Relationship between trap anatomy and function in Australian carnivorous bladderworts (*Utricularia*) of the subgenus *Polypompholyx*

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ABSTRACT

The suction traps of carnivorous *Utricularia* species are hollow bladders of foliar origin which trap and digest small organisms. In this paper, the trap wall anatomy and thickness in nine Australian *Utricularia* species belonging to three generic sections (*Pleiochasia*, *Polypompholyx*, *Tridentaria*) within the subgen. *Polypompholyx* are described. It is hypothesized that there is a close relationship between *Utricularia* trap wall anatomy and functional trap characteristics – species with more than two cell layers in their trap walls are less effective at trap firing (*i.e.*, water aspiration) and resetting (water pumping) than species with two-layered trap walls. The trap walls of all *Utricularia* species studied using light microscopy consisted of two to five cell layers. A distinct variation in the trap wall structure occurred not only within one generic *Utricularia* section, but even within one species and also different parts of one trap. Using an electronic position sensor to monitor trap thickness showed that in two clones of *Utricularia dichotoma*, both firing (41 ± 2 and $46 \pm 3\,\mu m\,mm^{-1}$) and resetting rates per unit trap length ($14 \pm 1\,\mu m\,mm^{-1}$ per 30 min) were markedly lower than the mean values for 13 aquatic *Utricularia* species obtained from the literature (73 ± 2 and $52 \pm 2\,\mu m\,mm^{-1}$, respectively). In conclusion, the number of cell layers in the trap wall may not in itself be decisive for the trap firing–resetting efficiency. The lag-period of water pumping by the trap, which can be caused by a different regulation mechanism of water pumping, is probably confined to the species from the *Pleiochasia* section in contrast to the aquatic species from the *Utricularia* section.

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1. Introduction

The carnivorous syndrome has evolved several times within flowering plants and more than 700 carnivorous plants are now known (Król et al., 2012). Within this ecological group, the root-less *Utricularia* L. (bladderwort, Lentibulariaceae) genus contains around 230 species (Taylor, 1989; Fleischmann, 2012). The defining characteristic of the bladderworts is their suction traps – hollow bladders of foliar origin that are used for trapping and digesting small organisms (Lloyd, 1942; Juniper et al., 1989; Guisande et al., 2007). These fluid-filled bladders are usually 1–5 mm long and have a mobile door. They contain several glands and trichomes on both their inner and outer walls (Juniper et al., 1989).

When set, the trap is ready for firing (we use the term 'firing' for the complex, very rapid process which involves the aspiration of the water and animal prey into reset traps) and a negative

pressure of *ca.* −16 kPa relative to the ambient medium is maintained inside (Sydenham and Findlay, 1973; Sasago and Sibaoka, 1985; Singh et al., 2011). When trigger hairs situated on the trap door are touched by a prey species the door opens, the prey is aspirated into the trap lumen and the door closes again; all in under 5 ms. This process is caused by the reversible buckling of the door (Vincent and Marmottant, 2011; Vincent et al., 2011a,b). It has recently been discovered that the traps can also fire spontaneously, *i.e.*, without any mechanical stimulation (Adamec, 2011a,b; Vincent and Marmottant, 2011; Vincent et al., 2011a,b). Additionally, all recent studies indirectly support a purely physical (mechanical) rather than an electrophysiological mechanism of *Utricularia* trap triggering (Adamec, 2012).

According to Lloyd (1942) and many other authors (*e.g.*, Juniper et al., 1989; Guisande et al., 2007), the flexible walls of *Utricularia* traps consist of two layers of cells. However, in the case of Australian *Utricularia* species, which were once considered by Lloyd (1942) as the separate genus *Polypompholyx* (now subgenus *Polypompholyx*), the traps walls consist of four layers of cells (two epidermal and two parenchymatic). Reifenrath et al. (2006) also

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observed four-layered walls in the traps of *Utricularia multifida* (syn. *P. multifida*). It may be assumed that these thicker trap walls could result in modified trap functioning characteristics. When comparing trap characteristics in 13 aquatic *Utricularia* species, Adamec (2011b) found that in *Utricularia volubilis* (subgen. *Polypompholyx*), both the trap firing and resetting rates per unit trap length were 2.5–9 times lower than the mean values for all 13 species. Moreover, during the first 10 min after firing, the kinetics of trap resetting in this single species showed a lag-period before water pumping commenced at a full rate.

