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RESEARCH PAPER

On the benefits of living in clumps: a case study on *Polytrichastrum formosum*

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ABSTRACT

- The study concerns the mechanics and water relationships of clumps of a species of endohydric moss, *Polytrichastrum formosum.*
- Anatomical and morphological studies were done using optical and scanning electron microscopy. Experiments on waterdrop capture and their distribution to adjacent shoots within a moss clump were performed with the experimental set-up for the droplet collision phenomena and ultra-high speed camera. The mechanical strength of the moss clump was tested on an electromechanical testing machine.
- During the process of moss clump wetting, the falling water drops were captured by the apical stem part or leaves, then flowed down while adhering to the gametophore and never lost their surface continuity. In places of contact with another leaf, the water drop stops there and joins the leaves, enabling their hydration. Mathematical analysis of anatomical images showed that moss stems have different zones with varying cell lumen and cell wall/cell radius ratios, suggesting the occurrence of a periodic component structure. Our study provides evidence that the reaction of mosses to mechanical forces depends on the size of the clump, and that small groups are clearly stronger than larger groups.
- The clump structure of mosses acts as a net for falling rain droplets. Clumps of *Polytrichastrum* having overlapping leaves, at the time of loading formed a structure similar to a lattice. The observed reaction of mosses to mechanical forces 3 indicates that this phenomenon appears to be analogous to the 'size effect on structural strength' that is of great importance for various fields of engineering.

INTRODUCTION

Mosses are one of the oldest groups of land plants and evolved more than 500 million years ago (Goffinet & Shaw 2008). With 25,000 species, they are the world's second largest group of embryophytes (Schaefer & Zryd 2001). These poikilohydric plants with little control over water loss inhabit almost all climate zones on Earth (Pressel 2006). As argued by some authors, this poikilohydry is an essential part of the complex of traits allowing bryophytes to be successful in many habitat types (Proctor & Tuba 2002; Proctor 2010). Most moss species grow in clumps and show clonal or colonial life forms (Bates 1998; Glime 2015). The growth and morphology of single plants within the clump are genetically determined. However, the structural organisation of this life form is influenced by environmental conditions and shows high plasticity (Gimingham & Birse 1957; Farge-England 1996; Bates 1998; Rossi *et al.* 2001). Living in clumps has many advantages and compensates for the lack of specialised anatomical structures typical of vascular plants. Such clumping structures allow two characteristics critically important for living on land: the transport and retention of water and mechanical stability.

Taking into account water transport, mosses can be divided into two groups: ectohydric mosses and endohydric mosses (Buch 1945, 1947; Glime 2015). Most mosses are ectohydric plants that rely on external water transport on the plant surface by capillarity. These plants usually have very thin non-waxy cuticles that can absorb water over the entire surface (Proctor 1979). Endohydric mosses have surfaces with water-resistant cuticles that reduce their ability to take in water through leaves. These mosses rely mostly on internal water and have some forms of conductive tissues (Proctor 1982). For mosses, moisture-holding capacity is the most commonly considered benefit of living in clumps. This is important because most of the organisms exchange water with the environment through their surface (Proctor *et al.* 2007; Elumeeva *et al.* 2011).

The shape of the clump significantly influences its moisture content in interactions with the wind (Proctor 1981; Zotz *et al.* 2000; Rice *et al.* 2001; Rice & Schneider 2004; Rice 2012). The irregular height of individual shoots within the clump, which increases its roughness, strongly stimulates the turbulent air flow, which increases evaporation (Proctor 1981; Rice & Schneider 2004). Mosses have the ability to adapt to environmental changes by changing the shape and organisation of their clumps depending on moisture conditions; this has been observed in *Polytrichum alpestre*, an endohydric moss, which in humid habitats shows looser clump structure and increased roughness (Birse 1957). In the case of moisture deficit, matted clumps with a relatively smooth surface are formed, and this limits turbulent flow and inner-clump wind penetration (Longton 1979; Guerra *et al.* 1992; Nakatsubo 1994). Additionally, the cushion life form characterises an increased ratio of volume to surface area and can be considered the optimal way to store water within the clump. However, other growth forms may be more effective in catching nutrients, allowing CO_2 diffusion to chloroplasts or competing for space with other organisms (*e.g.,* Bates 1989), and most mosses do not form cushions.

