devonian, when CO_2 atmospheric concentration declined importantly. Although their diversity is larger when extinct taxa are considered due to their abundance during Devonian and Carboniferous, they are currently limited to few taxonomic orders, i.e. Lycopodiales, Isoetales and Selaginellales (Shaw *et al.*, 2004; Saha *et al.*, 2014).

Although ferns and Spermatophytes (gymnosperms and angiosperms) are well-defined groups from a physiological perspective (Flexas *et al.,* 2012a; Carriquí *et al.*, 2015), photosynthesis in mosses and Selaginellales has been sparsely characterized as no more than 20 species have been analyzed. However, Brodribb *et al.* (2007) tested some mosses plus some lycophytes and concluded their maximum net photosynthesis (A_{max}) was comprised from 2 to less than 9 μmol CO_2 m^{-2} s^{-1} and from 1.6 to 6 μmol CO_2 m^{-2} s^{-1}, respectively. From the previous result, it appears that these groups have lower photosynthetic rates than ferns, gymnosperms and angiosperms which could be due to different facts. On the one hand, they are poikilohydric, so that tolerate desiccation to a certain point, but desiccate fast when ambient relative humidity falls below water saturation. As a consequence, there is a rapid cessation of photosynthesis (Alpert *et al.*, 1987). On the other hand, diffusional CO_2 limitations could also be involved in their lower photosynthetic efficiency. It has been a matter of debate if diffusional CO_2 limitations are more important than biochemical ones limiting photosynthesis amongst phylogenetic groups. For decades, biochemical limitations were thought to be much more essential than diffusional ones monitoring photosynthesis. However, it has been recently proved that diffusional CO_2 limitations are decisive (Flexas *et al.,* 2012a) and, in ferns, they play an even more important role than biochemistry in limiting photosynthesis (Carriquí *et al.*, 2015).

In general, two conductances determine diffusional CO_2 limitations and, consequently, photosynthesis: the stomatal (g_s) and the mesophyllic (g_m) conductances. Although g_m was crucial to regulate ferns photosynthesis, both conductances co-limited angiosperms' (Flexas *et al.,* 2012a; Carriquí *et al.*, 2015). Nevertheless, lycophytes and mosses are particular groups. Lycophytes have stomata, so their g_s has been studied. Although many studies demonstrated stomatal regulation of photosynthesis was not developed in lycophytes, Ruszala *et al.* (2011) disagreed. They proved that *Selaginella uncinata* (Desv. ex Poir.) Spring had stomatal regulation as closed once exposed to 0 – 25 μM of ABA. The same happened using CO_2 concentrations from 0 to 700 ppm. Additionally, Bowman (2011) showed how the moss *Physcomitrella patens* (Hedw.) Bruch&Schimp. had stomatal regulation in exposure to ABA, CO_2 and light. Specifically, stomata closed significantly when 50 – 100 μM ABA were reached as well as with 400 to 1000 ppm of CO_2. Moreover, genetical approaches proved that *P. patens* and *Selaginella moellendorffii* Hieron, a genetically equivalent species to *S. uncinata*, contained an *OST1* gene, which expressed in response to ABA and drought (Bowman, 2011; Ruszala *et al.,* 2011). However, there is still controversy whether lycohpytes' stomata can truly regulate photosynthesis. Both conductances will be considered here for *Selaginella denticulata* (L.) Spring, but its photosynthesis limitations will be mainly attributed to g_m. As mosses just have stomata during the sporophyte stage, photosynthesis

limitations could just be attributed to g_m because they mainly rest under gametophyte phase. Nonetheless, lycophytes are compounded by microphylls and mosses by phyllidia in spite of leaves, so do not exactly have a mesophyll. However, the term g_m is used here to simplify referring to the CO_2 conductance from the absorption surface to the sites of carboxylation.

The importance of the g_m as a limiting fact for photosynthesis has been highlighted in recent years. Now this parameter has been estimated for more than 100 species, so it is becoming possible to seek for evolutionary patterns among plant groups. Most studies focused on Spermatophytes and ferns, with only few data for liverworts and hornworts (Flexas *et al.,* 2012a). In angiosperms, leaf anatomical characteristics are determinants for the potential g_m and photosynthesis (Tomás *et al.*, 2013) and could explain why they have higher photosynthesis rates than other groups (Brodribb *et al.*, 2007). The same pattern has been recently shown in ferns, where lower g_m was due to high cell wall thickness (T_{cw}) and lower chloroplasts intercellular air spaces per leaf area (S_c/S) (Carriquí *et al.*, 2015; Tosens *et al.,* 2015), limiting parameters which affect the CO_2 diffusion through the mesophyll. Moss species analyzed thus far had in general lower photosynthesis rates than ferns (Brodribb *et al.,* 2007), so g_m is believed to be even lower than ferns' because of variations of the same structural parameters.

No previous study has addressed the analysis of g_m and anatomy in mosses nor Selaginellales growing under non-stress conditions. The aims of this study are (1) to compare how lower is the photosynthetic capacity of mosses and *S. denticulata* as compared with that reported in ferns and Spermatophytes; (2) to assess the physiological determinants for this lower capacity, specifically how important is g_m and; (3) to analyze the potential implication of leaf anatomical traits in establishing maximum photosynthesis in these two studied groups.

Materials and Methods

Studied plants and growth conditions

The present study combined physiological measurements developed from February to April in Spain and from June to July in Estonia, where anatomical characterization was also performed from July to August.

In Mallorca, Spain (39º45'51"N, 2º42'33"E, 47 m above sea level; a.s.l), the mean annual precipitation was 320.8 mm and the wettest month was November, with mean annual min/max temperatures of 14.4/21.8ºC (Balearsmeteo, 2016). In Tartu, Estonia (58º49'22"N, 26º43'21"E, 72 m a.s.l), the mean annual precipitation was 650 mm and the wettest months ranged from September to October, with mean annual min/max temperatures of 1.0/8.0ºC (Tosens *et al.,* 2015).

In the two countries, plants were grown under favourable conditions to avoid any effect of the site or stress conditions. In Spain, species were subjected to daily light fluctuation as were set outdoors in a shelter that protected them from strong wind and excessive solar radiation. *Polytrichum formosum* Hedw.*, Pseudoscleropodium purum* (Hedw.) M. Fleisch.*, Plagiomnium undulatum* (Hedw.) T. J. Kop. and *S. denticulata* were sown in one tray, respectively, that contained soil. Nevertheless, *Thuidium tamariscinum* (Hedw.) Schimp. was set in a shaded place of the shelter as it showed different requirements. Plants were watered twice a day to maintain optimal water status and vigour. Thus, a timing irrigation system was provided in shady periods to avoid dehydratation.

In Estonia, species were let in a plant growing room at 23ºC with 14/10 hours light/darkness daily fluctuation after being set in an individual tray. Trays for *Plagiomnium elatum* (Bruch, Schimp. & W. Gümbel) T. J. Kop. and *Pleurozium schreberi* (Brid.) Mitt. contained soil. Specimens were handly watered twice a day and protected from excessive radiation using a plastic cover.

The complete list of studied species is shown in Table 1. It also indicates at which sites species were collected from and the university where the measurements were performed. Figure 1 provides a visual characterization of all these species.

Table 1. List of the seven physiological and anatomical characterized species. Phylogenetic classification was established according to Goffinet *et al.* (2004). Habitats were generalized according to Ingerpuru *et al.* (1994) and HVMO (2007).

Division	Class	Order	Species	Habitat	Collection region	Measuring university
Lycopodiophyta	Selaginellopsida	Selaginellales	*Selaginella denticulata*	Wet shady rocky places	Sóller (Mallorca, Balearic Islands, Spain)	UIB (Mallorca, Balearic Islands, Spain)
Bryophyta	Polytrichopsida	Polytrichales	*Polytrichum formosum*	Coniferous and deciduous forests on humus-rich soils, more rarely on stones covered with humus	Galicia (Spain)	UIB (Mallorca, Balearic Islands, Spain)
	Bryopsida	Hypnales	*Thuidium tamariscinum*	In the ground, tree bases and decaying wood of shady wet forests	Galicia (Spain)	UIB (Mallorca, Balearic Islands, Spain)
	Bryopsida	Hypnales	*Pseudoscleropodium purum*	Pine forests and wooded meadows	Sóller (Mallorca, Balearic Islands, Spain)	UIB (Mallorca, Balearic Islands, Spain)
	Bryopsida	Hypnales	*Pleurozium schreberi*	Forests, meadows and bogs on the ground and decaying wood	Vapramäe (Tartu, Estonia)	EMÜ (Tartu, Estonia)
	Bryopsida	Bryales	*Plagiomnium undulatum*	Wet forests, covered by shady bushes and near bogs' edges	Sóller (Mallorca, Balearic Islands, Spain)	UIB (Mallorca, Balearic Islands, Spain)
	Bryopsida	Bryales	*Plagiomnium elatum*	Transitional mires, fens and wooded meadows	Vapramäe (Tartu, Estonia)	EMÜ (Tartu, Estonia)

Figure 1. Representative pictures of each studied species. Specimens A, B, C, D, E, F and G represent *S. denticulata, P. formosum, T. tamariscinum, P. purum, P. schreberi, P. undulatum* and *P. elatum,* respectively.

Gas exchange and fluorescence measurements

• Measurements in Spain

Fully expanded microphylls/phyllidia (hereafter "leaves") were selected for the simultaneous measurement of gas exchange and chlorophyll *a* fluorescence using an infrared gas analyser (IRGA) LI-6400XTR coupled with the fluorometer (Li-6400-40; Li-Cor Inc., Lincoln, NE, USA). Leaves were clamped into a 2 cm^2 cuvette. For all measurements, parameters were adjusted to those that species need naturally. Leaf temperature was fixed between 19-22ºC according to the requirements each species had. The vapour pressure deficit (VPD) was kept around 1.5 kPa. Humidity levels were maintained between 65-85%. Leaf steady-state conditions were induced at saturating photosynthetic photon flux density (PPFD 1500 µmol m^{-2} s^{-1}, 90-10% red-blue light) and

400 µmol CO_2 mol^{-1} air. The flow rate within the chamber was regulated from 150 to 200 mmol air min^{-1} in order to ensure CO_2 delta values were >4 µmol CO_2 mol^{-1} air between sample and reference IRGAs, allowing for photosynthetis rates. Steady-state conditions were usually reached after 5-10 minutes.