In this paper, the trap wall anatomy in nine Australian *Utricularia* species belonging to three generic sections (*Pleiochasia*, *Polypompholyx*, *Tridentaria*) within the subgen. *Polypompholyx* is described. Particular emphasis is placed on the number of cell layers in the trap walls. We hypothesize that there is a close relationship between trap wall anatomy and trap characteristics, *i.e.*, species with traps walls more than two cell layers thick are less effective at firing and resetting than species with two-layered trap walls. To verify this relationship, we measured the firing–resetting trap characteristics in two populations of *Utricularia dichotoma* with thicker trap walls.

2. Materials and methods

2.1. Plant material

Species from the subgen. *Polypompholyx*, section *Pleiochasia* were: *U. volubilis* R.Br. (from SW Australia), *U. dichotoma* Labill. (clone from the Botanic Garden of Jagiellonian University/BGUJ/in Cracow, Poland), *U. dichotoma* (robust clone from Newcastle, N.S.W., Australia), *U. dichotoma* (smaller clone from Katoomba, N.S.W.), *Utricularia* cf. *beaugleholei* Gassin, *Utricularia uniflora* R.Br., *Utricularia paulineae* Lowrie, *Utricularia inaequalis* A.DC. and *Utricularia menziesii* R.Br. From the section *Polypompholyx*: *U. multifida* R.Br. and from *Tridentaria*: *Utricularia westonii* P.Taylor, cultivated in the Institute of Botany of the Academy of Sciences of the Czech Republic at Třeboň, from the Prague Botanical Garden, the Botanical Garden of Jagiellonian University in Cracow and from the private collection of Kamil Pásek (http://www.bestcarnivorousplants.net/). Aquatic species (*U. volubilis, U. dichotoma* robust and smaller clone) were grown in 3–20 L aquaria floating or standing in a 300 L plastic container for cooling. The cultures were partly shaded within a naturally lit greenhouse. *Carex* litter or peat and sand was used as a substrate. From the concentration of nutrients and humic substances, the water in these cultures was considered oligotrophic and humic (for all details, see Sirová et al., 2003; Adamec, 2011b). The other *Utricularia* species were grown either in pots in a mixture of peat and sand (*ca.* 2:1, v/v) in a greenhouse or in sterile meristem tissue cultures under fluorescent lighting (see Adamec and Pásek, 2009).

2.2. Light microscopy

Two different techniques were used for the anatomical studies on 11 species or clones of *Utricularia* (see Table 1): a clearing technique and trap sectioning using traps which were embedded in Technovit/resin. 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). Cleared traps were examined using a Nikon Eclipse 80i or Olympus BX60 light microscope, both equipped with Nomarski interference contrast optics.

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 infiltered in 3:1, 1:1, and 1:3 (v/v) mixtures of absolute ethanol and Technovit (Technovit 7100 2-hydroxyethyl-methacrylate; Heraeus Kulzer; Konieczny et al., 2012) 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.

A 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. Typical images are always presented. For each species, 10–30 traps originating from different stolons/rhizoids of 2–4 different plants were generally used. Terminology of trap morphology is as per Taylor (1989) and Reifenrath et al. (2006). The sidewalls and rear walls of the traps, excluding the place where vascular bundles occurred, were analyzed.

The trap wall thickness of cleared traps was measured on semi-thin, longitudinal sections under a microscope. Measurements of the thickness along the dorsal and ventral trap walls were taken at approx. 100 μm intervals with the use of NIS-Elements D software and three main positions were distinguished for each trap side: near the peduncle, central position, and near the door. As anatomical parameters were estimated in fixed and halved traps, measurements of trap size were not conducted. Nevertheless, the trap size within each species varied only slightly as traps of a relatively homogeneous size were used.

