Anatomic adaptation of stems of aquatic *Utricularia* species dragging turions to the bottom of their habitats

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Abstract: Turions are vegetative, dormant, overwintering storage organs formed in aquatic plants. We have observed that shoots with maturating turions of three robust aquatic *Utricularia* species possess an anatomical adaptation of the central cylinder in apical shoot segments. The senescent mother shoots drag turions in these species to the bottom of their habitat. This adaptation leads to impregnation of the central cylinder by substances preventing the stems from early decomposing. Using anatomical staining methods, we have aimed to elucidate the substances impregnating the central cylinder – partly lignin and mainly polyphenols.

Introduction

Turions (winter buds) are vegetative, dormant storage organs produced by perennial aquatic plants in response to unfavorable ecological conditions in subtropical to polar zones, usually at the beginning of autumn. They form at least in 14 genera of vascular aquatic plants, especially in submerged (or amphibious) and free-floating species (Sculthorpe 1967; Adamec 1999, 2018). They also form in temperate species or populations of both rootless aquatic carnivorous genera, monotypic *Aldrovanda vesiculosa* (Droseraceae), and in many *Utricularia* species (Lentibulariaceae, Adamec 2018, 2020). Their purpose is to avoid fragile summer shoots being embedded in ice. Turions are formed by extreme shortening of apical shoot internodes with modified shortened leaves or scales (Figs. 1, 2, front and back covers). Turions of most aquatic plants, including those of carnivorous taxa, usually overwinter at the bottom of aquatic sites in darkness and under hypoxic or anoxic conditions, but they can also overwinter at the water surface or on a wet substrate, and the turions of these species exhibit frost resistance (Sculthorpe 1967; Adamec & Kučerová 2013; Adamec 2018). In contrast with bottom-rooted species, turions of submerged rootless carnivorous species form, maturate, germinate, and sprout in light and warmer water at the water surface. However, two distinct ecophysiological strategies of autumnal turion sinking

and spring rising (floating up, buoyancy) have been identified in carnivorous plants (Adamec 2008, 2018).

Figure 1: Mature turions of *Utricularia australis* formed in outdoor culture and stored in the fridge; January 2024. Distance between ticks indicate 1 mm.

Figure 2: Mature turions of *Utricularia macrorhiza* formed in outdoor culture; late November 2018.

In *Aldrovanda* (and also in non-carnivorous *Hydrocharis morsus-ranae*), an active mechanism of autumnal turion sinking and spring rising has developed. Within this group, mature autumnal turions usually break from the dying mother shoots in a dehiscence zone and sink gradually to the bottom as the voluminous air spaces in turion leaflets are filled with water. In the spring in warmer water during the break of the imposed dormancy, the water in the air spaces is expelled by a CO_2-enriched gas evolving from increased respiration, and the turions rise again (Adamec 2003, 2008, 2018). Turions of several robust *Utricularia* species (*U. vulgaris*, *U. australis*, *U. macrorhiza*, *U. tenuicaulis*) are usually less dense than water, and in the autumn these buds, which are firmly connected to the mother shoots, are dragged to the bottom. By early spring, the turions usually separate and rise to the surface, though small turions can separate as early as in autumn and then float at the surface over winter (Adamec 2018). Turions of several smaller species usually growing in very shallow water (*U. minor*, *U. bremii*, *U. intermedia*, *U. ochroleuca*, *U. stygia*) may develop the dehiscence zone and separate in autumn or may be attached to senescent shoots for several weeks.

The observation that in some robust *Utricularia* species, autumnal stems adjacent to maturating turions form a specific anatomic adaptation to drag the mature turion to the bottom (Fig. 3), motivated this study. This paper aims to describe the anatomic stem adaptation preceding turion maturation in three robust aquatic *Utricularia* species with monomorphic shoots (*U. vulgaris*, *U. australis*, and *U. macrorhiza*), in contrast with a species with dimorphic shoots (*U. stygia*) forming the turion dehiscence zone.

Materials and methods

Plants of *Utricularia vulgaris* L. (from S. Moravia, Czech Rep.), *U. australis* R.Br. (from S. Bohemia, Czech Rep.), *U. macrorhiza* Le Conte (from Canada) and *U. stygia* Thor (from S. Bohemia) were grown outdoors in 85- to 1500-L plastic containers mimicking natural dystrophic (humic) waters and *Carex* litter or brown peat were used as the main substrate (see Adamec *et al.* 2020, 2024). It has been repeatedly observed in the last years that stem segments just below maturating large turions in robust *Utricularia* species (*U. vulgaris*, *U. australis*, *U. macrorhiza*) are mechanically firm and rigid and that the central cylinder of these apical stem segments is markedly colored brown (Fig. 3). On 14 October 2022, a subapical stem internode ca. 5 mm long, between the first and second leaf node ca. 0.5–1 cm below the maturating turion, was cut off from several robust and long shoots of *U. vulgaris*, *U. australis*, and *U. macrorhiza* for anatomical investigations. Simultaneously, for comparison with the summer growing stem anatomy, a 5-mm long stem segment 50 cm below the turion in *U. vulgaris* (or 40 cm in *U. australis* and *U. macrorhiza*), was cut off from each plant. These stem segments were formed at the peak of the growing season (July to mid-August) and represent typical summer stem anatomy. Similarly, on 26 October 2022, subapical (3–6 mm below turion) and basal (10 cm below turion) stem segments were sampled from photosynthetic shoots of *U. stygia* as the comparison with a species forming dehiscing turions. All sampled stem segments were first fixed in 70% ethanol until anatomic processing.

