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## Anatomical adaptations in aquatic and wetland dicot plants: Disentangling the environmental, morphological and evolutionary signals

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### ABSTRACT

Understanding how plants adjust their internal structures to withstand adverse environmental conditions is vital for predicting their responses to ongoing environmental change. Plants have repeatedly evolved small water transporting conduits and large storage parenchyma tissues to cope with anoxia, freezing- or drought-induced damages. However, how these adaptations evolved in unrelated taxa across hydrological and thermal gradients, remains unclear. Here we show that stem anatomical variations in 212 European aquatic and wetland dicots are driven by thermal and hydrological constraints via control over plant size, growth form, and leaf traits, while phylogenetic constraints have only a weak effect. Phylogenetic comparative analyses controlling for confounding factors showed that both waterlogging (anoxia) and low-temperature promote smaller plants with reduced vessel conduits and limited lignification, but extended parenchyma and hence storage and tissue renewal capacity to secure resilience to biomass loss induced by running water or frost disturbances. Decreasing water depth and anoxia promote larger wetland plants with thick-walled libriform fibers, large vessels with simple perforation plates securing high hydraulic efficiency, and semi-ring porous xylem with wide earlywood vessels in spring and narrow latewood vessels in summer, providing both efficiency and safety in water transport. The aquatic environment promotes plants with a large cortex zone with photosynthetic chlorenchyma and starch-storing parenchyma cells along with extensive air spaces that provide aeration and buoyancy. Low temperatures promote short-stature forbs with smaller vessels, scalariform perforation plate, extended parenchyma, resulting in reduced embolism risk. Although most anatomical variation was explained by differences between aquatic and wet terrestrial growth forms, environmental gradients, plant size, and leaf properties exerted a significant control on plant tissue structures not confounded by phylogenetic inertia. Distinct habitats, spread across broad thermal and hydrological gradients, harbor unrelated species with different evolutionary histories that have converged to similar anatomical and hence morphological structures.

### 1. Introduction

During their evolution, vascular plants have developed specific adaptations to withstand adverse environmental conditions and utilize to their maximal benefit the nutrients and other conditions prevailing therein. While plant strategies for coping with adverse conditions are usually characterized by morphological, ecophysiological, and reproductive adaptations (Kleyer et al., 2008), plant anatomy has been

studied less frequently (Rascio, 2002; Schweingruber et al., 2020). Detailed knowledge of plant anatomical characteristics and their variation across species and habitats is both rare and limited mainly to woody species (e.g. Greguss, 1945; Schweingruber, 1990; Neumann et al., 2001; Schweingruber et al., 2013). Anatomical analysis of herbs has recently become of interest (Schweingruber et al., 2013; Crivellaro and Schweingruber, 2015; Schweingruber and Berger, 2019), as many herbs also develop annual rings as they age. However, these studies

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focus mainly on plants in terrestrial habitats, usually in drier (Dória et al., 2019) or colder places (Doležal et al., 2018), where plants usually grow slowly and live long.

The stem anatomy in aquatic (hydrophytes) and wetland plants (helophytes, Sculthorpe, 1967; Pfandenbauer and Klötzli, 2014) was studied mainly in terms of adaptation to increasing water depth, which is the most important environmental gradient in wetland habitats. Schenk (1886) performed the first synthesis of the anatomy of aquatic plants and later began more detailed studies. Many halophytes are anatomically close to aquatic plants (Grigore et al., 2014). However, the anatomical structure and the differentiation between aquatic and wetland plants are unclear for many taxa (see Ogden, 1974; Schweingruber and Berger, 2019). Many authors have described the aerenchyma in aquatic and wetland plants and its formation (Evans, 2003; Seago et al., 2005; Jung et al., 2008). However, a comprehensive assessment of the anatomical variability of plant structure in aquatic and wetland species across different environments and evolutionary linkages is still rare (Willby et al., 2000).

Variations in the anatomical structure should reflect differences in ecophysiology (Baas et al., 2004) and also specific adaptations of plants to environmental conditions (Hacke et al., 2016; Morris et al., 2018; Garcia-Giron et al., 2020). For example, the main adaptive features of submerged plants are a relatively low proportion of root biomass (about 10–30 % of total plant biomass) or completely rootless stems (genera *Ceratophyllum*, *Utricularia*, *Aldrovanda*), which leads to reduced mechanical and vascular tissue in stems at the expense of lacunar tissues (aerenchyma). Similarly, shallow-water emergent plants, rooted in waterlogged sediments with permanent hypoxia, have developed large air spaces and canals for the efficient transport of oxygen from emergent stems and leaves to submerged roots and rhizomes (diffusive and convective ventilation, Sculthorpe, 1967; Armstrong and Beckett, 1987; Armstrong et al., 1996). On the other hand, in large-stature terrestrial wetland forbs, due to resistance to mechanical stress, erect stems, and vertical leaves are strengthened by the development of sclerenchyma and lignified vascular bundles (Puijalon et al., 2011; Hamann and Puijalon, 2013). All these differences in the vascular system and the extent of lignification or aeration tissues have been repeatedly demonstrated in a limited number of model species (Jackson and Armstrong, 1999), but it has never been shown how these anatomical traits differ across a large number of aquatic and wetland species in different environments nor have these anatomical traits been tested for various plant functional groups or in a phylogenetic context.

Variation in anatomical features such as the presence of aerenchyma or the extent of lignification can be primarily explained by plant life-forms (aquatic submerged vs. free-floating plants, terrestrial rhizomatous vs. taprooted plants) as determined by prevailing hydrological conditions (Armstrong et al., 1991). Other features such as the structure of conductive and storage systems, plant longevity, and growth can be rather related to low-temperature constraints (Doležal et al., 2018). Elevation-related cooling can have strong control over conductive and storage systems by reducing the vessel conduit size and extending the parenchyma fraction to better resist freezing-induced embolism and cavitation as seen in alpine plants (Doležal et al., 2019). However, some anatomical features may be determined by other factors such as evolutionary constraints. Phylogenetic relatedness can cause similar 'blueprints' among taxa that share a part of their evolutionary history (Adams, 2014a). Numerous examples are linking anatomical variation among trees and terrestrial herbs to phylogeny, size, and environmental constraints (Schweingruber et al., 2013; Lens et al., 2016), but similar studies in aquatic and wetland species are lacking.

To fill this knowledge gap, we examined stem anatomical variation in a large number of dicot plant species within a wide taxonomic and ecological spectrum of aquatic and wetland environments in the European temperate zone (Hejný, 1960), including natural lakes, ponds, alluvial pools and oxbows, rivers and streams, sand-pit lakes, reservoirs, wet meadows, mountain peat bogs, and fens, many of them are the

species-richest communities threatened by current land-use changes, climate warming, drought and eutrophication (Hejný et al., 1998). These habitats cover wide thermal and hydrological gradients that can manifest in different plant anatomical structures due to different evolution and adaptation to specific environmental conditions (Carlquist, 2012).

Using phylogenetic comparative models (Adams et al., 2014b), we investigated how plant anatomical features differed according to plant size, growth form, thermal and hydrological conditions, and taxonomical origin, and whether individual habitats harbor unrelated taxa that have converged into similar morphological and anatomical structures. The study is based on 212 dicot species and 118 stem anatomical features, many of which serve as a proxy for ecophysiological adaptations which are more difficult to study for a large number of species. Our main goals were to disentangle the role of evolutionary, environmental, and morphological drivers of plant anatomical adaptations in the Central European aquatic and wetland species. Specifically, we studied how anatomical settings are related to plant size, growth form, leaf traits and thermal and hydrological constraints.

## 2. Materials and methods

### 2.1. Aquatic and wetland plant species and their environment

Altogether, we collected 212 dicot aquatic and wetland vascular species (including four species of basal angiosperms, Nymphaeaceae, and one species of Ceratophyllaceae, a sister family to eudicots according to the *Angiosperm Phylogeny Group*, 2016), growing naturally or as naturalized in seven Central European countries (Switzerland, Germany, Austria, Czechia, Slovakia, Hungary, and Poland) and belonging to 124 genera from 51 families. Most of the studied species are perennial clonal hemicryptophytes (143), followed by short-lived annual or biennial species (40), perennial non-clonal polycarpic forbs (11), perennial non-clonal monocarpic forbs (9), and shrub and tree species (9). The plant material for this study was collected from various habitats (fishponds, natural lakes, alluvial backwater pools and oxbows, rivers and streams, sand-pit pools, wet meadows, peat bogs, and fens). Protected species were usually collected from outdoor cultures in the Collection of Aquatic and Wetland Plants at the Institute of Botany CAS in Treboň, Czech Republic.

### 2.2. Anatomical description

To describe plant anatomy, age, and size, we analyzed more than three individual specimens of each species collected in 2013–2015. Each plant specimen was measured for its height. Microscopic structures were studied in the oldest part of perennial plants in the transition between the hypocotyl and the primary root. From each perennial plant, short segments (ca. 2–8 cm long) were cut either from root collars in taprooted forbs or from distal parts of a rhizome in rhizomatous herbs. Only in short-lived or rootless aquatic plants, the anatomical assessment was done on non-perennial stem fragments. The collected root collars, rhizomes, and stem segments were stored in plastic bags with 40 % ethanol to avoid drying artifacts before sectioning. Transverse, tangential, and radial sections were cut (thickness of sections c. 20–30 µm) from all individuals using a sledge microtome, double-stained using aqueous Astrablue and Safranin, and embedded in Canada Balsam (Gärtner and Schweingruber, 2013; Schweingruber et al., 2020). In few dozens of submerged species (e.g. *Aldrovanda*, *Ceratophyllum*, *Montia*, *Utricularia*), tiny fragile stems were fixed in FAA, embedded in paraffin, and sectioned. In *Utricularia* species with dimorphic shoots, the photosynthetic shoots were collected. Double staining gave us information on the distribution of vessels and lignified and unlignified (parenchymatic) parts. The slides were examined using an Olympus BX53 microscope, Olympus DP73 camera, and cellSense Entry 1.9 software. We evaluated 118 anatomical features grouped into 22 variables (Table A1 and

Fig. A1) including annual ring width, ring counts, vessel arrangement and porosity, vessel perforation plates, inter-vessel pit arrangement, earlywood vessel number and diameter, fiber type, and fiber wall thickness, rays, and axial parenchyma types, secondary cambial variants, bark secretory elements and aerenchyma air spaces (Fig. 1). However, we did not investigate some more detailed anatomical features such as gas-space connections across cambia, secondary aerenchyma, or distribution of chloroplasts in the secondary cortex. Finally, we calculated the proportion of four basic tissues on double-stained cross-sections to provide quantitative information on the extent of parenchymatic (storage), water conductive (transport), lignified (mechanical support), and aerenchymatic (aeration) tissues. By randomly drawing 100 polygons, each covering a quarter of the stem cross-sectional area, we calculated average xylem conduits area (white), fiber area (red-stained), and parenchyma (blue-stained), and cortical aerenchyma, using ImageJ image analysis software (Doležal et al., 2019).

