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REGULAR ARTICLE

Living between land and water – structural and functional adaptations in vegetative organs of bladderworts

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Abstract

Aims The carnivorous *Utricularia* (Lentibulariaceae) has an anatomically simple and seemingly rootless vegetative body. It occupies a variety of wetlands and inland waters and shows a broad range of life forms. Here, we aimed to elucidate structural and functional traits in various hydric conditions. Furthermore, we intended to evaluate morpho-anatomical adaptations in correlation with life forms.

Methods Morpho-anatomical characteristics typical for hydrophytes of all life forms were investigated by light microscopy on 13 *Utricularia* taxa, compared to one *Pinguicula* and two *Genlisea* taxa, and assessed by multivariate analyses.

Results Vegetative structures of *Utricularia* and *Genlisea* showed reduced cortical, supporting, and vascular tissues. With increasing water table, leaves were thinner, and narrower or dissected, and submerged organs tended to contain chloroplasts in parenchymatic and epidermal cells. In some main stolons, an endodermis with Casparian strips was visible. Large gas chambers, including a novel 'crescent' and a special 'hollow' aerenchyma pattern, were found in amphibious to free-floating taxa.

Conclusions The evolutionary transfer of carnivory from aerial to subterranean organs in *Genlisea*, and even more in *Utricularia*, coincides with a highly simplified anatomy, which is adapted to a broad variety of hydric conditions and compensates for structural innovations in the uptake of nutrients.

Keywords *Utricularia* · Lentibulariaceae · Hydrophyte · Carnivorous plants · Life forms · Aerenchyma · Casparian strips · Anatomy

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Introduction

Life forms of hydrophytes

Hydrophytes are plants living "in water or on a substrate that is saturated at a frequency and duration during the growing period sufficient to affect plant occurrence" (Tiner 2017). Water plants constitute about 1–2 % of the angiosperms today, while vascular hydrophytes (macrophytes) evolved from terrestrial plants of hundreds of families and genera at several points in time (Cook 1999; Chambers et al. 2008).

The life forms (habits) of aquatic plants range from terrestrial but occasionally inundated taxa, with anchorage in the substrate and with leaves and reproductive organs exposed to the air, to unattached, free-floating taxa with all parts under water. Between the extremes, gradually different forms exist, contingent upon water regimes and adaptive responses of the plants to environmental changes such as flooding and unflooding, precipitation, or evaporation (cf. Sculthorpe 1967; Braendle and Crawford 1999; Tiner 2017). Many hydrophytes have adapted to various habitats and hence show more than just one life or growth form (cf. Sculthorpe 1967; Cook 1999).

Classification of hydrophytes

Authors engaged in the research of carnivorous plants and the Lentibulariaceae in particular (e.g. Taylor 1989; Guisande et al. 2007; Reut and Płachno 2020) designate most species within the Lentibulariaceae as "terrestrials", although the majority colonizes wet, waterlogged or temporarily submerged habitats. According to Taylor (1989), "terrestrial" plants are those flowering when the soil is humid or saturated but being submerged in wet periods, which coincides with Cook's (1999) definition of 'tenagophytes'. Taylor (1989) delimits "terrestrials" from affixed aquatics and (freely) suspended (inflated) aquatics but in some instances also from subaquatics. Affixed aquatics are mostly anchored in the substrate (Guisande et al. 2007) and possibly represent a transition towards suspended (free-floating) hydrophytes (Jobson et al. 2003). Subordinating subaquatic in "terrestrial" habits (e.g. Guisande et al. 2007) or subaquatic in affixed aquatic habits (e.g. Jobson et al. 2018) leads to results which are difficult to compare. This attempt of a classification of life forms in carnivorous plants does not seem to be suitable for a classification of hydrophytes in general and needs to be adjusted.

In the current paper, we follow a simple classification of habits of wetland and aquatic plants as outlined by Sculthorpe (1967) and Hutchinson (1975), differentiating between 'anchored hydrophytes' (encompassing 'emergent hydrophytes', 'floating-leaved hydrophytes', and 'submerged hydrophytes') and 'free-floating hydrophytes'. However, since 'amphiphytes' are adapted to a terrestrial as well as a (periodically) submerged life, i.e. to aerobic and anaerobic conditions, we rank this group among anchored hydrophytes. They are often found in the transitional zone between land and water where

flooding and unflooding occur (Braendle and Crawford 1999; Crawford 2008). Extending the scope of hydrophytes by adding amphiphytes enables the identification of ecological and evolutionary transects (i.e. various grades and combinations of morphological and anatomical specializations) within hydrophytic plants, and the concurrent delimitation of this group from purely terrestrial plants (Barrett et al. 1993).

Morpho-anatomical characters

Early anatomical works on hydrophytes provide insights into structural characteristics of species and genera across various plant families (e.g. Schenck 1886; Arber 1920). In his book on aquatic vascular plants, Sculthorpe (1967) connects morpho-anatomical traits with physiological and functional aspects. Experimental and observational studies, mainly at the species level, led to the conclusion that the life in a transitional zone between land and water, and in fluctuating water conditions entails a variety of adaptive responses, and hence a greater phenotypic plasticity, especially in amphibious and emergent hydrophytes (Barrett et al. 1993; Crawford 2008; Lusa et al. 2011). In these groups, heterophylly is widespread, exhibiting submerged and aerial leaf forms adapted to the respective milieu (Sculthorpe 1967; Mommer et al. 2005; Li et al. 2019). In the relatively stable and homogeneous environment of the water column, however, the diversification of obligate hydrophytes is generally low (Barrett et al. 1993; Hidalgo et al. 2015). Overall, hydrophytes show heterogeneous combinations of morphological, anatomical, and functional traits (Table 1) that differ from those of strictly terrestrial plants.

Comparing species of various life forms within one genus may uncover adaptations to habitats and hence also evolutionary aspects rather than just phenotypic responses to changes in the environment. However, to date, a comparative morpho-anatomical study on a hydrophytic plant genus with a broad range of life forms is missing. One reason may be that only few aquatic genera offer a large variety of life forms (cf. Cook 1999). One of these is the carnivorous *Utricularia* L.

Lentibulariaceae

Members of the bladderwort family (Lentibulariaceae: Lamiales) with its three genera *Pinguicula* L., *Genlisea* A.St.-Hil. and *Utricularia* L. are among the

Table 1 Common morphological and anatomical characters and their functions in herbaceous hydrophytes

	Morphological / anatomical adaptation	Function
1)	Reduced or absent roots in some (submerged) species	BIO
2)	Tendency to develop narrow leaves (submerged form) or dissected leaves (in several submerged or free-floating aquatics) increasing the ratio of surface to volume	ABS, FLE, LIG
3)	Thin leaves without palisade parenchyma, and with reduced or no mesophyll, losing dorsiventrality	FLE, LIG
4)	Epidermis of submerged organs/leaves with chloroplasts	LIG
5)	Delicate submerged organs with a thin cuticula	ABS
6)	Reduced xylem elements	BIO
7)	Prominent intercellular spaces (between spongy parenchyma in leaves) or lacunar / aerenchymatous anatomy	GAS
8)	Occurrence of hydropotes or glandular types of trichomes in the epidermis, e.g. in submerged species of *Potamogeton*, *Ceratophyllum*, and *Cryptocoryne*	ABS
9)	Reduced or absent supporting tissue (sclerenchyma, collenchyma)	FLE
10)	Limitation of the vascular strand to a central cylinder in roots (and stems); endodermoid layer, channelizing the flow of water through reduced xylem	BAR, BIO
11)	Reduction, inactivity, or absence of stomata (especially in submerged leaves); in some plants replaced by secretory structures	BIO, STO
12)	Development of adventitious roots	STA, ABS

Abbreviations of functions: ABS = Enhancement of absorption of nutrients, and dissolved CO_2 and O_2; GAS = Passage for an increased diffusion of gases, responding to gaseous pressure; buoyancy; BAR = Barrier to restrict radial oxygen loss (ROL) to the soil; BIO = Optimization of biomass investment (alternative structures for the uptake or transport of nutrients and dissolved gases); FLE = Flexibility in water movements; LIG = Enhancement of the collection of light in lower light conditions and in every organ orientation; STA = Stabilization of the plant (rosette) position in the water; STO = Redundancy of stomata (gas exchange in the air) in submerged organs; hydathodes for guttation (water secretion by root pressure) on floating leaves

Summarized from overviews by Sculthorpe (1967), Wetzel (1988), Wiegleb (1991), Jung et al. (2008), Colmer et al. (2011), Krähmer (2016), and Tiner (2017)

most interesting herbaceous angiosperms because of their carnivorous nature, unique vegetative bodies, small genome sizes, and several more biological peculiarities (cf. Ibarra-Laclette et al. 2013; Rutishauser 2020; Silva et al. 2020a).