Detection of lignin (polyphenols)

The apical and basal stem segments were placed in 96% ethanol for a limited time, then properly washed in phosphate-buffered saline and post-fixed overnight at 4°C in Bouin-Hollande solution without acetic acid but supplemented with mercuric chloride (Levine *et al.* 1995). Standard

procedures were used for tissue dehydration, embedding in paraplast, sectioning to 7–10 µm, deparaffinization, and rehydration in 70% ethanol. Lignin was stained with Safranin O/Astra Blue dyes combination (Diapath, Martinengo BG, Italy) for 10 min according to the manufacturer‘s protocol. The stained sections were washed in distilled water, dehydrated and embedded in DPX medium (Fluka, Buchs, Switzerland) and viewed and imaged under the Olympus BX51 microscope equipped with an Olympus DP80 CCD camera (Olympus Corporation, Tokyo, Japan).

Detection of phenols

The apical and basal stem segments were placed in 96% ethanol for a limited time, then hand cut into thin cross sections, which were then washed in sodium borate buffer pH 8.5 and stained with 0.1% toluidine blue (Sigma-Aldrich, Inc., St. Louis, Missouri, USA) in acidic sodium borate buffer pH 4.0 for 5 min. Sections were briefly rinsed in acidic borate buffer and then in distilled water. The samples were mounted in 100% glycerol, and blue-green staining of toluidine blue, or the autofluorescence emission in the green spectra, was imaged under the microscope BX63 (Olympus, Japan) equipped with the DP74 CMOS camera (Olympus, Japan).

Results and discussion

As seen in the photos of the anatomical adaptation of apical segments in *U. vulgaris* stems, the central cylinder, both in maturating turions (Fig. 3A) and in mature ones (13 Nov 2022, Fig. 3B), is brown and, unlike the decomposing cortex, it remains intact and is not subject to rapid decomposition. In this way, it helps keep the mature turions on the bottom until spring. Otherwise, the anatomy of apical and basal stem segments of *U. vulgaris*, *U. australis*, and *U. macrorhiza* is very similar. However, the basal stem segments grew in the summer, whereas the apical ones grew simultaneously with early turion maturation in September (Adamec *et al*. 2024). The stem of these species is round-shaped and has a diameter of ca. 1.5–1.8 mm (Figs. 4–6). The parenchymatic cortex with regular, radially arranged large air spaces is separated by an endodermis with weakly developed Casparian strips from the central cylinder (cf. Schweingruber *et al*. 2020). The xylem and phloem within the central cylinder are difficult to distinguish. In apical and basal stem segments, the proportion of the diameter of the central cylinder to the total stem diameter is the same, around 25–35%. The central cylinder in both parts of the stems consists mainly of thick-walled cells. These cells are locally and variably stained red by Safranine (Figs. 4–6) and the whole cylinders are stained blue by toluidine blue (Figs. 4–6) in both parts of the stems. Still, the staining in apical segments by both stains is usually somewhat stronger. In line with this, the blue-light-excited fluorescence of stems stained by toluidine blue (*sensu* Hutzler *et al.* 1998; Sulborska *et al*. 2020) led to marked green coloration (Figs. 4–6), which was stronger in apical segments. In apical *U. vulgaris* stem segments, staining by acidic phloroglucinol for lignin detection resulted in weak staining, and staining by lipidic Sudan Red 7B for suberin (cork) gave negative results (A. Soukup, unpubl. results). Apical and basal stem segments of *U. stygia* were only around 1 mm in diameter and their central cylinder was relatively very thin (only around 17–18% of the total stem diameter). Staining by Safranine or toluidine blue was very weak in both segments of the stem (Fig. 7).

As it follows from the microphotos, the anatomy of apical stem segments bordering with turions in *U. vulgaris*, *U. australis*, and *U. macrorhiza* with robust shoots is not too different from that in basal segments. The results may be interpreted (e.g. after Bond *et al*. 2008; Baldacci-Cresp 2020;

Schweingruber *et al*. 2020) that–at least in *U. australis* and *U. macrorhiza*–apical stem segments are more lignified in their central cylinder than in basal ones. Still, the level of lignification is not high. Moreover, except for *U. australis*, the central cylinder, as well as the adjacent inner layers of the cortical cells in the apical segments in the other two robust *Utricularia* species (especially in *U. vulgaris*), contain more phenolic compounds than the basal ones (e.g. after Hutzler *et al.* 1998;

