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Vascular tissue in traps of Australian carnivorous bladderworts (*Utricularia*) of the subgenus *Polypompholyx*

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ABSTRACT

Utricularia (bladderworts) are rootless carnivorous plants forming small suction traps which are hollow discoid bladders. There is some controversy surrounding the understanding of trap vascularization in *Utricularia* species and most of the knowledge in the literature is based on aquatic *Utricularia* from section *Utricularia*. In this study, we investigated trap vascularization in 9 *Utricularia* species or clones from the subgenus *Polypompholyx* using several light microscopy staining techniques. Both xylem and phloem elements were found in the traps of all investigated species or clones. The pattern of trap vascular bundles from the subgenus *Polypompholyx* was similar to that reported for subgenus *Bivalvaria,* but different from that of aquatic *U. vulgaris* from the subgenus *Utricularia*. The system of trap vascularization in the members of the subgenus *Polypompholyx* was different from that found in the traps of *Genlisea*, which is a closely related genus (both Lentibulariaceae). The structure of trap vascular bundles was, however, similar in *Genlisea* and *Polypompholyx*. Possible utilization of xylem elements in *Utricularia* traps is discussed.

1. Introduction

In most carnivorous plant traps, vascular bundles or their elements (tracheids) are closely associated with digestive glandular structures (e.g., Heslop-Harrison, 1975; Parkes, 1980; Juniper et al., 1989; Owen and Lennon, 1999; Płachno et al., 2007). In some carnivorous genera, vascular elements are even components of digestive glands; e.g., tracheids and associated phloem occur in the stalked glands (tentacles) in *Drosophyllum* and *Triphyophyllum*, and tracheids occur in stalked glands of *Drosera* (Green et al., 1979; Juniper et al., 1989). This close association is inter alia due to the tracheal elements transporting water needed for producing both digestive fluid and mucilage (where glands produce both substances). Additionally, vascular bundles also play an important role in nutrient translocation from the place of prey-derived nutrient absorption to other trap parts and plant organs. This is well documented in some carnivorous genera (e.g., Heslop-Harrison and Knox, 1971; Owen et al., 1999; Schulze et al., 1999).

The suction traps of *Utricularia* are hollow, discoid bladders ca. 1–6 mm long and usually have walls two cell-layers thick (Lloyd, 1942; Płachno et al., 2015). *Utricularia* traps capture a wide range of invertebrates as prey but they also house other commensal microorganisms (e.g. bacteria, protozoa, algae), which may form a miniature food web (e.g. Sirová et al., 2009; Płachno et al., 2012 and the references cited therein).

In traps of aquatic *Utricularia* species (trap type *Utricularia vulgaris*), a single vascular bundle traverses the stalk into the trap body and later branches into two bundles (Lloyd, 1942). One of them runs along the dorsal side of the trap and continues to the upper part of the entrance (trap door). The other runs up to the threshold and finally branches into two bundles. According to Poppinga et al. (2016), these bundles run laterally, then upwards and parallel to the trap opening, and terminate in the antennae. The vascular bundle consists mostly of phloem and sometimes xylem. However, in contrast with these results, Slinger (1954) observed only one vascular bundle in the *Utricularia livida* trap. This bundle traverses the stalk into the trap body and later runs along the dorsal side of the trap up to the upper part of the entrance. A similar observation was made by Compton (1909) in *U. brachiata*. Detailed knowledge of *Utricularia* trap vascularization is essential to fully understand the trap physiology, including: digestive enzyme secretion, nutrient transport from digested prey and also water pumping from the traps. Moreover, Sirová et al. (2010, 2011) found that aquatic *Utricularia* shoots supplied a great amount of photosynthetic carbon to the

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trap fluid for supporting the microbial commensals. All of these extensive processes require the functions of the trap vascular tissue.

