

ARE TRAPS OF *UTRICULARIA MULTIFIDA* AND *U. WESTONII* ACTIVE
AND DO THEY CREATE NEGATIVE PRESSURE?

LUBOMÍR ADAMEC • Institute of Botany of the Czech Academy of Sciences • Dukelská 135 • CZ-379
01 Třeboň • Czech Republic • lubomir.adamec@ibot.cas.cz

SIMON POPPINGA • Botanical Garden of the Technical University of Darmstadt • Schnittpahnstraße
3-5 • 64287 Darmstadt • Germany • simon.poppinga@tu-darmstadt.de

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Abstract: *Utricularia multifida* and *U. westonii* are Australian species classed recently with the subgenus *Polypompholyx*, which is comprised of the most evolutionary basal species. Their traps bear all functional structures necessary for negative pressure generation and suction (“firing”), i.e., active prey catching, but traps from tissue-culture-raised plants are not known to fire. However, presumably, plants *in situ* are active. Traps of terrestrial plants of both species raised from tissue culture were used for measurement of the critical negative pressure to further elucidate whether or not they could fire. According to our results, traps of *U. multifida* can be considered closed (i.e., no water flow between the trap inside and outside takes place) while those of *U. westonii* can be considered open. This study confirms great functional differences between the underwater traps in these species grown naturally (in an aquatic state) and those grown terrestrially in tissue culture.

Introduction

Suction traps of aquatic *Utricularia* (Lentibulariaceae) species are hollow discoid bladders ca. 1–5 mm large, the lumen of which is hermetically closed from the ambient water by a flexible mobile trapdoor (Juniper *et al.* 1989; Poppinga *et al.* 2016; Westermeier *et al.* 2017; Adamec 2018). Two types of glands occur inside the traps – numerous quadrifid and bifid glands – and are principal for trap physiology: prey digestion, nutrient uptake, and water pumping. The active water pumping processed by bifid glands situated on the trapdoor maintains a negative pressure relative to the ambient medium of ca. -0.16 bar in a fully set trap. After a prey touches sensory hairs on the trapdoor, it opens and water with the prey is sucked inside. Traps also fire spontaneously. The process of trap firing lasts only 3–5 ms and is associated with profound trap volume changes. After firing, resetting lasts ca. 20–40 min until the trap can fire again (Poppinga *et al.* 2016; Westermeier *et al.* 2017; Adamec 2018). These active trap functioning traits have been known mainly for all tested aquatic or amphibious *Utricularia* species from the evolutionary advanced *Utricularia* section *Utricularia* with efficient traps (Adamec 2011; Plachno *et al.* 2015) but obscurity persists for trap functioning of some evolutionary most basal (“primitive”) species from the subgenus *Polypompholyx* – *U. multifida* and *U. westonii* (cf. Plachno *et al.* 2019; Poppinga *et al.* 2020; Horstmann *et al.* 2021 and the literature therein). Overall, direct evidence of active *Utricularia* trap functioning (active water flows and negative pressure creation) can follow either from recordings of suction action (Poppinga *et al.* 2020),

findings of large animal prey caught inside traps (e.g. Horstmann *et al.* 2021), or from biophysical measurements of consequences of water flows (Adamec 2011; Adamec & Poppinga 2016; Poppinga *et al.* 2016, 2020; Westermeier *et al.* 2017; Plachno *et al.* 2019).

Utricularia multifida R.Br. (Fig. 1) and *U. westonii* P.Taylor (Figs. 2, 3) are two Australian species classed recently with the subgenus *Polypompholyx* which comprises phylogenetically ancestral species. The former species belongs to the *Utricularia* section *Polypompholyx* and the latter to *Tridentaria* (Jobson *et al.* 2017). Both annual species are endemic to a small territory of SW Australia with a Mediterranean climate and grow affixed in very shallow water only a few cm deep, so they can be considered semiterrestrial or semiaquatic or amphibious (Taylor 1989; Poppinga *et al.* 2020; Horstmann *et al.* 2021). In the last decade, various studies have been conducted on traps in both species to elucidate directly or indirectly whether or not the traps can create negative pressure and actively catch their prey by the suction mechanism during firing (Plachno *et al.* 2015, 2017, 2019; Westermeier *et al.* 2017; Poppinga *et al.* 2020; Horstmann *et al.* 2021). Unfortunately, most of these studies were conducted on fixed, dead traps and/or plants grown terrestrially in tissue culture (TC; Fig. 2). For the TC-raised plants, the ability to catch prey is greatly reduced.

Unlike the typical traps of species from the *Utricularia* section *Utricularia*, traps of both *Utricularia* species discussed here are triangular in cross-section with rigid trap walls consisting of four (three) cell layers. However, the total wall thicknesses are comparable to other aquatic species (Plachno *et al.* 2015; Westermeier *et al.* 2017). In sum, despite the different shapes of traps in both



Figure 1: *Utricularia multifida* *in-vitro* plants transplanted to a floating jar. Polyurethane, acidic clay, and sedge litter were used as substrates. Photo by Lubomír Adamec.