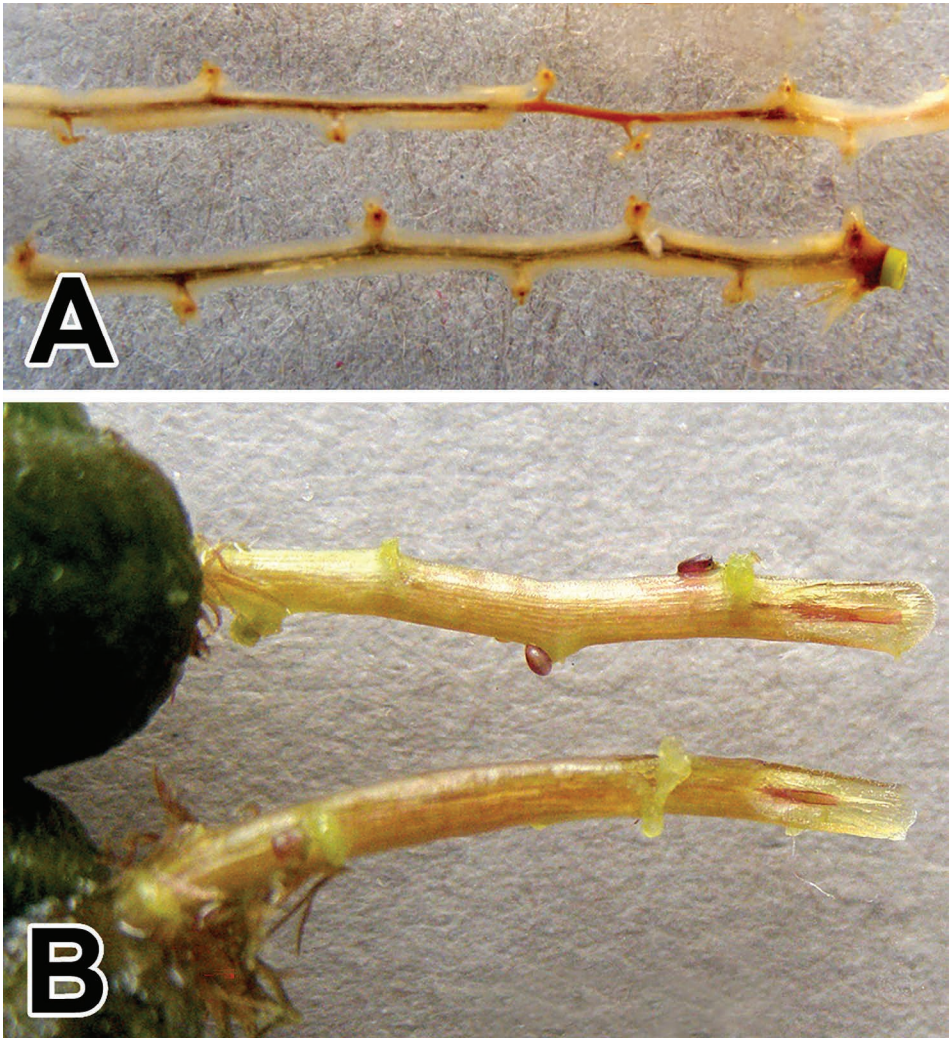

Figure 3: (A) Decomposing apical segments of stems of *Utricularia vulgaris*, which were attached to mature turions and which had dragged the turions to the bottom of an outdoor container; 13 November 2022. Note that the stem cortex is rather decomposed, but the brown central cylinder is quite intact. (B) Mature turions with adjacent stems and brown central cylinder in odd stem sections, 14 October 2022.