Müller et al. (2006) proposed classifying *Utricularia* species into three subgenera: *Polypompholyx*, *Bivalvaria* and *Utricularia*. The most basal in the evolutionary sense (with "primitive" characteristics occurring) are species from the subgenus *Polypompholyx*. As mentioned above, there is only fragmented knowledge on vascular tissue in *Utricularia* traps and the existing knowledge is confined to a few species: *U. vulgaris* – sect. *Utricularia,* subgenus *Utricularia; U. brachiata* – sect. *Phyllaria,* subgenus *Bivalvaria; U. livida* – sect. *Calpidisca,* subgenus *Bivalvaria*. There are some differences between the trap anatomy and physiology of species from different subgenera (e.g., Reifenrath et al., 2006; Adamec, 2011; Płachno et al., 2015Adamec and Poppinga, 2016). Thus, are there differences in trap vascularization between the subgenera, too?

The aim of this study was to investigate the trap vascularization in nine clones from seven *Utricularia* species from the subgenus *Polypompholyx* using several light microscopic methods. Special attention is paid to the occurrence of xylem elements.

2. Materials and methods

2.1. Plant material

The following species from the subgenus *Polypompholyx*, section *Pleiochasia,* were used: *Utricularia volubilis* R.Br. (from SW Australia), *U. dichotoma* Labill. (clone from the Botanic Garden of Jagiellonian University/BGUJ/in Kraków, Poland), *U. dichotoma* (robust clone from Newcastle, N.S.W., Australia), *U. dichotoma* (smaller clone from Katoomba, N.S.W.), *U. cf. beaugleholei* Gassin, *U. uniflora* R.Br., *U. paulineae* Lowrie, *U. inaequalis* A.DC. and *U. menziesii* R.Br. From the section *Polypompholyx*: *U. multifida* R.Br., and from *Tridentaria*: *U. westonii* P.Taylor. These species were cultivated in the Institute of Botany of the Czech Academy of Sciences at Treboň, the Prague Botanical Garden, Czech Republic, the BGUJ in Kraków, or in the private collection of Kamil Pásek in Ostrava, Czech Republic (http://www.bestcarnivorousplants.net/).

2.2. Methods

Three different microscopic techniques were used for the anatomical studies; the terminology of trap morphology follows Taylor (1989), Reifenrath et al. (2006) and P & achno et al. (2015).

2.2.1. The clearing technique

For the clearing technique, whole or halved mature traps were fixed in FAA (40% formaldehyde/glacial acetic acid/70% ethanol, 5:5:90, v/v) for 48 h and stored in 70% ethanol. The traps were then dehydrated in 70%, 80%, 90% ethanol (one change) and 100% ethanol (three changes) for 1 h and incubated for 1.5 h in one change of 1:1 ethanol/methyl salicylate, one change of 1:3 ethanol/methyl salicylate and two changes of 100% methyl salicylate (Young et al., 1979; Płachno et al., 2015). Cleared traps were examined using a Nikon Eclipse 80i or Olympus BX60 light microscope, both equipped with Nomarski interference contrast optics.

2.2.2. Technovit method

Traps were also fixed in 5% buffered (0.1 M phosphate buffer, pH 7.2) glutaraldehyde at room temperature for 2 h, washed in the same buffer four times, dehydrated in a graded ethanol series at each concentration step for 15 min and kept overnight in absolute ethanol. Later, the samples were treated with 3:1, 1:1, and 1:3 (v/v) mixtures of absolute ethanol and Technovit (Technovit 7100 2-hydroxyethyl-methacrylate; Heraeus Kulzer) for 1 h each and then stored in pure Technovit for 12 h. The resin was polymerized with the addition of the hardener. The material was sectioned to 5 μm with a rotary microtome (Microm, Adamas Instrumenten), stained with 0.1% toluidine blue O (TBO), mounted in Entellan synthetic resin (Merck) and analyzed using a Nikon Eclipse 80i microscope.

2.2.3. Epoxy resin method

Part of the material was also fixed in 2.5% formaldehyde and 2.5% glutaraldehyde in a 0.05 M cacodylate buffer (pH 7.0) for 2 days. The material was postfixed in 1% OsO_4 in a cacodylate buffer at 4 °C for 24 h, rinsed with the same buffer, treated with 1% uranyl acetate in distilled water for 1 h, dehydrated with acetone and embedded in an Epoxy Embedding Medium Kit (Fluka). Semi-thin sections (700 μm) were stained with methylene blue and examined using an Olympus BX60 microscope. Sections were cut at 70 nm for transmission electron microscopy (TEM) using a Leica ultracut UCT ultramicrotome, stained with uranyl acetate and lead citrate and examined using a Hitachi H500 transmission electron microscope at an accelerating voltage of 75 kV.