### 2.3. Species phylogeny, growth form, and habitat preferences

A dated, ultrametric phylogeny was prepared for the 212 studied vascular plant species by pruning the DaPhnE 1.0 supertree of the Northwest and Central European vascular plant species (Durka and Michalski, 2012) (Fig. A3). To obtain a robust estimate of the species' climatic optima, we calculated species affinities for 19 bioclimatic CHELSA variables (Karger et al., 2016) based on species geographic occurrences in the GBIF (Global Biodiversity Information Facility, [www.gbif.org](http://www.gbif.org)) database. Since the GBIF distribution data may not encompass the entire species geographic ranges to precisely characterize species climatic niches, we additionally used the Ellenberg ecological indicator

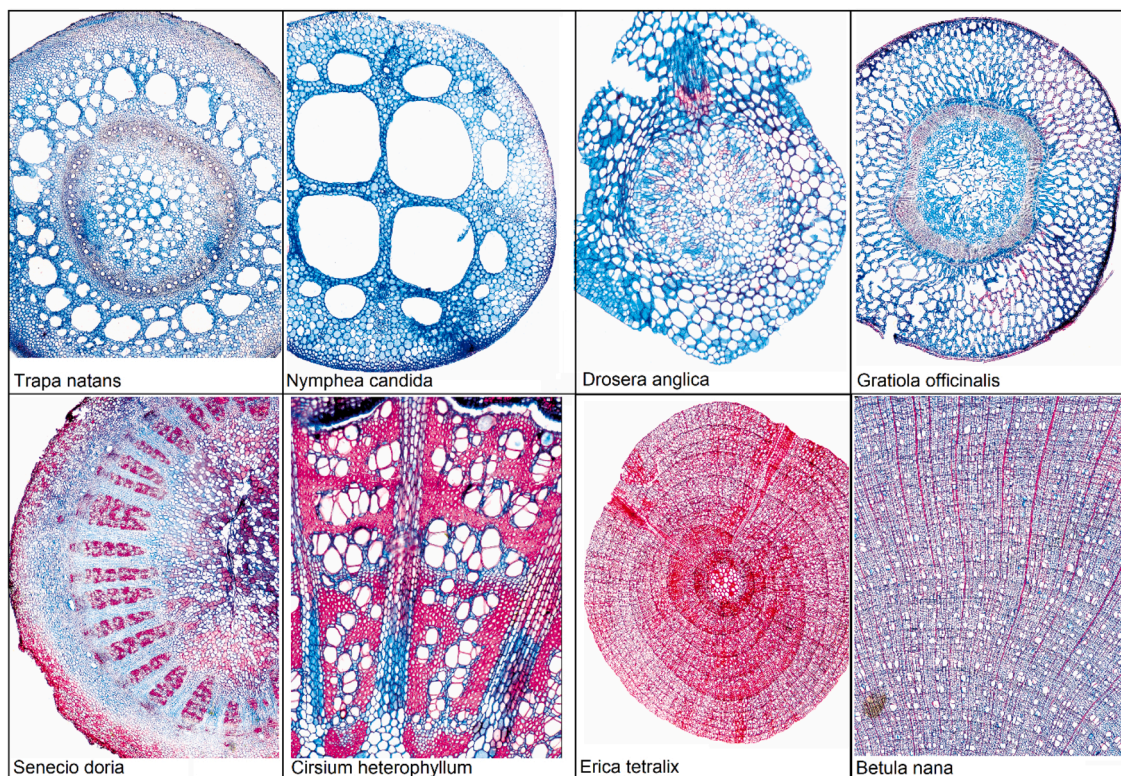
values (EIV, Chytrý et al., 2018) to characterize species habitat preferences (Table 1).

To determine how much variation in anatomical structure is due to morphological and growth form differences, for each plant we gathered information on specific leaf area (SLA) and leaf dry matter content (LDMC) (Kleyer et al., 2008), and each plant was classified as an aquatic or terrestrial wetland plant. Aquatic plants were further subdivided based on their relation to water level and rooting into 1) submerged plants with all their photosynthetic parts underwater, 2) rooted floating-leaved plants with many floating leaves and a few submerged, and 3) emergent plants with thick bottom-rooted rhizomes thriving in water depth not exceeding 1.5–2 m, with most leaves and stems emerged and in contact with the air.

Terrestrial wetland plants, which usually do not experience regular long-term waterlogging, were classified into several growth forms based on woodiness, perennality, and belowground organs of a stem- or root-origin according to Klimešová et al. (2017). The examined terrestrial wetland dicots had taproots, tubers, stolons, rhizomes, or were annual or woody (Table 1). Rhizomatous plants were classified into one of two rhizome categories: 1) long (>10 cm between mother and daughter ramet) mostly hypogeogenous rhizomes, i.e., perennating clonal growth organ of a stem origin formed below ground, 2) short (<10 cm between mother and daughter ramet) epigeogenous rhizome, i.e., perennating clonal growth organ of a stem origin formed above ground, with distal parts covered by soil and litter or pulled into the soil by contraction of roots (Klimešová et al., 2017).

### 2.4. Data analyses

To evaluate the effect of species' temperature and moisture



**Fig. 1.** Transverse sections of selected aquatic and wetland plant species. *Trapa natans* basal part of the whole rosette shoot with large aerenchyma spaces in the cortex. *Nymphaea candida* round air ducts. *Drosera anglica* root collar cross-section consists of a central cylinder (stele) with vascular bundles and a large cortex. *Gratiola officinalis* basal part of the stem consists of a large pith, small xylem, and phloem, and large cortex with honeycomb aerenchyma. *Senecio doria* large pith of the rhizome is surrounded by solitary vascular bundles, a cortex, and a small pith. *Cirsium heterophyllum* xylem consists of vessels, thin-to thick-walled fibers and paratracheal parenchyma. *Erica tetralix* semiring-porous xylem with distinct annual growth rings, solitary vessels, and thick-walled fibers. *Betula nana* diffuseporous xylem, with growth rings marked by flat latewood fibers, separated by uniseriate rays.

**Table 1**

Aquatic and wetland plant life and growth form categories and their selected bioclimatic optima for temperature (T) and precipitation (P), ecological preferences (Ellenberg ecological indicators), and selected functional traits (LDMC – leaf dry matter content, SLA – specific leaf area, clonality index and belowground rhizome lateral spread).

Growth forms	Aquatic plants			Terrestrial wetland plants							
	floating	submerged	emergent	short-rhizomatous	long-rhizomatous	stoloniferous	with tubers	tap-rooted	root sprouting	woody	short-lived
<i>Number of species</i>	10	23	30	41	35	12	4	11	11	3	32
<i>Mean annual T</i>	9.4	9.4	9.5	6.7	8.0	7.8	9.6	9.1	9.5	6.8	10.7
<i>Coldest month min T</i>	-1.2	-0.6	-0.3	-4.5	-2.9	-2.8	-3.4	-0.7	-1.2	-3.7	0.7
<i>Wettest quarter T</i>	17.9	17.3	17.3	15.4	16.7	16.5	18.8	17.0	18.0	15.5	18.4
<i>Coldest quarter T</i>	1.6	2.1	2.4	-1.2	-0.1	-0.1	0.5	1.9	1.4	-0.8	3.6
<i>Annual precipitation</i>	760	810	775	902	754	775	898	793	695	890	761
<i>Warmest quarter P</i>	214	220	215	241	215	212	245	215	192	223	205
<i>Plant height (cm)</i>	63.5	55.1	45.8	36.3	56.5	30.4	50.0	26.4	62.9	447.1	33.5
<i>LDMC [mg g<sup>-1</sup>]</i>	163.2	126.0	148.2	172.6	214.4	179.2	178.3	166.4	246.2	305.1	163.2
<i>Seed mass [mg]</i>	5.4	0.4	1.1	1.1	2.3	2.4	1.8	1.5	21.8	0.6	2.1
<i>SLA [mm<sup>2</sup> mg<sup>-1</sup>]</i>	29.9	47.8	31.3	28.2	26.1	33.9	28.6	21.4	25.2	14.8	34.8
<i>Clonality index</i>	3.6	5.5	3.3	3.2	4.7	4.5	4.3	0.0	4.1	5.7	0.0
<i>Lateral spread (cm)</i>	14.1	18.0	9.0	4.0	13.5	13.6	11.8	0.0	16.4	15.4	0.0
<i>EIV_light</i>	7.7	7.1	7.1	6.8	6.9	6.6	6.7	7.2	6.9	7.3	7.8
<i>EIV_temperature</i>	6.1	5.8	5.7	4.3	4.9	4.9	5.0	5.5	6.4	4.3	6.2
<i>EIV_moisture</i>	10.5	11.0	8.8	7.2	7.9	8.2	7.3	7.5	7.4	7.9	7.7
<i>EIV_soil_reaction</i>	5.8	5.5	6.2	5.5	5.7	5.3	6.7	5.8	6.1	5.0	5.9
<i>EIV_nutrients</i>	5.9	4.6	5.8	4.7	5.4	5.3	6.3	4.4	5.3	4.0	6.1
<i>EIV_salinity</i>	0.3	0.4	0.9	0.7	0.9	0.6	1.0	1.8	0.7	0.1	1.0