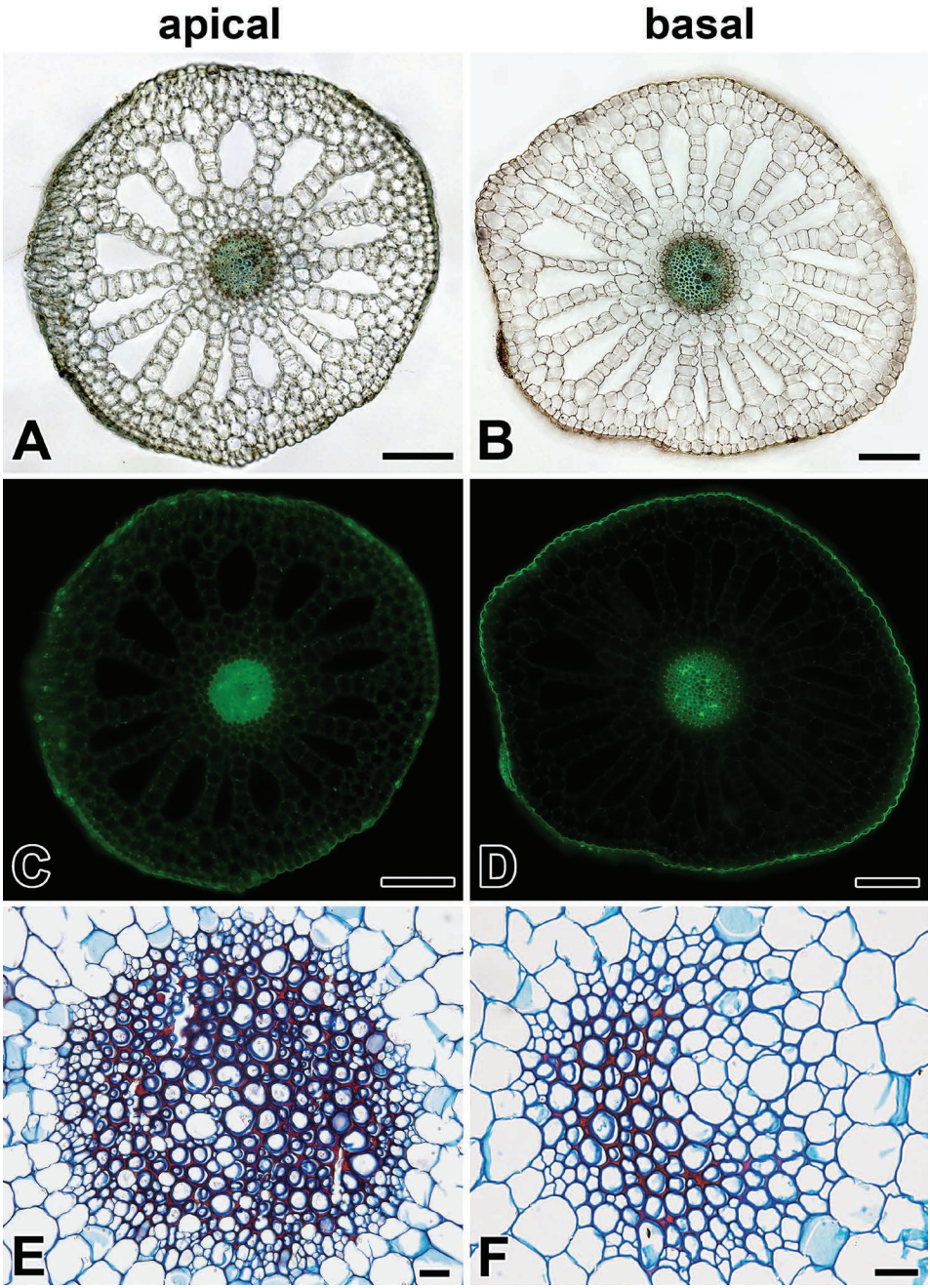

Figure 4: Cross-sections through apical (A, C, E) and basal (B, D, F) stem segments of *Utricularia australis*. (A-B) stained by toluidine blue for phenolic substances; (C-D) a fluorescence induced by blue light; (E-F) details of central cylinder stained by Safranin/ Astra Blue for lignin. Scale bar: (A-D) 200 µm; (E-F) 20 µm.