2.2.4. Evaluation of data

For all methods, typical images are always presented. For each species, 10–30 traps originating from different adult stolons/rhizoids of 2–4 different plants were generally used. The parameters such as trap height, width, their ratio, thickness of trap wall, and the diameter of vascular bundles and tracheal elements in the dorsal trap wall position (see Fig. 1), were estimated for these traps (cf. Płachno et al., 2015). The clearing technique allows observations of internal tissue structure (e.g. vascular elements; Supplementary Fig. 1). Obtained photographs, showing longitudinal cross section of the traps, were analyzed with the use of NIS-Elements Basic Research software (Nikon). Trap width and height were estimated as the distance between the two most distant points of the trap body. Trap wall lengths were measured from the peduncle up to the trap door hinge and the threshold for dorsal and ventral part, respectively. Trap wall thickness was measured along the dorsal and ventral walls on the cross section: 10 separate measurements were taken along each trap wall of 10–30 traps. The diameter and penetration of vascular elements within trap walls were also estimated in cleared traps. The measurements were made similarly to those of the trap wall length and thickness.

Linear regression models were used for the whole dataset of 9 species or clones (n = 266) and also for each species or clone (except for *U. dichotoma* small clone; n = 10-30) to determine the statistical significance of relationships between trap sizes and the diameter of vascular bundles or tracheal elements. Means ± SD intervals and the range of values are shown.

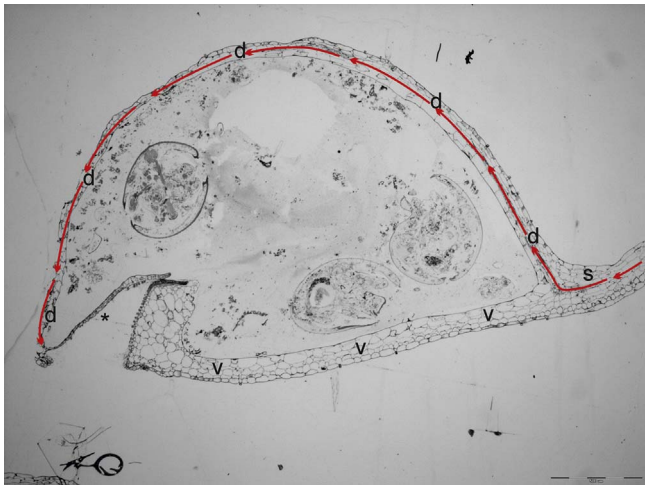

Fig. 1. Longitudinal section through *U. volubilis* trap showing trap anatomy and occurrence of vascular bundle: D – dorsal part; V- ventral part; * – entrance, arrow – vascular bundle, s – stalk, bar = 500 μm.

Table 1
Results of the anatomical study on *Utricularia* traps. The results of direct measurements are combined with data on maximal trap width from Taylor (1989). For explanation of anatomical parameters see the text. Means ± SD interval are shown; n = 10–30. For some parameters, the range of values (in italics in the lower line) is also shown.