A_N-C_a response curves were performed considering six atmospheric CO_2 concentration (C_a) steps, starting with 400, decreasing to 50 and reaching 800 µmol CO_2 mol^{-1} air. A range from eight to twelve replicates per species was performed to ensure enough data. While measurements were being developed, the rest of the sample outside the chamber got easily dehydrated. Thus, it was covered with a plastic bag and water-dampened. In all cases, measurements of the values of A_N and steady-state fluorescence (F_s) were registered after the stabilization of the gas exchange rates in a given C_a in a range of 120-180 seconds to avoid dehydratation. Then, a saturating white light flash around 8000 µmol m^{-2} s^{-1} was applied to determine the maximum fluorescence (F_m'**)**. From these values, the electron transport rate (*ETR*) through PSII was calculated after calibration in low O_2 conditions (Valentini *et al.,* 1995).

The respiration in the dark (R_d) was measured in five replicates per species after leaving plants in all-night darkness conditions. To approach the measuring conditions to the environmental ones, CO_2 atmospheric concentration was fixed in the chamber. Five measurements were recorded five by five seconds for each replicate.

Following Flexas *et al.* (2007), cuvette CO_2 leakage curves were used to correct A_N-C_a curves as well as R_d values for all species.

Once measurements finished, samples were treated before results were finally obtained. As leaves did not totally cover the area of the cuvette, a digital picture of them inside the chamber's gasket was taken to know how much sample had been measured. *P. formosum* and *P. undulatum* were defoliated. *S. denticulata, T. tamariscinum* and *P. purum* were placed under a Petri dish to fix them, avoiding overlapping of their structures. After the appropriate treatment was done according to species characteristics, a definitive photograph was took to recalculate their area with the image analysis software ImageJ (Wayne Rasband/NIH, Bethesda, MD, USA).

For all the species, estimations for the maximum velocity of carboxilation ($V_{c,max}$) were performed as well as for g_m. The first parameter was obtained according to Farquhar *et al.* (1980). The mesophyll conductance was calculated following the method of Harley *et al.* (1992) with the equation:

$$g_m = \frac{A_N}{C_i - \frac{\Gamma^*(ETR+8(A_N+R_d))}{ETR-4(A_N+R_d)}}$$

However, some parameters had to be reconsidered as mosses had specific particularities. The CO_2 concentration at the substomatal cavity (C_i) was replaced for the atmospheric CO_2 concentration (C_a) during the performance of the measurements as mosses lack stomata. Thus, the remodelled and finally applied equation was:

$$g_m = \frac{A_N}{C_a - \frac{\Gamma^*(ETR+8(A_N+R_d))}{ETR-4(A_N+R_d)}}$$

Furthermore, the Rubisco specificity factor (τ) or the CO_2 compensation point (Γ*) was assumed to be the one for *Nicotiana tabacum* L., i.e. 42.5 (Galmés *et al.,* 2006) because no reliable values were available for mosses. As this value is at 25ºC, its recalculation according to the leaf temperature during the measurements needed to be done following Bernacchi *et al.* (2002). Obtained values were used to transform the A_N-C_a curves into A_N-C_c with the equation:

$$g_m = \frac{A_N}{C_a - C_c}$$

Moreover, as we assume that the actual Γ* for analyzed species could vary ± 30% from the recalculated one according to measurements' temperature, a sensitivity analysis was performed and two additional g_m's (*g_m and **g_m) were also determined.

• Measurements in Estonia

Leaves were selected following the same criteria as in Spain for the simultaneous measurement of gas exchange and chlorophyll *a* fluorescence using a Walz portable gas-exchange and fluorescence system (GFS-3000) coupled with the ED-Array/PAM-Fluorometer 3055-FL (Heinz, Walz, Germany). Leaves were clamped into an 8 cm^2 cuvette, with fixed temperature at 20ºC. Humidity level was maintained at 75%. Leaf steady-state conditions were induced at saturating photosynthetic photon flux density (PPFD of 1500 µmol m^{-2} s^{-1}, 90-10% red-blue light) and 400 µmol CO_2 mol^{-1} air. The flow rate within the chamber was 750 mmol air min^{-1}. Steady-state conditions were reached usually after 2-3 minutes.

Five or six replicates per species was done to perform A_N-C_a response curves considering same atmospheric CO_2 concentration (C_a) steps than in Spain. While measurements were being developed, the rest of the sample outside the chamber was treated as described above. Measurements of the values of A_N and steady-state fluorescence (F_s) were registered after the stabilization of the gas exchange rates in a given C_a in a range of 120-180 seconds to avoid dehydratation. Then, a saturating white light flash around 8000 µmol m^{-2} s^{-1} was applied to determine the maximum fluorescence (F_m'**)**. From these values, the real quantum efficiency of photosystem II (Φ_{PSII}) was recorded in the equipment and corresponded to:

$$\Phi_{PSII} = \frac{(F_m' - F_s)}{F_m'}$$

With the previous value, the electron transport rate (ETR) through PSII was calculated as:

$$ETR = \alpha\beta \cdot PAR \cdot \Phi_{PSII}$$

where αβ was determined from the relationship between Φ_{PSII} and Φ_{CO2} once obtained by varying the light intensity under non-photorespiratory conditions provided by an atmosphere which contained 1% O_2. The PAR value corresponded to the saturating quantum flux density applied during the measurement and Φ_{PSII} was obtained as previously described (Martins *et al.,* 2013).

R_d was measured at CO_2 concentration of 400 µmol CO_2 mol^{-1} air after plants had been let under same conditions that those in Spain. Previously described methodology was followed to obtain estimations for same number of replicates per species.

Cuvette CO_2 leakage curves were performed as explained above to correct A_N-C_a curves and R_d estimations for all species.

Recalculation of the area was done using the same methodology as in Spain. *P. elatum* was defoliated, but *P. schreberi* was treated as *T. tamariscinum, S. denticulata* and *P. purum.*

Leaf mass per unit area

The total area of microphylls/phyllidia area (hereafter “leaf area”) from five replicates per species was determined analyzing images with the image analysis software ImageJ (Wayne Rasband/NIH, Bethesda, MD, USA). Caulidia regions of replicates of *P. formosum, T. tamariscinum, P. undulatum* and *P. elatum* were removed. However, *S. denticulata, P. purum* and *P. schreberi* structures did not allow the separation between leaves and stems/caulidia, so their structure was considered as a whole during the analysis. Every replicate was set in a labelled envelope and oven-dried at 70ºC for, at least, 48 hours. After reaching constant weight, the dry leaf mass per unit leaf area (LMA) was calculated.

Anatomical measurements

In Estonia, anatomical analysis was performed to determine leaves structure and to assess the four structural traits most likely affecting g_m: the fraction of mesophyll occupied by intercellular air spaces (f_{ias}), the cell wall thickness (T_{cw}) and the fraction of mesophyll and chloroplasts surface area exposed to intercellular air spaces per leaf area (S_m/S and S_c/S, respectively) (Tomás *et al.*,

2013; Carriquí *et al.,* 2015; Tosens *et al.,* 2015).

Following Tomás *et al.* (2013), Carriquí *et al. (*2015) and Tosens *et al.* (2015), who adjusted the methodology described in Evans *et al.* (1994) and Syvertsen *et al.* (1995), three replicates per species which measured 1 x 1 mm were cut between main structures such as veins. They were fastly fixed under vacuum pressure using a prepared chemical solution that contained glutaraldehyde 4% and paraformaldehyde 2% in a 0.01 M phosphate buffer (pH = 7.4). Then, each replicate was set in a labelled eppendorf which contained 1 ml, approximately, of this fixing solution. In Estonia, replicates were post-fixed in 2% buffered osmium tetroxide during one hour and dehydrated by ethanol series. Obtained dehydrated replicates were placed in one mould, respectively, and embedded in LRwhite resin (London Resin Company) avoiding bubbles formation. They were solidified once let in an oven at 60ºC for 24 hours.

For each replicate, semi-fine cross-sections of 1 µm for light microscopy were cut with an ultramicrotome (Leica UC6, Vienna, Austria). They were dyed with 1% toluidine blue solution and viewed in bright-field using an Olympus BX60 optic microscope. One picture of each replicate was taken at 100x, 200x and 400x magnifications to characterize the species and indentify which structures belonged to leaves. Once identified, two pictures of each replicate were taken at 1000x magnifications with a digital camera (U-TVO.5XC; Olympus, Tokyo, Japan) after covered with immersion oil to be seen with the microscope.

For *P. purum* and *T. tamariscinum*, one randomly selected micrograph from each picture at 1000x magnifications was analyzed as structures were smaller than those of other species, where the whole picture was analyzed. Nevertheless, *P. elatum* anatomy was determined using the same methodolgy described above but at 400x magnifications as did not require more amount of magnificaions. From these micrographs/pictures values for f_{ias}, S_m/S and S_c/S were obtained by averaging the six values from analyzed pictures using the ImageJ software (Wayne Rasband/NIH, Bethesda, MD, USA).

Following Evans *et al.* (1994) and Syvertsen *et al.* (1995), f_{ias} was obtained as:

$$f_{ias} = 1 - \frac{\sum S_s}{t_{mes}\, W}$$

where ΣS_S was the total cross-sectional area of the mesophyll cells, t_{mes} was the mesophyll thickness between two epidermis and W was the width of the analyzed section. S_m/S was determined with the equation:

$$S_m S = \frac{L'_{mes}}{W} \cdot F$$

where L'_{mes} represented the length of mesophyll cells exposed to intercellular air space, W represented the same mentioned above and F was the curvature correction factor, taken as 1.36. This was important to convert cross-section lengths to surface areas as cell surfaces were not uniformly perpendicular to the plane of the sections. S_c/S was calculated as:

$$S_c S = \frac{L'_c}{L'_{mes}} \cdot S_m S$$

where L'_c corresponded to the length of chloroplasts exposed to intercellular air space and L'_{mes} and S_m/S represented same parameters explained above. From the calculation of these two previous parameters, the ratio S_c/S_m was obtained. Finally, as T_{cw} required more accurated measurement, three cells per picture/micrograph were analyzed and averaged to obtain one final value per replicate. T_{cw} per species was obtained averaging the calculated six ones for all pictures.