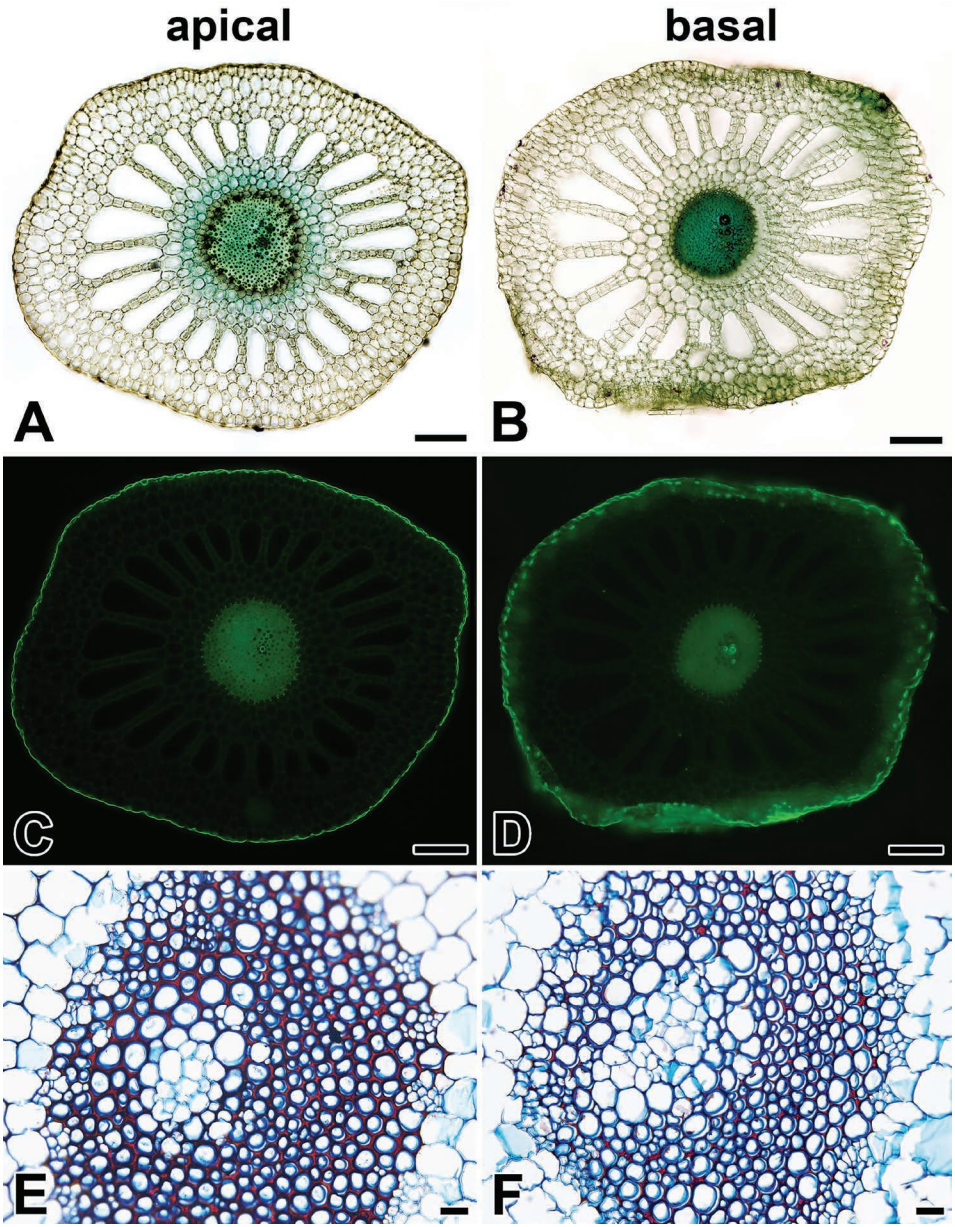

Figure 5: Cross-sections through apical (A, C, E) and basal (B, D, F) stem segments of *Utricularia macrorhiza*. For stainings and legends, refer to Fig. 4.

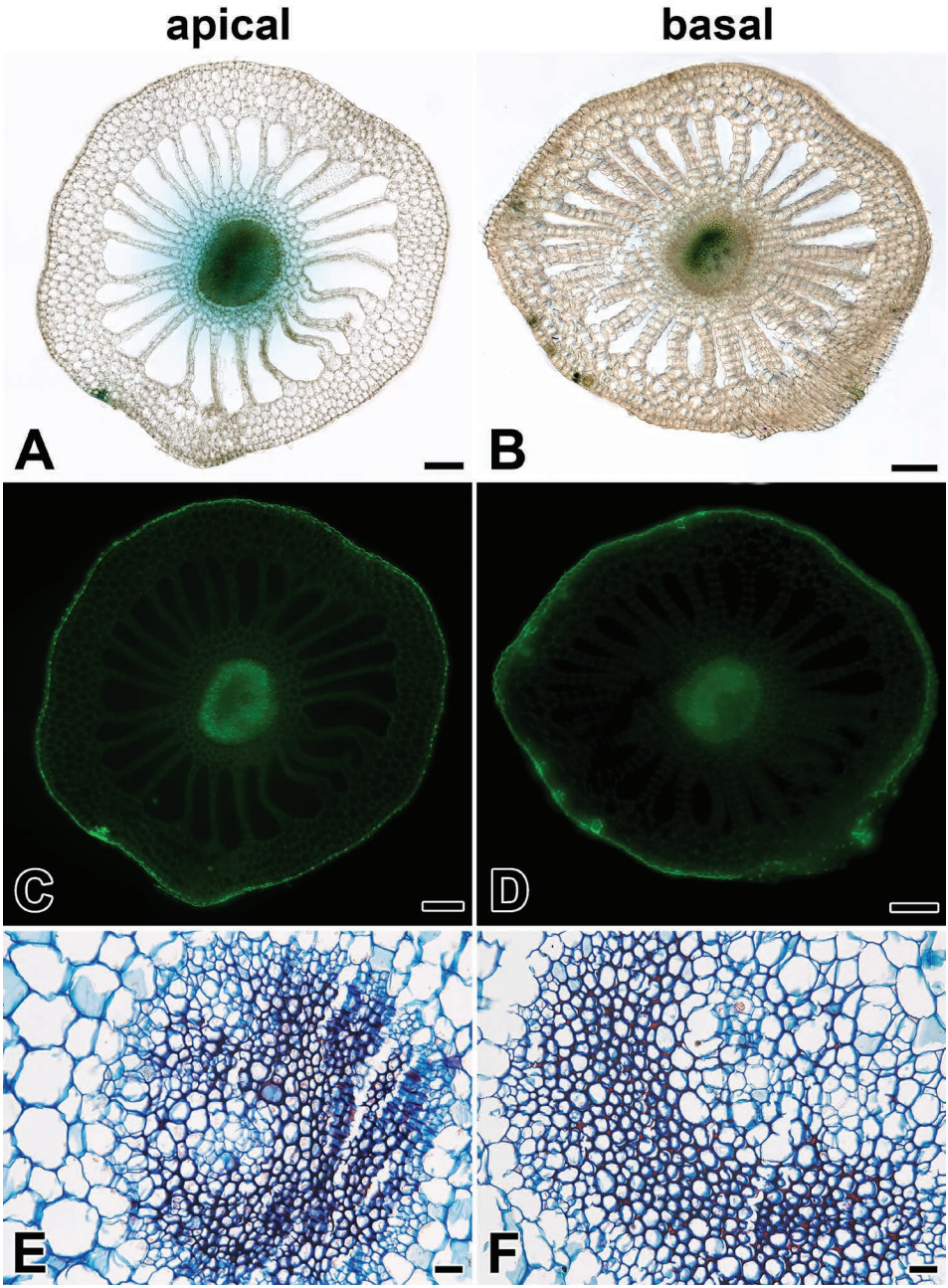

Figure 6: Cross-sections through apical (A, C, E) and basal (B, D, F) stem segments of *Utricularia vulgaris*. For stainings and legends, refer to Fig. 4.