Section	Species	Habitat	Trap shape	Trap opening position	Trap width[a] (mm)	Trap height (mm)	Trap width (mm)	Trap W:H ratio	Dorsal trap wall length (mm)	Ventral trap wall length (mm)	D:V trap wall length ratio	Dorsal trap wall thickness (µm)	Ventral trap wall thickness (µm)
Poly-pom-pholyx	*U. multifida*	Terr.	Ovoid, triangular in transverse section	–	2–2.5	–	–	–	–	–	–	80 ± 18.1 *(76–105)*	120 ± 20.0 *(102–140)*
Triden-taria	*U. westonii*	Terr.	Ovoid, triangular in transverse section	Basal	3–4	1.7 ± 0.15	1.8 ± 0.11	1.06	4.0 ± 0.80	0.5 ± 0.05	8.0	75 ± 17.0 *(72–130)*	80 ± 4.0 *(69–89)*
Pleiochasia	*U. beaugleholei*	Terr.	Globose, ovoid	Lateral	N.M.	1.6 ± 0.99	2.1 ± 0.26	1.31	3.0 ± 0.08	0.8 ± 0.07	3.5	110 ± 4.1 *(105–120*	105 ± 5.0 *(96–108)*
	U. dichotoma	Terr.	Globose, ovoid	Lateral	1–5	1.3 ± 0.19	1.5 ± 0.25	1.17	3.2 ± 0.53	0.8 ± 0.13	4.2	110 ± 12.7 *(92–140*	130 ± 25.7 *(75–176)*
	U. dichotoma, small clone, Katoomba	Affix. aquat.	Globose, ovoid	Lateral	N.M.	–	–	–	–	–	–	82 ± 15.0 *(76–125)*	86 ± 18.3 *(85–87)*
	U. dichotoma, robust clone, Newcastle	Affix. aquat.	Globose, ovoid	Lateral	N.M.	1.9 ± 0.34	2.5 ± 0.52	1.34	4.2 ± 0.83	1.3 ± 0.27	3.2	104 ± 35.5 *(70–200)*	113 ± 31.6 *(107–139)*
	U. inaequalis	Terr., affix. aquat.	Obliquely ovoid, polymorphic	Lateral	1–6	1.5 ± 0.28	2.2 ± 0.64	1.46	3.0 ± 0.80	1.2 ± 0.40	2.5	100 ± 18.7 *(98–136)*	105 ± 26.0 *(93–140)*
	U. menziesii	Terr.	Obliquely ovoid	Terminal	0.6–1.6	1.4 ± 0.24	2.4 ± 0.64	1.70	3.6 ± 0.77	2.1 ± 0.37	1.7	194 ± 58.0 *(108–250)*	190 ± 39.8 *(96–233)*
	U. paulineae	Terr.	Globose	Basal	N.M.	0.7 ± 0.16	0.8 ± 0.15	1.09	1.7 ± 0.30	0.2 ± 0.04	7.7	65 ± 15.8 *(54–92)*	80 ± 17.7 *(67–110)*
	U. uniflora	Terr.	Subulate to narrowly deltoid	Basal	0.8–1.5	0.7 ± 0.15	0.8 ± 0.17	1.16	1.7 ± 0.39	0.3 ± 0.05	5.8	60 ± 18.1 *(54–110)*	70 ± 24.7 *(50–120)*
	U. volubilis	Affix. aquat.	Obliquely broadly to narrowly ovoid, polymorphic	Lateral	1–6	1.3 ± 0.50	1.9 ± 0.80	1.49	2.7 ± 0.90	1.3 ± 0.50	2.0	105 ± 30.9 *(60–190)*	140 ± 31.8 *(100–215)*

Abbreviations used in the table: H – height, W – width; D – dorsal, V – ventral; Terr. – terrestrial, Affix. aquat. – affixed aquatic; N.M. – not mentioned.

[a] Data according to Taylor (1989).