Statistical analysis

Statistical analysis was done using the software package IBM SPSS 19.0 (SPSS, Chicago, IL, USA). Two independent one-way analysis of variance (ANOVA's) were performed to reveal

differences between physiological and anatomical traits, respectively. The differences between analyzed groups' means and pairs of species' means were detected by Tukey's honest significant difference tests. In all cases, significant statistical differences were found when $P < 0.05$. Additionally, a Pearson correlation matrix was performed for all species to determine the correlation between paramameters, being considered to be significant when $P < 0.05$ and $P < 0.01$.

Results

Photosynthetic comparison between *S. denticulata* and mosses

Figure 2 represents A_N-C_a response curves for each species. All studied species showed typical A_N-C_a response curves.

At ambient CO_2, the net CO_2 assimilation (A_N) was more than three times higher for *S. denticulata* than for averaged value of studied mosses, the difference being significant (Table 2). Specifically, statistically significant differences were found between *S. denticulata* and each single moss species except *P. formosum*, which was the one with highest A_N (2.65 μmol CO_2 m^{-2} s^{-1}). In contrast, the lowest values corresponded to *P. schreberi* and *P. elatum* (0.31 μmol CO_2 m^{-2} s^{-1}) and representing eight times less CO_2 assimilation as compared to *P. formosum*. A_N for the rest of mosses were comprised between this range and were generally lower than 1 μmol CO_2 m^{-2} s^{-1}. Therefore, *P. formosum* net CO_2 assimilation statistically differed from that of any other moss species.

In reference to mesophyll conductance, a sensitivity analysis was performed considering that the actual Γ* for analyzed species could vary ± 30% from the recalculated one according to measurements' temperature. These additional g_m's were $*g_m$ and $**g_m$, respectively. It is important to highlight that for the three calculated ones ($*g_m$, g_m, $**g_m$), *S. denticulata* values were more than four times higher than that averaged for mosses (Table 2). Hence, significant difference was found testing both values. Specifically, statistical differences were found between *S. denticulata* and *P. schreberi, P. undulatum* and *P. elatum* in the three g_m's. In fact, these species were the ones with lowest values for these parameters. Furthermore, testing the moss with highest values for $*g_m$, i.e. *P. formosum,* statistical differences were found with *P. schreberi* and *P. elatum.*

According to Table 2, although average respiration in the dark (R_d) for mosses was higher than for *S. denticulata,* no significant differences were found between them. Besides *P. elatum,* R_d of *S. denticulata*'s was the lowest in this study. Moreover, the species with highest value, *P. formosum*, differed statistically with *S. denticulata, P. schreberi* and *P. elatum*. Same happened analyzing the second and the third ones with highest values, *T. tamariscinum* and *P. purum*, respectively, both differing with *P. elatum*.

Focusing on the characters driving the photo- and biochemical capacity of photosynthesis in plants, $V_{c,max}$ and *ETR,* both were higher for mosses (Table 2). Although significant statistical difference was found analyzing averaged value for mosses and that one of *S. denticulata* for $V_{c,max}$, no difference was obtained testing values for *ETR*. In reference to $V_{c,max}$, the highest value was obtained for *P. elatum* (62.0 μmol CO_2 m^{-2} s^{-1}), followed by *P. schreberi* and *P. formosum*. Thus, statistical differences were found testing *P. elatum* with the rest of species. Additionally, *P. schreberi* and *P. formosum* differed with *S. denticulata, P. undulatum* and *T. tamariscinum,* the species with lowest values. Moreover, *P. purum* and *P. schreberi* also statistically differed. Considering *ETR*, *P. elatum* was the species with highest value, being 79.3 μmol m^{-2} s^{-1}. Thus, statistical significant differences were found between it and the rest of species. Additionally, the second species with highest value, i.e. *P. formosum,* also statistically differed with *P. undulatum,* the one with lowest *ETR.*

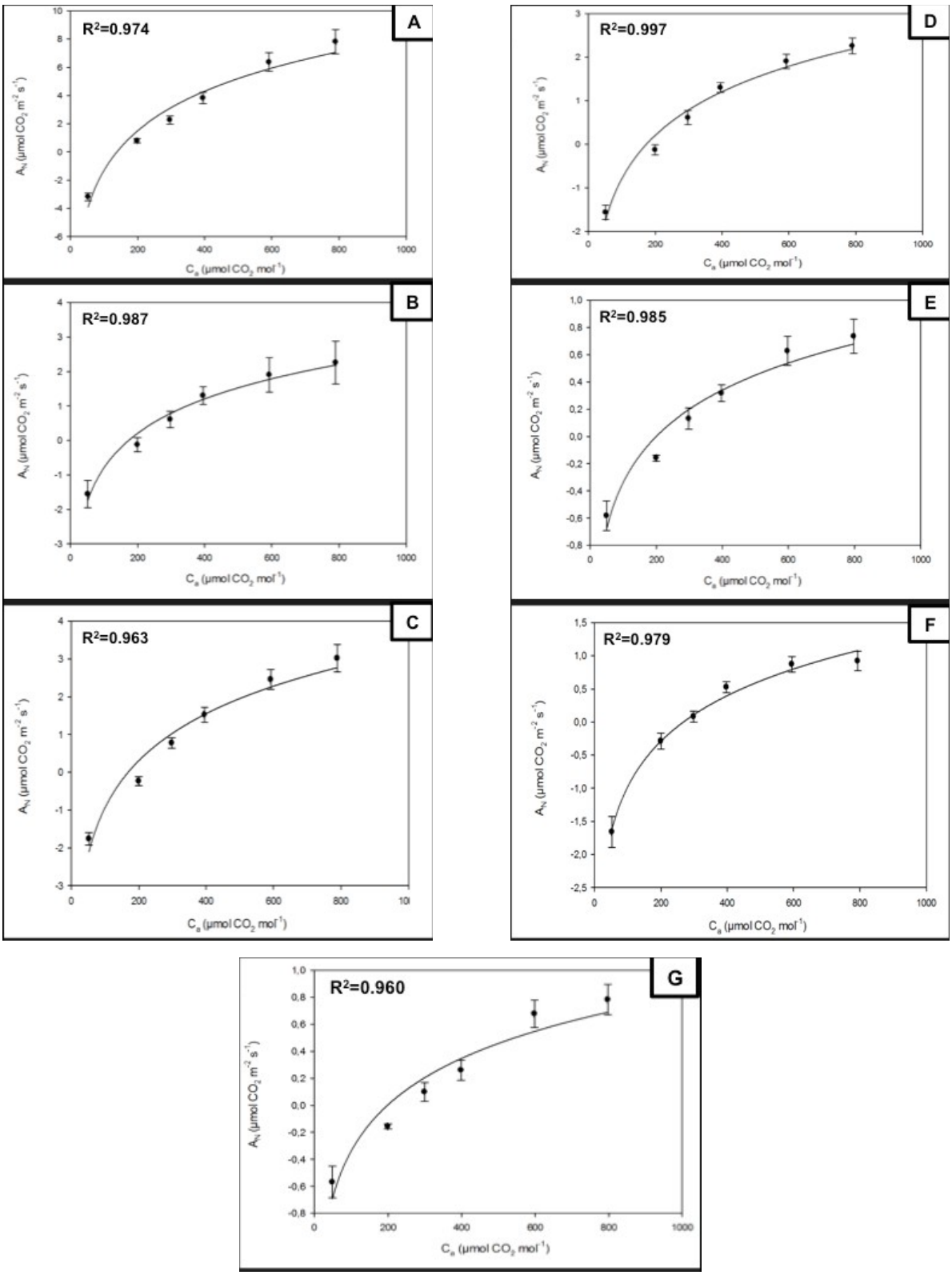

Figure 2. A_N-C_a response curves. A, B, C, D, E, F and G represent *S. denticulata, P. formosum, T. tamariscinum, P. purum, P. schreberi, P. undulatum* and *P. elatum* A_N-C_a response curves at 20, 19, 22, 21, 20, 20 and 20ºC, respectively, and 800 µmol photons m^{-2} s^{-1} for all of them excepting *P. undulatum,* where 400 µmol photons m^{-2} s^{-1} were used. Six atmospheric CO_2 concentrations (C_a) were considered. Symbols show averages ± standard error (SE) in both axes (errors in X-axis are not seen due to scale adjustment). n=12, 10, 8, 11, 5, 8 and 6, respectively. Data were fitted by logarithmic function.

Table 2. Photosynthetic characteristics for each studied specie. Average values ± SE are shown for CO_2 net assimilation (A_N), stomatal conductance (g_s), mesophyll conductance (g_m) – $^*g_m$ and $^{**}g_m$ considered <30% Γ* and >30% Γ*, respectively –, maximum carboxylation rate on a C_c basis ($V_{c,max}$), electron transport rate (*ETR*) and dark respiration rate (R_d).