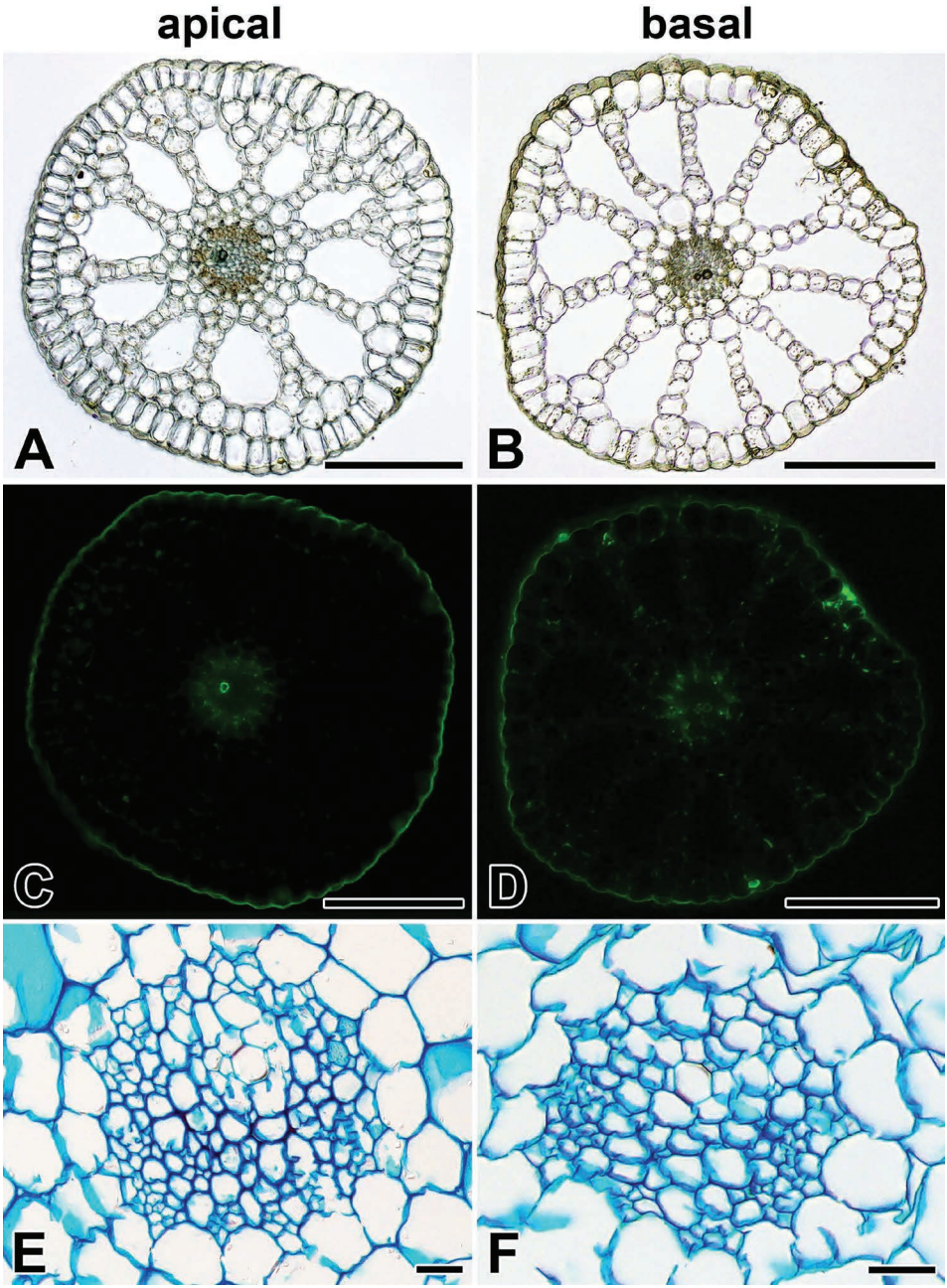

Figure 7: Cross-sections through apical (A, C, E) and basal (B, D, F) stem segments of *Utricularia stygia*. For stainings and legends, refer to Fig. 4.

Sulborska *et al*. 2020). In the apical stem segments fixed in 70% ethanol for ca. 15 months, the intensity of the brown coloration of the central cylinder (Fig. 3) was not changed, and the brown substances were not solubilized or washed out. Presumably, these substances are polyphenols. In contrast, mature *U. stygia* turions usually dehisce from mother stems and their stems do not drag them to the bottom. Moreover, they usually grow in very shallow waters. Their apical stem segments have a very thin central cylinder without lignification or polyphenol substances (Fig. 7). Moreover, the anatomical stem adaptation was not observed in other Central European aquatic carnivorous plant species, which more or less dehisce their maturating turions from mother stems (*Aldrovanda vesiculosa*, *U. bremii*, *U. minor*, *U. intermedia*, *U. ochroleuca*; L. Adamec, unpubl. observ.).