Anatomy is an important feature determining the mechanical properties of plant tissues (Hébant 1977; Rossi *et al.* 2001; Niklas *et al.* 2006; Frenzke *et al.* 2011; Atala & Alfaro 2012; Vincent 2012; Zajączkowska *et al.* 2015). The biomechanics of mosses are usually omitted in studies, even though their mechanical stability is important for such aspects as resistance to snow cover in winter (Kennedy 1993; Woolgrove & Woodin 1996). There are a few studies examining the mechanics of single stems (Frenzke *et al.* 2011; During *et al.* 2015); however, the most important determinant for the functioning of a species is the clump. Another approach that focuses on single stems is to express moss size in the context of the concept of the allometric evolution of land plants, mainly focusing on their small body size and resulting from the lack of a xylem (Carlquist 1975; Niklas 1997). However, there are no studies describing moss anatomy from a mechanical point of view. Cells forming moss gametophores typically show a hydrostatic structure. Stiffness of the gametophore depends not only on cell hydration, but also on the ratio of cell wall thickness to cell radius (R). A cell acts biomechanically as a hydrostatic structure when cell wall thickness does not exceed 20% of the cell radius, and at higher values cell wall thickness becomes the most important factor in the biomechanical control (Niklas 1992). The vast majority of mosses have a unistratose stem epidermis without stomata. Epidermal cells are similar in diameter or slightly thicker than cell walls in sclerodermal cells; thus, they can more actively participate in mechanical stress transmission. In the subclass *Polytrichidae*, the conducting strand is formed in the central part of the stem. It consists of water-conducting cells (hydroids), which are surrounded by a leptoid cell cylinder that conducts assimilates. In comparison with tracheids in tracheophytes, cell walls of mosses have no secondary wall layers; they are imperforated, non-lignified, with tapering ends and easy to deform in a dehydrated state (Ligrone *et al.* 2000; Raven 2003; Vanderpoorten & Goffinet 2009).

Although there is extensive literature about how water is handled by mosses (*e.g.*, Brodie 1951; Bates 1988; Davey & Ellis-Evans 1996; Mulder *et al.* 2001; Rixen & Mulder 2005; Sand-Jensen & Hammer 2012), so far, however, there are no experimental data documenting the process of capturing water drops and distributing them to adjacent shoots within a moss clump. This study attempts to describe this phenomenon based on the endohydric moss species *Polytrichastrum formosum*. In this paper, we also tried to answer the question of how the construction of a clump of mosses in conjunction with anatomical and morphological structures of individual shoots affects the mechanical strength of the clump.

MATERIAL AND METHODS

In our study, we used the moss species commonly found in temperate coniferous forests: *Polytrichastrum formosum* (Hedw.) G. L. Sm. Specimens were sampled from a suboceanic fresh pine forest *Leucobryo-Pinetum* W, Mat. in the Chojnów Forest District (central Poland; 52°01′54.7″ N 21°02′50.8″ E). We focused on the clumps formed by the gametophytes of this species. Twelve clumps of size 50 × 50 cm were dug up, and with special attention to maintain integrity, were placed into plastic boxes, and in fresh state transferred to the laboratory.

Anatomical and morphological studies

The structure of moss clumps was visualised using scanning electron microscopy (FEI QUANTA 200 at 25 kV), which enabled samples to be observed without a previous coating process. For anatomical studies, stem samples were fixed for 2 h in a mixture of glutaraldehyde (5%) and paraformaldehyde (4%) in a sodium cacodylate buffer (0.1 M, pH 7.2). Subsequently, the material was rinsed, dehydrated with ethanol and embedded in glycidyl ether epoxy resin (Epon 812, SERWA). The 1 to 5-μm thick sections were then cut with a Leica UC7 ultramicrotome and stained with 1% safranin dye. Observations and micrographs were performed using an Olympus BX-61 optical microscope in white and UV light.

Analysis of cell lumen area and cell wall thickness

We analysed the distribution of the cell lumen surface size and cell wall thickness in *Polytrichastrum* from cross-sections of the six moss stems. To test whether it is possible to distinguish parenchyma zones with similar cell lumen dimensions in stems, we performed the following steps: (i) a microscopic image (taken from the middle part of the stem) and binarisation using the Otsu method (Otsu 1979); (ii) morphological opening (this operation helped in removing the smallest objects in the image which may be treated as noise); (iii) cell clustering. For clustering, we used the K-means method. All segmented objects of the image described by their areas are assigned to K clusters with the K-means algorithm (Hartigan & Wong 1979). The aim of the K-means algorithm is to divide the image area into clusters so that the within-cluster sum of squares of values of area parameters is minimised. In our experiments, we used four clusters. This means that each object in the cluster is more similar to an object in the same cluster than to other clusters. Based on these results, we estimated groups of cells with a trigonometric polynomials function. The main problem here was that different objects may belong to the same cluster, but may be placed in totally different regions, and we wanted to include only objects with similar distances from the centre. To solve this problem, we estimated the mean value of all areas. The new cluster begins where the approximated function crosses the mean. This trigonometric approximation is given by the following equation:

$$y = a_0 + \sum_{i=1}^{m} \left(a_i \times \cos\frac{2\pi i}{n}x + b_i \times \sin\frac{2\pi i}{n}x\right)$$

where m = 6 (degree of polynomial), n is the number of extracted cells, a_0, a_i, b_i, are coefficients, which are given by the following equations:

$$a_0 = \frac{1}{n}\sum_{j=1}^{n} y_j;\; a_i = \frac{2}{n}\sum_{j=1}^{n} y_j \cos\frac{2\pi ij}{n}$$

$$b_i = \frac{2}{n}\sum_{j=1}^{n} y_j \sin\frac{2\pi ij}{n}$$

Cell wall thickness was measured in images using ImageJ software (https://imagej.nih.gov/ij/). To be more accurate these characteristics were measured in seven different directions. Each measurement was saved for further processing and combined as a single dataset. The datasets were visualised and approximated by polynomial approximations, which can be given using the following equation:

$$P_n(x) = a_0 + a_1x + a_2x^2 + \cdots + a_nx^n = \sum_{i=0}^{n} a_ix^i$$

where n = 6 (degree of polynomial), $a_0, a_1, a_2, \ldots, a_n$ are coefficients.

In order to find the regions within the moss stem where the cell biomechanical properties might depend mostly upon cell hydration (Niklas 1992, 2000), the results of estimation of cell lumen area and cell wall thickness were also used for calculation of the ratio between cell wall thickness and cell radius (R) to determine regions where this value exceeded 20%.

Water transfer

Fluorescein diacetate (FDA), which is commonly used in viability tests, was applied as an indicator of water transfer into moss cell protoplasts. An FDA stock solution (C-7521; Sigma-Aldrich, Saint Louis, MO, USA) was prepared by dissolving 5 mg FDA in 1 ml acetone. The solution was diluted in water to a ratio of 1:100. Drops of the solution were applied to the top of the fresh shoot of *P. formosum* and the spread of dye in the moss was then observed using a fluorescence stereo Leica M165 FC microscope with a digital Leica DFC 425 camera. Twelve replicate tests were conducted.

Wetting of mosses by falling drops of water

The main aim of this experiment was to analyse the behaviour of falling water drops and their contact with the surfaces of leaves and stems of *P. formosum*. The studies were conducted on 12 samples of fresh plants placed vertically in normal upright position, using an experimental set-up for the droplet collision phenomenon study (Fig. 1) at the Division of Aerodynamics, Warsaw University of Technology, Poland (Bobinski *et al.* 2014). We observed and filmed the collision process of drops with a high-speed Photron FASTCAM SA-Z digital camera (Photron, Tokyo Japan), working at a speed of 50,000 frames per second. The camera was operated with integrated PFV (Photron FASTCAM Viewer) software. Operations such as setting camera options, shooting and saving recorded data to the PC were performed with the help of PFV. A fibre optic light source with variable intensity was used to backlight the water drop impact events. The size of the drops depended on the parameters defined in the pneumatic generator drops. In the first variant, the diameter of the discharge nozzle was 0.2–0.5 mm with 0.9–1.0 m·s^{-1} – drop initial velocity; in the second variant, we manually released large water drops using a pipette to simulate rain drops from a height equalling 50 mm. These droplets were 2–4 mm in diameter and their velocity was 5 m·s^{-1} just before contact with the plant. Size of the drops used in experiment was similar to that found in nature, which usually range from 0.5 mm for light rains up to 5.0 mm for heavy showers (Best 1950; Brandt 1989; Yakubu *et al.* 2016). All experiments were conducted at an ambient temperature of 20 °C. Captured material was then further analysed (droplet diameter and velocity) with software based on LABoratory Virtual Instrument Engineering Workbench, LabVIEW (Twelve replicate tests were performed).

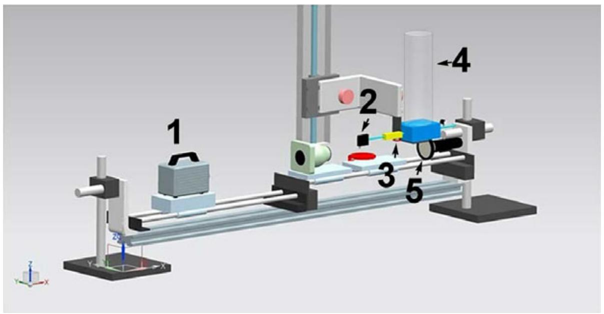


Fig. 1. The experimental set-up for the droplet collision phenomenon study. 1 – fastcam; 2 – discharge nozzle; 3 – quick exhaust valve; 4 – expansion tank; 5 – light source.