preferences (CHELSA bioclimatic and Ellenberg EIV variables) and plant morphology (height, SLA, LDMC, and growth form) on their anatomy, we first performed Principal Component Analysis (PCA) and distance-based Redundancy Analyses (db-RDA) (ter Braak and Šmilauer, 2012) to find major axes of variation in anatomical structure and to quantify the variation explained by environment and morphology. To find a parsimonious subset of environmental predictors of variation in anatomical composition, an interactive forward selection was used based on adjusted explained variation (corresponding to  $R^2_{adj}$  measure used in linear regression). During stepwise selection of explanatory variables, estimated Type I errors were adjusted by transforming them into FDR (false discovery rates, Verhoeven et al., 2005). Statistical tests were based on 999 permutations. Second, to quantify how much variation in anatomical settings is explained by individual predictors after controlling for phylogenetic inertia, we used a distance-based generalized least-squares model (db-GLS) which is suitable for highly multivariate datasets unlike its likelihood-based alternatives (Adams, 2014b; Adams and Collyer, 2018). Anatomy consisted of 22 categorical features. From anatomical features, we calculated a distance matrix among 212 species (*Pinguicula alpina* was excluded from these analyses since its moisture EIV was not available) using a simple matching coefficient (Legendre and Legendre, 1998):

$$\text{Distance} = 1 - (\text{Number of agreements} / \text{Number of variables})$$

The distance matrix was used as a dependent variable and temperature and moisture preferences (Ellenberg's values), the natural logarithm of plant height, and the growth form were used as predictors. To account for the phylogenetic signal to an appropriate degree (Revell, 2010), we ran the model repeatedly with the phylogenetic tree transformed using Pagel's lambda (Pagel, 1999) from 0 to 1 (by 0.01) and selected the model with the highest explained variability. For each predictor, we report its maximum explained variability and its explained variability after accounting for all other predictors. We used R package *geomorph* (version 3.2.1, Adams et al., 2019) to evaluate the model and package *geiger* (version 2.0.6.4, Harmon et al., 2008) to transform the phylogenetic tree. Because most of our predictors affected the anatomy of plants, we further explored each anatomical feature separately.

## 2.5. Individual models

We evaluated the model used for all anatomical features separately. The analyses were done in the same way as the main model by constructing a distance matrix, used the same four predictors (temperature and moisture preferences of species, the natural logarithm of height, and growth form), and estimated the lambda parameter scaling the phylogenetic tree. We report maximum explained variability per predictor, explained variability after accounting for all other predictors and corresponding p-values acquired by permutation tests with 999 iterations as implemented in function *procd.pgls* in package *geomorph* (Adams et al., 2019). FDR approach was used to adjusted Type I errors in multiple testing (Verhoeven et al., 2005).

To explore relationships among selected tissue components (parenchyma, lignin and conduits), the height of plants and their annual radial growth increment, we used phylogenetic reduced major axis regression with an estimation of phylogenetic signal (lambda; Pagel, 1999). As growth is often a multiplicative process and height and annual radial growth increment had skewed distributions, we transformed them using natural logarithm before the analyses. We estimated the selected relationship for terrestrial and aquatic plants separately. As the used implementation of phylogenetic reduced major axis regression does not allow testing the slope against zero (null hypothesis being zero slope), we tested against 0.01 (or -0.01 in case of negative relationships). Analyses were done in R package *phytools* (Revell, 2012).

## 2.6. Phylogenetic signal

We estimated the phylogenetic signal in our dataset of anatomical features. We used a distance matrix which we constructed for the model and estimated multivariate generalization of Blomberg's *K* (Blomberg et al., 2003; Adams, 2014a) using a function *physignal* from package *geomorph* (Adams et al., 2019). The difference of the estimated phylogenetic signal from the Brownian motion model of evolution was tested by permutation test with 999 iterations.

## 2.7. Regression trees

To find a parsimonious subset of environmental predictors for explaining the variation in proportions of four basic tissues: parenchymatic, conductive, lignified and aerenchymatic, conditional inference trees [a type of classification and regression tree (CART)] were employed using the R package *party* (Hothorn et al., 2006). The method belongs to non-parametric regressions, making a dichotomous tree, which can be used as a predictive model to gain insights into which abiotic and biotic factors control anatomical structure.

## 3. Results

### 3.1. Environmental and morphological determinants of anatomical variation

Plant height, growth form, leaf traits, and species temperature and moisture indicator values together accounted for 34.28 % of the variability in anatomical features of 212 plants (db-RDA,  $F = 7.3$ ,  $P < 0.001$ ). The predictors explaining the most variation in anatomical structures, based on the forward selection of a parsimonious subset of predictors (see Methods) included the woody plants (38.3 % contribution,  $P_{\text{adj}} = 0.006$ ), root-sprouters (10.5 %,  $P_{\text{adj}} = 0.02$ ), EIV\_nutrients (9.9,  $P_{\text{adj}} = 0.004$ ), EIV\_salinity (6.5 %,  $P_{\text{adj}} = 0.003$ ), short-lived plants (5.9 %,  $P_{\text{adj}} = 0.001$ ), long-rhizomatous forbs (3.6 %,  $P_{\text{adj}} = 0.001$ ), taprooted forbs (2.9 %,  $P_{\text{adj}} = 0.002$ ), submerged plants (2.2 %,  $P_{\text{adj}} = 0.008$ ), MAP (2.1 %,  $P_{\text{adj}} = 0.008$ ), MAT (2.4 %,  $P_{\text{adj}} = 0.009$ ), aquatic floating-leaved plants (2.0 %,  $P_{\text{adj}} = 0.018$ ), short-rhizomatous forbs (2.3 %,  $P_{\text{adj}} = 0.013$ ), and EIV\_light (2.1 %,  $P_{\text{adj}} = 0.014$ ).

The main anatomical differences among the studied species were associated with habitat preferences along moisture gradients, from freshwaters to drier shores and floodplain forest floors. Ordination of plant anatomical features by Principal Component Analysis (Fig. 2) showed that the first (horizontal) axis separated submerged plants, often with propagating turions and belonging to the families Ceratophyllaceae, Elatinaceae, Haloragaceae, and Lentibulariaceae, from perennial species with long rhizomes or species with partly or completely woody stems belonging mostly to Asteraceae, Betulaceae, Lamiaceae and Salicaceae (Fig. 2b). Submerged plants had anatomical structures characterized by a regular round lysigenous aerenchyma, missing or solitary vessels, scalariform intervessel pitting, pervasive parenchyma, preserved endodermis, mostly solitary sieve elements, and missing fibers and bark secretory elements. The anatomical structure of tall wetland forbs and woody root-sprouters at the opposite end of the moisture gradient was characterized by thick-walled libriform fibers, vessels with a tangential diameter of 50–100  $\mu\text{m}$  arranged in short radial multiples, alternate intervessel pitting holes, small aerenchymatic cavities, and diffusely porous rings. Anatomical changes along the second axis were associated with lower-growing season temperature and higher precipitation, from short-lived warm-adapted plants in well-irradiated drier habitats (e.g. Crassulaceae, Gentianaceae, Plantaginaceae) to cold-adapted, large wet meadow forbs with short rhizome and subshrubs typically from Ranunculaceae and Rosaceae. Short-lived warm-adapted plants had one ring and the endodermis was largely present. They had simple perforation plates, small vessel diameters, missing fibers or libriform fibers, and missing rays. Perennial forbs had distinct rings with average plant ages ranging from 2 to 10 years. They had large multi-seriate upright rays and small or missing aerenchymatic spaces, small vessels with a tangential diameter of 20–50  $\mu\text{m}$ , vasicentric paratracheal, and pervasive parenchyma and missing endodermis.

### 3.2. Phylogenetic anatomical–environmental analyses

In the db-GLS model controlling for the phylogeny, species temperature and moisture indicator values, plant height and growth form together accounted for 13.75 % of the variability in anatomical features

of 212 plants (Fig. A2). For the main model, we found non-significant phylogenetic dependence (Pagel's lambda was zero). All of our predictors when inspected individually affected anatomical traits ( $p < 0.003$ ). However, after accounting for other predictors, indicator values for temperature ( $p = 0.033$ ) and especially for moisture ( $p = 0.379$ ) had the weakest effects ( $p$ -value for growth form and plant height remained 0.001, Fig. 3).  $K_{\text{mult}}$  phylogenetic signal strength of anatomical traits was 0.2052, which was significantly different from 1 ( $p = 0.001$ ), i.e. different random patterns of trait evolution.  $K_{\text{mult}}$  lower than 1 denotes the power of the phylogenetic signal lower than that expected under the Brownian motion model of evolution.