2.3. Measurement of trap firing and resetting rates

Overall trap thickness measurements were used as a measure of water flow and trap volume change. Isolated traps of two clones of *U. dichotoma* (from Newcastle and Katoomba, N.S.W.) were used, as these clones tend to produce larger traps. This procedure also allowed an estimate of trap firing and resetting rates to be made. We define 'firing' as a sudden increase in trap thickness, while 'resetting rate' as a gradual decrease in trap thickness (*i.e.*, kinetics) over a time period (10, 30 or 60 min) after trap firing (see Adamec, 2011a,b). For both clones, young traps from the 2nd to 4th mature leaf node, as counted from the plant apex, were used. The traps were usually functional and their length was 2.4–3.8 mm. The intact traps were excised directly under water in the aquaria and trap firing was induced by shaking them. They were then transferred to the laboratory, washed in tap water, and carefully transferred to a 10 mL perspex chamber containing a solution composed of 0.1 mM KCl, 0.05 mM $CaCl_2$ and 0.2 mM $NaHCO_3$ of pH 7.3–7.4 (Adamec, 2011a,b). A freshly excised trap at the initial stage of resetting (2–5 min), and free from macroscopic animal prey or air bubbles, was inserted into the holder of an electronic position sensor to measure trap thickness changes (for all technical details, see Adamec, 2011a). The position sensor had a 1 μm resolution and the trap thickness was monitored at 30 s intervals; the chamber was partly covered by a perspex lid to minimize water evaporation. The water temperature was relatively stable during the measurements; mean values were within 21.5–23.5 °C for all measurements with the total temperature range being <1.5 °C for each clone. During the measurements, the temperature change was usually <1.0 °C and the

traps were in natural, dim daylight. Viewing with a loupe allowed the fixed traps to be mechanically stimulated to fire using a very fine brush which gently touched the sensitive trigger hairs, but the position of the fixed trap in the holder remained unchanged (Adamec, 2011a,b).

Measurements of trap thickness were made according to the following schedule: 2 h after the insertion of a fired trap into the holder (resetting period), the trap was fired by mechanical stimulation and this step was conducted a total of three times, each after a further 2 h. Three sets of firing–resetting data were thus usually obtained for each functional trap. In some measurements, the trap was then allowed to rest for 12–15 h and spontaneous firings were recorded (*cf*. Adamec, 2011a,b). Within each clone, 11–14 parallel traps of the same age and originating from different shoots, were used for the measurements. After this, trap lengths and widths were estimated to the nearest 0.1 mm using a ruler viewed with a loupe. For each clone, 9–28 firing–resetting records, both for stimulated and spontaneous firings, were obtained. The results for non-functional traps were discarded.

2.4. Evaluation of data and statistical analyses

The following parameters were evaluated from the measurements of each *U. dichotoma* clone (after Adamec, 2011a,b): initial maximum (fired) trap thickness just after insertion into the holder; change of trap thickness due to mechanically stimulated firing 2 h after insertion or during spontaneous firings; initial resetting rate after both types of firing over 10 min (expressed as trap thickness decrease between 0.5 and 10.5 min) and over 30 and 60 min; and the time between two spontaneous firings (and/or the time between the last mechanically stimulated firing and the first spontaneous firing). Within each *U. dichotoma* clone, the data for all traps were pooled, with outlying values discarded and presented as such. Repeated measures design of ANOVA could not be used for data evaluation as this step would lead to a substantial reduction of data. Moreover, as the traps of both clones differed slightly in their length and thickness, firing and resetting rates were also expressed per unit trap length. Adamec (2011b) has shown that for

13 *Utricularia* species, trap length usually correlates more strongly with the trap firing and resetting rates than trap thickness. The data were tested for normality (Kolmogorov–Smirnov test) and as the conditions of normality were not disturbed no data transformation was used. Differences in the parameters between the two *U. dichotoma* clones were evaluated by 1-way ANOVA (Tukey HSD test for unequal *n*). The same test was also used to look for significant differences in the same parameters between mechanically stimulated and spontaneous firings within each clone. The significant differences in the mean trap wall thickness between ventral and dorsal trap sides within each species were statistically tested in Statistica 9.0 with the use of a Tukey test preceded by Kolmogorov–Smirnov test. Throughout the paper, mean ± S.E. values are shown where possible.

3. Results

3.1. Trap wall anatomy

The trap walls of all studied species consisted of two to five cell layers. Great variability was found both between different species within the *Pleiochasia* section and between the dorsal and ventral trap sides for each species across all generic sections. Dorsal and ventral trap wall anatomy and thickness in examined *Utricularia* species are shown in Table 1 and in Figs. 1 and 2. The mean trap wall thickness usually ranged between 80 and 120 μm, except for the ventral sides of *U. menziesii* and *U. volubilis* which reached 173–193 μm. The mean trap wall thickness in *U. paulineae* and *U. uniflora* was significantly lower (by 6–98 μm) in comparison to most of the other species, ranging between 66 and 75 μm. In three species – *U. multifida*, *U. menziesii* and *U. volubilis* – the ventral trap wall was significantly thicker than the dorsal one.