Species	A_N (µmol CO_2 m^{-2} s^{-1})	g_s (mol CO_2 m^{-2} s^{-1})	g_m (mol CO_2 m^{-2} s^{-1})			$V_{c,max}$ (µmol CO_2 m^{-2} s^{-1})	*ETR* (µmol m^{-2} s^{-1})	R_d (µmol m^{-2} s^{-1})
			$^*g_m$	g_m	$^{**}g_m$			
Lycophytes								
Selaginella denticulata	3.02 ± 0.41^{a*}	0.077 ± 0.008	0.008479 ± 0.002374^{a*}	0.009504 ± 0.002797^{a*}	0.010836 ± 0.003388^{a*}	23.7 ± 2.8^{a*}	33.1 ± 3.7^{ab*}	-0.67 ± 0.04^{ab*}
Mosses								
Polytrichum formosum	2.65 ± 0.41^{a}	–	0.006804 ± 0.000929^{ab}	0.007244 ± 0.001253^{ab}	0.007747 ± 0.001136^{ab}	40.8 ± 3.5^{bc}	52.9 ± 4.1^{b}	-1.26 ± 0.14^{c}
Thuidium tamariscinum	1.01 ± 0.17^{b}	–	0.002450 ± 0.000003^{ab}	0.002674 ± 0.000057^{ab}	0.002948 ± 0.000143^{ab}	16.6 ± 8.0^{a}	22.8 ± 5.7^{a}	-0.97 ± 0.11^{bc}
Pseudoscleropodium purum	0.84 ± 0.15^{b}	–	0.001141 ± 0.000850^{b}	0.001197 ± 0.000894^{b}	0.001258 ± 0.000945^{ab}	25.3 ± 1.2^{ab}	30.9 ± 0.7^{ab}	-0.86 ± 0.09^{bc}
Pleurozium schreberi	0.31 ± 0.06^{b}	–	0.000834 ± 0.000162^{b}	0.000861 ± 0.000168^{b}	0.000891 ± 0.000174^{b}	43.3 ± 3.6^{c}	43.3 ± 4.1^{ab}	-0.77 ± 0.06^{ab}
Plagiomnium undulatum	0.38 ± 0.10^{b}	–	0.000646 ± 0.000096^{b}	0.000678 ± 0.000101^{b}	0.000713 ± 0.000106^{b}	19.5 ± 1.2^{a}	22.0 ± 2.0^{a}	-0.80 ± 0.20^{bc}
Plagiomnium elatum	0.31 ± 0.05^{b}	–	0.000839 ± 0.000132^{b}	0.000862 ± 0.000137^{b}	0.000890 ± 0.000140^{b}	62.0 ± 3.5^{d}	79.3 ± 6.8^{c}	-0.30 ± 0.08^{a}
Average	**0.92 ± 0.38****	–	**0.002119 ± 0.000974****	**0.002253 ± 0.001042****	**0.002408 ± 0.001120****	**34.6 ± 7.1****	**41.9 ± 8.9***	**-0.83 ± 0.13***

Superscript letters and asterisks indicate significant difference ($P < 0.05$) between different species and groups, respectively, both based on Tukey's multiple comparison test for each analyzed physiological parameter.

Anatomical comparison between *S. denticulata* and mosses

As each species had different anatomical characteristics, Figure 3 represents an approach to their traits and were some of those pictures where anatomical parameters were obtained from. For more details, see Annexed information.

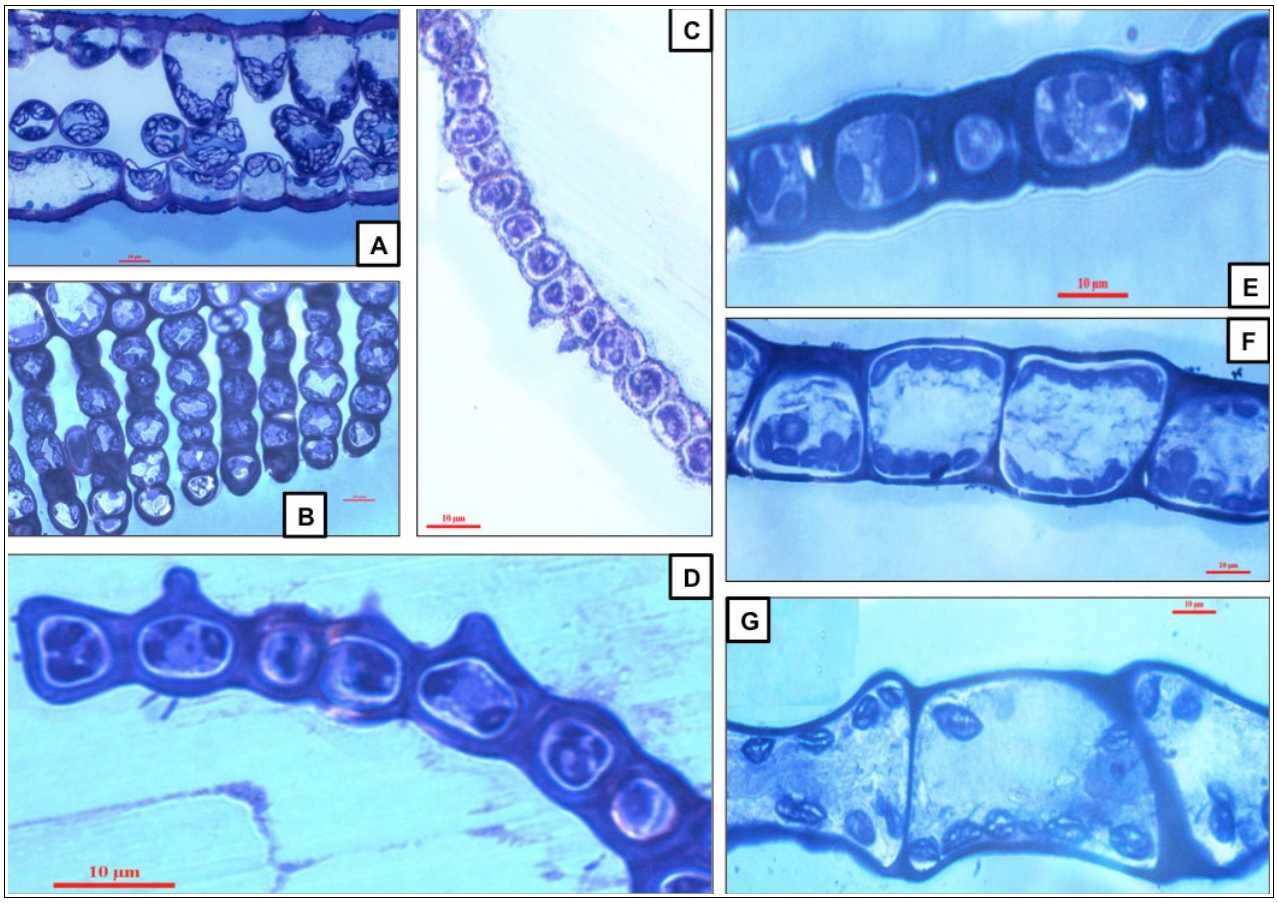

Figure 3. Anatomical representation of each species at 1000x magnifications. Specimens A, B, C, D, E, F and G represent *S. denticulata, P. formosum, T. tamariscinum, P. purum, P. schreberi, P. undulatum* and *P. elatum,* respectively.

Considering leaf mass per unit area (LMA), there were not significant differences between average value for mosses and the one of *S. denticulata*. However, statistical significant differences were found comparing *P. undulatum* and *P. elatum* with the rest of species as these two species showed values as low as 14.7 and 9.8 g m^{-2}, respectively (Table 3).

Regarding to the fraction of mesophyll occupied by the intercellular air spaces (f_{ias}), the only species where it could be measured was *S. denticulata* (Table 3) as a consequence of their traits.

Focusing on the cell wall thickness (T_{cw}), the averaged value for mosses statistically differed with that of *S. denticulata*. Particularly, highest and lowest values corresponded to *P. schreberi* and *S. denticulata,* being 2.17 and 0.47 µm, respectively. Hence, statistical differences were found testing these species between all others. Additionally, statistical differences were also detected analyzing *T. tamariscinum*, the second species with highest thickness of the cell wall with *S. denticulata*, *P. schreberi*, *P. elatum*, *P. formosum* and *P. purum. P. undulatum,* the third species with highest value, statistically differed with *P. formosum* and *P. purum* (Table 3).

Related to the mesophyll surface area exposed to intercellular air spaces per leaf area

(S_m/S), the highest value was obtained for *P. purum* and the lowest for *S. denticulata*, being 6.30 and 3.83 $m^2\,m^{-2}$, respectively (Table 3).

Regarding to the chloroplasts surface area exposed to intercellular air spaces per leaf area (S_c/S), the value of *S. denticulata* was five times higher than the average for mosses. In fact, *S. denticulata*'s one was the highest, being 11.88 $m^2\,m^{-2}$ (Table 3). As a consequence, statistically significant differences were found between and both each separate moss and their average. The same happened for S_c/S_m (Table 3).

Table 3. Anatomical characteristics of all studied species. Average values ± SE are shown for the dried leaf mass per unit area (LMA), fraction of the mesophyll occupied by the intercellular air spaces (f_{ias}), cell wall thcinkess (T_{cw}), mesophyll surface area exposed to intercellular air spaces per leaf area (S_m/S), chloroplasts surface area exposed to intercellular air spaces per leaf area (S_c/S) and the ratio S_c/S_m.

Species	LMA ($g\,m^{-2}$)	f_{ias} (%)	T_{cw} (µm)	S_m/S ($m^2\,m^{-2}$)	S_c/S ($m^2\,m^{-2}$)	S_c/S_m
Lycophytes						
Selaginella denticulata	57.1 ± 4.5^{a*}	8.8 ± 2.5	0.47 ± 0.04^{a*}	3.83 ± 0.51^{a*}	11.88 ± 2.74^{a*}	3.02 ± 0.54^{a*}
Mosses						
Polytrichum formosum	61.8 ± 3.5^{a}	–	0.99 ± 0.05^{b}	4.11 ± 0.04^{a}	2.94 ± 0.25^{b}	0.71 ± 0.06^{b}
Thuidium tamariscinum	57.7 ± 11.5^{a}	–	1.72 ± 0.15^{d}	4.31 ± 0.13^{a}	2.04 ± 0.12^{b}	0.47 ± 0.02^{b}
Pseudoscleropodium purum	61.7 ± 3.0^{a}	–	1.18 ± 0.09^{b}	6.30 ± 1.40^{a}	2.85 ± 0.25^{b}	0.52 ± 0.08^{b}
Pleurozium schreberi	48.1 ± 3.0^{a}	–	2.17 ± 0.08^{e}	3.99 ± 0.04^{a}	1.76 ± 0.08^{b}	0.44 ± 0.02^{b}
Plagiomnium undulatum	14.7 ± 1.0^{b}	–	1.58 ± 0.06^{cd}	4.26 ± 0.31^{a}	2.57 ± 0.16^{b}	0.61 ± 0.02^{b}
Plagiomnium elatum	9.8 ± 1.1^{b}	–	1.30 ± 0.07^{bc}	3.97 ± 0.23^{a}	1.56 ± 0.17^{b}	0.39 ± 0.02^{b}
Average	**42.3 ± 9.7***	–	**1.49 ± 0.17****	**4.49 ± 0.37***	**2.29 ± 0.24****	**0.52 ± 0.05****

Superscript letters and asterisks indicate significant difference ($P < 0.05$) between different species and groups, respectively, both based on Tukey's multiple comparison test for each analyzed anatomical parameter.