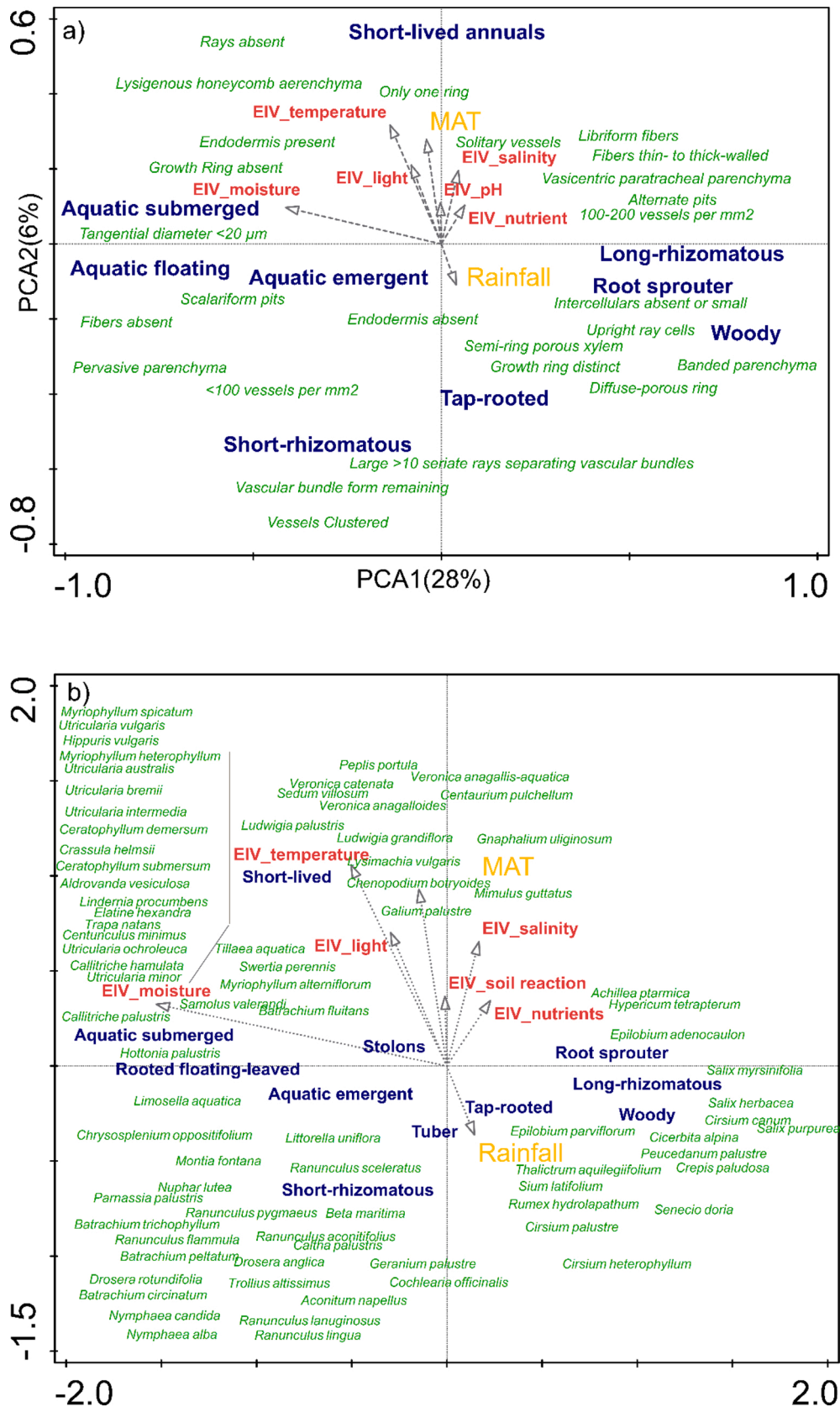
Plant height accounted for most interspecific variation in radial growth increments (growth ring widths) of studied species (phylogenetic reduced major axis regression,  $p < 0.01$ ,  $R^2 = 0.28$ , Fig. 4a). Most annual rings were between 0.1 and 0.5 mm wide. The smallest increments were found in short-rhizomatous plants and the highest in tall herbaceous taprooted forbs and long-rhizomatous forbs. Slow growth and longer life are characteristic of most rhizomatous forbs growing in cold montane wetlands with radial growth increments  $< 0.5$  mm per year. Plant age was determined in 148 species (70 % of studied species), in most taprooted species and root-sprouters (mostly woody species), and about half of the rhizomatous forbs. Most aquatic and many terrestrial wetland plants (e.g., Amaranthaceae, Haloragaceae, Nymphaeaceae, Ranunculaceae) had no countable rings. Among the studied species with countable rings, plants 2–10 years old predominated (91 %).

Growth forms and temperature preferences accounted for the most variation in the structure of the conductive system including vessel arrangement, ring porosity, and earlywood vessel size (Fig. 4b). These predictors retained significance after controlling for phylogeny and other confounding explanatory variables (Fig. 3). Diffusely-porose (56 %) and semiring-porose (42 %) vessels predominated, while ring-porose vessels were limited to woody taxa.

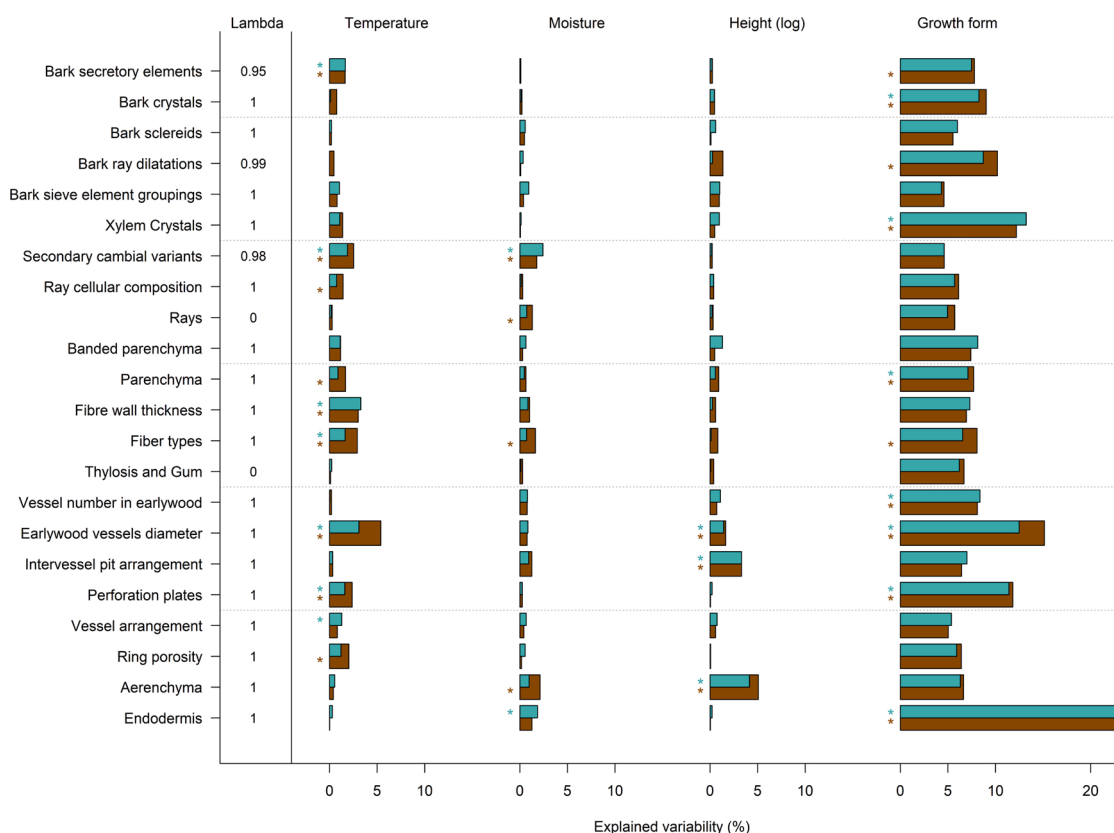
The submerged plants had a small vessel diameter  $< 20$   $\mu\text{m}$ , while taprooted and long-rhizomatous forbs had larger vessel diameters  $> 50$   $\mu\text{m}$ . Plant height had also a significant and positive effect on vessel diameter variations and a fraction of vessel conduits (Fig. 4a). Small annuals and short-rhizomatous alpine plants  $< 10$  cm tall had the smallest diameters, whereas larger wetland forbs  $> 60$  cm had the large vessel diameters (Fig. 5b). Solitary vessels usually occurred in submerged plants and terrestrial long-rhizomatous forbs, while vessels grouped in multiples were found in woody plants and short-rhizomatous and emergent forbs.

Growth forms and temperature accounted for the most variation in vessel perforation plate structure (Fig. 3), with simple plates predominating (81 %), while scalariform perforation plates occurred across unrelated genera, from rhizomatous herbs to heavily lignified subshrubs, mostly from cold environments (Fig. 5c). Plant height accounted for the most variation in intervessel pitting arrangement and retained significance after controlling for other confounding explanatory variables (Fig. 3). Plants with alternate intervessel pits predominated (52 %), followed by those with scalariform pits (35 %), which were over-represented in small aquatic plants and under-represented in large wetland forbs. The absence of tyloses or phenolic plugs in intervessel pits of most species suggests that all vessels are functional during their existence.

Variation in lignification (fiber types and fiber wall thickness) was primarily related to temperature, while the net effect of growth form was insignificant (Fig. 3). There was a clear relationship between fiber content, temperature optimum, and plant size (Fig. 5). Smaller plants tended to be fiberless, mostly short-lived or alpine plants (Fig. 5d). Fiber-tracheids occurred in larger forbs and subshrubs of cold marshes and peat bogs with the ability of clonal propagation either through lignifying rhizomes or root-suckers. Thick-walled fibers were most common in tall-rhizomatous forbs and root-sprouting forbs and shrubs from lower-elevation wetlands (Fig. 5e), while gelatinous fibers



**Fig. 2.** Bi-plots of Principal Component Analysis (PCA) showing intercorrelations among (a) plant anatomical features of aquatic and wetland plant species (b) to plant growth and life forms, ecological indicator values (EIV), mean annual temperature (MAT) and annual precipitation, which were fitted ex-post to the PCA ordination axes.



**Fig. 3.** Explained variability from db-GLS models in individual anatomical features by temperature, moisture, height (log), and growth form. Brown bars denote explained variability without accounting for the other three predictors, azure bars denote explained variability after accounting for other predictors. Stars show the p-value below 0.05. The estimated strength of the phylogenetic signal in each model (Pagel's lambda) is given in the left column.

occurred in woody plants. The high number of plant studied were free of fibers (42 %), which can be explained by their aquatic habitat and the largest amount of biomass allocated to submerged/floating organs or below-ground organs and only a small proportion of aboveground/emerging shoots and leaves, resulting in a limited need for mechanical stem support.