Following Schweingruber *et al.* (2020), in summer stems of three out of four species used here, the xylem and phloem within the central cylinder are difficult to distinguish and have a small proportion, as typical for rootless aquatic species. In three robust *Utricularia* species in the present study, the central cylinder of apical stem segments with mature turions probably has non-functional vascular bundles as these are impregnated by phenolic substances. By the next spring, the brown central cylinders of apical stem segments decompose and release turions that rise to the surface. However, it is interesting that the described anatomical adaptation of apical stem segments in three robust aquatic *Utricularia* species occurs only in larger turions: stems bearing turions as small as 2–4 mm usually do not form the adaptation, and these mature turions are detached from the dying mother shoots as early as in the autumn (L. Adamec, unpubl. observ.). The ecological purpose might be that the smallest turions of all *Utricularia* species have the highest probability of being attached to water birds' feathers and transported to another potential aquatic site than large turions. On the other hand, the small, free-floating turions are also more subject to being grazed by water birds and embedded in ice. However, it is not known what the mortality of free-floating *Utricularia* turions is over the winter period, but considerable frost resistance of all turions has been observed (Adamec 2018).

In conclusion, our research reveals that maturating large turions of three robust *Utricularia* species are firmly attached to senescent mother stems due to an anatomical adaptation of the central cylinder. Polyphenolic substances mainly impregnate the central cylinder in apical stem segments, thus preventing rapid decomposition of the stem at the bottom. Species that dehisce their turions from dying mother stems do not exhibit the adaptation.

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References

Adamec, L. 1999. Turion overwintering of aquatic carnivorous plants. Carniv. Pl. Newslett. 28: 19-24. https://doi.org/10.55360/cpn281.la532

Adamec, L. 2003. Ecophysiological characterization of dormancy states in turions of the aquatic carnivorous plant *Aldrovanda vesiculosa*. Biol. Plant. 47: 395-402. https://doi.org/10.1023/B:BIOP.0000023883.62127.5e

Adamec, L. 2008. Respiration of turions and winter apices in aquatic carnivorous plants. Biologia 63: 515-520. https://doi.org/10.2478/s11756-008-0073-4

Adamec, L. 2018. Ecophysiological characteristics of turions of aquatic plants: A review. Aquat. Bot. 148: 64-77. https://doi.org/10.1016/j.aquabot.2018.04.011

Adamec, L. 2020. Biological flora of Central Europe: *Utricularia intermedia* Hayne, *U. ochroleuca* R.W. Hartm., *U. stygia* Thor and *U. bremii* Heer ex Kölliker. Perspect. Plant Ecol. Evol. Syst. 44: e125520. https://doi.org/10.1016/j.ppees.2020.125520

Adamec, L., and Kučerová, A. 2013. Overwintering temperatures affect freezing temperatures of turions of aquatic plants. Flora 208: 497-501. https://doi.org/10.1016/j.flora.2013.07.009

Adamec, L., Kučerová, A., and Janeček, Š. 2020. Mineral nutrients, photosynthetic pigments and storage carbohydrates in turions of 21 aquatic plant species. Aquat. Bot. 165: 103238. https://doi.org/10.1016/j.aquabot.2020.103238

Adamec, L., Plačková, L., and Doležal, K. 2024. Characteristics of turion development in two aquatic carnivorous plants: Hormonal profiles, gas exchange and mineral nutrient content. Plant Direct 8: e558. https://doi.org/10.1002/pld3.558

Baldacci-Cresp, F., Spriet, C., Twyffels, L., Blervacq, A.-S., Neutelings, G., Baucher, M., and Hawkins, S. 2020. A rapid and quantitative safranin-based fluorescent microscopy method to evaluate cell wall lignification. The Plant J. 102: 1074-1089. https://doi.org/10.1111/tpj.14675

Bond, J., Donaldson, L., Hill, S., and Hitchcock, K. 2008. Safranine fluorescent staining of wood cell walls. Biotech. Histochem. 83: 161-171. https://doi.org/10.1080/10520290802373354

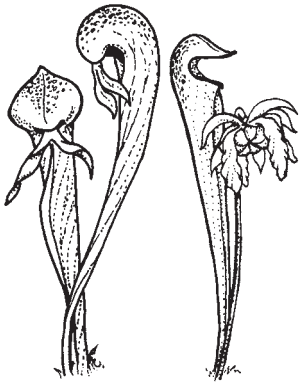
Hutzler, P., Fischbach, R., Heller, W., Jungblut, T.P., Reuber, S., Schmitz, R., Veit, M., Weissenböck, G., and Schnitzler, J.-P. 1998. Tissue localization of phenolic compounds in plants by confocal laser scanning microscopy. J. Exp. Bot. 49: 953-965. https://doi.org/10.1093/jxb/49.323.953

Levine, J.D., Sauman, I., Imbalzano, M., Reppert, S.M., and Jackson, F.R. 1995. Period protein from the giant silkmoth *Antheraea pernyi* functions as a circadian clock element in *Drosophila melanogaster*. Neuron 15: 147-157. https://doi.org/10.1016/0896-6273(95)90072-1

Schweingruber, F.H., Kučerová, A., Adamec, L., and Doležal, J. 2020. Anatomic Atlas of Aquatic and Wetland Plant Stems. Springer Nature Switzerland AG, Cham, Switzerland. https://doi.org/10.1007/978-3-030-33420-8

Sculthorpe, C.D. 1967. The Biology of Aquatic Vascular Plants. Edward Arnold, London, U.K.