Water retention by a clump of shoots in normal upright and inverted position

In order to test architectural adaptations (especially the leaf–stem angle effect) of the *Polytrichastrum* gametophore for water retention by the moss clump, we examined the difference in retention of water drops falling into a clump of fresh moss shoots placed vertically in normal upright or inverted position. The experiment was performed with seven uppermost, 4-cm long portions of moss shoots placed in a support stand in the form of a ring of 2.5-cm diameter. The density of moss shoots within the ring was identical to that in natural clumps of *P. formosum*. Plants mounted in the support stand were inserted into a beaker and placed on the scale of an Ohaus EX124 analytical balance with 0.1 mg readability (Emcee Electronics, Venice, FL, USA). Twenty drops of water with a total weight of 1 g were put on the upper surface of the moss clump. The water drops were released manually using a pipette from a height equalling 50 mm. Their velocity was 5 m·s^{-1} just before contact with the plants. Three minutes after the termination of dropping, plants were taken out and the weight of water remaining in the beaker was determined. The experiment was conducted at an ambient temperature of 20 °C. Twenty replicate tests were performed for each of the experimental variants with shoots in normal and inverted positions.

Mechanical testing

Mechanical testing of cuboid-shaped specimens was performed on an electromechanical testing machine (Electromechanical 10 kN; MTS Insight, Minneapolis, MN, USA) using a 500 N

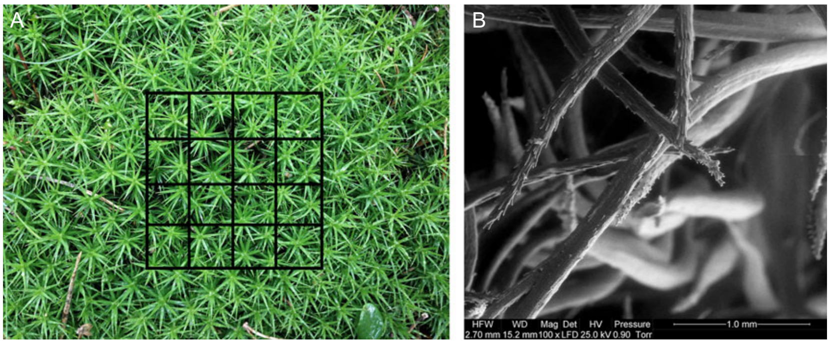


Fig. 2. Morphology of *Polytrichastrum formosum* gametophores. (A) Typical latticed structure of the clump; length of the side single element of the matrix – 1 cm. (B) Overlapping leaves from neighbouring shoots with hook-like tops; Scale bar = 1000 µm.

load cell (model 569328-03; MTS). The specimens were cut out from fresh moss clumps into cuboid-shaped blocks with four different dimensions in their upper surface: 10 × 10, 5 × 5, 5.0 × 2.5 and 1 × 1 cm. Samples were prepared from the selected part of the moss clumps with regular flat upper surfaces. This ensured smooth contact between the specimen and compression platen during the test. The set of four specimens was used in the three replicates tests.

The prepared specimens were tested with a uniaxial compression test. The specimens were loaded with platens with a compression force (force speed 2 mm·min^{-1}) until the upper plate displacement reached a limit of 15 mm. The relationships of (i) force–displacement; and (ii) stress–displacement were analysed. Stress value was calculated by dividing the compression force by the surface area of the specimen. Displacement was defined as the standard displacement of the crosshead. This method of displacement measurement is appropriate when the high level of stiffness of the testing machine on tested samples is taken into account.

RESULTS

Morphology

The studied gametophores of *P. formosum* are characterised by scattered, 10-cm tall, unbranched shoots. The subsquarrose leaves (5 to 8-mm long) are broadly spreading and overlapping. As a result, a regular matrix of lattice-like structure of the clump is formed (Fig. 2A). Scanning microscopy showed that the leaves have a hook-like appendage on their tops (Fig. 2B).

Analysis of cell lumen area, cell wall thickness and the ratio of cell wall thickness to cell radius (R)

Measurements of cells in the moss stems revealed that their lumen areas were not equal and varied within the stem cross-section (Fig. 3A). We found four zones (clusters) that significantly differed in size (Fig. 3B). In contrast, a relatively constant gradient of cell wall thickness across stem radius, from 0.7 to 1.0 µm in the core to the 2.5 µm in the epidermis, was observed. As far as lumen area is concerned, we found a tendency for a gradual decrease in this feature from the core to the epidermis. In the moss stem cross-sections, four zones can also be distinguished that differ with respect to the 20% ratio of cell wall thickness and cell radius (Fig. 3C). The R > 20% zones in the centre and outer parts of the stem cover much more than 50% of the moss stem cross-section area.