Correlation between characters

A Pearson correlation matrix was performed for some analyzed parameters combining the seven species studied in this work (Table 4). The largest correlations were generally found between CO_2 net assimilation (A_N) and the mesophyll conductance (g_m), the cell wall thickness (T_{cw}) and the chloroplasts surface area exposed to intercellular air spaces per leaf area (S_c/S). These high correlations were also detected when plotting the data (Fig. 4), where positive linear trends were detected between A_N and g_m, T_{cw} and S_c/S. However, higher correlation between parameters ($P < 0.01$) was found testing A_N with g_m ($r = 0.988$), which indicates that photosynthetic lower trends were due to CO_2 diffusive limitations. In fact, those parameters related to biochemistry, the electron transport rate (*ETR*) and the velocitiy of maximum carboxylation ($V_{c,max}$), were not positively correlated with the CO_2 net assimilation. This lower mesophyll conductance and, as a consequence, lower photosynthesis was because of positve correlation between A_N and the anatomical parameters T_{cw} and S_c/S, indicating these traits were those involved in lower photosynthetic capacity of analyzed species.

Table 4. Pearson correlation matrix of the physiological and anatomical parameters measured for the seven species. Values in italics and bold indicate significant ($P < 0.05$) and highly significant ($P < 0.01$) correlation coefficients, respectively. Parameter abbreviations as in Tables 2 and 3.

	A_N	g_m	$V_{c,max}$	*ETR*	R_d	LMA	T_{cw}	S_m/S	S_c/S
A_N		**0.988**	0.211	0.076	0.423	0.593	*0.801*	0.215	*0.755*
g_m			0.173	0.043	0.328	0.527	*0.782*	0.336	*0.803*
$V_{c,max}$				**0.971**	0.463	0.407	0.084	0.297	0.320
ETR					0.462	0.388	0.102	0.300	0.224
R_d						0.681	0.002	0.178	0.096
LMA							0.237	0.319	0.312
T_{cw}								0.035	*0.759*
S_m/S									0.214
S_c/S									

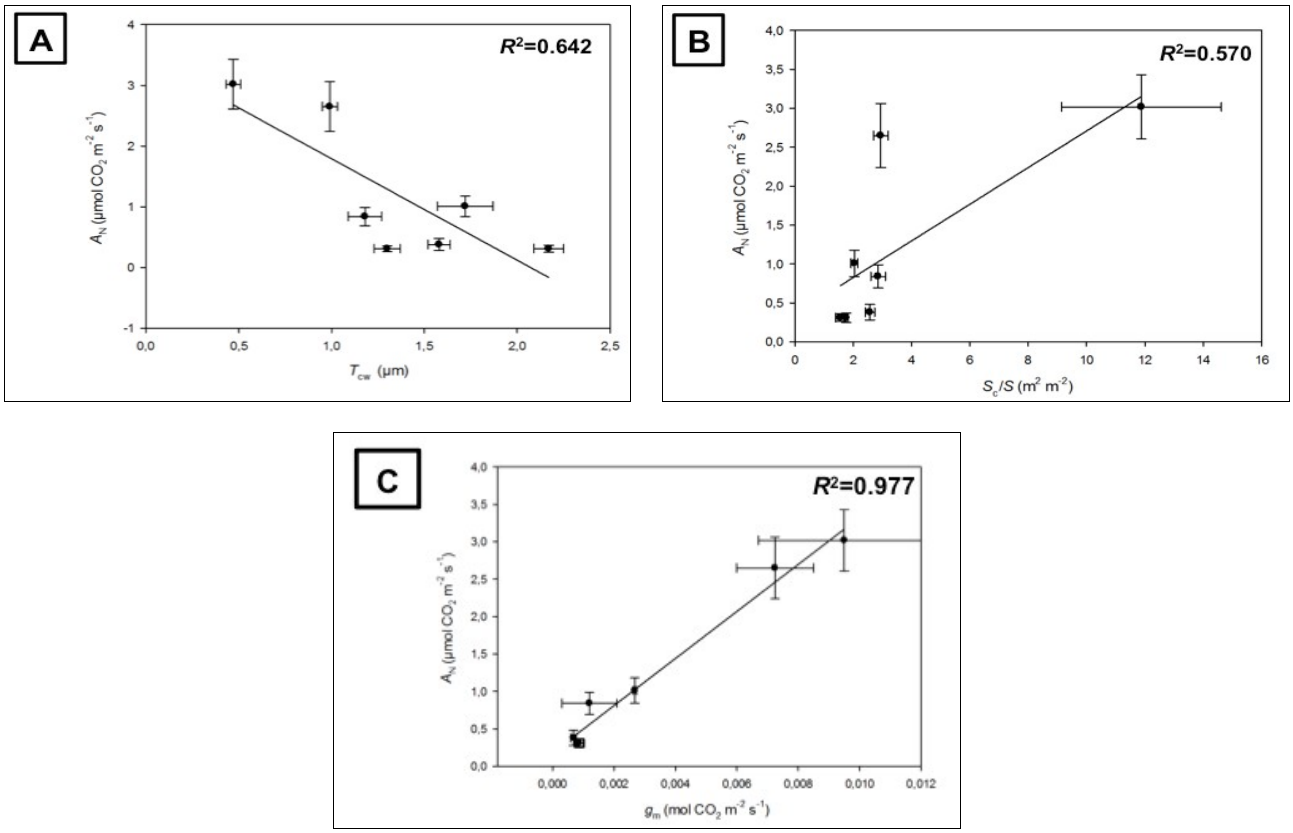

Figure 4. Correlation between net CO_2 assimilation (A_N) and (A) cell wall thickness (T_{cw}), (B) chloroplasts surface area exposed to intercellular air spaces per leaf area (S_c/S) and (C) mesophyll conductance (g_m). Values are means ± SE.

Discussion

While few studies have reported gas exchange rates in mosses (Alpert *et al.,* 1987; Brodribb *et al.,* 2007; Waite *et al.,* 2010) and lycophytes (Brodribb *et al.,* 2007; Soni *et al.,* 2012), to the best of our knowledge this is the first attempt to determine their photosynthetic capacity,

mesophyll conductance (g_m) and anatomical determinants in specimens grown under optimal conditions.

The first objective of this study was to compare how lower was the photosynthetic capacity of mosses and *S. denticulata* with that of ferns and Spermatophytes. Although all the characterized species were studied under conditions selected to favour their maximum photosynthesis, in general the photosynthetic capacity of mosses was lower than that of *S. denticulata* (Table 2). Only in *P. formosum*, net CO_2 assimilation (A_N) did not differ significantly from *S. denticulata*. Anyway, while the A_N for *S. denticulata* was well within the range documented for Lycophytes by Brodribb *et al.* (2007), it was far lower than the rates observed in another *Selaginella, S. bryopteris*, by Soni *et al.* (2012). It is thus possible that our *Selaginella* was not at its maximum photosynthetic capacity during the study for whatever reason. As for mosses, the values obtained in the present study were generally lower than those in Brodribb *et al.* (2007). Nevertheless, it has to be noticed that in their study they just tested one phylogenetic order, the Polytrichales. This group of mosses can be considered special as they do present highly developed "pseudo-vascular" system, with connections between the conducting tissues of the stem and that of the phyllidia (Hébant, 1977). In addition, it has been reported that Polytrichales present a "pseudo-mesophyll" that increases the available area for CO_2 uptake and, as a consequence, their photosynthesis is higher than the one of other orders. Therefore, they are considered as a particular group among mosses, being placed in higher phylogenetic position than other orders (Bell *et al.,* 2010). Indeed in the present study, the A_N of the only representative of Polytrichales we analyzed, *P. formosum,* was the highest among studied mosses (2.65 μmol CO_2 m^{-2} s^{-1}, Table 2) and well within the range described by Brodribb *et al.* (2007). Contrary to photosynthesis, the respiration in the dark (R_d) was generally higher in mosses than in *S. denticulata* (Table 2), although not significantly.

From Peguero-Pina *et al.* (2012), Tomás *et al.* (2013), Tosens *et al.* (2015) and Carriquí *et al.* (2015), net CO_2 assimilation characterization of ferns and Spermatophytes (gymnosperms and angiosperms) were compiled and are, on average, 5.83, 9.45 and 10.37 μmol CO_2 m^{-2} s^{-1}, for ferns, gymnosperms and angiosperms, respectively. In comparison with these values, *S. denticulata* and studied mosses had much lower net CO_2 assimilation (Table 2). Particularly, mosses are the plant group were the lowest average A_N was achieved (0.92 μmol CO_2 m^{-2} s^{-1}, Table 2), followed by *S. denticulata* (3.02 μmol CO_2 m^{-2} s^{-1}, Table 2), the only representative of Lycophytes we studied. This means that mosses have on average six times less CO_2 net assimilation than ferns and eleven times less than angiosperms. From these results, we can establish a phylogenetic trend in terms of net CO_2 assimilation, starting with the lowest rates for mosses and raising progressively in lycophytes, ferns and gymnosperms, to finally reach the highest values in angiosperms.

The second objective of this study was to determine the physiological causes for the lowest photosynthetic capacity exhibited by tested groups in comparison to other plant groups, focusing on the importance of mesophyll conductance (g_m), as the latter has been shown to the most important determinant for photosynthesis in ferns, the phylogenetic group immediately above the lycophytes (Carriquí *et al.,* 2015; Tosens *et al.,* 2015). Photosynthetic limitations can be due to diffusive or biochemical limitations. In relation to the first ones, stomatal conductance (g_s) just could be determined in *S. denticulata* as mosses lack them during the main part of their life cycle (Table 2). Nevertheless, g_s in *S. denticulata* was far largerer than its g_m, for which the latter can be considered as the main diffusional limitation in this species and the only one in mosses. Concerning biochemical limitations, they are related to the functioning of Rubisco, which regulates the velocity of carboxylation ($V_{c,max}$) and/or to the functioning of the photochemical activity linked to the Calvin cycle, which is expressed by the electron transport rate (*ETR*). About these two mentioned parameters, although statistical differences were found testing $V_{c,max}$ between groups, non significant differences were found considering averaged *ETR* for mosses with that of *S. denticulata* (Table 2). This could mean that, even more CO_2 carboxylation was found in mosses due to higher biochemical activity of Calvin cycle, their averaged *ETR* was not enough different from *S. denticulata*'s one. Therefore, if we consider all species achieved their maximum $V_{c,max}$ and *ETR*, biochemical capacity for mosses still makes them unable to reach the photosynthetic traits of *S. denticulata*. However, Table 4 and Figure 4 prove this lower photosynthetic capacity of mosses was not because of these biochemical parameters as no positive correlation was found between A_N and them.