3.2. Firing–resetting characteristics

The traps of both *U. dichotoma* clones differed non-significantly in trap length from each other but significantly (at $p < 0.05$) in their trap thickness just after firing (Table 2). The Newcastle clone was

Table 1
Dorsal and ventral trap wall anatomy and thickness in examined *Utricularia* species.

Section/Species		Trap side	Cell layers in the trap wall	Trap wall thickness [μm] ± SE			
				Near the peduncle (*n* = 4–14)	Central position (*n* = 8–40)	Near the door (*n* = 8–16)	**Mean value (*n* = 20–64)**
Polypompholyx	*U. multifida*	Dorsal	3	105 ± 1.7	80 ± 5.3	76 ± 18.5	**82 ± 5.3***
		Ventral	4	102 ± 3.9	140 ± 6.6	113 ± 13.7	**126 ± 6.5**
Tridentaria	*U. westonii*	Dorsal	4	75 ± 5.7	72 ± 4.3	97 ± 6.9	**78 ± 3.7***
		Ventral	4	69 ± 4.2	81 ± 2.0	89 ± 4.9	**81 ± 2.7***
Pleiochasia	*U. dichotoma*	Dorsal	2	106 ± 6.0	108 ± 07.0	114 ± ±4.7	**108 ± 3.7**
		Ventral	2	114 ± 8.8	132 ± 10.8	109 ± 18.1	**122 ± 8.5**
	U. dichotoma (robust clone from Newcastle)	Dorsal	3	106 ± 5.5	104 ± 2.1	100 ± 04.0	**104 ± 1.9***
		Ventral	3–4	139 ± 4.8	116 ± 7.9	107 ± 14.6	**119 ± 6.0***
	U. dichotoma (small clone from Katoomba)	Dorsal	2	76 ± 6.3	81 ± 6.2	125 ± 4.3	**88 ± 9.7***
		Ventral	2	87 ± 2.0	86 ± 2.5	85 ± 5.0	**86 ± 1.5***
	U. inequalis	Dorsal	2	114 ± 6.3	100 ± 3.6	98 ± 02.5	**103 ± 3.0***
		Ventral	3	120 ± 5.0	115 ± 7.2	93 ± 12.0	**109 ± 8.5***
	U. menziesii	Dorsal	3	166 ± 21.7	112 ± 4.9	108 ± 12.6	**124 ± 9.3***
		Ventral	5	233 ± 18.5	189 ± 5.8	180 ± 11.2	**193 ± 8.5**
	U. volubilis	Dorsal	3–4	133 ± 16.5	129 ± 7.2	96 ± 05.3	**117 ± 5.4***
		Ventral	4	168 ± 08.7	161 ± 4.5	218 ± 22.0	**173 ± 7.0**
	U. beaugleholei	Dorsal	2	79 ± 2.1	85 ± 2.9	83 ± 8.8	**82 ± 2.9***
		Ventral	2	87 ± 1.5	89 ± 3.1	83 ± 6.0	**87 ± 2.2***
	U. paulineae	Dorsal	2	69 ± 4.6	65 ± 3.5	66 ± 3.8	**66 ± 2.4***
		Ventral	2	75 ± 2.9	79 ± 3.1	71 ± 1.9	**75 ± 1.8***
	U. uniflora	Dorsal	2	77 ± 06.3	46 ± 1.1	93 ± 9.5	**72 ± 9.2***
		Ventral	2	88 ± 18.5	59 ± 8.9	52 ± 4.0	**66 ± 8.9***