Water transfer between moss shoots

The FDA solution applied to the top of the shoot spread throughout the leaves within 1–2 min, and it was possible to

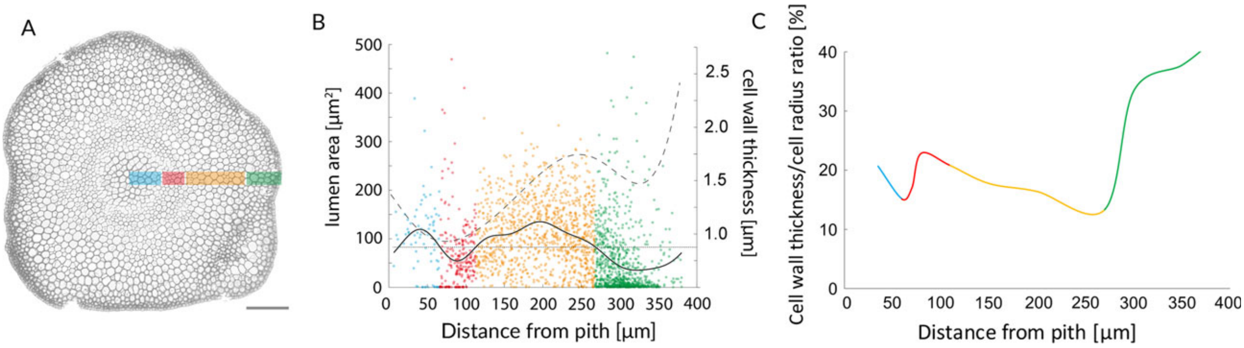


Fig. 3. The results of *Polytrichastrum formosum* stem cross-section division (A) into zones from the cell lumen area and cell wall thickness (B) and cell wall thickness/cell radius ratio (C) with respect to their distance to the core. (A) Width of rings corresponding to a cluster is marked with a rectangle of the given colour on the cross-point of the analysed stem. (B) Results of lumen area approximation are presented with a black line; in places where the black line crosses the global mean (horizontal dashed line), a new zone or cluster begins, represented by a different colour; dashed lines show cell wall thickness. (C) Cell wall thickness/cell diameter radius ratio (R) is represented with a multicoloured line; different colours correspond to clusters shown in (B and C); horizontal line shows value 20% for the cell wall thickness/cell diameter ratio (R = 20%); mechanical strength of cells with R > 20% depends mostly on cell turgor, and the strength of cells with R < 20% depends mostly on cell wall modifications. Scale bar = 100 µm.

clearly observe fluorescence in the stem-forming cells and in the lamellae of leaves, indicating cell hydration (Fig. 4A). Water run off along the stem was stopped not only on the surface of a leaf at the same stem, but also distributed to a leaf on the neighbouring stem (Fig. 4B). Water that accumulated at an angle between a leaf and the stem was distributed throughout the plant (Fig. 4C).

Wetting of moss by falling water drops

The large water drop at the *Polytrichastrum* apical part of the gametophore fully filled the uppermost leaves and then slowly flowed down while adhering to the shoot. In every case, a large drop adhered to the plant body, and it never lost its surface continuity (Fig. 5A–D). Smaller drops were captured by leaves and flowed down following the bend of the leaf either towards the stem or a place of contact with another leaf; however, in this case, the drop adhered to the place of contact and did not continue to flow (Fig. 5E–H). Large drops falling on contact with leaves pressed them, which resulted in leaf bending, this enabled more effective wetting of the leaf axils (Fig. 5I–L). Large water drops in contact with the lateral leaves flowed towards the stem and then ran off along the stem. We observed neither interruption of the water surface tension nor, consequently, water falling outside the plant (Fig. 5M–P). The video recording of the wetting process in *P. formosum* is presented in Video S1.