Rosulate and rhizomatous growth forms are generally found in terrestrial or amphibious species of the Lentibulariaceae, while stolonifery occurs within all habits. In the current paper, we use the terms 'rhizome' and 'stolon' group-specifically. Both are generally horizontally growing shoots, while rhizomes tend to be shorter, often having storage function and giving rise to roots. Stolons are considered to have longer internodes. 'Anchor stolons' ('rhizoids', Taylor 1989) of several *Utricularia* species are assigned to unbranched and much shorter organs that grow geotropically positive and generally into the soil (Reut and Płachno 2020).

The rosulate butterworts (*Pinguicula*) contain around 100 species of which most of them are mesophytes, amphiphytes and lithophytes, and only a few are true

epiphytes (Fleischmann and Roccia 2018; Casper 2019). Insects are trapped on the foliage leaves of homophyllous butterworts above ground throughout the year, while heterophyllous species are effectively carnivorous only during wet periods (e.g. Legendre 2000; Fleischmann and Roccia 2018). *Pinguicula* species have rhizomes (Casper 2019) or short stems (Legendre 2000), and few produce runner stolons.

The corkscrew plants (*Genlisea*) comprise 31 species of amphiphytes and few emergent hydrophytes (Fleischmann 2012, 2018; Silva et al. 2020b). *Genlisea* is considered being heterophyllous, having non-carnivorous rosettes of green leaves above ground and subterranean "rhizophylls" that function like an eel-trap (Reut 1993; Fleischmann 2018; Płachno and Muravnik 2018). *Genlisea* shows short vertical stems or horizontal rhizomes (Fleischmann 2018). Since the rhizomes in *G. repens* are elongated (Reut 1993), they will hereafter be called stolons in accordance with Fleischmann (2018).

Finally, the bladderworts (*Utricularia*) are the most diverse within the Lentibulariaceae. They consist of about 240 species and show the widest range of life forms within the family, from mesophytes to free-floating aquatics, in addition to rheophytes, lithophytes, and epiphytes (cf. Guisande et al. 2007; Jobson et al. 2018; Jobson and Baleeiro 2020). Like *Genlisea*, "terrestrial" *Utricularia* species have leaves for photosynthesis and subterranean leaf-like organs for carnivory. In *Utricularia*, the prey is actively captured by tiny suction bladders, functioning like a mousetrap, while the tubular trap of *Genlisea* is considered being passive. However, abundant water is required for an effective operation of both trap types (Guisande et al. 2007; Jobson et al. 2018; Płachno et al. 2019a, b). Most species of *Utricularia* are stoloniferous (Taylor 1989; Jobson et al. 2003). The term "stolon" has established in *Utricularia* and prevails for reasons of consistency (Taylor 1989), also because in some species it grows above ground and up to several meters in length.

Anatomy of vegetative organs in the Lentibulariaceae

Early anatomical examinations of vegetative parts of carnivorous plants were done e.g. by Kamieński (1891), Lang (1901) and Merl (1915), but they largely failed to imply comparative discussions on functional aspects in a broader context of e.g. carnivory or adaptations to aquatic habitats. Later anatomical studies on vegetative organs in the Lentibulariaceae were mainly focusing on trap tissues (e.g. Lloyd 1942; Płachno et al. 2014; Płachno et al. 2017) and the functional ultrastructure of digestive glands (e.g. Płachno and Jankun 2004; Płachno et al. 2019a). Most recently, Płachno et al. (2020b) described structures of tubers in one *Genlisea* and two *Utricularia* species showing adaptation to abiotic stress (e.g. seasonal drought or fire). Reut and Płachno (2020) examined the anatomy of stolons, bladder stalks and leaves of *U. dichotoma sensu lato* clones, originating from five amphibious to emergent populations of Australia and New Zealand, and found simple and lacunate characters, which are basically homogenous throughout the subterranean/submersed organs and across the sources.

Aims of the study

In the current study, we use *Utricularia* (Lentibulariaceae) as model genus to investigate differences and commonalities of morpho-anatomical characters between species of several hydrophytic life forms, as this genus yields probably the greatest richness of habits amongst hydrophytes. We aim to elucidate by which structural means *Utricularia* handles water, nutrients, and gases in various hydric conditions. By applying multivariate analyses, we assess the clustering of life forms and the grade of adaptations to the level of submergence, in correlation to combinations of morpho-anatomical characters of vegetative organs of several amphibious, submerged, and free-floating taxa of *Utricularia* and chosen taxa of *Pinguicula* and *Genlisea*. Results are discussed in relation to functional aspects of traits, the carnivorous syndrome, and the evolution of the Lentibulariaceae.

Materials and methods

Plant material and preparation

In our morpho-anatomical investigation, we selected a total of 16 species from the Lentibulariaceae genera *Pinguicula*, *Genlisea* and *Utricularia* (see Table 2 for a full description, including taxonomic ranking, growth form and sources).

The plant material was studied with respect to several morpho-anatomical characters as outlined in Table 1. Examinations were performed on leaves, roots (*Pinguicula* only), trap stalks, and several types of stolons (where present). The anatomy of leaves was investigated on *P. gigantea*, *G. hispidula*, *U. volubilis*, *U. paulineae*, *U. uniflora*, *U. delicatula*, and *U. stygia*. Detailed data of all investigated Lentibulariaceae species with traits of leaves, stolons, roots, anchor stolons, and trap stalks is provided in the Supplementary Information (Online Resource) 1.

Plant samples used for light microscopy were fixed in a mixture of 2.5 % glutaraldehyde and 2.5 % formaldehyde in a 0.05 M cacodylate buffer (Sigma; pH 7.2) overnight or for up to four days. After fixation, the material was washed three times in a 0.1 M sodium cacodylate buffer followed by post-fixation in 1 % osmium tetroxide solution at room temperature for 1.5 h. The samples were dehydrated in graded ethanol series to 95 %, infiltrated and embedded using an epoxy embedding medium kit (Fluka). After polymerisation at 60 °C, sections were cut using a Leica Ultracut UCT ultramicrotome. Semi-thin sections (0.9-1.0 μm thick) were prepared and stained with aqueous methylene blue /

Table 2 Plant material used for anatomical examinations

Genus
  Subgenus
    Section
	Species	Habit, growth	Source

Pinguicula			
Isoloba			
Agnata	*Pinguicula gigantea* Luhrs	t (l), sto	Ex origin Oaxaca, Mexico[1]*
Genlisea			
Genlisea			
Genlisea	*Genlisea hispidula* Stapf	a, ros	Ex origin Africa[1]**
	Genlisea repens Benj.	a, sto	Ex origin South America[2]**
Utricularia			
Polypompholyx			
Polypompholyx	*Utricularia multifida* R.Br.	a, ros	Ex origin Western Australia[3]*
Pleiochasia	*Utricularia volubilis* R.Br.	(e-)s, ros	Ex origin SW Western Australia[4]***
	Utricularia paulineae A.Lowrie	a, sto	Ex origin SW Western Australia[5]**
	Utricularia oppositiflora R.Br.†	a(-e), sto	Ex origin Newcastle, New South Wales, Australia[4]**
	Utricularia dichotoma subsp. *aquilonia* R.W.Jobson†	a, sto	Ex origin Katoomba, New South Wales, Australia[1]**
	Utricularia tubulata F.Muell.	f	Ex origin Australia[6]****
Lasiocaules	*Utricularia uniflora* R.Br.	a, sto	Ex origin Australia[5]**
Bivalvaria			
Australes	*Utricularia delicatula* Cheeseman	a, sto	Ex origin Whangamarino wetlands, New Zealand[5]**
Utricularia			
Foliosa	*Utricularia tricolor* A.St.Hil.	a, sto	Araraquara, São Paulo State, Brazil[7]
Utricularia	*Utricularia stygia* Thor	s, sto	Třeboň, Czech Republic[8]
	Utricularia breviscapa Wright ex Griseb.	f, sto, flo	Araraquara, São Paulo State, Brazil[7]
Vesiculina	*Utricularia cucullata* A.St.Hil. & Girard	f, sto	a) Itirapina, São Paulo State, Brazil; b) Delfinópolis, Minas Gerais State, Brazil[9]
	Utricularia purpurea Walter	(s-)f, sto	Ex origin unknown[4]****