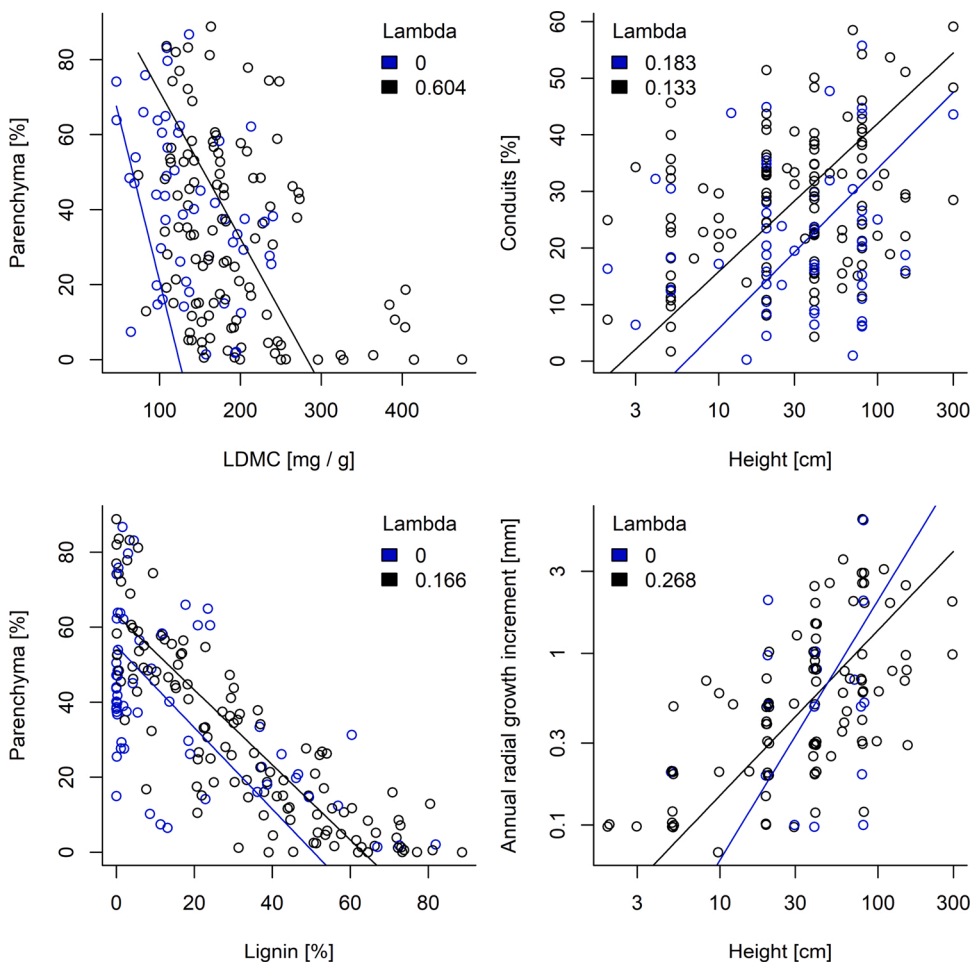
Variation in the structure of the storage system was most strongly related to growth form, but also temperature variation. In general, axial parenchyma cells, rays, and cortex made up the storage system in the studied plants. Most species had large multiseriate ray zones between permanent vascular bundles (41 %), or pervasive parenchyma (30 %). Plants living in colder wetlands had wider rays, which were mostly formed by upright cells (67 %), and in many cases, confluent with the axial parenchyma. Multiseriate rays separating vascular bundles were an almost exclusive feature in short-rhizomatous alpine wetland forbs (Fig. 5f). Exclusively uniseriate rays were found in trees, shrubs, and large lignified perennial forbs. The aquatic submerged and floating-leaved plants had large unligified cortex zones. Unligified pervasive parenchyma predominated also among emergent and terrestrial short-rhizomatous clonal plants. Vasicentric paratracheal parenchyma (35 %) or combined pervasive and vasicentric parenchyma (17 %) occurred in tall, long-rhizomatous forbs. Vasicentric paratracheal parenchyma was a common type of parenchyma in annual and biennial species, while apotracheal parenchyma was a rare (3%) feature belonging to trees and shrubs.

Variation in aerenchymatic tissue was primarily related to plant height and moisture preferences. Lysigenous aerenchyma, which is caused by cell death and collapse either radially or tangentially, was present in 41 % of species with aerenchyma, mostly aquatic submerged plants, with either regular round (21 %), or large irregular (21 %) cavities. Schizogenous aerenchyma (28 %), which is caused by cell

separation without cell death, driven by cell divisions and cell expansions, predominated in terrestrial, perennial wetland plants such as alpine short-rhizomatous, while expansigenous honeycomb aerenchyma (28 %) was the most widespread type among aquatic floating-leaved plants.

### 3.3. Determinants of tissue type proportions

Regression trees provided comprehensive information on external and internal determinants of tissue type proportions (Fig. 6). Leaf dry matter content (LDMC) accounted for most variation in parenchymatic fraction (Fig. 4c). Plants with softer leaves (LDMC < 143 mg g<sup>-1</sup>) had a higher proportion of parenchyma, on average 51 %, such as in submerged (*Aldrovanda*, *Batrachium*, *Ceratophyllum*, *Utricularia* spp.) and emergent (*Cicuta virosa*, *Berula erecta*, *Rorippa amphibia*, *Lycopus europaeus*, *Oenanthe* spp.) aquatic plants, but also short-rhizomatous cold wetland plants (*Adenostyles alliariae*, *Pinguicula alpina*, *Viola biflora*) and saline forbs (*Lysimachia maritima*, *Plantago maritima*). Among plants with tougher leaves (LDMC 143–273 mg g<sup>-1</sup>), a higher parenchyma fraction was found in plants with lower specific leaf area (SLA < 26 mm<sup>2</sup> mg<sup>-1</sup>) and limited clonality. The lowest fraction of parenchyma (<16 %) occurred in heavily lignified woody species with tough leaves (LDMC > 273 mg g<sup>-1</sup>). Life form, LDMC, lateral spread, and maximum temperature of the warmest month were the best predictors of the lignified tissue fraction, with submerged and floating-leaved plants with a low lignified fraction (5.5 % on average), while high fractions occurred in large wetland forbs and woody taxa possessing leaves with higher LDMC, and growing in habitats with lower summer temperature maxima. Plant height and temperature seasonality (the difference between the annual maximum and minimum temperatures) were the best predictors of the conductive tissue fraction, with taller plants >110 cm



**Fig. 4.** Relationships between selected pairs of traits for aquatic (blue) and terrestrial (black) plants, fitted by phylogenetic reduced major axis regression with the estimated strength of phylogenetic signal ( $\lambda$ ). In the figure showing the relationship between height and annual radial growth increment, small values were added (jitter, only for visualization) to observations to visualize overlapping points. The number of species in analyses was: 51, 59, 59, 17 for aquatic and 110, 122, 124, 106 for terrestrial plants. There was a positive or negative relationship in all explored pairs of variables for both terrestrial and aquatic plants ( $p$ -values < 0.001). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

having larger conduit fractions (34.5 % on average), while smaller plants growing in habitats with high-temperature seasonality (higher embolism risk) had the lowest fraction, 16.1 % on average. Life form, LDMC, and climatic variables were the best predictors of the aerenchymatic tissue fraction, with submerged and floating-leaved plants having a high fraction, especially large water plants with tougher leaves ( $LDMC > 128 \text{ mg g}^{-1}$ ) such as *Nuphar lutea*, *Nymphaea alba*, and *Trapa natans*, while much lower fractions were typical for terrestrial wetland plants.