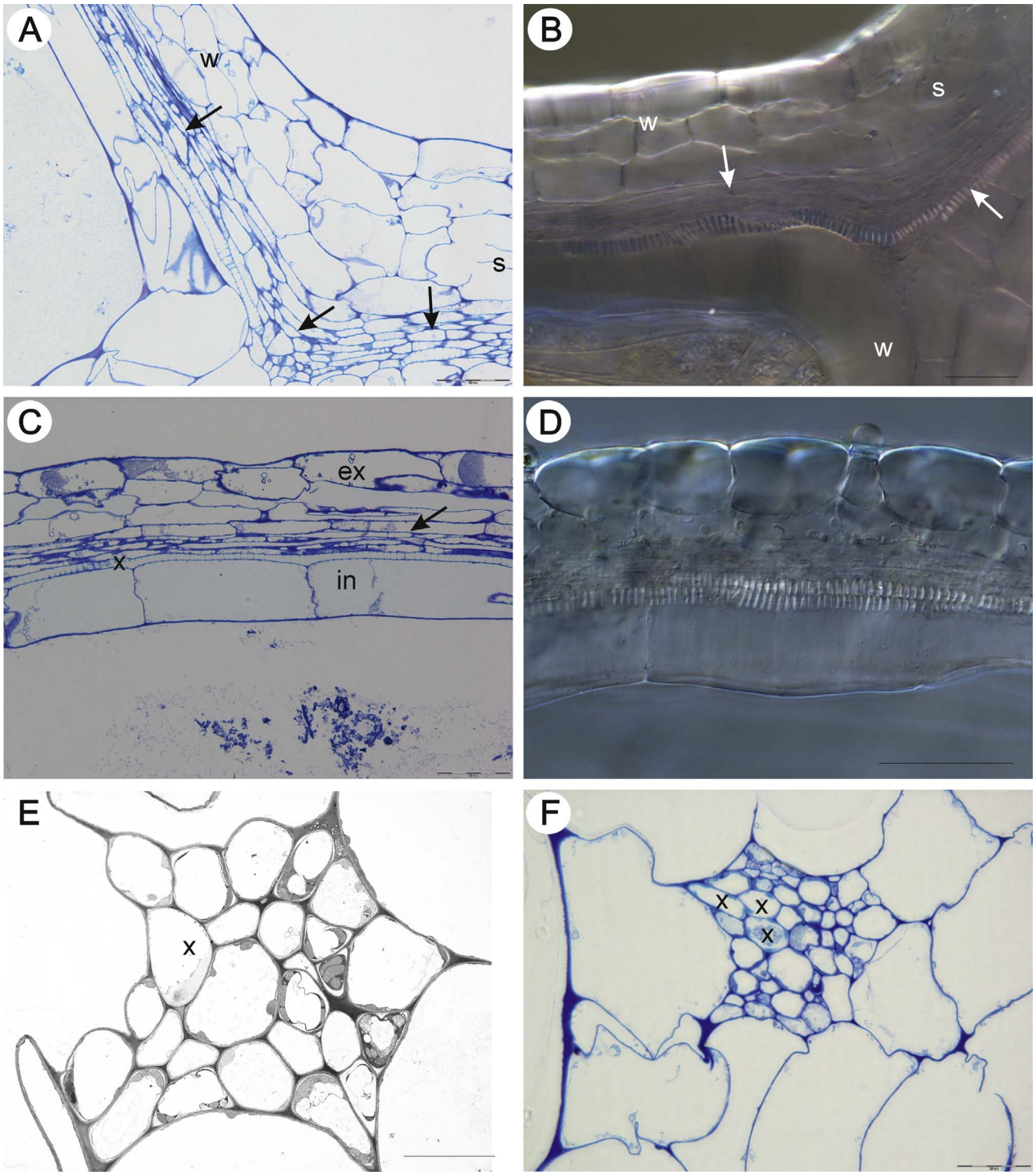

Fig. 2. Vascular bundle occurrence and anatomy of traps of *U. volubilis*: (A-B) Passage of the vascular bundle from the stalk into the trap body: w – trap wall, s – stalk, arrow – vascular bundle, bar = 50 μm. (C) Longitudinal section through the trap in the dorsal line of the trap; arrow – vascular bundle, x – xylem tracheid, ex – external trap epidermis, in – internal trap epidermis, bar = 50 μm. (D) Trap wall with vascular bundle after using clearing technique, note the occurrence of two tracheids in the vascular bundle, bar = 50 μm. (E) An electronogram showing a transverse section through vascular bundle in the trap wall, x – xylem tracheid, bar = 10 μm. (F) A transverse section through the vascular bundle in the trap wall, note three xylem tracheids (x), bar = 20 μm.

3. Results

The trap size parameters are shown in Table 1. In all species, both tracheal elements and phloem were detected in the trap bundle (Figs. 1 and 2, Supplementary Figs. 1 and 2). With the exception of *U. paulinae* and *U. uniflora* which had a much reduced vascular bundle length within the traps (only 0–6%), the vascular bundle in all other *Utricularia* species from the subgenus *Polypompholyx* penetrated up to the trap entrance (Table 2). The mean diameter of the vascular bundles was only 13–14 μm in *U. paulinae* and *U. uniflora*, but was the largest (31 and 28 μm) in *U. menziesii* and *U. dichotoma* (robust clone). Regression analyses between trap size and/or trap wall thickness and vascular bundle diameter for the whole data set revealed highly significant ($P < 0.00001$) linear correlations between these parameters (Fig. 3) and the linear correlation between the diameter of tracheal elements and vascular bundles was also highly significant ($n = 204$, $r^2 = 0.19$,

Table 2
Results of the anatomical study on *Utricularia* traps.