Sulborska, A., Konarska, A., Matysik-Woźniak, A., Dmitruk, M., Weryszko-Chmielewska, E., Skalska-Kamińska, A., and Rejdak, R. 2020. Phenolic constituents of *Lamium album* L. subsp. *album* flowers: Anatomical, histochemical, and phytochemical study. Molecules 25: e6025. https://doi.org/10.3390/molecules25246025



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Front Cover: *Utricularia macrorhiza*, late season turions, preparing for winter dormancy at a high elevation site (Plumas County, California, USA). Photo by Barry Rice. Article on page 128.

Back Cover: *Utricularia macrorhiza*, late season turions at a circumneutral pH bog (Berkshire County, Massachusetts, USA). Photo by Barry Rice. Article on page 128.

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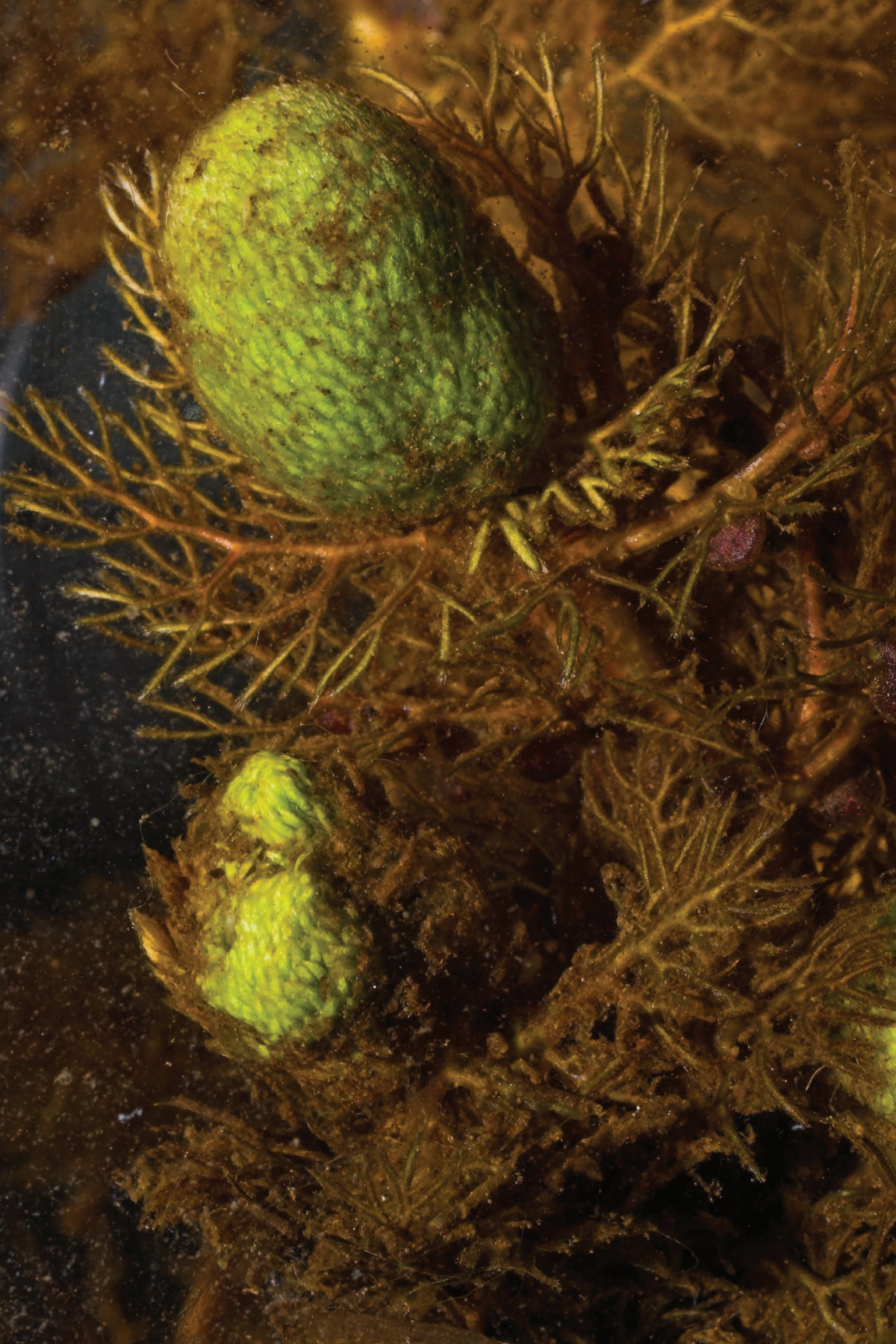
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