The taxonomy of genera, subgenera, sections and species follows Cieslak et al. (2005), Fleischmann (2012), Jobson et al. (2018), and Jobson and Baleeiro (2020)

†Treated as *U. dichotoma sensu* Taylor (1989) in Reut and Płachno (2020), but subsequently underwent taxonomic revision by Jobson and Baleeiro (2020)

Habit of sample (alternative/usual life form in situ in brackets): amphiphyte (a); emergent hydrophyte (e); free-floating (suspended) hydrophyte (f); lithophyte (l); submerged hydrophyte (s); terrestrial plant (t). Growth form: with floats (flo); rosulate (ros); stoloniferous (sto)

Sources, ex situ: [1] Cultivation in the Botanic Garden of Jagiellonian University in Kraków, Poland. [2] Cultivation in the Department of Plant Cytology and Embryology, Institute of Botany, Jagiellonian University in Kraków, Poland. [3] Collection of Kamil Pásek, Ostrava, Czech Republic, http://www.bestcarnivorousplants.net. [4] Collection of Dr. Lubomír Adamec, Institute of Botany of the Czech Academy of Sciences at Třeboň, Czech Republic. [5] Collection of Nigel Hewitt-Cooper, UK, http://www.hccarnivorousplants.co.uk. [6] Collection of Corin Gardiner, New Zealand. *Grown in humid soil. **Grown in wet to saturated soil. ***Grown under water in an aquarium, anchored in soil. ****Grown free-floating

Sources, collected in situ: [7] Farm near Araraquara, São Paulo State, Brazil; Fazenda Palmeiras, swamp c. 50 m from the road; V.F.O.Miranda et al. 2856. [8] Near Třeboň, Czech Republic. [9] From two populations: (a) road Itirapina-Brotas, São Paulo State, Brazil; swamp c. 10 m from the road; V.F.O.Miranda et al. 2859; (b) Delfinópolis, Minas Gerais State, Brazil; flooded area c. 250 m from the road; V.F.O.Miranda et al. 2877

azure II (MB/AII) for 1–2 min (Humphrey and Pittman 1974). Additional material was fixed as above, embedded in Technovit 7100 (Kulzer, Germany) and processed as in Lustofin et al. (2020). Sections were examined and photographed using a Nikon Eclipse E400 microscope with a Nikon DS-Fi2 camera and NIS-Elements D 4.00.00 4.0 software (Nikon, Tokyo, Japan) and using an Olympus BX60 microscope with an Olympus XC50 digital camera and cellSens Standard Software (Olympus, Tokyo, Japan).

Multivariate analyses

To quantitatively evaluate morpho-anatomical characters and their correlation with life forms in the Lentibulariaceae, we selected characters 1–9 from Table 1, as only those provided variability across the chosen taxa. The traits were recorded from 14 species with comparable organs (leaves for the morphological and stolons for anatomical data collection). To visualize how the species can be optimally clustered, if life forms can be suitably grouped, and which morpho-anatomical features contribute most to the groupings, the data dimensionality was reduced through several computing techniques while maintaining the most important information. We created three sets of variables for multivariate analyses on the 14 taxa. Dataset I contained 'life forms' and 'location of stolons' in addition to the

morpho-anatomical characters 1–9. The trait 'life forms' was selected to assess correlations of life forms with all other traits. 'Location of stolons' was included in dataset I to evaluate how far the stolon related traits were influenced by the location of the stolons in the respective substrate. In dataset II we excluded 'life forms', and dataset III contained the morpho-anatomical traits only. All biometric traits and their values used in the analyses are presented in Table 3.

We ran a graph-based clustering with BioVinci (BioTuring Inc) version 3.0.9 to calculate the "best fit" dimensionality reduction method with highest Silhouette Score [-1, 1]. In addition, the tool identified suitable traits supporting the evaluated hierarchical clusters in a decision tree by applying cost complexity pruning criteria such as Gini index and Entropy. The same methods were used when clusters were validated according to life forms ('life form clustering'). We ran Principal Component Analyses (PCA) by exclusion vs. inclusion of pre-processing with scaling, and by applying singular value decomposition (SVD) with imputation to calculate principal components. Some PCA scatter plots were overlayed with vectors (traits), adding information on the correlations of traits (direction of vectors) and the influence of traits (lengths of vectors). PCA statistics were taken from BioVinci (BioTuring Inc) version 1.1.5. The computing and illustration of

Table 3 Biometric traits and their values used in multivariate analyses

	Trait (variable)	Value
L)	Life form	terrestrial plant (1), amphiphyte (2), submerged hydrophyte (4), free-floating (suspended) hydrophyte (5)
0)	Stolon location	above ground, aerial (1), subterranean, few mm to few cm in the soil (2), submersed, few cm under water - in soil or floating (3)
1)	Anchoring organs	roots present (1), anchor stolons (rhizoids) present (2), roots/rhizoids absent (3)
2)	Leaf form	large-fleshy obovate (1), small spathulate-circular (2), (small) narrow-linear (3), dissected (4)
3)	Leaf thickness	mesophyll layers: > 10 (1), 8–10 (2), 4–7 (3), < 4 (4)
4)	Stolon chloroplasts	none identified (1), in parenchyma (2), in parenchyma and epidermis (3)
5)	Stolon diameter	thickness, in mm: > 0.56 (1), 0.41–0.56 (2), 0.25–0.40 (3), < 0.25 (4)
6)	Stolon xylem	Xylem tracheary element (vessel), diameter in µm: > 20 (1), 14–20 (2), < 14 (3), none identified or xylem lacuna only (4)
7)	Stolon intercellular spaces	nil (1), slight intercellular spaces (2), moderate/extensive intercellular spaces to wheel-shaped aerenchyma (3), crescent to hollow aerenchyma (4)
8)	Stolon external glands	absent (1), present (2)
9)	Stolon supporting tissue	present (1), absent (2)

confidence ellipses (CI = 95 %) of clusters in PCA were done with ClustVis (Metsalu and Vilo 2015).

Additional data can be found in Table 5, which was used for multivariate analyses.

Results

Morpho-anatomy of leaves and main stolons

We characterized the anatomical traits of leaves and main/runner stolons (see Table 4 for a full description, including references to figures).

Morpho-anatomy of other vegetative organs

The stoloniferous *P. gigantea* showed adventitious roots arising from a short vertical stem, without branching or root cap, but with unicellular hairs. Vegetative organs of *Genlisea* and *Utricularia* did not exhibit external root structures and adventitious roots. Some *Utricularia* species developed other anchoring organs (so called anchor