Figure 2: *Utricularia westonii* in-vitro plants. The diameter of the colony is ca. 3 cm. Photo by Lubomír Adamec.

species and relatively thick and rigid trapdoors, their traps (though usually collected from TC) contain all structures essential for active prey capture: bifid glands for pumping out the water, trigger hairs for sensing prey, and pavement epithelium (velum) facilitating hermetical trap closure (Fig. 4; Westermeier *et al.* 2017; Płachno *et al.* 2019). However, a simple test of aspiration of an air bubble due to trap firing (see Adamec & Poppinga 2016) in TC-raised traps in both species led to ambiguous results and did not confirm active prey capture (Płachno *et al.* 2019). On the other hand, Poppinga *et al.* (2020) presented a direct visualization of model prey capture by aquatic traps of nearly naturally grown *U. westonii*. In line, Horstmann *et al.* (2021) investigated a snapshot prey spectrum in aquatic traps of naturally growing *U. multifida* in SW Australia. They found many prey items of various taxa inside the traps with the dominance of ostracods and cyclopooids. However, how these partly relatively large prey items (with lengths exceeding 1 mm) entered the traps remains unknown.

Due to very obscure ecological requirements and the annularity of both species, it is nearly impossible to grow them in a relatively natural setting, on a peaty or sandy substrate (see Figs. 1 and 3; L. Adamec, unpubl. data). They can be more successfully grown from seeds (Poppinga *et al.* 2020) or in TC (Płachno *et al.* 2019), in an unsubmerged, terrestrial setting in a TC environment. While this study explores the functioning (the critical negative pressure in traps) of these *Utricularia* species, we recognize that TC-raised, terrestrial plants may not be a proper substitution for naturally grown aquatic plants.



Figure 3: *Utricularia westonii* in-vitro plants transplanted to a floating jar. Photo by Lubomír Adamec.

Methods

Utricularia multifida (UM) and *U. westonii* (UW) were grown in a TC in a gerlite-solidified, half-strength Murashige-Skoog medium with sucrose, at $19\pm 1^\circ\text{C}$ in white fluorescent light at a 14 h photoperiod and PAR irradiance of $30\text{--}50\ \mu\text{mol m}^{-2}\ \text{s}^{-1}$. The adult UM plants used for experiments were ca. three months and UW plants six months old. Their terrestrial, rose to pink traps grown above the medium were ca. 2 mm large in UM (Fig. 1) and 2.0–2.8 mm in UW (Fig. 2) and fluid-filled. Freshly excised traps were used to measure the critical negative pressure as a measure of the “maximal” negative pressure that leads to a spontaneous firing (for all methodical details, see Adamec & Poppinga 2016). Shortly, an excised trap was impaled underwater by a glass capillary trap connected to an accurate electronic pressure sensor and a peristaltic pump. The capillary was sealed with the impaled trap above water by a rapid cyanoacrylate underwater adhesive. The pump was gradually generating negative pressure which was monitored electronically. As soon as the magnitude of the negative pressure inside the impaled trap exposed in the air reached the critical value for spontaneous trap firing, the trap inspired an air bubble and the negative pressure was recorded. In UW traps underwater, positive pressure was also applied by the pump and the first water flow through the trap was detected by a rapid water movement in the capillary. Water movement by a water-filled capillary with the tip exposed underwater or above water was also measured, using both negative and positive pressure, as an estimate of the surface tension and/or the capillary force of the

capillary tip. It is possible to assume that during trap firing, the diameter of the leaks of the open, firing trap is roughly comparable with that of the capillary tip.

Both species from TC were planted semiterrestrially in 580 mL glass jars and cubes of foamy polyurethane, acidic clay, and sedge litter were used as a substrate (Figs. 1, 3) to mimic natural conditions. The jars covered with Petri dishes floated on the surface of a 2 m² plastic container for cooling and ensured maximal sunshine. In May 2019, the afternoon temperatures did not exceed ca. 20°C. The water was kept very shallow so that the rhizoids were underwater but the other plant organs lay only at the surface and could not dry out.

Results and discussion

The critical negative pressure in *U. multifida* traps was -449 ± 28.5 mbar (Table 1). Adamec and Poppinga (2016) measured in traps of 15 *Utricularia* species values from -69 to -346 mbar (mean -195 mbar) but all used species possessed active traps and exhibited firing. Therefore, due to the very high absolute value of the critical negative pressure in UM traps, it is possible to assume that the UM traps are closed. Even if the measured high critical negative pressure is theoretically generated inside the traps to open the very rigid trapdoor spontaneously, the magnitude of the trapdoor leak could be very low to enable a catch of any larger prey and only microorganisms could be caught. On the other hand, *U. westonii* traps exhibited a very low absolute value of the critical negative underpressure of -25.4 ± 2.6 mbar and this absolute value was nearly the same as that for the positive pressure (27.2 ± 2.5 mbar). These results imply that UW traps are open and do not function as a one-way pressure valve as it is standard in all *Utricularia* species with active traps. This conclusion is supported by the fact that a similar small critical pressure (both negative and positive) was measured for the capillary alone in the air (Table 1), which included mainly the surface tension of water in the capillary tip.