The asterisk indicates significant differences in the mean trap wall thickness between dorsal and ventral part of the wall within the species at $p < 0.05$ (Tukey test).
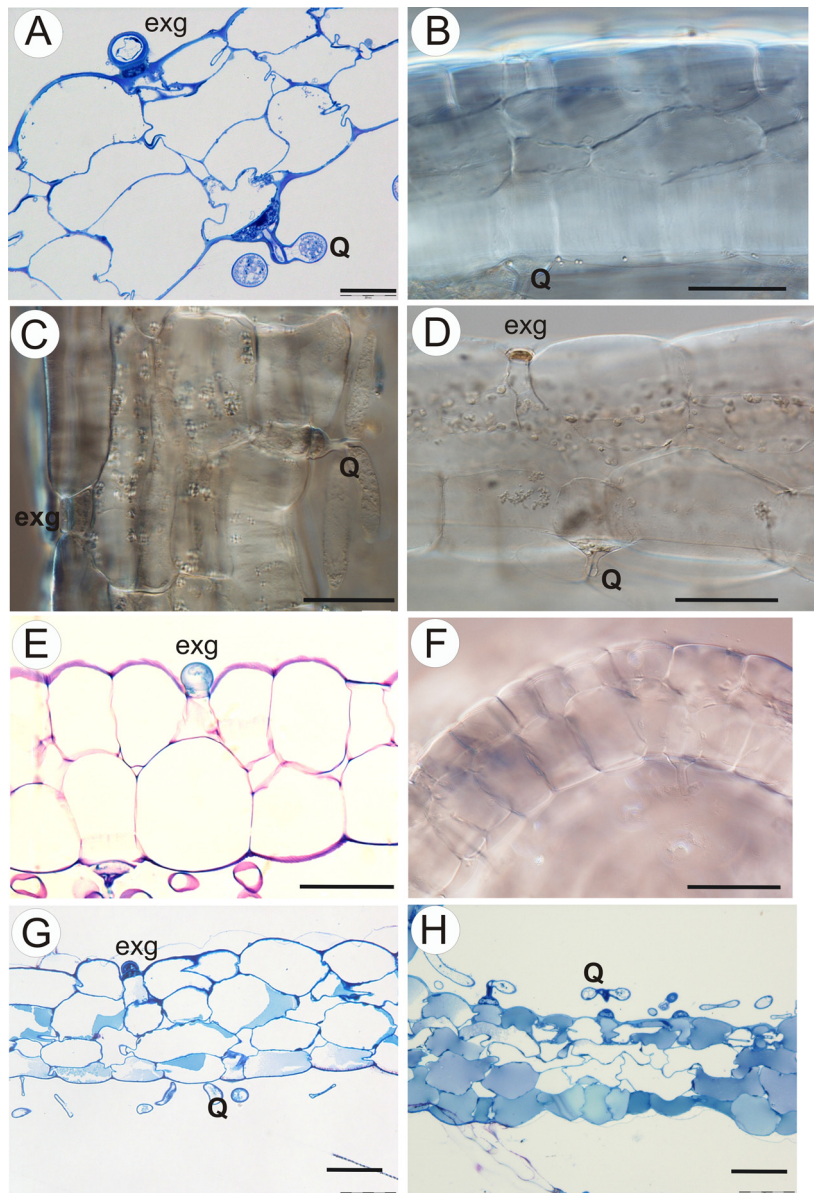
Fig. 1. Trap wall anatomy of different *Utricularia* species from the subgenus *Polypompholyx*. (A) *U. volubilis*, bar = 20 μm. (B) *U. dichotoma* robust plants from Newcastle, bar = 20 μm. (C) *U. menziesii*, bar = 20 μm. (D) *U. inaequalis*, bar = 20 μm. (E) *U. dichotoma*, bar = 20 μm. (F) *U. paulineae*, bar = 50 μm. (G) *U. westonii*, bar = 50 μm. (H) *U. multifida*, bar = 50 μm. Q, quadrifid hair; exg, external gland.

thicker. In line with this, the magnitude of trap firing of the more robust Newcastle clone was significantly ($p < 0.01$) higher than that of the smaller Katoomba clone, both for stimulated and spontaneous firings on the absolute scale and also for spontaneous firings when related to trap length (Table 3). In total, the mean firing magnitude was low and only ranged from 95 to 165 μm. On both absolute and relative scales, only the 10 min resetting rate was significantly greater in the Katoomba clone. The absolute 30 and 60 min resetting rates of the Newcastle clone were always somewhat greater, but this difference was not statistically significant. On the relative scale, these differences were ambiguous.