Species	Vascular bundle structure		
	Tracheal elements/Phloem penetration along dorsal trap wall (%)[a]	Vascular bundle diameter (μm)	Tracheal elements diameter (μm)
U. westonii	100	27 ± 8.4 *(22–29)*	6.1 ± 1.1 *(5–7)*
U. beaugleholei	100	20 ± 5.5 *(11–23)*	5.4 ± 1.2 *(3–7)*
U. dichotoma	100	24 ± 4.7 *(16–33)*	5.7 ± 2.2 (2–11)
U. dichotoma, small clone, Katoomba	100	20 ± 5.0 *(15–30)*	5.2 ± 1.8 *(3–13)*
U. dichotoma, robust clone, Newcastle	100	28 ± 8.2 *(14–48)*	6.9 ± 1.9 *(3–10)*
U. inaequalis	100	24 ± 5.5 *(14–30)*	7.4 ± 1.6 *(5–10)*
U. menziesii	100	31 ± 5.5 *(28–35)*	7.2 ± 2.0 *(5–8)*
U. paulineae	0–6	13 ± 4.8 *(6–26)*	5.3 ± 1.8 *(3–8)*
U. uniflora	0–5	14 ± 5.6 *(6–32)*	3.3 ± 1.0 *(2–5)*
U. volubilis	100	23 ± 7.6 *(14–43)*	6.8 ± 3.1 *(3–9)*

Vascular bundles in the trap were investigated in the dorsal trap position. In all species, both tracheal elements and phloem were detected in the bundle. Means ± SD interval and the range of values (in italics) are shown where possible; n = 10–30.

[a] Percentage shows how far tracheal elements or phloem cells penetrate the dorsal part of a trap wall (from stalk up to the trap entrance).

$P < 0.0001$; data not shown). However, only several regression models calculated separately for each species (n = 10–30) indicated significant correlation ($P < 0.00001$–0.022) between these parameters (data not shown). Trap wall thickness positively correlated with vascular bundle diameter in *U. dichotoma* (robust clone) and *U. volubilis*, the latter parameter correlated with trap size in *U. dichotoma* (basal and robust clone), *U. uniflora* and *U. volubilis*, while the diameters of tracheal elements and vascular bundles only correlated in the latter species.

In all examined species regardless of their taxonomic position and for all used techniques, only one vascular bundle was observed in the trap (Figs. 1 and 2, Supplementary Figs. 1 and 2). This bundle traverses the stalk into the trap body, later runs along the dorsal side of the trap and continues up to the upper part of the entrance (Fig. 1A, Supplementary Fig. 1A). In most species, xylem vessels occur individually (Supplementary Fig. 1C, E, F and H) but rarely in pairs (Fig. 2D). In some robust traps of *U. volubilis*, three vessels were observed exceptionally on the cross-section of the bundle (Fig. 2F). In all species, xylem vessels have spiral or annular thickening of their walls (Supplementary Fig. 1). A thin-walled group of cells consisting of parenchyma cells and phloem elements occurs near to the xylem tracheal elements (Fig. 2F). In *U. inaequalis,* a small vessel was observed between the epidermal cells of the inner trap epidermis (Supplementary Fig. 1I).

In *U. uniflora* and *U. paulineae,* vessels occur in the vascular bundle in the trap stalk and in the place where the bundle traverses from the stalk into the trap body (Supplementary Fig. 2). In the dorsal part of the traps of these species, the bundle does not contain xylem tracheal elements (Supplementary Fig. 2F).