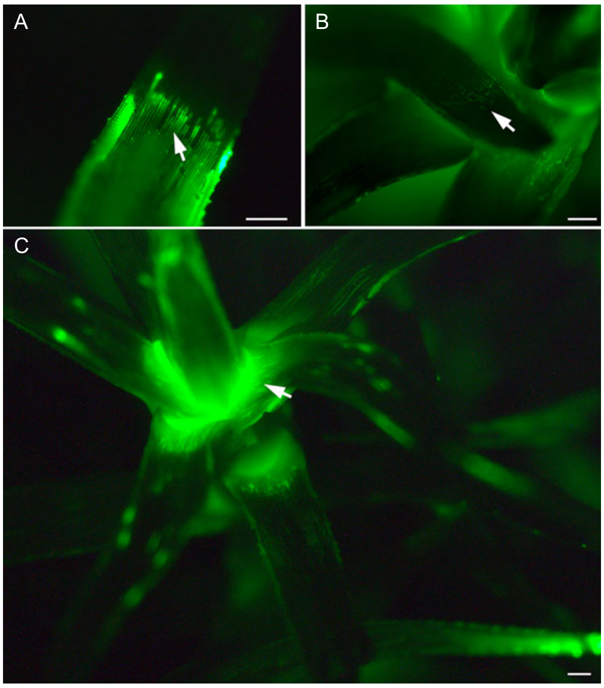


Fig. 4. Water transfer from the top of the shoot of *Polytrichastrum formosum* into protoplast and micro-capillaries of the lamella cells; FDA (fluorescein diacetate) test. (A) Translocation of dye from the stem along the leaf, observed 1 min after dye application; (B) FDA fluorescence in the leaf hydrated with solution running off along the stem and on the surface of its neighbouring top leaf. Penetration of the solution between leaves from two shoots is visible (see arrow); (C) water retention in leaf axils where water transfer towards leaves begins. Scale bars 100 μm.

Water retention by a clump of shoots in normal upright and inverted position

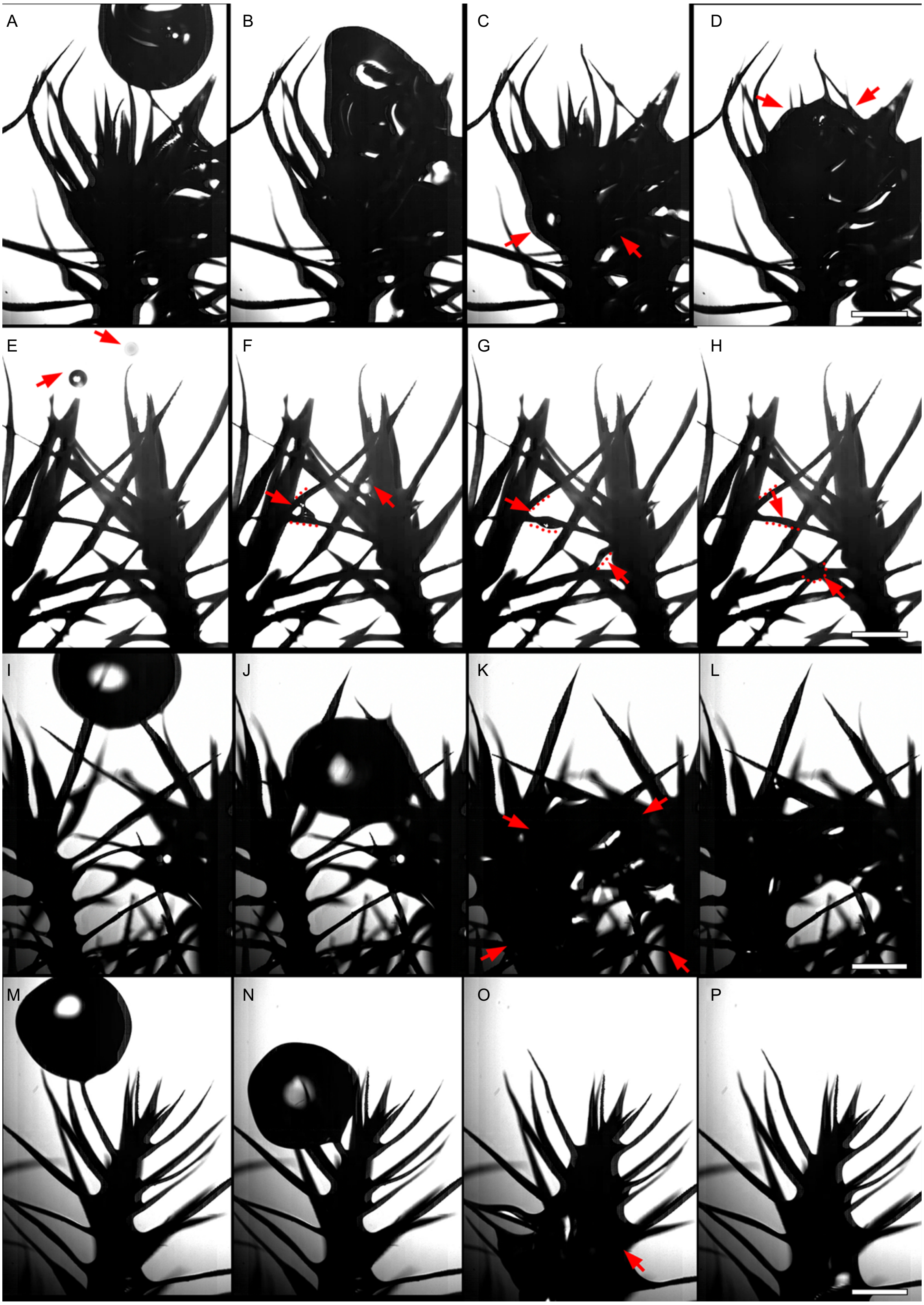
Three minutes after the water drops were put into the upper parts of vertically oriented moss shoots, the amount of water flowing down into the beaker was significantly higher in the variant with inverted shoots as compared to those in normal position. Clumps with normally oriented shoots retained almost 60% of the applied water (Mean = 58.9 ± 1.4%; ±SEM), whereas clumps with inverted shoots retained less than 40% (Mean = 37.5 ± 2.6%). Observations of shoots revealed that just after the dropping treatment, water started to flow downwards along the shoot and the process ceased after about 2 min. After a period of 3 min, the presence of water drops was observed much more often in those shoots placed in normal vertical position as compared to those in inverted shoots. In the latter case, most of the supplied water drops quickly flowed downwards to the bottom of the beaker.