Table 4 Summary of morpho-anatomical characters of leaves and stolons

	Leaves (Fig. 1a-e)	Stolons (Figs. 1f-h and 2)
Organization	Simple	Simple
Cross-section	Dorsiventral (but circular in trap bearing *U. volubilis* leaves)	Circular
Epidermis	Uniseriate	Uniseriate
Cuticle*	Cuticularized	Cuticle seemingly thin (e.g. Figs. 1f and g and 2e) but somewhat thicker in *U. volubilis* (Fig. 2d)
Stomata	Stomatous (except for submersed leaves)	No stomata found
Glands	*P. gigantea*: Stalked and sessile glands *Genlisea* and *Utricularia*: button-like glands	*Genlisea* and *Utricularia*: button-like glands abundant (Fig. 2e) with generally one head cell (but with two head cells in *U. breviscapa*)
Mesophyll (leaf) / cortex (stolon)	Palisade parenchyma absent. *P. gigantea*: prominent water storage tissue *Genlisea* and *Utricularia*: Spongy parenchyma Intercellular spaces or lacunae present (Fig. 1c-e)	Pentagonal to polygonal, thin-walled and unlignified parenchymatic cells (more irregular cell shapes in *P. gigantea*, Fig. 1g). Intercellular spaces: none (*P. gigantea*, Fig. 1g), small (e.g. *G. repens*, Fig. 1f), or frequently aerenchymatic (Fig. 2a, c-i), predominantly lysigenous No hypodermis/exodermis found
Chloroplasts	*P. gigantea*: adaxially in mesophyll, abaxially in patches, and in bundle sheaths; occasionally with amyloplasts *Genlisea* and *Utricularia*: in spongy parenchyma; in submerged and free-floating *Utricularia* species also in the epidermis (Fig. 1e)	None or very few to numerous in parenchyma; in some species also in the epidermis (Fig. 2i) *G. repens*. Occasionally with amyloplasts (Fig. 1f)
Vascular tissue	*P. gigantea*: bundle shape almost a closed circle, with 3 to >13 lignified xylem tracheary elements (vessels) scattered towards the centre and 3 to >13 phloem groups towards the abaxial side (Fig. 1a, b) *Genlisea* and *Utricularia*: 1–2 xylem tracheary elements (vessels) and 1–3 phloem groups (e.g. Fig, 1c), but occasionally no xylem found (Fig. 1d, e)	Ectophloic central cylinder with unlignified pith cells and without pericycle. ≤4 lignified xylem tracheary elements and ≤12 or more phloem groups (Figs. 1f-h and 2). Xylem vessels not found in some stolons of *U. stygia* and *U. breviscapa*, or central (xylem) lacuna occasionally present (Fig. 2a, g). Phloem generally present but not identifiable in *U. uniflora* (Fig. 2 f).
Supporting tissue	Absent	Generally absent, but present internal to parenchyma sheath / endodermis in *G. repens* and *U. tricolor* (Fig. 1f, h)

*not conclusively demonstrated by the applied methods

Table 5 Data matrix of species and their values of biometric traits used in multivariate analyses

Species	Dataset I											
		Dataset II										
			Dataset III									
	L	0	1	2	3	4	5	6	7	8	9
PGI	1	1	1	1	1	1	1	2	1	1	2
GRE	2	2	3	2	2	3	1	2	2	2	1
UVO	4	3	2	3	3	2	2	3	3	2	2
UPA	2	2	2	2	3	1	3	3	4	2	2
UOP	2	2	2	3	3	2	1	2	4	2	2
UDI	2	2	2	2	3	3	3	3	4	2	2
UTU	5	3	3	3	4	3	3	4	3	2	2
UUN	2	2	2	2	3	1	4	3	4	2	2
UDE	2	2	2	3	2	1	4	3	3	2	2
UTR	2	2	2	2	3	2	2	1	3	2	1
UST	4	3	2	4	4	2	2	2	3	2	2
UBR	5	3	3	4	4	3	1	4	3	2	2
UCU	5	3	3	4	4	3	3	3	3	2	2
UPU	5	3	3	4	4	3	2	3	3	2	2

Species (samples): *G. repens* (GRE); *P. gigantea* (PGI); *U. breviscapa* (UBR); *U. cucullata* (UCU); *U. delicatula* (UDE); *U. dichotoma* subsp. *aquilonia* (UDI); *U. oppositiflora* (UOP); *U. paulineae* (UPA); *U. purpurea* (UPU); *U. stygia* (UST); *U. tricolor* (UTR); *U. tubulata* (UTU); *U. uniflora* (UUN); *U. volubilis* (UVO)

Traits (variables, further defined in Table 3): Life form (habit) (L), stolon location (0), anchoring organ (1), leaf form (2), leaf thickness (3), stolon chloroplasts (4), stolon diameter (5), and stolon xylem (vessels / diameter) (6), stolon intercellular spaces (7), stolon external glands (8), stolon supporting tissue (9)

stolons or "rhizoids") with geotropically positive growth (see also Table 5).

In the root of *P. gigantea*, parenchyma was densely packed. Except for *U. volubilis*, all examined trap stalks and the anchor stolon of *U. multifida* showed spacious ('hollow type') aerenchyma, occupying nearly the whole interior of the organ, and leaving only a bridge of parenchyma between the vascular strand and the epidermis.

Anatomy of the central cylinder

The central cylinder was generally bordered by a uniseriate parenchyma sheath in roots and in main stolons (Figs. 1f and 2d, e, g and i), in which an endodermis (Fig. 1h, 2a-c, h) with Casparian strips (Fig. 2b, h) was visible in *U. tricolor*, *U. oppositiflora*, *U. delicatula*, and *U. stygia*. The parenchyma sheath and the thin-walled endodermis with Casparian strips did not differ greatly in shape from cortex parenchyma. In the runner

stolon of *P. gigantea*, the sheath structure was less apparent but implied by smaller parenchyma cells irregularly surrounding and interlacing the vascularized central zone (Fig. 1g).

Multivariate analyses

The results of cluster analyses of datasets I-III are presented in Table 6, showing Silhouette Scores, clusters of species, and decisive traits for the branching (clustering of species) for selected dimensionality reduction and clustering methods. A selection of multivariate analyses of datasets I-III is visualized in Fig. 3, illustrating correlations (distances) and clusters of species.

Even though UMAP is not an ideal method for cluster analysis, as the data tend to be uniformly distributed, the separation of two clusters (terrestrial-amphibious and submerged-free-floating) with graph-based clustering of dataset I was distinctive and received support by PCA (Table 6; Fig. 3a). The
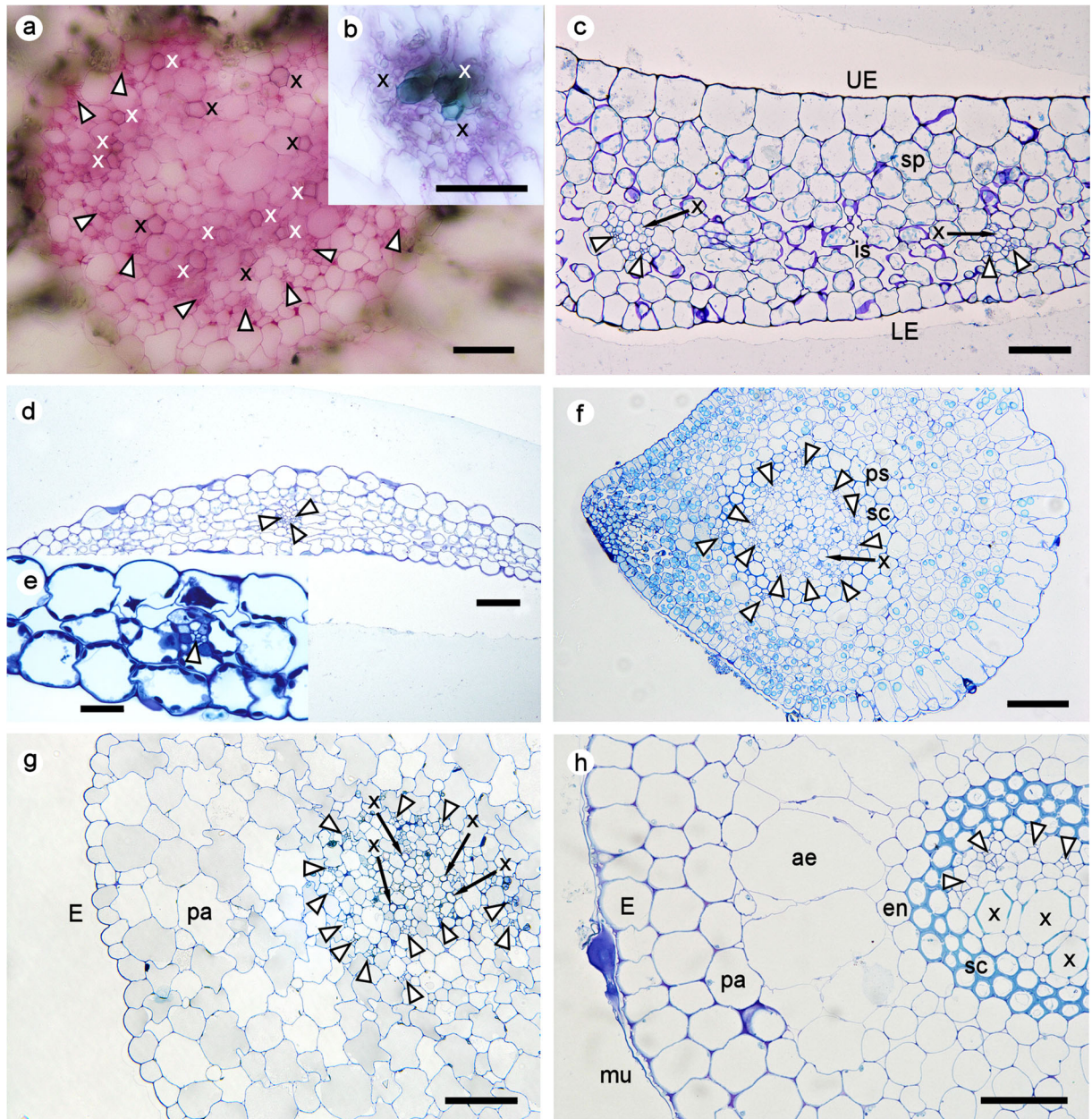
Fig. 1 Cross sections through foliage leaves and stolons of Lentibulariaceae. Main vascular strand of *P. gigantea* with (**a**) xylem tracheary elements (vessels) (x) arranged in an arc and bordered by phloem (arrowheads) and (**b**) occasionally grouped together. (**c**) *Genlisea hispidula* showing the general anatomy of leaves of *Genlisea* and *Utricularia* with upper (UE) and lower (LE) epidermis, spongy parenchyma (sp), intracellular spaces (is), and vascular bundles with xylem tracheary elements and phloem. (**d**) Vascular bundle in *U. paulineae* with three phloem groups but no visible xylem elements. (**e**) Leaflet of dissected leaves in the submerged *U. stygia* containing chloroplasts in single layered parenchyma and epidermal cells. In the vascular bundle, only