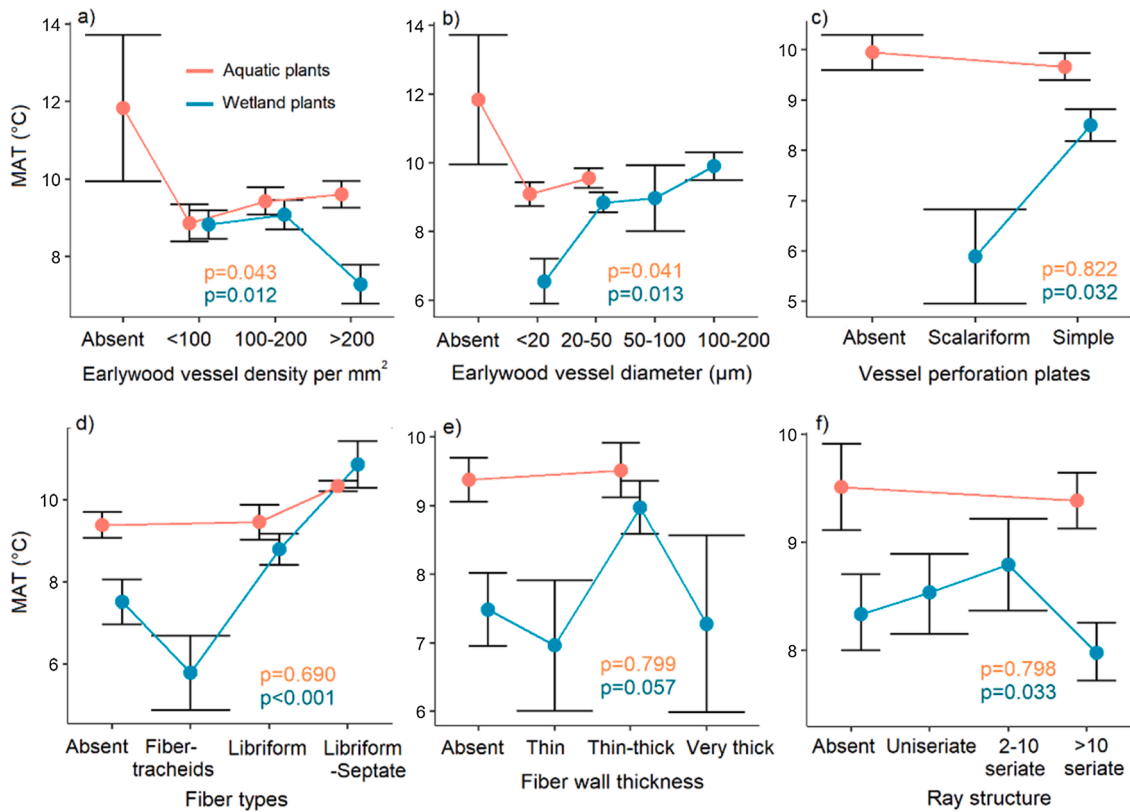
#### 4. Discussion

In this study, we assessed dozens of stem anatomical features across most aquatic and wetland dicots in Central Europe, representing key components of natural and artificial aquatic and wetland habitats. The stems of the studied species were essentially composed of conductive, mechanical, storage, and aeration tissues providing efficient structural support and water, nutrient, and air transport. Their proportions varied according to plant morphology, thermal optimum, hydrological conditions, and taxonomic origin. We were able to disentangle the direct contributions of different internal (plant phylogeny, plant size and growth form) and external (moisture and temperature gradients) drivers affecting interspecific variation in stem structural adaptations that assist plants to thrive in aquatic and wetland conditions. Although most of the anatomical variation was explained by growth form differences between aquatic and wet terrestrial conditions, the environmental gradients (temperature and soil moisture) had a significant unique effect on anatomical divergence not confounded by evolutionary inertia. Due to the high plant stature variation (0.02–10 m), many anatomical features

were related to plant height (vessel conduit size), leaf properties (parenchyma storage capacity), while others were directly related to temperature (lignification) or waterlogging (aerenchyma) gradients.

We described two major axes of variation in anatomical structures. The first axis is related to the water level gradient, separating strictly aquatic plants from terrestrial wetland taxa. Stem anatomy in aquatic plants is characterized by a large cortex zone formed by the photosynthetic chlorenchyma and starch-storing parenchyma cells along with extensive aerenchymatic air spaces that provide aeration, buoyancy and help aquatic plants to float (Armstrong et al., 1991), lignified endodermis that does not allow gas bubbles to enter the xylem and helps prevent embolisms (Taiz and Zeiger, 2015), xylem with absent or solitary vessels embedded in pervasive parenchyma, and largely absent fibers and bark secretory elements. Extensive aerenchymatic spaces in the stems of all submerged plants serve as mechanical support for their long flexible stems in a water column. Nevertheless, the presence of air spaces limits the occurrence of aquatic vascular plants to shallower waters where hydrostatic pressure is still low and does not compress their aerenchymatic stems (Sculthorpe, 1967). At depths of more than 10 m, submerged vascular plants are replaced by aquatic mosses or charophytes without aerenchyma, which can grow up to 30 m (Pietsch, 1987), being limited only by light availability. With decreasing water depth, aquatic plants are replaced by perennial wetland forbs and woody taxa with larger stem stature supported by thick-walled libriform fibers instead of aerenchymatic spaces (Schweingruber et al., 2013), large, often grouped vessels with simple perforation plates securing high hydraulic efficiency (Baas et al., 2004), semi-ring porous xylem with wide earlywood vessels in spring and narrow latewood vessels in summer, providing both efficiency and safety of the water transport as well





**Fig. 5.** Differences in the species' climatic optima, calculated based on the CHELSA mean annual temperatures (MAT), between groups of taxa with different anatomical structures defined by (a) earlywood vessel number, (b) vessel diameter, (c) vessel perforation plates, (d) fiber type, (e) fiber wall thickness, and (f) parenchyma ray composition.

as a potentially longer growing season (Kitin and Funada, 2016), and alternate intervessel pitting helping plants with xylem safety to water stress-induced embolism (Sperry and Hacke, 2004).

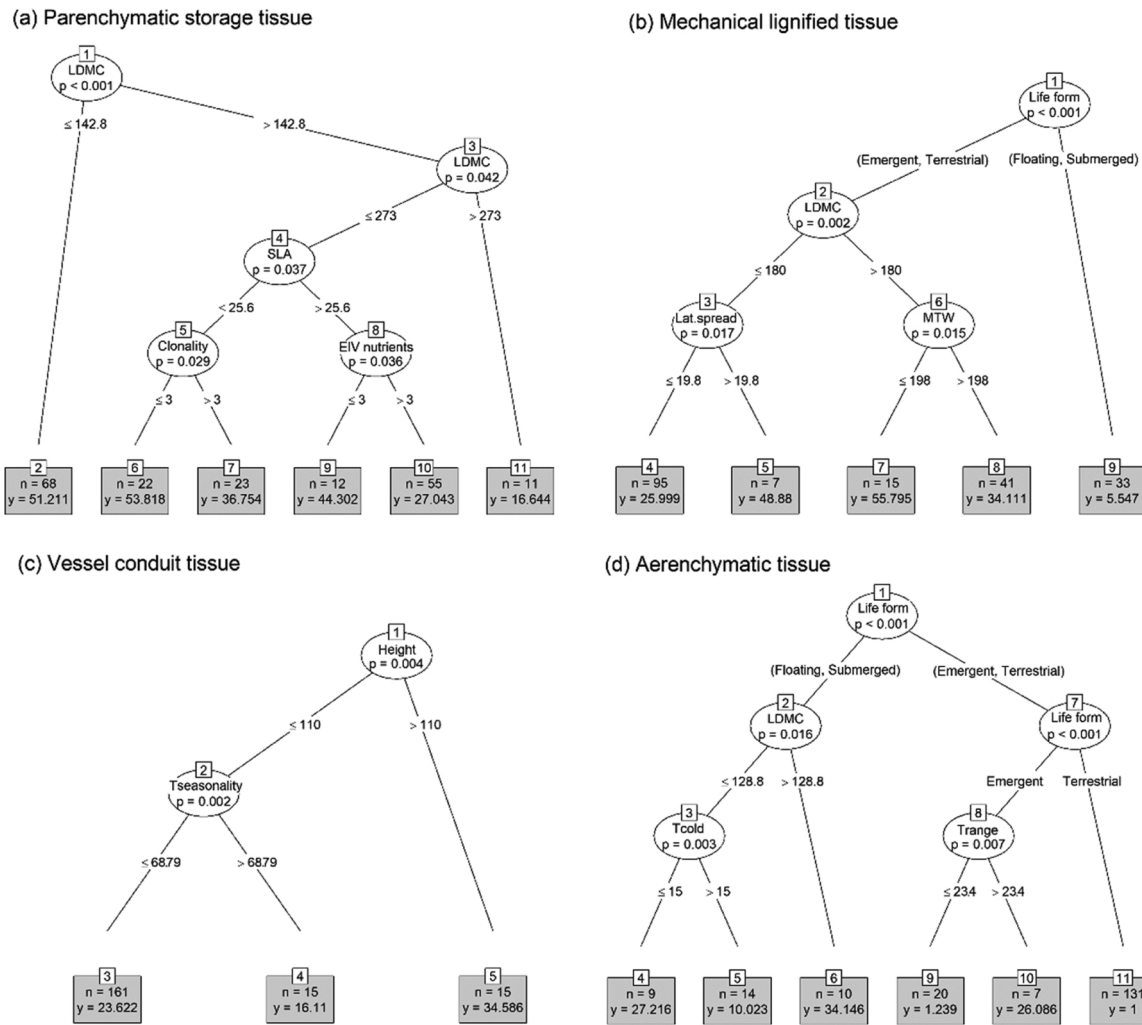
The second major axis of anatomical variation is associated with decreasing temperature of the growing season, separating terrestrial short-lived warm-adapted plants at lower elevations from cold-adapted short-rhizomatous forbs and subshrubs, with specific adaptations (slow growth, higher lifespan, extensive parenchyma) to cope with low-temperature stress. Decreasing temperature with increasing elevation reduces the plant size in both aboveground shoot and belowground rhizomes, and also exerts strong control over anatomical structures including slower growth rate (smaller radial increments) and longer lifespan, similarly as was observed in alpine plants along elevation gradients (Nobis and Schweingruber, 2013; Doležal et al., 2019). Elevation-related cooling affects the conductive system via reduced vessel sizes and extended storage tissues formed by multiseriate rays and axial pervasive parenchyma surrounding small vessels. Smaller conduits have repeatedly evolved in colder places to allow plants to cope with freezing-induced embolism and cavitation (Foster and Gifford, 1974). Xylem cavitation reduces plant capacity for water transport and impairs carbon fixation by inducing stomatal closure to prevent further cavitation and leaf drying (Davis et al., 1999; Sperry, 2003). In response to harsh conditions, most of the xylem tissue in studied cold-adapted wetland plants is composed of small vessels embedded with unlignified, fiberless parenchymatic cells (Spicer, 2014) with a high capacity for storing starch, fructans, and other important cryoprotective substances that decrease the freezing point (Valluru and Van den Ende, 2008), and thus increase the freezing resistance of tissues (Liu and Osborne, 2013).

The occurrence of gelatinous fibers in woody wetland species (*Frangula alnus*, *Salix* spp.) may be associated with the tension wood where they play an important role in the tensile stress mechanism of the

reaction wood inside the fully developed stem (Mellerowicz and Gorshkova, 2012). We also found gelatinous fibers in a few herbaceous species (*Epilobium* and *Lythrum*). Similarly, Piva et al. (2019) observed the gelatinous fibers in three species of *Ludwigia* (Onagraceae) growing in wet or periodically waterlogged soils. They interpreted the presence of gelatinous fibers along with vascular tissues in herbs as an adaptation associated with short-term water storage, similar to Carlquist (2014).