The comparison of the 10, 30 and 60 min resetting rates from stimulated and spontaneous firings, both on the absolute and relative scales in both clones, revealed a distinct lag-period during the first 10 min (Tables 2 and 3). A detailed plot shows that this lag-period lasted about 6 min (Fig. 3). In the smaller Katoomba clone, the lag-period was less distinct (data not shown). Spontaneous firings occurred during the 12–15 h resting period following the three

firing–resetting cycles for most traps of both clones. Their occurrence was highly irregular, from after 51 min to *ca.* 10 h after the last firing in the Newcastle clone and after 30 min to *ca.* 10 h in the Katoomba clone. No significant difference in any firing–resetting characteristics was found between mechanically stimulated and spontaneous firings within each clone (1-way ANOVA, data not shown).

4. Discussion

Of the nine Australian *Utricularia* species studied, only *U. volubilis* is a true aquatic plant. The others are typically amphibious, growing either in very shallow waters or in wet soils (see Taylor, 1989). The vast majority of papers on trap physiology and prey capture focus on aquatic *Utricularia* species from the *Utricularia* subgenus (*U. purpurea* and species from the *Utricularia* section; *e.g.* Richards, 2001; Sirová et al., 2003, 2009, 2010; Guisande et al., 2007; Vincent et al., 2011a,b; Płachno et al.,

Table 2
Trap firing characteristics measured as trap thickness changes in two clones of *Utricularia dichotoma*.

Spec.	Trap length (mm)	Initial max. trap thickness (μm)	Trap thickness increase (μm) during firing within		Resetting rate (μm) after firing during					
					10 min		30 min		60 min	
			MSF	SF	MSF	SF	MSF	SF	MSF	SF
A	$3.23^{ns} \pm 0.52$	$855^{*} \pm 38$	$145^{**} \pm 8$	$165^{**} \pm 11$	$10.4^{ns} \pm 1.7$	$7.06^{**} \pm 0.76$	$45.6^{ns} \pm 4.0$	$42.0^{ns} \pm 2.5$	$82.4^{ns} \pm 7.1$	$74.2^{ns} \pm 6.0$
B	2.71 ± 0.04	722 ± 29	111 ± 6	95.2 ± 5.3	10.7 ± 0.9	11.5 ± 1.3	38.5 ± 2.0	38.6 ± 1.8	66.2 ± 3.7	64.0 ± 3.4

A, robust plants from Newcastle, N.S.W., Australia; B, smaller plants from Katoomba, N.S.W. Trap thickness changes during mechanically stimulated firing (MSF) or spontaneous firing (SF) and subsequent resetting rates are shown. Means ± SE intervals are shown; n, 9–28 measurements on 11–14 different traps from different plants. The asterisks denote statistically significant difference in a given parameter between A and B clones (** $p < 0.01$; * $p < 0.05$; ns, $p > 0.05$; 1-way ANOVA).
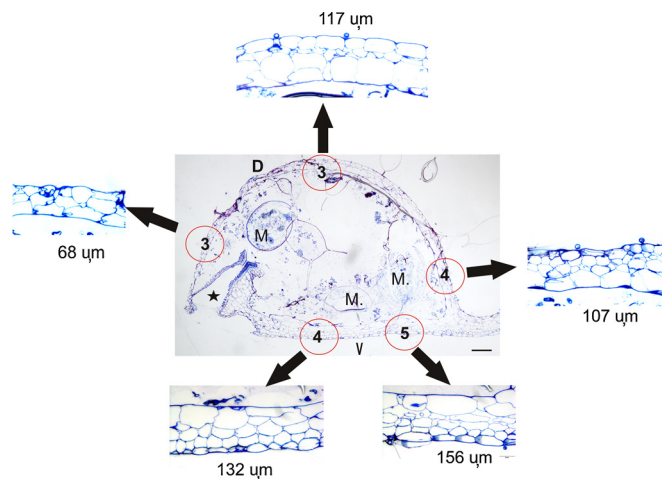

Fig. 2. Longitudinal section through the *U. volubilis* trap showing trap wall thickness and anatomy. Numbers represent the number of cell layers. D, dorsal part; V, ventral part; *, entrance; M, ostracods, bar = 200 μm.