phloem but no xylem is apparent. (**f**) Runner stolon of *G. repens* exhibiting abundant amyloplasts and an ectophloic central cylinder within a sclerenchyma ring (sc), surrounded by a parenchyma sheath (ps). (**g**) Runner stolon of *P. gigantea* showing a uniseriate epidermis (E), irregularly shaped parenchyma cells (pa) and numerous phloem groups around several vessels in the centre. (**h**) Main stolon of *U. tricolor* with an epidermis covered by mucilage (mu), radially developing lysigenous aerenchyma (ae), and a central cylinder characterized by large vessels and phloem groups and bordered by 2–3 layers of conspicuous sclerenchyma cells within an endodermis (en). Scale bars = 0.1 mm in **a-d**, **f**, **g**; 0.05 mm in **h**; 0.02 mm in **e**
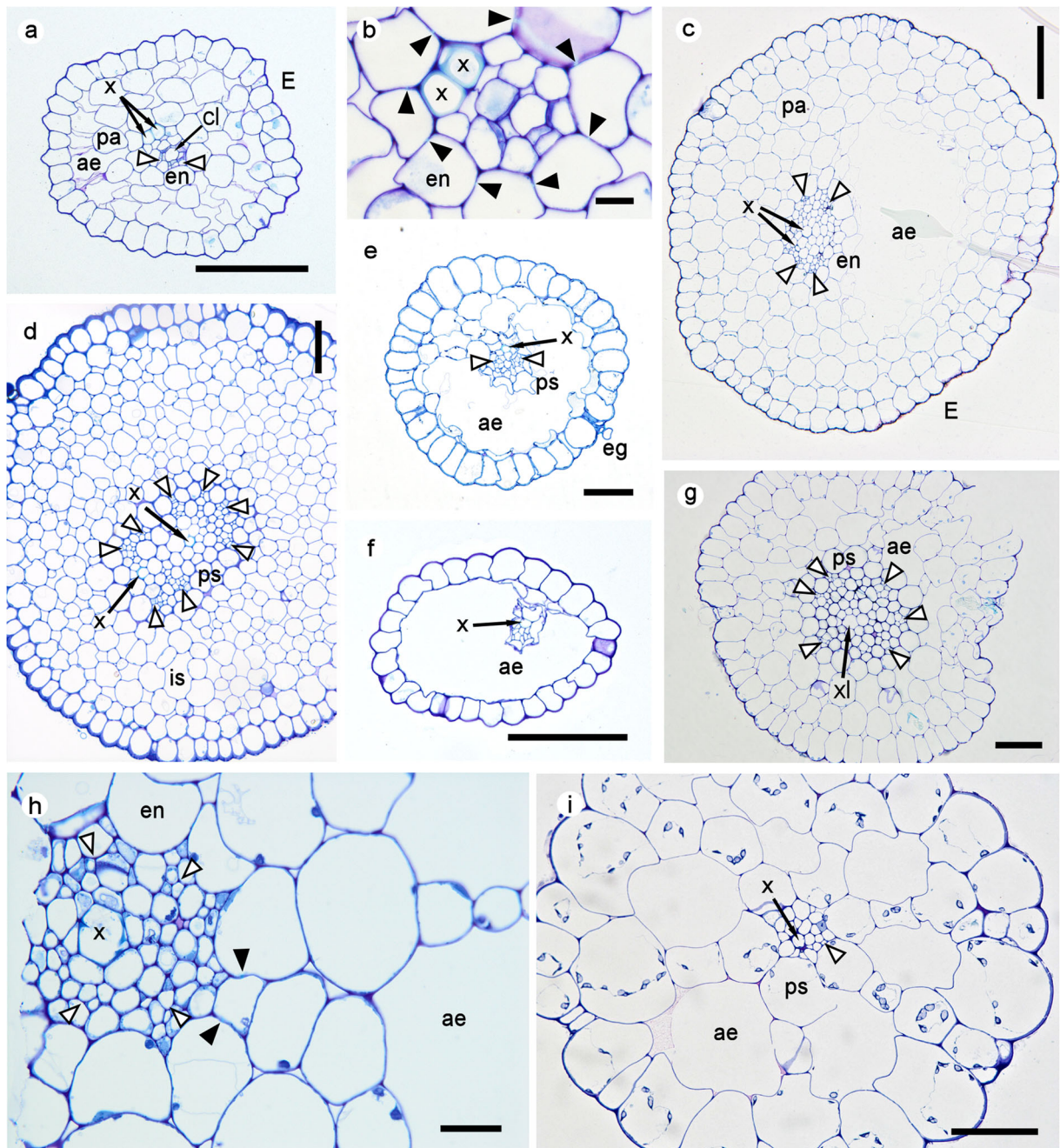
primary branching leading to these clusters were, however, mainly influenced by the two non-morpho-anatomical traits 'life form' and 'stolon locality' (Table 6). While 'life form' as trait is obviously a bias of life form clustering, 'stolon location' has an affinity to the life form (by the milieu) but also to growth and consequently to morphology. As shown by the biplots (Fig. 3a, c), 'stolon location' correlated to some extent to stolon related traits, namely to 'chloroplast occurrence' and 'xylem diameter', while the contribution of 'occurrence of external glands' was weak (Fig. 3a, c). Hence, in

◀ **Fig. 2** Anatomical structures of stolons of *Utricularia*. **a** Main stolon of *U. delicatula* with a uniseriate epidermis (E), aerenchyma (ae) between shrinking parenchyma cells (pa), and an endodermis (en) encompassing a vascular strand with two adjacent xylem tracheary elements (x) and a central lacuna (cl) in a phloem ring (arrowheads). **b** Close-up of the central cylinder of **a**, showing lignified Casparian strips (black arrowheads) in the endodermis. **c** Runner stolon of *U. oppositiflora* with half-moon shaped aerenchyma in one sector, representing a unique distribution, designated as "crescent" pattern. **d** Main stolon of *U. volubilis* containing moderate intracellular spaces (is) and a central cylinder with the appearance of a two-part parenchyma sheath (ps), accommodating a double vascular strand with one xylem tracheary element and 4 phloem groups each. **e** *Utricularia dichotoma* subsp. *aquilonia* showing extensive tangentially lysigenous aerenchyma dominating the interior of the runner stolon. **f** Main stolon of *U. uniflora* exhibiting an advanced decay of cortex cells, forming 'hollow type' aerenchyma. In the vascular tissue, phloem is indistinct and only one xylem tracheary element is visible. **g** Main stolon of *U. tubulata* with schizogenous aerenchyma and a central xylem lacuna (xl) in the vascular strand. Thick-walled cells are abundant within the pith. **h** Detailed view into the stele of a main stolon of *U. stygia* revealing an endodermis with Casparian strips encircling the vascular cylinder. **i** Main stolon of *U. cucullata* showing plastids in the epidermis and in scanty parenchyma. The aerenchyma pattern is wheel-shaped. The vascular bundle exhibits one xylem tracheary element and a sieve tube (arrowhead). Scale bars = 0.1 mm in **a**, **c**, **f**; 0.05 mm in **d**, **e**, **g**, **i**; 0.02 mm in **h**; 0.01 mm in **b**

submerged and free-floating species, main stolons tended to develop chloroplasts in parenchyma and epidermis, and smaller xylem tracheary elements.