Additional mechanical support and the necessary transport of oxygen to the roots of most aquatic and many wetland plants is achieved by creating conspicuous aerenchymatic tissue (cavities, lacunae, air spaces) present in 44 % of the studied taxa. Expansigenous honeycomb aerenchyma (Seago et al., 2005) is the most widespread type among aquatic floating-leaved plants, being present across different families such as Nymphaeaceae (*Nuphar lutea*, *N. pumila*), Menyanthaceae (*Nymphoides peltata*), Onagraceae (*Ludwigia grandiflora*, *L. palustris*), Primulaceae (*Hottonia palustris*) and Lythraceae (*Trapa natans*), but less frequently in wetland terrestrial herbs such as rhizomatous forbs (*Gratiola officinalis*, *Mentha aquatica*, *Myosotis rehsteineri*, *Pulicaria dysenterica*) and short-lived annuals (*Bidens cernuus*, *Limosella aquatica*, *Veronica anagalloides*). The lysigenous aerenchyma evolved in many phylogenetically unrelated aquatic submerged plants including all studied *Callitriche*, *Ceratophyllum*, *Utricularia*, and *Myriophyllum* species, and also *Aldrovanda vesiculosa*, *Crassula helmsii*, and *Elatine hexandra*. Schizogenous aerenchyma, which is caused by cell divisions and expansions without cell death, predominated in short-lived small herbs (*Centunculus minimus*, *Myosurus minimus*, *Sedum villosum* and *Veronica catenata*), root sprouters (*Cnidium dubium*), some aquatic emergent plants (e.g. *Veronica beccabunga*, *Nasturtium officinale*, *Myosotis scorpioides*), alpine short-rhizomatous (e.g. *Ranunculus aconitifolius*, *Rumex alpinus*) and long-rhizomatous (*Glaux maritima*) forbs.

Besides storage and osmoprotection, parenchyma cells have the potential for renewed meristematic activity resulting in wound repair and



**Fig. 6.** Conditional inference trees showing a significant effect of internal and external drivers on the fraction of individual tissue types. In each split of the tree, all predictors are tested and the one that best discriminates between higher and lower values is selected. Each split of the tree is described by the factors associated with the split (ovals), the permutation-based significance of the split (p-value) (ovals), and the level at which the split occurs (the line between ovals and boxes). The mean percent tissue fraction (y) and the number of species (n) is given at each terminal node. Lifeform – four categories (aquatic submerged, emergent, floating-leaved, and terrestrial wetland), LDMC – leaf dry matter content, SLA – specific leaf area, Latspe – lateral spread, MTW – max. temperature of warmest month, TemSeas – temperature seasonality, Tcold – mean temperature of coldest quarter, Trange – temperature annual range.

tissue protection. A characteristic feature of some phylogenetically unrelated perennial aquatic and terrestrial wetland species is root segmentation as a result of senescence. If the need to support water and nutrient transport and stem metabolism is out of balance, living parenchyma cells within the xylem change their storage mode to a meristematic mode to form an inter-xylary periderm towards the pith, separating the metabolic active outer part from a dead central part (Crivellaro and Schweingruber, 2015). This process can occur several times with all segregated parts forming a new phellem around the stem (Doležal et al., 2018). Tertiary meristematic activities resulting in the formation of a secondary intra-xylary periderm towards the pith were observed in only 4% of species such as *Clematis integrifolia*, *Gratiola officinalis*, *Hottonia palustris*, and *Succisa pratensis*, while successive cambia simultaneously producing xylem and phloem were observed in 3% of species such as *Senecio doria*, *Petasites hybridus*, *Ranunculus lingua*, and *Nymphaea candida*.

Besides decreasing conduit size and increasing parenchyma share, the variation in vessel perforation plate structure, with the presence of scalariform plates in species of cold temperate waterlogged habitats such as *Menyanthes trifoliata*, *Erica tetralix*, *Ledum palustre*, *Rubus chamaemorus*, *Vaccinium uliginosum*, *V. oxycoccus*, and *Betula nana*, probably

reflect plant adaptations to low-temperature stress. There is still controversy over the adaptive role of scalariform perforation plates (Lens et al., 2016), but it has been shown that the closely spaced bars in the perforations can trap small freezing-induced air bubbles, thereby preventing detrimental embolism levels (Zimmermann, 1983). It is assumed that evolution from scalariform vessel perforations (ancestral) to simple vessel perforations (derived) was driven by the need for a higher hydraulic efficiency when plants moved from ever-wet or cold habitats to (seasonally) dry habitats (Carlquist, 1975). Simple perforations are the most common type in studied dicots, typically in perennial forbs of wetland habitats with a dry late summer period. It is thus assumed that seasonal or permanent demand for high hydraulic efficiency in drier and/or warmer areas has triggered terrestrial plants to evolve simple perforations, allowing more efficient long-distance water transport and thus, a higher CO<sub>2</sub> fixation (e.g. Baas et al., 2004; Jansen et al., 2004).

In conclusion, we show that the entire anatomical spectrum across various aquatic and wet environments can only be evaluated and grasped by using the analysis of numerous species belonging to many different taxonomic and ecological units. By combining life-form classification with plant ecological preferences and stem anatomical

structures in more than two hundred aquatic and terrestrial wetland vascular plants, we disentangled the contributions of phylogeny, temperature, and water level gradients as well as plant morphology and size to interspecific variation in stem structural adaptations. Environmental gradients had a strong influence on plant anatomy after controlling for confounding factors (phylogenetic and growth form variation), with extreme conditions related to either waterlogging (anoxia) or low-temperature (freezing damage) leading consistently to smaller conduit sizes and limited lignification, but extended parenchyma and hence storage and tissue renewal capacity resulting in higher resistance and resilience to biomass loss induced by running water, snow and frost disturbances. Being distributed along a wide elevation and water level gradients, the major aquatic and wet habitats harbor unrelated species with different evolutionary histories that have converged to similar anatomical and hence morphological structures. However, further comparative studies based on a large number of taxa will be necessary to fully understand the evolution of plant anatomical adaptations in major aquatic and wetland ecosystems worldwide.

## Contributions

All authors participated in the conception and the design of the study. J.D., A.Ku., V.J. and F.S. collected the data. J.D. and A.Kl. analyzed the data. J.D. wrote the manuscript. All authors reviewed and approved the final manuscript.

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## Declaration of Competing Interest

The authors report no declarations of interest.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.envexpbot.2021.104495>.