The dominant role of g_m in limiting maximum photosynthesis in the two plant groups studied here (Table 4 and Fig. 4) is in agreement with such values being much lower than those found in ferns, gymnosperms and angiosperms. For ferns, an average of 0.057 mol CO_2 m^{-2} s^{-1} was obtained for mesophyll conductance (Tomás *et al.,* 2013; Carriquí *et al.,* 2015), which is six times that of *S. denticulata* and 25 times the averaged in mosses. Considering gymnosperms, an averaged 0.108 mol CO_2 m^{-2} s^{-1} was determined (Flexas *et al.,* 2012b; Peguero-Pina *et al.,* 2012) and for angiosperms, 0.154 mol CO_2 m^{-2} s^{-1} (Tomás *et al.,* 2013; Carriquí *et al.,* 2015). That is, mosses have on average 68 times lower g_m than angiosperms. These results suggest that the evolution of g_m may have been crucial to support the evolution towards larger photosynthetic rates along terrestrial plant phylogeny (Flexas *et al,* 2012a).

The central role of the mesophyll conductance in establishing a more limited photosynthetic capacity of mosses in comparison with the one of *S. denticulata* and other plant groups leads to the question of which are the causes for these differences. In Spermatophytes, there is evidence that those leaf anatomical parameters which determine the length and the nature of the CO_2 pathway until its carboxylation site play a dominant role (Peguero-Pina *et al.*, 2012; Tomás *et al.,* 2013; Tosens *et al.,* 2015). Therefore, analyzing the anatomical traits that are more related to this issue was the third objective of our study. Thus, we measured those ones that had been revealed as the most important for g_m in Spermatophytes: LMA, f_{ias}, S_c/S and T_{cw} (Tomás *et al.,* 2013) plus S_m/S and the ratio S_c/S_m.

According to Table 3, obtained values of leaf mass per unit area (LMA) did not statistically differ between the two tested groups. The fraction of the mesophyll occupied by intercellular air spaces (f_{ias}) just could be measured in *S. denticulata* as it was the only species which traits allowed its determination (Table 3 and Fig. 3). Either statistical significant differences were found comparing the mesophyll surface area exposed to intercellular air spaces per leaf area (S_m/S) (Table 3) between the two groups. However, significant statistical difference were detected within the two groups analyzing the cell wall thickness (T_{cw}), the chloroplasts surface area exposed to intercellular air spaces per leaf area (S_c/S) and the ratio S_c/S_m (Table 3 and Fig. 3). Focusing on the two first parameters, our results were not surprising because anatomical differences between *S. denticulata* and mosses can be detected at first sight (Fig. 3). *S. denticulata* has a leaf structure more similar to a parenchyma than those of mosses, where just series of cells are found. For this reason and due to intercellular air space, more cells can be placed in the *S. denticulata*'s mesophyll. As a consequence of major number of cells, higher ratio of chloroplasts surface area exposed to intercellular air spaces per leaf area were found. Contrary, mosses have less chloroplasts as contain less cells in their mesophyll and CO_2 entrance is even more complicated because of thicker cell wall. Finally, significant differences for the S_c/S_m ratio were found as a consequence of the variation in S_c/S as S_m/S did not differ between tested groups (Table 3).

From all the results mentioned above, it is concluded that main differences regulating g_m and, consequently, photosynthetic traits in the studied groups are mostly due to variations of T_{cw} and S_c/S, where higher correlations were found (Table 4 and Fig. 4). Considering the first parameter and regarding to Figure 4, our results do not differ from those found in Peguero-Pina *et al.* (2012), Tosens *et al.* (2012ab), Tomás *et al.* (2013) and Carriquí *et al.* (2015), where main differences enabling higher ratios of photosynthesis were attributed to variations in the thickness of the cell wall. In fact, a phylogenetic trend consisting of a reduction of the cell wall thickness while an increase of g_m is experimented has been established. It starts with thickest cell walls of Bryophytes, with values comprised between 1 and 5 μm (Waite *et al.,* 2010) reaching thinest ones in angiosperms, with values raging from 0.1 to 0.5 μm (Tomás *et al.* 2013; Carriquí *et al.,* 2015), being ferns the intermediate group with values of 0.2-0.8 μm (Carriquí *et al.,* 2015). From our results, *S. denticulata* could be placed between Bryophytes and ferns as its T_{cw} was 0.47 μm (Table 3). Nonetheless, more Lycophytes should be analyzed to get enough data to correctly locate them in this phylogenetic scale.

Besides photosynthetic traits, it is important to highlight that cell wall particularities have been also related with desiccation tolerance. In this sense, as we were testing poikilohydric species, it is not strange that their cell wall is thicker than in the rest of phylogenetic groups. For this reason, changes in cell wall particularities among plant phylogeny might operate towards favouring desiccation tolerance and optimizing photosynthesis. From this point of view, Bryophytes

and Lycophytes represent the first plants that adapted to terrestrial conditions, being desiccation-tolerant but with low photosynthetic capacity due to their thick cell wall in comparison with angiosperms, non-desiccation tolerant but with high photosynthetic capacity because of thinner cell wall (Proctor *et al.,* 2002). Nevertheless, although the role of the cell wall thickness is highly relevant, others such as S_c/S are also important to understand differences in photosynthetic capacity along the plant phylogeny (Table 4 and Fig. 4).

Conclusions

A specific comparative study of physiological traits in *S. denticulata* and mosses have been performed once species were grown under optimal conditions.

1. *S. denticulata* had three times more photosynthetic capacity than the averaged for mosses. In comparison with other plants groups, mosses had six and eleven times less CO_2 assimilation than ferns and angiosperms, respectively.
2. In order to assess which limitations were the most determining for the previous result (the biochemical or the diffusive ones), it appeared that the CO_2 diffusional limitations, with mesophyll conductance playing the most important role, was the main fact. In fact, *S. denticulata* showed a mesophyll conductance which was four times higher than that averaged for mosses. Additionally, mosses had 25 and 68 times lower mesophyll conductance than ferns and angiosperms, respectively.
3. The reason why mesophyll conductance was lower in mosses was because of leaves structural differences between them and *S. denticulata* plus other plant groups. Particularly, the cell wall thickness as well as the chloroplasts surface area exposed to intercellular air spaces per leaf area were those issues more implicated in photosynthetic differences, but additional facts may be also involved.

Nevertheless, more studies are required to test more species and a more complete set of anatomical parameters to reach a better understanding of the facts involved in the low g_m detected in mosses.

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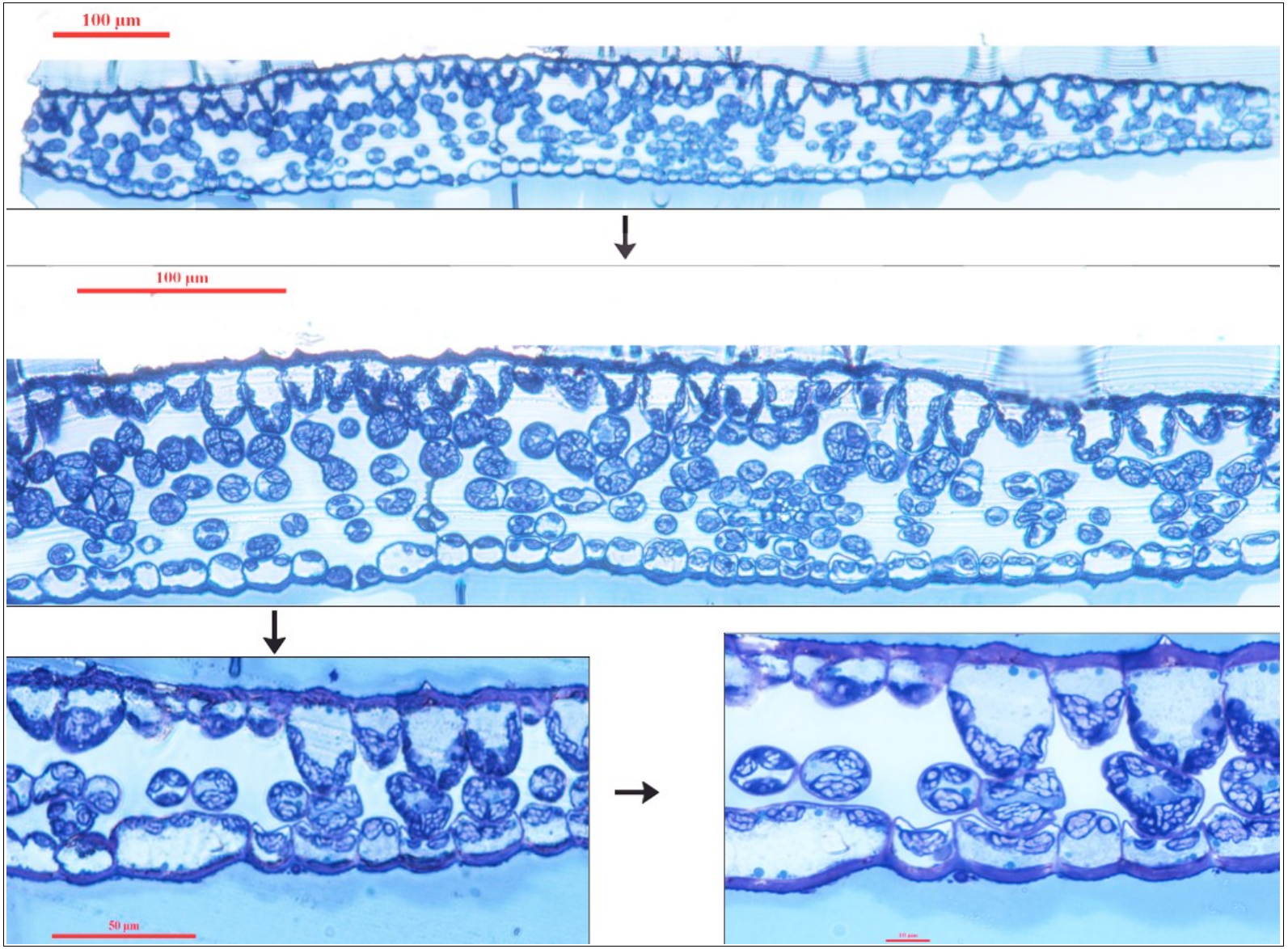
Annexed information