Mechanical tests

The uniaxial compression test of moss clump specimens of different size clearly showed that the crosshead displacement increased with increasing force and was progressively higher in those specimens with smaller surface area (Fig. 5A). Special attention, however, should be given to the results concerning stress, which were calculated by dividing the compression force by the surface area of the moss clump specimen (Fig. 5B). In this case, the highest stress was determined in the variant with the smallest surface area (1 × 1 cm), in which the stress was many times higher than in other variants with larger contact surfaces. However, in larger specimens (10 × 10, 10 × 5, 5 × 2.5 cm) we found no clear effect on stress caused by a decreased surface area, and these samples were characterised by similar stress values. Detailed results concerning the individual measurements of samples are presented in Figure S1.

DISCUSSION

It is vital for mosses to maintain moisture at the plant's surface and the clump's internal space as long as possible (Vitt *et al.* 2014). In the extensive literature on the importance of the clump structure in water management among mosses, there are no studies that have shown the absorption of falling water drops by individual shoots and distributing them through the leaves into the neighbouring shoots. In our experiments, we are the first to precisely observe a drop's behaviour in the clump structure leading to water distribution between neighbouring shoots. The lattice structure of *P. formosum*, an endohydric moss, acts as a net of leaves where water can run off onto adjacent shoots. The second aspect concerns leaf bending caused by the force of a falling drop of water, which results in an increased angle between stem and leaf. In solitary shoots, such drops would not be stopped on the surface because of plant instability and evidence that the *Polytrichastrum* leaf has insufficient bending strength to counteract stresses; therefore, the plant would lose the drop of water. We therefore consider the lattice-like structure and its self-supporting qualities to be a resilient net for falling water. The results of the experiment of applying the FDA solution on top of the shoot of *P. formosum* indicated that this endohydric species needed an average of



2 min for cell hydration to occur in leaf lamellae. According to Clayton-Greene *et al.* (1985), endohydric Polytrichaceae do not move water along leaf lamellae, which are structured to exclude water and improve gas exchange. It should be noted, however, that endohydric bryophytes can be facultative ectohydrics (Glime 2015). Bayfield (1973) showed that *Polytrichum*

Fig. 5. Stages of wetting in *Polytrichastrum formosum* by large (A–D and I–P) and small (E–H) water drops. (A) Large drop falling down and connecting with tops of leaves; (B) drop begins to fill the uppermost whorl of leaves; (C) drop begins to fill the whorl of leaves, but does not leak out; (D) drop has filled and wetted all leaves in the whorl; arrows point to increased meniscus of water in comparison with the previous stage. (E–H) Small drops falling on leaves and wetting them, while running off due to the position of the leaves; arrows show drops and dotted line shows place of hydration. (I–L) A large drop falling on leaning leaves of two neighbouring plants. The drop is stopped by leaves, forms a membrane (K) and spreads onto the leaves (L), which break the surface tension of the water drop and complete water flow towards leaves and stem; arrows indicate boundary of the membrane. (M–P) Process of large water drop collection by leaves on the stem; the drop does not break while in contact with leaves, but hydrates them, covers the stem (red arrow) and runs off. Scale bars = 500 μm.