4. Discussion

The pattern of trap vascular bundles of species of the subgenus *Polypompholyx* found in this study was similar to that described in the literature for species in the subgenus *Bivalvaria* (Compton, 1909; Slinger, 1954), but different from that in *U. vulgaris* from the subgenus *Utricularia* (Lloyd, 1942; Poppinga et al., 2016). The pattern of vascular bundles in traps could be associated with taxonomical position; however, more data (from different species of various sections of the three subgenera) are needed to prove this. The pattern of vascular bundles in traps could also be associated with different trap anatomy, physiology, or biophysics and/or the different life form and ecophysiological traits of the studied species rather than with taxonomic differences per se (e.g., Adamec, 2013; Płachno et al., 2015; Adamec and Poppinga, 2016). There are, after all, aquatic, epiphytic, terrestrial and lithophytic species of Utricularia (Taylor, 1989). Out of all of the species investigated for trap vascular bundles here or in the literature so far, only *U. vulgaris* (section *Utricularia*) and *U. volubilis* (section Pleiochasia) are strictly submerged aquatic species. The others are mostly amphibious species living usually in very shallow water or terrestrially with *U. brachiata* and *U. livida* being lithophytic (Taylor, 1989). Submerged

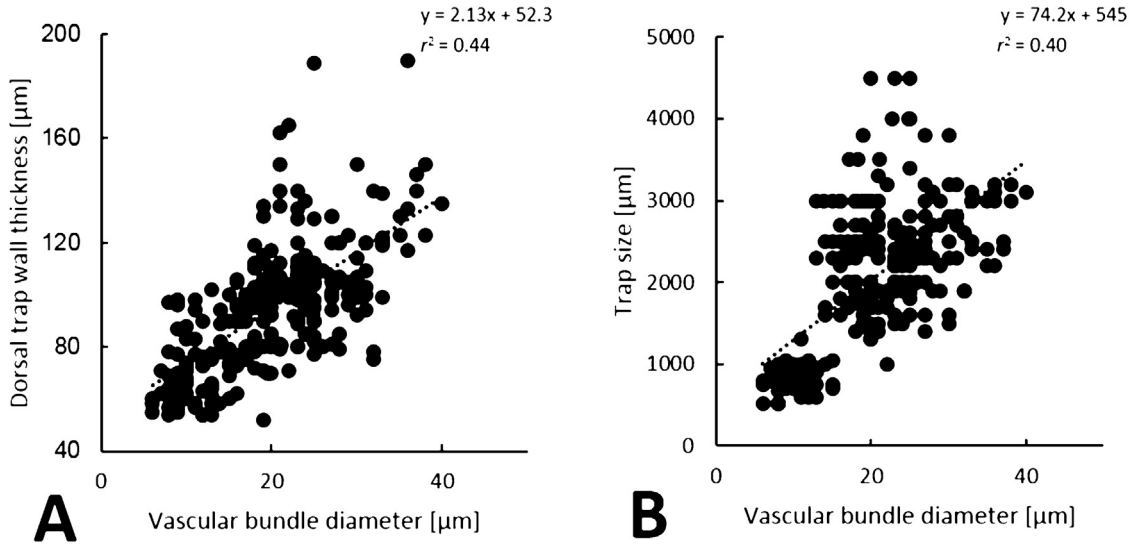

Fig. 3. Linear regression analyses of vascular bundle diameter in relation to trap wall thickness (A) and trap size (B) for the whole data set of 9 *Utricularia* species or clones (n, 266; $P < 0.00001$; r^2, coefficient of determination).

species from the section *Utricularia* usually show very rapid apical growth and their traps typically have a very short life-span when compared to species from the section *Pleiochasia*, which grow much more slowly and their traps generally have much longer life-spans (Friday, 1989; Adamec, 2013). Moreover, traps of the former section function much more efficiently in terms of their firing or resetting rates than those from the latter section (cf. Adamec, 2011; Płachno et al., 2015). This suggests that traps from the faster growing *Utricularia* section may have greater transport needs than those of the other, more slowly growing generic sections with amphibious or terrestrial life forms.