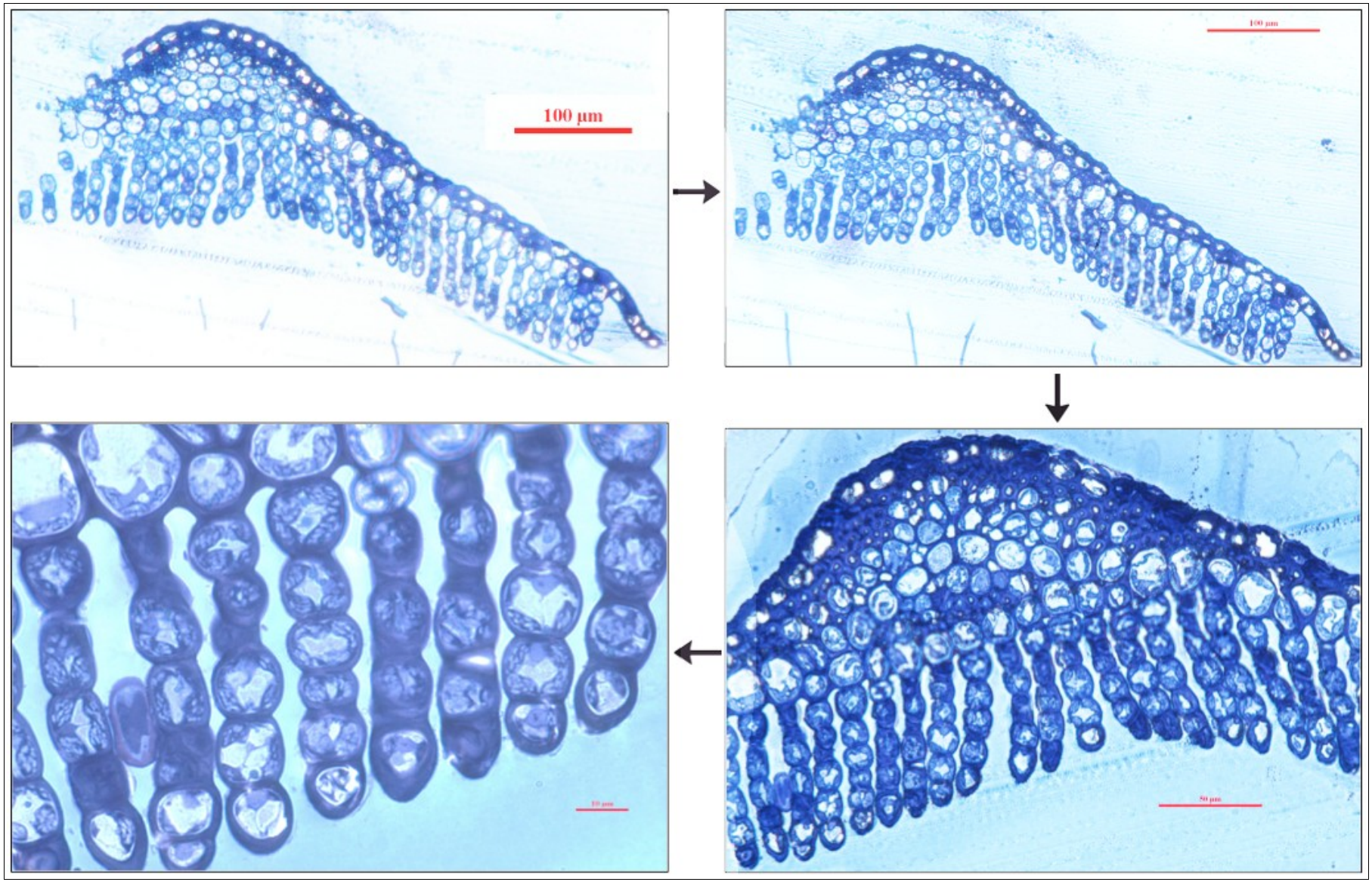
Anatomical characterization of each studied species. According to appropiated scale, each picture was taken at 100x, 200x, 400x or 1000x magnifications. Arrows indicate increasing magnification.

[1] One asterisk (*) means pictures include venation regions (those “rounded-shaped” ones).
[2] Double asterisk (**) means pictures include caulidia regions (those different foliar structures which are bigger and lack chloroplats).