The exclusion of the traits 'life form' and 'stolon location' led to a certain decrease of distances of clusters in PCA (compare Fig. 3a, c, d), which became also evident by lower Silhouette Scores (Table 6). The correlation of species was not extremely altered by excluding these traits from the datasets, especially because 'anchoring organ', 'leaf form' and 'leaf thickness' were directly correlated to 'life form' and 'stolon location' and, therefore, likewise contributed to the same (PC1) direction of distribution. In the PCA of all datasets, this approximately horizontal distribution followed a "life form transect" from free-floating to submerged to amphibious species towards the terrestrial *P. gigantea*.

By considering the decisive characters across the PCA of datasets I-III, it appeared that the separation of free-floating and submerged species (with or without *U. volubilis*) from other clusters was strongly influenced

by 'stolon location' or 'leaf thickness', and that free-floating species split from submerged species mainly driven by 'anchoring organ' and 'chloroplast occurrence' (Table 6).

The vertical (PC2) distribution was generally related to 'stolon thickness' with some assistance from 'stolon intercellular spaces' (Fig. 3a, c). This was mainly apparent in the amphibious group and reflected in the direction of the corresponding confidence ellipse in Fig. 3d. Being transversal to the horizontal transect, stolon thickness was not correlated to life forms. The vertical scattering of amphibious species represented a gradient of species with thin main stolons containing crescent to hollow aerenchyma (e.g. *U. uniflora*) to species with thick main stolons (e.g. *G. repens*). However, using graph-based clustering with datasets II and III generated a separation of a group around *U. delicatula* from a diffuse group around *U. tricolor* (Fig. 3b-d; Table 6). The split was diminished when a few theoretical terrestrial species similar to *P. gigantea* were added and 'stolon thickness' was omitted from the datasets. In the PCA scatter plot of the accordingly modified dataset III, *U. tricolor* was much closer to *U. delicatula*, and *U. oppositiflora* was between *U. dichotoma* and *U. paulineae*/*U. uniflora* (not shown here).

By applying Kernel PCA (KPCA) in dataset II and III, the correlations of species in the datasets differed mainly in the cluster assignment of *U. volubilis* (Table 6), which affiliated with *U. stygia* and the free-floating group or with the terrestrial-amphibious group (Fig. 3b), respectively. When data were pre-processed by unit variance scaling (leading to more or less equally strong traits), the density of clusters was increased and *U. oppositiflora* was much closer to the submerged taxa than to *U. tricolor* (e.g. Fig. 3d vs. a and c).

Discussion

Morpho-anatomical traits support life form clustering of species in Lentibulariaceae

The applied multivariate analyses show that life form clustering is acceptably supported by adding some terrestrial species and discarding the interfering character 'stolon thickness', even though the submerged group (*U. stygia*, *U. volubilis*) tends to overlap with the free-floating and/or the amphibious group. The

Table 6 Cluster analyses of datasets

Dataset (variables)	Method (Silhouette Score)	Decision tree (cluster branching) Clusters (c)	Decisive traits for cluster separation
I (L, 0, 1–9)	Graph-based clustering: UMAP (0.7), PCA (0.5)	Branching 1: S+F (c0), A+PGI (c1)	L (Gini or Entropy≤0.04) or 0 (Entropy>0.04)
	Life form clustering: PCA (0.4)	Branching 1: S+F (c0), A+PGI (c1) Branching 2: S (c0a), F (c0b), A (c1a), PGI (c1b)	L/0 L/1/4 Several
II (0, 1–9)	Graph-based clustering: KPCA (0.7), PCA (0.5)	Branching 1: S+F (c0), others (c1+c2) Branching 2: PGI, GRE, UTR, UOP (c1), UDI, UDE, UPA, UUN (c2)	0 5/6
	Life form clustering: PCA (0.2)	Branching 1: S+F (c0), A+PGI (c1) Branching 2: S (c0a), F (c0b), A (c1a), PGI (c1b)	0 1/4 Several
III (1–9)	Graph-based clustering: KPCA (0.6), PCA (0.5)	Branching 1: F+UST (c1), others (c0+c2) Branching 2: PGI, GRE, UTR, UOP, UVO (c0), UDI, UDE, UPA, UUN (c2)	3 5
	Life form clustering: PCA (0.1)	Branching 1: F+UST (c1), others (c0+c2) Branching 2: F (c1a), UST (c1b), PGI (c0a), A+UVO (c0b)	3 1/4/6 Several

Abbreviations: Kernel PCA (KPCA); Uniform Manifold Approximation and Projection (UMAP); Principal Components Analysis (PCA)

Species: *G. repens* (GRE); *P. gigantea* (PGI); *U. delicatula* (UDE); *U. dichotoma* (UDI); *U. oppositiflora* (UOP); *U. paulineae* (UPA); *U. stygia* (UST); *U. tricolor* (UTR); *U. uniflora* (UUN); *U. volubilis* (UVO)

Plant groups with the same life forms: amphiphytes (A); free-floating hydrophytes (F); submerged hydrophytes (S)

Clusters after branching, e.g.: S + F (c0) = S and F build together cluster 0. S (c0a), F (c0b) = S and F are two subclusters (a and b) of cluster 0

Decisive traits (further defined in Table 3): Life form (habit) (L), stolon location (0), anchoring organ (1), leaf thickness (3), stolon chloroplasts (4), stolon diameter (5), and stolon xylem (vessels / diameter) (6)

Traits, e.g.: 1/4 = traits 1 and 4 are more or less interchangeably decisive on the branching

datasets do not contain any emergent species. However, *U. volubilis* and *U. oppositiflora* occur as emergent hydrophytes in some natural habitats (Taylor 1989; Jobson and Baleiero 2020). As expected, both species show a strong correlation and have affinities with the amphibious as well as with the submerged habit.