2012); work on terrestrial species is very rare (*e.g. U. uliginosa*; Jobson and Morris, 2001). Additionally, studies on even the basic anatomy of other *Utricularia* species are very rare. Notable excetions are Lloyd (1942) and the recent work of Reifenrath et al. (2006), who analyzed the traps of 14 of the 230 species of *Utricularia* representing 11 of the 35 sections. This study revealed that only *U. multifida* has a multi-layered trap wall. Our work shows that a variation in the trap wall structure occurs within a single species in the *U. dichotoma* complex. The multi-layered trap walls were found in *U. volubilis*, a species which was considered "primitive" (see Taylor, 1989), because traps are formed in a terminal position on the "leaves" (phylloclades). This character is perhaps basal, especially
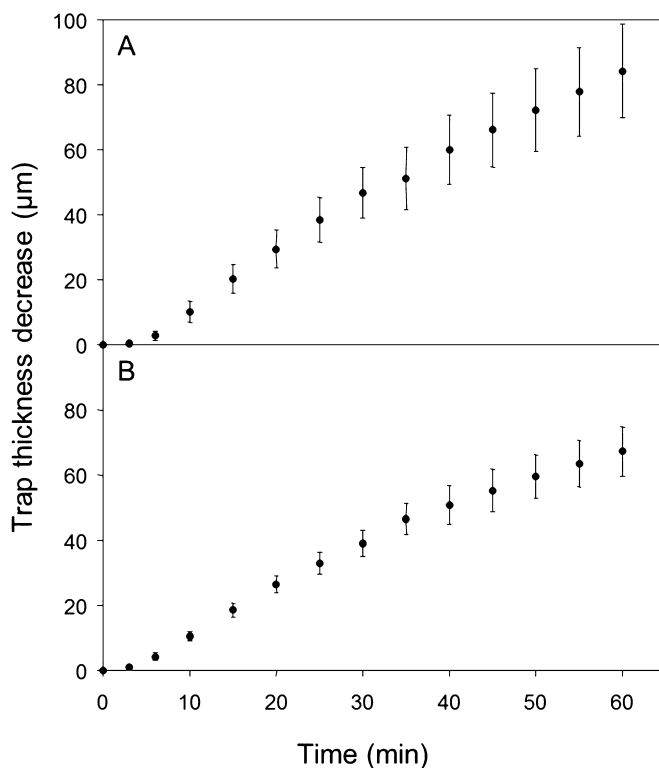

Fig. 3. Time-course of resetting *U. dichotoma* traps recorded immediately after mechanically stimulated firings. Trap resetting was measured as trap thickness changes. (A) Robust clone from Newcastle, N.S.W.; (B) smaller clone from Katoomba, N.S.W. Means ± 2SE intervals are shown; n = 9–15.

Table 3
Trap firing characteristics measured as trap thickness changes in two clones of *Utricularia dichotoma*.

Spec.	Trap thickness increase/Length (μm mm^{-1}) during firing within		Resetting rate/Length (μm mm^{-1}) after firing during					
			10 min		30 min		60 min	
	MSF	SF	MSF	SF	MSF	SF	MSF	SF
A	$45.8^{ns} \pm 2.5$	$50.8^{**} \pm 3.0$	$3.30^{ns} \pm 0.54$	$2.17^{**} \pm 0.24$	$14.4^{ns} \pm 1.2$	$12.9^{ns} \pm 0.8$	$26.2^{ns} \pm 2.2$	$22.6^{ns} \pm 1.7$
B	40.8 ± 2.2	34.8 ± 1.9	4.09 ± 0.28	4.19 ± 0.47	14.1 ± 0.7	14.1 ± 0.6	24.3 ± 1.4	23.4 ± 1.2

A, robust plants from Newcastle, N.S.W., Australia; B, smaller plants from Katoomba, N.S.W. Trap thickness changes expressed per unit trap length during mechanically stimulated firing (MSF) or spontaneous firing (SF) and subsequent resetting rates are shown. Means ± SE intervals are shown; *n*, 9–28 measurements on 11–14 different traps from different plants. The asterisks denote a statistically significant difference in a given parameter between A and B clones ($^{**}p < 0.01$; $^{*}p < 0.05$; ns, $p > 0.05$; 1-way ANOVA).

when a multi-layered trap wall has been found in members of the *Polypompholyx* and *Tridentaria* sections. On the other hand, traps in some clones of *U. dichotoma* (a species evolutionarily more advanced than *U. volubilis*) also possess trap walls which are three cell layers thick. Moreover, Reifenrath et al. (2006) noted that in general, the rear walls of *Utricularia* traps consisted of a number of disordered cells and were thicker than the sidewalls. Similar differences between the dorsal and ventral parts of the traps were also observed here (Table 1). For five of the examined species – *U. multifida*, *U. dichotoma* (robust clone), *U. inequalis*, *U. menziesii* and *U. volubilis* – the ventral part of the trap wall consisted of a larger number of cell layers than the dorsal part, but this only correlated with greater trap wall thickness in *U. volubilis*, *U. multifida* and *U. menziesii*. A thicker ventral part of the trap wall was observed in both large traps (>2 mm) of *U. volubilis* and *U. multifida* and in the smaller traps (<2 mm) of *U. menziesii*.