## References

- Adams, D.C., 2014a. A generalized K statistic for estimating phylogenetic signal from shape and other high-dimensional multivariate data. *Syst. Biol.* 63, 685–697.
- Adams, D.C., 2014b. A method for assessing phylogenetic least squares models for shape and other high-dimensional multivariate data. *Evolution* 68, 2675–2688.
- Adams, D.C., Collyer, M.L., 2018. Multivariate phylogenetic comparative methods: evaluations, comparisons, and recommendations. *Syst. Biol.* 67, 14–31.
- Adams, D.C., Collyer, L., Kalliontzopoulou, A., 2019. Geomorph: Software for Geometric Morphometric Analyses. R Package Version 3.1.3.
- Angiosperm Phylogeny Group, 2016. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. *Bot. J. Linn. Soc.* 181, 1–20.
- Armstrong, W., Beckett, P.M., 1987. Internal aeration and development of stela anoxia in submerged roots. *New Phytol.* 105, 221–245.
- Armstrong, W., Justin, S.H.F.W., Beckett, P.M., Lythe, S., 1991. Root adaptation to soil waterlogging. *Aquat. Bot.* 39, 57–73.
- Armstrong, W., Armstrong, J., Beckett, P.M., 1996. Pressurised ventilation in emergent macrophytes: the mechanism and mathematical modelling of humidity-induced convection. *Aquat. Bot.* 54, 121–135.
- Baas, P., Ewers, F.W., Davis, S.D., Wheeler, E.A., 2004. Evolution of xylem physiology. In: Poole, L., Hemsley, A. (Eds.), *Evolution of Plant Physiology*. Elsevier Academic Press, pp. 273–295.
- Blomberg, S.P., Garland, T., Ives, A.R., 2003. Testing for signal in comparative data: behavioral traits are more labile. *Evolution* 57, 717–745.
- Carlquist, S., 1975. *Ecological Strategies of Xylem Evolution*. University of California Press, Berkeley, p. 259.
- Carlquist, S., 2012. How wood evolves: a new synthesis. *Botany* 90, 901–940.
- Carlquist, S., 2014. Fibre dimorphism: cell type diversification as an evolutionary strategy in angiosperm woods. *Bot. J. Linn. Soc.* 174, 44–67.
- Chytrý, M., Tichý, L., Dřevojan, P., Sádlo, J., Zelený, D., 2018. Ellenberg-type indicator values for the Czech flora. *Preslia* 90, 83–103.
- Crivellaro, A., Schweingruber, F.H., 2015. *Stem Anatomical Features of Dicotyledons. Xylem, Phloem, Cortex and Periderm Characteristics for Ecological and Taxonomical Analysis*. Verlag Kessel, Remagen-Oberwinter, Germany.
- Davis, S.D., Sperry, J.S., Hacke, U.G., 1999. The relationship between xylem conduit diameter and cavitation caused by freeze-thaw events. *Am. J. Bot.* 86, 1367–1372.
- Doležal, J., Dvorský, M., Borner, A., Wild, J., Schweingruber, F.H., 2018. *Anatomy, Age and Ecology of High-mountain Plants in Ladakh, the Western Himalaya*. Springer, Cham.
- Doležal, J., Klimeš, A., Dvorský, M., Říha, P., Klimešová, J., Schweingruber, F., 2019. Disentangling evolutionary, environmental and morphological drivers of plant anatomical adaptations to drought and cold in Himalayan graminoids. *Oikos* 128, 1576–1587.
- Dória, L.C., Meijs, C., Podadera, D.S., del Arco, M., Smets, E., Delzon, S., Lens, F., 2019. Embolism resistance in stems of herbaceous Brassicaceae and Asteraceae is linked to differences in woodiness and precipitation. *Ann. Bot.* 124, 1–13.
- Durka, W., Michalski, S.G., 2012. Daphne: a dated phylogeny of a large European flora for phylogenetically informed ecological analyses. *Ecology* 93, 2297.
- Evans, D.E., 2003. Aerenchyma formation. *New Phytol.* 161, 35–49.
- Foster, A.S., Gifford, E.M., 1974. *Comparative Morphology of Vascular Plants*, 2nd edn. WH Freeman & Co.
- García-Giron, J., Heino, J., Baastrup-Spohr, L., Bove, C.P., Clayton, J., de Winton, M., Feldmann, T., Fernandez-Alaez, M., Ecke, F., Grillas, P., Hoyer, M.V., Kolada, A., Kosten, S., Lukacs, B.A., Mjelde, M., Mormul, R.P., Rhazi, L., Rhazi, M., Sass, L., Xu, J., Alahuhta, J., 2020. Global patterns and determinants of lake macrophyte taxonomic, functional and phylogenetic beta diversity. *Sci. Total Environ.* 723, 138021.
- Gärtner, H., Schweingruber, F.H., 2013. *Microscopic Preparation Techniques for Plant Stem Analysis*. Verlag Kessel, Remagen-Oberwinter.
- Greguss, P., 1945. *Bestimmung der mitteleuropäischen Laubbölder und Sträucher auf xylogomischer Grundlage*. Verlag des Ungarischen Naturwissenschaftlichen Museums, Budapest.
- Grigore, M.N., Ivanescu, L., Toma, C., 2014. *Halophytes: An Integrative Anatomical Study*. Springer.
- Hacke, U.G., Spicer, R., Schreiber, S.G., Plavcová, L., 2016. An ecophysiological and developmental perspective on variation in vessel diameter. *Plant Cell Environ.* 40, 831–845.
- Hamann, E., Puijalón, S., 2013. Biomechanical responses of aquatic plants to aerial conditions. *Ann. Bot.* 112, 1869–1878.
- Harmon, L.J., Weir, J.T., Brock, C.D., Glor, R.E., Challenger, W., 2008. GEIGER: investigating evolutionary radiations. *Bioinformatics* 24, 129–131.
- Hejný, S., 1960. *Ökologische Charakteristik der Wasser- und Sumpfpflanzen in den slowakischen Tiefebene (Donau- und Theissgebiet)*. Verlag der Slowakischen Akademie, Bratislava.
- Hejný, S., Segal, S., Raspopov, I.M., 1998. General ecology of wetlands. In: Westlake, D. F., Květ, J., Szczepański, A. (Eds.), *The Production Ecology of Wetlands*. Cambridge University Press, Cambridge, pp. 1–77.
- Hothorn, T., Hornik, K., Zeileis, A., 2006. Unbiased recursive partitioning: a conditional inference framework. *J. Comput. Graph. Stat.* 15, 651–674.
- Jackson, M.B., Armstrong, W., 1999. Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding and submergence. *Plant Biol.* 1, 274–287.
- Jansen, S., Baas, P., Gasson, P., Lens, F., Smets, E., 2004. Variation in xylem structure from tropics to tundra: evidence from vested pits. *Proc. Natl. Acad. Sci. U. S. A.* 101, 8833–8837.
- Jung, J., Lee, S.C., Choi, H.K., 2008. Anatomical patterns of aerenchyma in aquatic and wetland plants. *J. Plant Biol.* 51, 428–439.
- Karger, D.N., Conrad, O., Böhner, J., et al., 2016. CHELSA Climatologies at High Resolution for the Earth's Land Surface Areas, Version 1.1. World Data Center for Climate at DKRZ. [https://doi.org/10.1594/WDC/CHELSA\\_v1.1](https://doi.org/10.1594/WDC/CHELSA_v1.1).
- Kítin, P., Funada, R., 2016. Earlywood vessels in ring-porous trees become functional for water transport after bud burst and before the maturation of the current-year leaves. *IAWA J.* 37, 315–331.
- Kleyer, M., Bekker, R.M., Knevel, I.C., et al., 2008. The LEDA Traitbase: a database of life-history traits of the Northwest European flora. *J. Ecol.* 96, 1266–1274.
- Klimešová, J., Danihelka, J., Chrtěk, J., de Bello, F., Herben, T., 2017. CLO-PLA: a database of clonal and bud-bank traits of the Central European flora. *Ecology* 98, 1179.
- Legendre, P., Legendre, L., 1998. *Numerical Ecology*, 2<sup>nd</sup> English edn., Vol. 20. Elsevier Science.
- Lens, F., Vos, R.A., Charrier, G., et al., 2016. Scalariform-to-simple transition in vessel perforation plates triggered by differences in climate during the evolution of Adoxaceae. *Ann. Bot.* 118, 1043–1056.
- Liu, M.Z., Osborne, C.P., 2013. Differential freezing resistance and photoprotection in C3 and C4 eudicots and grasses. *J. Exp. Bot.* 64, 2183–2191.
- Mellerowicz, E.J., Gorshkova, T.A., 2012. Tensional stress generation in gelatinous fibres: a review and possible mechanism based on cell-wall structure and composition. *J. Exp. Bot.* 63, 551–565.

- Morris, H., Gillingham, M.A.F., Plavcová, L., et al., 2018. Vessel diameter is related to amount and spatial arrangement of axial parenchyma in woody angiosperms. *Plant Cell Environ.* 41, 245–260.
- Neumann, K., Schoch, W., Détienne, P., Schweingruber, F.H., 2001. Woods of the Sahara and the Sahel. Paul Haupt Verlag, Bern, Stuttgart, Wien.
- Nobis, M.P., Schweingruber, F.H., 2013. Adult age of vascular plant species along an elevational land-use and climate gradient. *Ecography* 36, 1076–1085.
- Ogden, E.C., 1974. Anatomical Patterns of Some Aquatic Vascular Plants of New York. Bulletin 424. University of the State of New York, State Education Dept., Albany.
- Pagel, M., 1999. Inferring the historical patterns of biological evolution. *Nature* 401, 877.
- Pfandenhauer, J.S., Klötzli, F.A., 2014. Vegetation der Erde. Grundlagen, Ökologie, Verbreitung. Springer-Verlag, Berlin, Heidelberg.
- Pietsch, W., 1987. Zur Vegetation der Charetea-Gesellschaften der Mitteleuropäischen Tiefebene. *Studia Phytologica Nova* 69–86.
- Piva, T.C., Machado, S.R., Scremin-Dias, E., 2019. Anatomical and ultrastructural studies on gelatinous fibers in the organs of non-woody xerophytic and hydrophytic species. *Botany* 97, 529–536.
- Puijalon, S., Bouma, T.J., Douady, C.J., van Groenendael, J., Anten, N.P.R., Martel, E., Bornette, G., 2011. Plant resistance to mechanical stress: evidence of an avoidance–tolerance trade-off. *New Phytol.* 191, 1141–1149.
- Rascio, N., 2002. The underwater life of secondarily aquatic plants: some problems and solutions. *Crit. Rev. Plant Sci.* 21, 401–427.
- Revell, L.J., 2010. Phylogenetic signal and linear regression on species data. *Methods Ecol. Evol.* 1, 319–329.
- Revell, L.J., 2012. Phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol. Evol.* 3, 217–223.
- Schenk, H., 1886. Vergleichende Anatomie der submersen Gewächse. *Bibliotheca Botanica. Abhandlungen aus dem Gesamtgebiet der Botanik, Bd. 1.* Verlag Theodor Fischer.
- Schweingruber, F.H., 1990. Anatomy of European Woods. Paul Haupt Verlag, Bern, Stuttgart, Heidelberg.
- Schweingruber, F.H., Berger, H., 2019. Anatomy of Culms and Flower Stalks of Monocotyledonous Plants, Vol. 3. Verlag Kessel, Remagen-Oberwinther.
- Schweingruber, F.H., Börner, A., Schulze, E.D., 2013. Atlas of Stem Anatomy in Herbs, Shrubs and Trees, Vol. 2. Springer-Verlag, Berlin, Heidelberg.
- Schweingruber, F.H., Kučerová, A., Adamec, L., Doležal, J., 2020. Anatomic Atlas of Aquatic and Wetland Plant Stems. Springer.
- Sculthorpe, C.D., 1967. The Biology of Aquatic Vascular Plants. Edward Arnold, London.
- Seago, J.L., Marsh, L.C., Stevens, K.J., Soukup, A., Votrubová, O., Enstone, D.E., 2005. A re-examination of the root cortex in wetland flowering plants in respect to aerenchyma. *Ann. Bot.* 96, 565–579.
- Sperry, J.S., 2003. Evolution of water transport and xylem structure. *Int. J. Plant Sci.* 164, 115–127.
- Sperry, J.S., Hacke, U.G., 2004. Analysis of circular bordered pit function I. Angiosperm vessels with homogenous pit membrane. *Am. J. Bot.* 91, 369–385.
- Spicer, R., 2014. Symplastic networks in secondary vascular tissues: parenchyma distribution and activity supporting long-distance transport. *J. Exp. Bot.* 65, 1829–1848.
- Taiz, L., Zeiger, E., 2015. Plant Physiology and Development. Sinauer Associates, Sunderland, Massachusetts.
- ter Braak, C.J.F., Šmilauer, P., 2012. CANOCO Reference Manual and Users's Guide: Software for Ordination (version 50). Microcomputer Power, Ithaca, NY.
- Valluru, R., van den Ende, W., 2008. Plant fructans in stress environments: emerging concepts and future prospects. *J. Exp. Bot.* 59, 2905–2916.
- Verhoeven, K.J., Simonsen, K.L., McIntyre, L.M., 2005. Implementing false discovery rate control: increasing your power. *Oikos* 108, 643–647. <https://doi.org/10.1111/j.0030-1299.2005.13727.x>.
- Willby, N.J., Abernethy, V.J., Demars, B.O.L., 2000. An attribute-based classification of European hydrophytes and its relationship to habitat utilisation. *Freshw. Biol.* 43, 43–74.
- Zimmermann, M., 1983. Xylem Structure and the Ascent of Sap. Springer, Berlin.