Leaf traits

Our results demonstrate that leaves tend to be thinner and narrower from terrestrial to free-floating taxa. On one end of the transect, the anatomy of the fleshy *P. gigantea* leaf is largely consistent with the succulent leaves of several Mexican *Pinguicula* species of
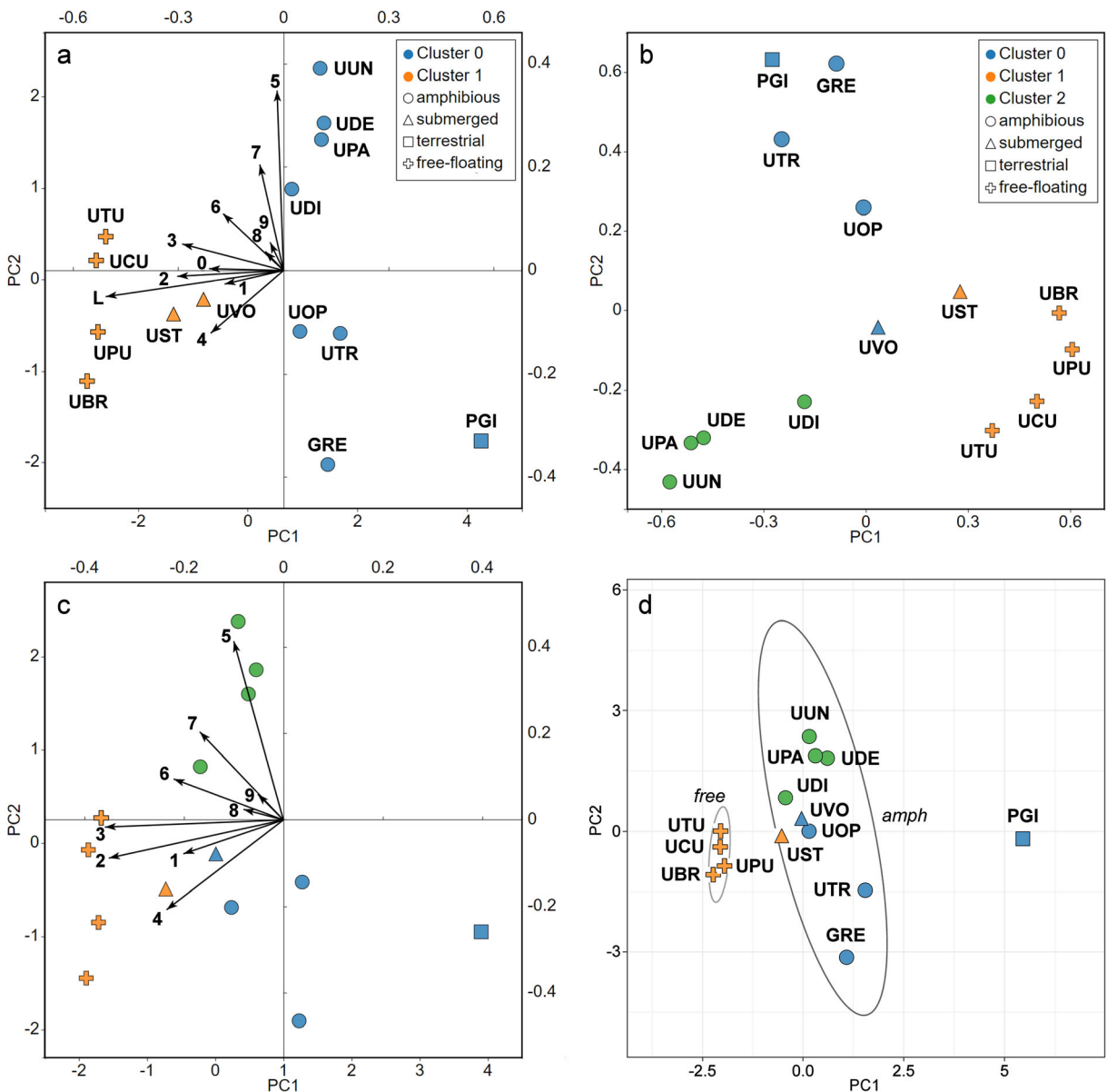
Fig. 3 Visualization of multivariate analyses with graph-based clustering of stoloniferous Lentibulariaceae species. **a** PCA biplot of dataset I, showing two clearly separated clusters. The submerged UST and UVO are between amphibious and free-floating species. Variances PC1 = 58.71 %, PC2 = 20.01 %, PC1-3 = 86.4 %. **b** Scatter plot of species according to KPCA (best fit) of dataset III with three clusters. **c** PCA biplot of dataset III with the same clusters as in **b**. PC1 = 44.38 %, PC2 = 28.30 %, PC1-3 = 82.64 %. **d** PCA scatter plot of dataset II with unit-variance scaling as pre-processing. Amphiphytes (*amph*) and free-floating hydrophytes (*free*) are circumscribed by confidence (prediction) ellipses (CI = 95 %). Both submerged species are imbedded in the

amphibious group. PC1 = 47.89 %, PC2 = 21.00 %, PC1-3 = 82.61 %. No scaling was applied to analyses **a-c**. Species: *G. repens* (GRE); *P. gigantea* (PGI); *U. breviscapa* (UBR); *U. cucullata* (UCU); *U. delicatula* (UDE); *U. dichotoma* subsp. *aquilonia* (UDI); *U. oppositiflora* (UOP); *U. paulineae* (UPA); *U. purpurea* (UPU); *U. stygia* (UST); *U. tricolor* (UTR); *U. tubulata* (UTU); *U. uniflora* (UUN); *U. volubilis* (UVO). Traits (vectors, further defined in Table 3): Life form (habit) (L), stolon location (0), anchoring organ (1), leaf form (2), leaf thickness (3), stolon chloroplasts (4), stolon diameter (5), and stolon xylem (vessels / diameter) (6), stolon intercellular spaces (7), stolon external glands (8), stolon supporting tissue (9)

subgenera *Isoloba* and *Pinguicula* that occupy wet and periodically dry habitats and may use C_4 carbon fixation (cf. Studnička 1991). On the other end, in line with e.g. Sculthorpe (1967) and Colmer et al. (2011), our observations on Lentibulariaceae confirm that obligate hydrophytes differ distinctively from emergent, amphibious or terrestrial herbaceous plants by having thin leaves.

Free-floating aquatics are limited to sections *Utricularia* and *Vesiculina* (both of subgenus *Utricularia*) and *U. tubulata* of subgenus *Polypompholyx* (Taylor 1989). The two former sections carry leaves (if present) divided into capillary segments, whilst the morphologically unique *U. tubulata* shows verticillate and undivided leaves (Taylor 1989), reminding of e.g. the submerged and freely suspended *Hydrilla verticillata* (cf. Yeo et al. 1984) or the carnivorous free-floating *Aldrovanda vesiculosa* with terminal traps on its leaves (cf. Adamec 2018). A combination of dissected leaves and a verticillate phyllotaxy is known from e.g. the free-floating (rarely submerged) *Ceratophyllum*, which is also considered to be rootless (Sculthorpe 1967; Cook 1999).

Our results corroborate that narrow and thin leaves are common features in amphibious and emergent plants as adaptive response to submergence, as shown in e.g. *Rumex palustris* (Mommer et al. 2005) and *Melaleuca cajuputi* (Tanaka et al. 2011). In the aquatic environment, the absorption of light and dissolved gases is enhanced by an increased surface area to volume ratio, which is accomplished by thinner, lobed, and dissected leaves (e.g. Sculthorpe 1967; Colmer et al. 2011).

Chlorophyllous tissues

In accordance with our results, simple anatomical structures without a palisade layer are a common characteristic of submerged leaves (e.g. Wiegleb 1991; Catian and Scremin-Dias 2015; Krähmer 2016), indicating organ flexibility and/or reduced light conditions (cf. Sculthorpe 1967; Gotoh et al. 2018). A diminished photosynthesis rate is partly compensated by chloroplasts occurring in both parenchymatic and epidermal cells of submersed organs (Catian and Scremin-Dias2015), as indicated in our study by e.g. *U. stygia* and *U. cucullata*. Results of our multivariate analyses corroborate that aquatic life forms relate to a higher abundance of chloroplasts in tissues of submerged runner stolons (cf. Sculthorpe 1967; Wetzel 1988; Colmer et al. 2011). However, responding to (periodic) submergence or shady habitats, chloroplasts are occasionally observed in the epidermis of leaves (*Genlisea* sp.; Lang 1901) or in runner stolons (e.g. *G. repens*) of amphibious species, although in *G. repens*, plastids may develop into amyloplasts under drier conditions. In *Pinguicula*, the absence of a chloroplast-rich palisade parenchyma may provide more flexibility to the leaf for carnivorous mechanisms (Heslop-Harrison 2004).

Anchoring organs

In the Lentibulariaceae, *Pinguicula* still shows "typical" roots (although partly reduced in some species such as *P. gigantea*). In *Utricularia*, "rhizoids" (anchor stolons) seem to take on the anchoring function from roots. Our analyses show that the presence/absence of anchor stolons is a delimitation between submerged and free-floating life forms in *Utricularia*. However, the base of peduncles in some suspended *Utricularia* species (e.g. *U. australis*, *U. gibba*) develop rhizoids (Taylor 1989), which may anchor the plant when flowering.

Structures of absorption and secretion

In the absence of an effective root system, the surfaces of other organs adopt the uptake of water and nutrients (cf. Schenck 1886; Adlassnig et al. 2005; Babourina and Rengel 2010). As in most submerged hydrophytes, the cuticle of organs of the examined species seems to be generally thin, since the mechanical protection is of less importance in the water. On the outer surface of all vegetative organs across the examined *Genlisea* and *Utricularia* species, we found numerous button-like trichomes, which were considered being hydropotes (Lloyd 1942; Płachno et al. 2005) with absorptive and/or secretory function, depending on their ontogeny (Fineran 1980). On traps of *Utricularia* and *Genlisea*, mature external glands seem to expel water to the substrate (Fineran 1985; Fineran and Reut unpublished data), while some glandular trichomes on leaves and subterranean organs of *Genlisea* and *Utricularia* produce mucilage, e.g. to protect from drying, as defensive compound against herbivores, as source of nutrients for micro-organisms, or as lubricant for penetrating the soil (cf. Lang 1901; Płachno et al. 2005; Rivadavia et al. 2013). Since only *Pinguicula* lacks external bottom-like trichomes on vegetative organs, the occurrence of these glands does only marginally contribute to the life form clustering in the present study.