In this study, the firing–resetting trap characteristics were measured in two clones of *U. dichotoma*, which usually grow in water 5–10 cm deep (Jobson, pers. comm.). When the values of the firing magnitude both on the absolute and relative scales (Tables 2 and 3) are compared with those estimated for 13 aquatic *Utricularia* species from different continents (Adamec, 2011b), they are markedly lower than the average for all 13 species ($216 \pm 5 \mu$m or $73 \pm 2 \mu$m mm^{-1}, respectively). This difference is even more distinct when comparing the mean 10 and 30 min resetting rates for all 13 species (62 ± 2 or $150 \pm 5 \mu$m on the absolute scale and 22 ± 1 or $52 \pm 2 \mu$m mm^{-1} on the relative scale, respectively; Adamec, 2011b). On the other hand, all the present low values of both firing and resetting rates of the two clones of *U. dichotoma* are very similar to those for *U. volubilis* (cf. Adamec, 2011b) from the same generic *Pleiochasia* section. Moreover, these values for *U. volubilis* were clearly the lowest out of all 13 species measured. In addition, as with the two clones of *U. dichotoma* (Fig. 3), the distinct lag-period of water pumping occurred in this species during the first 10 min after firing. This was not found in the other species tested.

In this study, 3–4 cell layers were found in the trap walls of *U. volubilis* (Fig. 1A), while only 2–3 layers were found in the two clones of *U. dichotoma* (Fig. 1B). Nevertheless, the quantitative functional trap characteristics of these taxa are very similar. This indicates that the number of cell layers in the trap walls in itself may not be decisive for the trap firing–resetting efficiency. This conclusion is further supported by the comparison of both *U. dichotoma* clones. Although traps from the more robust Newcastle clone have three cell layers and the smaller Katoomba clone has two, the firing–resetting characteristics on the relative scale are very similar to each other, with two statistically significant exceptions (Table 3). Firstly, the traps of the Newcastle clone fired significantly more than the traps of the Katoomba one. Secondly, the lag-period of water pumping after firing occurred in both clones, but was less distinct in the Katoomba one which has thinner trap walls. This means that a thicker trap wall (*i.e.*, with a higher mechanical stiffness) may not limit the magnitude of firing, but can limit the subsequent resetting rate for several minutes after firing. In contrast,

Adamec (2011b) found no lag-period of water pumping after trap firing for 12 aquatic *Utricularia* species from the generic sections other than *Pleiochasia* (presumably exclusively with 2 cell layers) and postulated the existence of 'permanent water pumping'. It is therefore probable that the lag-period of water pumping, which may be caused by a different regulation mechanism, is confined to species from the *Pleiochasia* section.

Both the formation of relatively large traps (up to 4–6 mm) in most aquatic *Utricularia* species and their ease of manipulation within their growing medium have meant that all studies on trap function have been confined to these species (Sydenham and Findlay, 1973; Sasago and Sibaoka, 1985; Adamec, 2011a,b; Adamec, 2011a,b; 2012; Singh et al., 2011; Vincent and Marmottant, 2011; Vincent et al., 2011a,b). Aquatic and/or amphibious *Utricularia* species represent only *ca.* 20–22% of the total species richness (Fleischmann, 2012), which challenges researchers to study the trap function in terrestrial species that have a different ecology. *U. dichotoma* is known as a wide-ranging, polymorphic species (Taylor, 1989) and nowadays some distinct populations are considered as new species (Jobson, 2013). It might thus be reasonable to use the number of cell layers in the trap wall as a possible taxonomic criterion.

Finally, we suggest that the number of cell layers in the trap walls may not, in itself, be decisive for determining the trap firing–resetting efficiency.

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