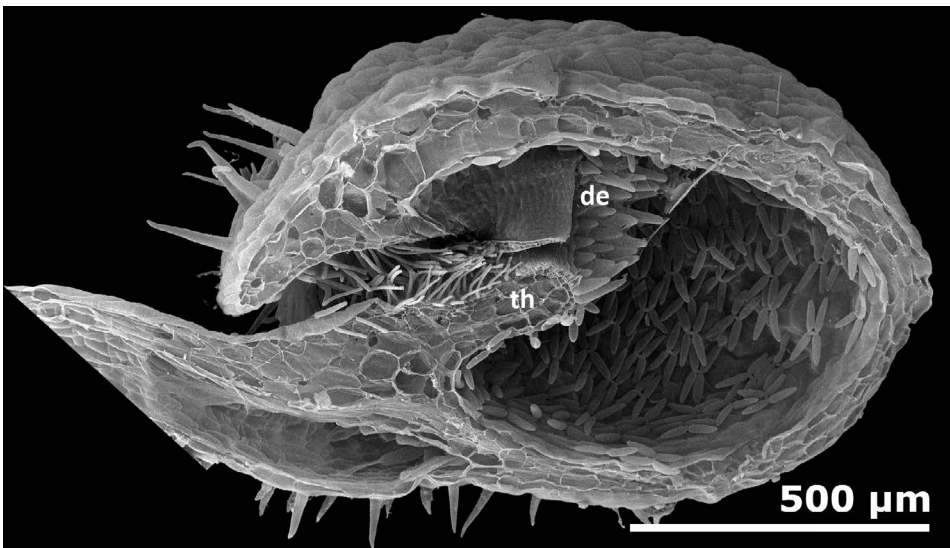


Figure 4: SEM micrograph of a dissected *Utricularia multifida* trap. The plant was grown from seed. Abbreviations: de, door edge; th, threshold. Image modified from Westermeier *et al.* (2017).

Table 1. Results of measurements of the critical negative and positive pressure in traps of *Utricularia multifida* and *U. westonii* from the TC and a glass capillary filled with water (outer tip diam. ca. 120 µm) positioned either in water or in the air as a blank. Means ±SE intervals are shown, number of parallels in parentheses.

Species or conditions	Critical pressure of traps or capillaries (mbar)	
	Negative pressure	Positive pressure
<i>U. multifida</i>	-449.2±28.5 (14)	–
<i>U. westonii</i>	-25.4±2.6 (13)	27.2±2.5 (9)
Capillary in water	–	3.61±0.19 (8)
Capillary in the air	-19.0±0.6 (8)	17.2±0.3 (6)

In both *Utricularia* species raised from TC, their traps were grown terrestrially. The results can be interpreted as that the functional traits of TC-raised terrestrial traps are quite different from underwater traps grown in natural conditions. While TC-raised UM traps in this study are functionally closed, those grown underwater under natural conditions can efficiently catch a wide spectrum of zooplankton (Horstmann *et al.* 2021). TC-raised UW traps in our study are permanently open, while Poppinga *et al.* (2020) demonstrated firing and active prey capture in traps in cultivated, nearly natural plants. That is why the simple biophysical test of trap firing conducted on both TC-raised species was quite ambiguous (Plachno *et al.* 2019). Based on our results, it can be concluded that TC-raised terrestrial traps of both UM and UW do not represent functional traits in naturally underwater-raised traps in these species but rather artificial results, though the anatomical appendages of the TC-raised traps include all necessary functional parts (trapdoor, bifid glands, trigger hairs, velum) as in naturally grown plants (Plachno *et al.* 2015, 2017, 2019). Generally, all naturally grown aquatic or semiterrestrial *Utricularia* species from *Utricularia* sections *Polypompholyx*, *Tridentaria*, *Pleiochasia*, *Orchidioides*, *Utricularia*, and *Vesiculina* with underwater traps can actively catch prey (Adamec 2011; Plachno *et al.* 2015; Adamec & Poppinga 2016, Westermeier *et al.* 2017; Poppinga *et al.* 2020; Horstmann *et al.* 2021).

Our attempt at growing adult, TC-raised plants of both species *ex-vitro* to simply substitute natural conditions in the floating jar was unsuccessful (Figs. 1, 3). Although the UM plants were able to complete their flowering from *in-vitro*-initiated flower buds in the jar (Fig. 1), neither species grew and the plants died within a few weeks. This unsuccessful attempt supports the strictly annual character of both species and proves that *in-vitro*-raised plants cannot be easily transplanted to an *ex-vitro* culture. Any *ex-vitro* culture must thus start from seeds (Poppinga *et al.* 2020). It is evident that such a growth limitation strongly impedes any research on the trap functioning of these most basal *Utricularia* species.

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