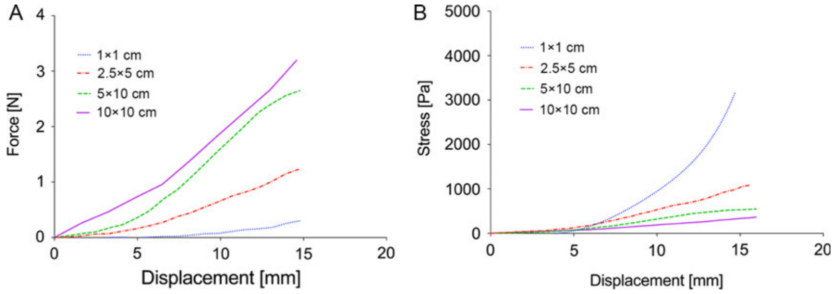


Fig. 6. Results of uniaxial compression tests of *Polytrichastrum formosum* compression depending on sample size, in the form of force-displacement curves (A) and stress-displacement curves (B). A time lag in reaction of the resistance, expressed by a flattened line in the early stage of the test, is clearly noticeable.

commune was ectohydric under moderate moisture, whereas in dry air it was predominantly endohydric. Taking into account that all of our experiments were performed with the fresh plants, it is possible that studied *P. formosum* gametophytes could also manifest some ectohydric features. In this context it is also worth noting that in nature rhizoids can facilitate water transport along the stem in a capillary action phenomenon. However, in the case of the endohydric moss *Polytrichum*, this route of water transport is probably less important than water uptake from the plant surface (Smith 1988; Jones & Dolan 2012).

Mosses are plants with a relatively simple anatomical structure; and their mechanical strength depends on the dimensions of the cell lumen and cell wall thickness. On the *Polytrichastrum* stem cross-sections cell lumen surface areas are not equal and create alternate zones of smaller and larger cells. We found that cell wall thickness to cell radius ratio (R) is not equal along the radius of moss stem cross-section area. The ratio exceeds 20% in cells surrounding the stem central region formed by hydroids and leptoids. This suggests that such zones (R > 20%) can perform strengthening functions (see Niklas 1992) for the central part of the stem, consisting of thin-walled and non-lignified conductive cells. It should be noted that the major part of the moss stem tissue is characterised by cells with typical hydrostat parameters (R < 20%), unlike cells near the outer part of the stem, which have thicker cell walls with smaller cell wall lumens, which causes higher R ratios. These results may serve as evidence that cell geometry is an important factor influencing biomechanical stability, especially in plants without typical supporting tissues. It is worth noting that in the case of alternating layers of stiff and soft structures we are dealing with *periodic component materials* (Vincent 2012) that commonly occur in many biological structures, providing better mechanical features (Dunlop *et al.* 2011; Fratzl *et al.* 2016). Such structures are characterised by the alternating order of components with different mechanical properties, which, in general, increases the strength of the whole structure. Alternating zones of small diameter cells with thicker cell walls (stiff layer) and wide diameter cells, with thin walls (soft layer) observed in the *Polytrichastrum* stems, can serve as an example of such a structure. It is also worth noting that thicker cell walls in the outermost zones of a stem increase its second moment of area, and are connected with higher bending strength (Niklas 1992; Spatz *et al.* 1998).

Leaves in moss clumps can be in direct contact with one another, as well as using water surface tension. This was shown using FDA fluorescence and the video recorded observations. Thus, due to the water surface tension connecting neighbouring leaves, clumps of mosses reach their optimal form and might increase their mechanical strength. Single shoots create a much rarer structure, and larger side movements during compression are possible. The shoots of *Polytrichastrum* have overlapping leaves with a hook-like appendage on their tops that, at the time of loading, can form a structure similar to the space frame structure. Our results show that the shape and spatial geometry of moss clumps, plants of simple and modular construction, make up a form characterised by a set of features necessary for the functioning of separate shoots. In comparison to individual shoots, clumps make up different and very interesting life forms that allow optimisation of the behaviour of the whole group under diverse environmental conditions. In this context of particular interest are the results from biomechanical tests, which show that small moss groups (1 × 1 cm) are clearly stronger than larger groups. This phenomenon appears to be analogous to the so-called ‘size effect on structural strength’, which is of great importance for various fields of engineering. It concerns structures made of the same material with the same microstructure and results from the effect of size on the nominal strength of a structure with a similar geometry. This could be explained with structure strength randomness or different energy release processes, when a final deformation develops before the maximum load is reached (Bažant 1999). From this point of view, the scaling problem (Stevens 1976) has never been studied in detail in mosses. Elaborating this issue may be important to challenge the question of what is the smallest size of a group of individuals that could be considered as minimal size of a clump that can be recognised as the 'unit' of a moss clump. Therefore, further studies examining this problem may open new perspectives for better understanding of the mechanisms of plant adaptation to environmental factors.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Video S1. Process of capture and translocation of raindrops in a clump of *Polytrichastrum formosum*.

Figure S1. Detailed results from biomechanical tests on clumps of *Polytrichastrum formosum*, based on samples of different sizes.

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