The occurrence of well-developed trap xylem elements might help in the understanding of how water is pumped out from traps. It is generally believed that water entering the internal trap glands (mainly bifids and probably also quadrifids) is driven by ion fluxes and is later released outside the traps by external glands (Sydenham and Findlay, 1975; Sasago and Sibaoka, 1985a,b); part of the water is removed from the cells of the pavement epithelium glands by the turgor pressure through a very leaky plasmalemma (Sydenham and Findlay, 1975). Thus, one might expect that the vascular bundle should occur in the threshold area where both bifid glands and pavement epithelium glands occur. Moreover, the occurrence of xylem elements in the trap vascular system might indicate that some part of the water absorbed by internal glands is transported using vascular bundles to other plant organs. However, in submerged aquatic plants, reduced xylem elements are used not only for water transport but mainly for mineral nutrient transport from roots to shoots (Pedersen, 1993; Pedersen and Sand-Jensen, 1993). In terrestrial plants, xylem can also conduct organic substances (e.g., Zimmermann, 1983). Is it thus plausible that the trap xylem elements passing through the stalk serve mainly for transporting the mineral and organic nutrients needed for trap construction when the trap is young. It is evident that all trap mineral and organic nutrients must come from the shoots before the trap becomes functional. The finding of significant linear correlations between the trap size parameters and the diameter or vascular bundle or tracheal elements in the whole dataset supports the view that vascular bundles are highly functional in *Utricularia* traps, not rudimental. Based on the Poiseuille's law that a pressure-driven flow through a capillary depends on the fourth power of capillary diameter, even small interspecific differences of the tracheal element diameter might be physiologically important. The morphometric data (Table 1) show that the variability of trap parameters such as shape, entrance position, height, width and dorsal and ventral trap length does not change substantially dorsal and ventral trap wall thickness in various species from different sections. Moreover, our results indicate that regardless of the variability of all these parameters, a similar pattern of distribution and structure of vascular bundles occurs in traps of all species of the *Tridentaria* and *Pleiochasia* sections (cf. Table 2).

In ripe, functional traps, xylem could mainly transport great amounts of organic substances as respiratory substrates for the trap (Adamec, 2006) and to support trap commensals (Sirová et al., 2009, 2010, 2011). In terrestrial and lithophytic *Utricularia* species, which have aerial phylloclades with stomata, limited water transport from the shoot to cover the evaporative losses from traps might also be assumed. Juang et al. (2011) used carboxyfluorescein diacetate tracer to show the symplasmic pathway in traps of *U. gibba*. The tracer was transported from quadrifid glands to the trap wall and later to the trap stalk. This result suggests that nutrients could be transported via phloem in *U. gibba*, because carboxyfluorescein is used to trace phloem translocation.

Concerning the phylogeny of the Lentibulariaceae family, it is crucial to compare how trap vascularization is developed in the genus *Genlisea*, a sister lineage to *Utricularia* (Jobson and Albert, 2002; Jobson et al., 2003; Müller et al., 2004). According to Lloyd (1942) and Reut (1993), in the *Genlisea* trap, one vascular strand divides at the distal end of the footstalk into two strands, dorsal and ventral, before entering the trap chamber. These vascular strands divide once more at the trap branching zone, thus each trap arm contains two vascular strands. Thus, in the basal group of *Utricularia,* one should expect a similar pattern of vascularization as in *Genlisea*. However, as shown here the system of trap vascularization in the subgenus *Polypompholyx* is different from that in *Genlisea*. Yet, the structure of vascular bundles is similar in *Genlisea* and *Polypompholyx* as in both taxa, the well-developed xylem elements occur. It seems that occurrence of well-developed xylem elements in traps may be an ancestral character. This suggestion is in line with some authors that the subgenus *Polypompholyx* is the most basal ("primitive") taxon within the genus *Utricularia*, while the section *Utricularia* is considered advanced (e.g. Taylor, 1989; Müller and Borsch 2005). However, to fully understand evolutionary aspects of both structure and localization of trap vascular bundles, more *Utricularia* species from various sections should be studied.

In conclusion, the pattern of trap vascular bundles of species of the subgenus *Polypompholyx* is similar to that in the subgenus *Bivalvaria,* but different from that in aquatic *U. vulgaris* from the subgenus *Utricularia.* Moreover, it is different from the genus *Genlisea*, which is closely related to *Utricularia*. A future study should include experiments which help to understand the functionality of trap vascularization in Lentibulariaceae.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.aquabot.2017.06.003.

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