Further histochemical and ultrastructural work (including tracers) on the epidermis, cuticle, and external glands of *Genlisea* and *Utricularia* of various habits would be helpful to increase the knowledge on permeability and the various functions of external glands in relation to their position on the organ and their developmental stages.

Conductive tissues

A general simplification of xylem tissue in subterranean and submersed organs was evident in the species studied. However, in the aquatic environment, the reduction of xylem and lignified elements does not necessarily impede the acropetal flow of water and nutrients (Sculthorpe 1967; Pedersen and Sand-Jensen 1993; Lusa et al. 2011). Moreover, in some main stolons of *Utricularia*, we were able to identify an endodermis with Casparian strips (CS), which directs and controls the passage of water and minerals (cf. Barberon 2017). Although an endodermis with CS mainly occurs in roots, it is not uncommon in aerial shoots and leaves (Lersten 1997; Seago 2020) but more often established in subterranean or submersed stems (Dalla Vecchia et al. 1999). In the "rootless" bladderworts, the conductive function of the endodermis has been seemingly transferred from roots into stolons that manifest dynamic developmental processes for roots, shoots, and leaves (Rutishauser 2016, 2020; Reut and Płachno 2020).

Due to the thin cell walls of the parenchyma sheaths, the capability to visualize the CS was limited. Therefore, even if the CS could not be detected, the presence of CS cannot be conclusively ruled out for the remaining species of the study. Other staining and fluorescence techniques should be applied to improve the identification of CS and to gain insights into their composition (see e.g. Dalla Vecchia et al. 1999; Naseera et al. 2012; Seago 2020).

Aerenchyma

To enhance the exchange of gases within and between roots, stems, and leaves, plants develop intercellular cavities or chambers of various sizes (Takahashi et al. 2014), whereby the enlarged space is called 'aerenchyma' (Seago et al. 2005; Jung et al. 2008), which additionally facilitates buoyancy (Krähmer 2016). Although aerenchyma is typical for hydrophytes, it is also known from some terrestrial plants caused by several forms of

stress (Evans 2003; Jung et al. 2008). The morphoanatomical differences between emergent and submerged taxa studied reflect a big step from slight or periodic inundation to complete and permanent submergence. Colonization of habitats with standing water (e.g. swamps, pools, lakes) has evolved with the abundance of large air cavities in submerged organs, supporting an efficient diffusion of gases (cf. Wetzel 1988).

Lysigenous aerenchyma results from programmed cell death and cell collapse, which can be induced by hypoxia, nutrient deficiency and/or ethylene accumulation (Evans 2003; Striker 2012). Since lysigenous aerenchyma formation is dominant in the material studied, these factors may all be present in the preferred habitats of *Genlisea* and *Utricularia*, in which their carnivorous strategy (partly) compensates the limited availability of certain nutrients (cf. Adamec 1997). Lysigenous aerenchyma was also reported by Schweingruber et al. (2020) from stems of submerged *Utricularia* species and the carnivorous free-floating *Aldrovanda vesiculosa*.

Our results indicate that the 'wheel-shaped' aerenchyma pattern is abundant in main stolons of submerged and free-floating *Utricularia* species, which is supported by earlier observations on shoots of *U. tenuicaulis* (syn. *U. australis*) and *U. gibba* of section *Utricularia* by Jung et al. (2008) and Chormanski and Richards (2012), respectively. Within the same order Lamiales, it was reported from the submerged *Limnophila sessiflora* by Jung et al. (2008).

The unique 'crescent' type aerenchyma, observed in runner stolons of *U. oppositiflora*, seems to be due to the dorsiventral symmetry of these stolons with organs arising on nodes on the upper side of the runner (cf. Reut and Płachno 2020). The continuing decay of parenchymatic cells (seemingly by tangential lysigeny) leads to a special 'hollow type' aerenchyma, which is evident in several subterranean organs of the examined amphibious species of *Genlisea* (see also Lloyd 1942) and *Utricularia* subg. *Polypompholyx*.

Schizogeny, as visible in main stolons of *U. volubilis* and *U. tubulata* of section *Pleiochasia*, is developed by cell differentiation, but molecular mechanisms substantial for this formation type are not yet known (Yamauchi et al. 2018).

Supporting tissues

Our results confirm earlier observations on *Genlisea* (Merl 1915) and *Utricularia* (Schenck 1886; Kamieński

1891), whereupon the anatomy of thicker stems is characterized by a central cylinder and by an increasing number of vascular elements and the occurrence of supporting tissue, as indicated by sclerenchyma around the pith in runner stolons of *G. repens* and *U. tricolor*. Sclerenchyma was also reported below the epidermis of adhesive anchor stolons of the rheophytic *U. neottioides* by Płachno et al. (2020a). However, since only two amphibious species in our dataset showed sclerenchyma around the pith in runner stolons, this trait did not contribute greatly to the delimitation of life forms in the multivariate analyses.

Evolutionary aspects of the Lentibulariaceae

With respect to the morpho-anatomical features, our findings reveal that the reduction of roots and conductive tissues (especially xylem) are taxonomically common characteristics of all genera of the Lentibulariaceae, which points to an ancestor that was adapted to at least seasonally wet and/or periodically inundated habitats. The more free water is abundant, the less protection and stability of organs is required, which is apparently reflected in a loss of the root cap in some *Pinguicula* species (cf. Rutishauser 2016, 2020), the palisade layer in leaves, and supporting tissues in subterranean/submersed organs within the Lentibulariaceae.

Apart from an abandonment of several developmental processes for roots, *Genlisea* and *Utricularia* share other more hydrophilic features, such as (in general) more delicate organs with reduced cuticle and button-like glandular trichomes on the surface of leaves and subterranean/submersed organs, indicating a next level in the adaptation to submergence but also a shift of the carnivory from aerial leaves (in *Pinguicula*) to underground leaf-like organs (in *Genlisea-Utricularia*). In other words, costs for innovative structures used for the uptake of nutrients are compensated by less investment in other structures (e.g. cortex, vascular elements and supporting tissue), which seems to be facilitated in a watery milieu.

Most of the plants in our study were kept ex situ in their preferred conditions, and hence revealed more adaptive rather than reactive morpho-anatomical features. We recommend that future research on hydrophytes with a comparable plasticity takes the differentiation of emergent and submerged life forms into consideration. An experimental approach with variable settings (e.g. water levels, light) could unlock which morpho-anatomical changes are adaptations and which ones are responses.

Morpho-anatomical and functional adaptations to a life between land and water

The key findings of our study are:

– Life forms along the hydric transect in the Lentibulariaceae correlate directly to the location of the stolon in the substrate and to the shape and thickness of leaves, which aims at enhancing the absorption of sparse light, nutrients, dissolved gases as well as the flexibility of leaves with increasing depth of submergence of vegetative organs.

– Submerged and free-floating *Utricularia* species tend to develop chloroplasts in parenchymatic and epidermal cells of suspended main stolons, which supports the collection of light.

– Life forms in the Lentibulariaceae correspond with the reduction of the typical root morphology and with the presence and type of anchorage.

– Buoyancy and gas exchange are enhanced by large intercellular cavities that are abundant in stems and shoots of amphibious to free-floating taxa studied. Novel 'crescent' to special 'hollow' aerenchyma patterns develop in dorsiventrally organized stems and shoots of amphibious *Genlisea* and *Utricularia* species.

– Morpho-anatomical characters in vegetative organs of *Genlisea* and *Utricularia* strongly show adaptations to the hydric environment, which they share with many other aquatic plants. However, some of these features may have been partly driven by the evolutionary shift of carnivory from aerial to subterranean organs and hence by an alternative uptake of certain minerals in nutrient-poor and submerged habitats.

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