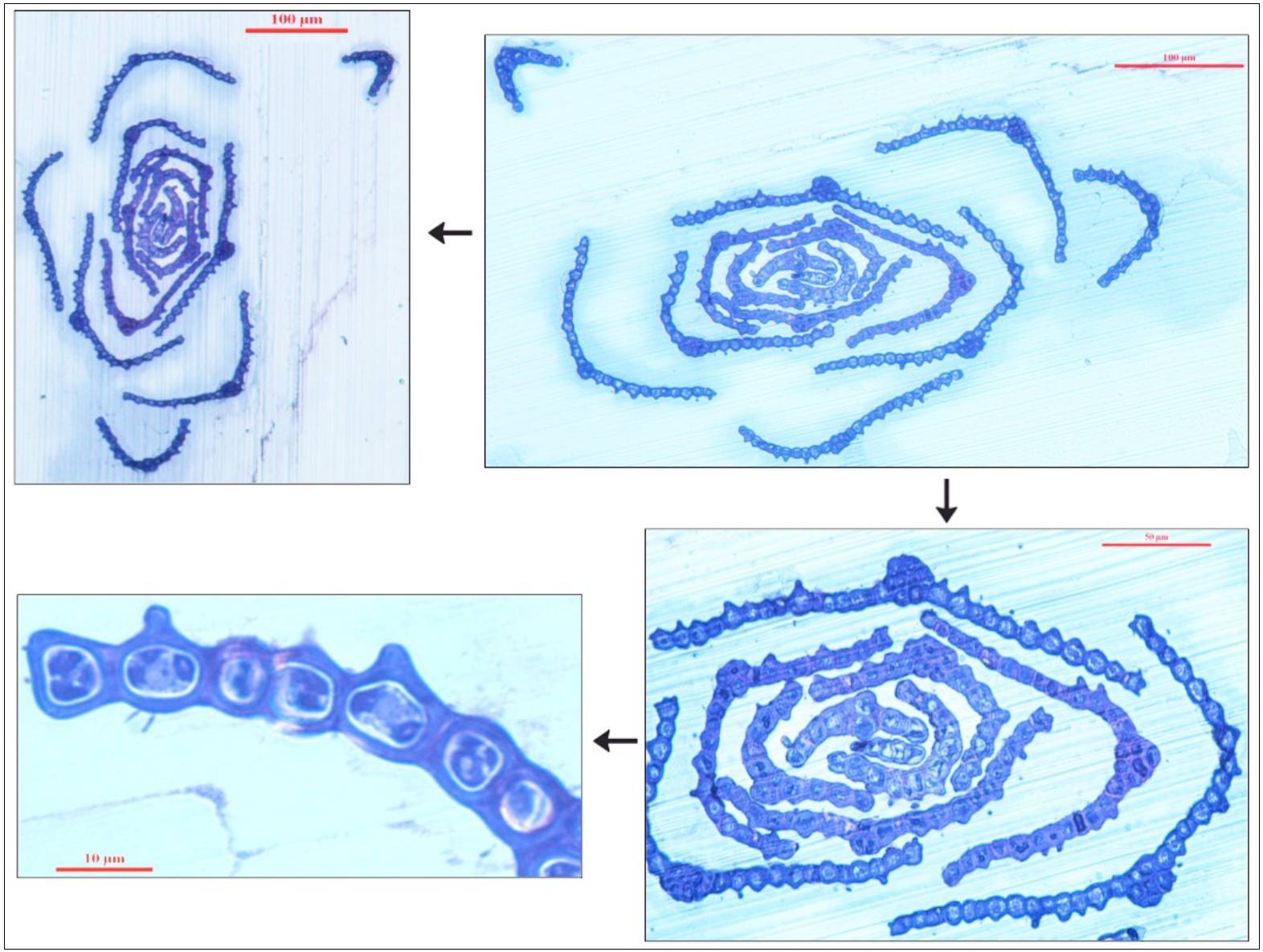
Selaginella denticulata



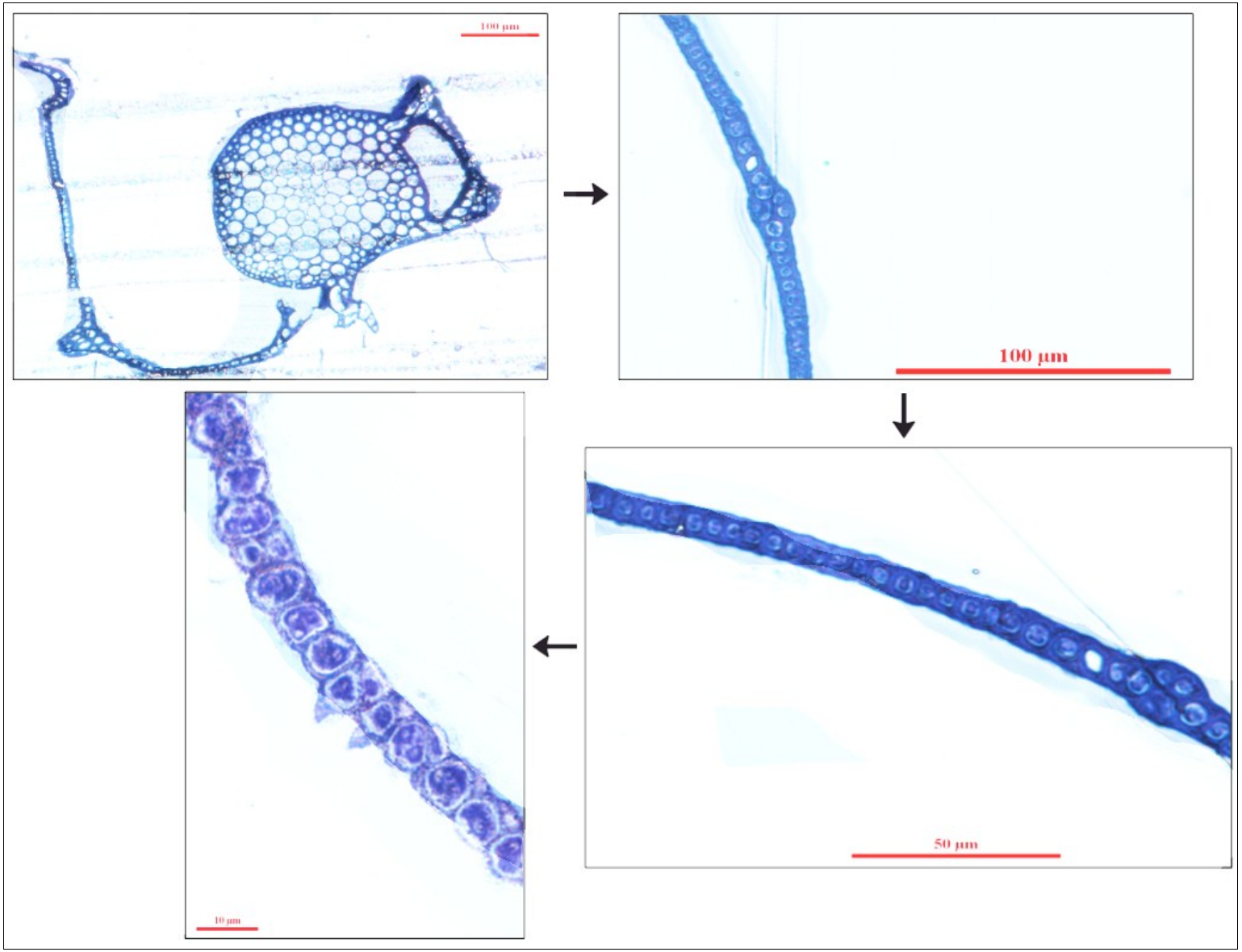
*Polytrichum formosum**



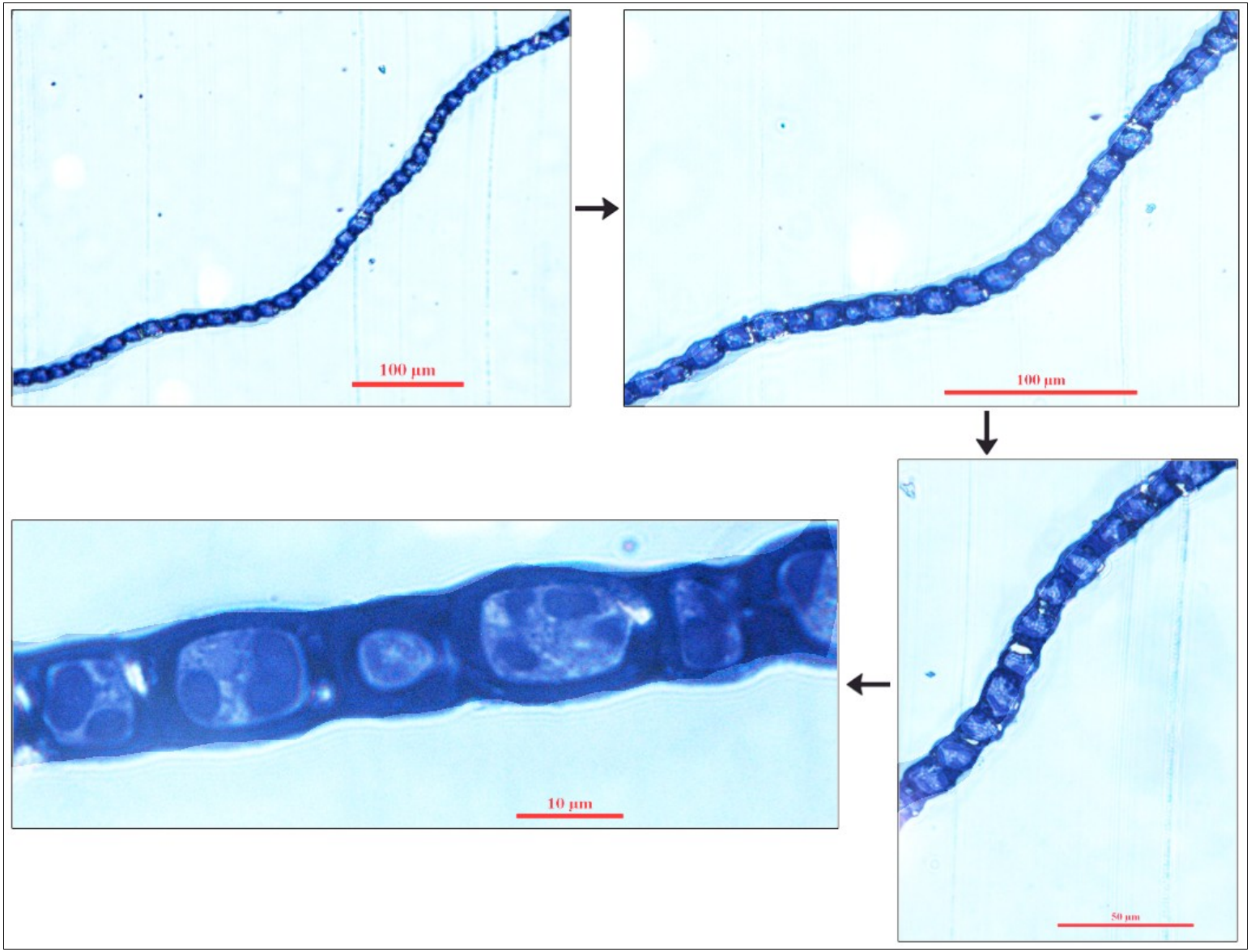
*Thuidium tamariscinum**



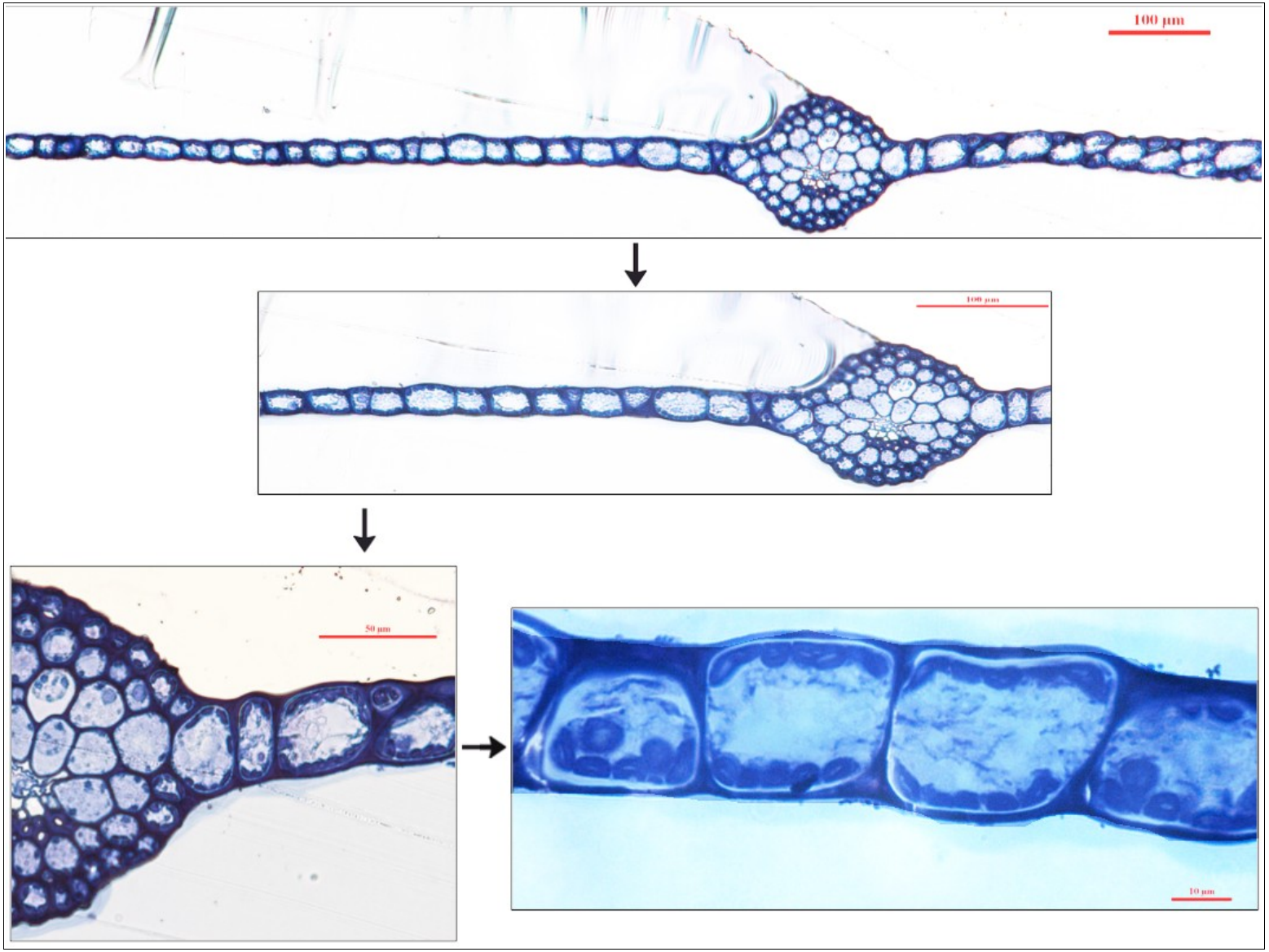
*Pseudoscleropodium purum***



Pleurozium schreberi



*Plagiomnium undulatum**



Plagiomnium elatum