Floral micromorphology and nectar composition of the early evolutionary lineage *Utricularia* (subgenus *Polypompholyx*, Lentibulariaceae)

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Abstract

*Utricularia* (Lentibulariaceae) is a genus comprising around 240 species of herbaceous, carnivorous plants. *Utricularia* is usually viewed as an insect-pollinated genus, with the exception of a few bird-pollinated species. The bladderworts *Utricularia multifida* and *U. tenella* are interesting species because they represent an early evolutionary *Utricularia* branch and have some unusual morphological characters in their traps and calyx. Thus, our aims were to (i) determine whether the nectar sugar concentrations and composition in *U. multifida* and *U. tenella* are similar to those of other *Utricularia* species from the subgenera *Polypompholyx* and *Utricularia*, (ii) compare the nectary structure of *U. multifida* and *U. tenella* with those of other *Utricularia* species, and (iii) determine whether *U. multifida* and *U. tenella* use some of their floral trichomes as an alternative food reward for pollinators. We used light microscopy, histochemistry, and scanning and transmission electron microscopy to address those aims. The concentration and composition of nectar sugars were analysed using high-performance liquid chromatography. In all of the examined species, the floral nectary consisted of a spur bearing glandular trichomes. The spur produced and stored the nectar. We detected hexose-dominated (fructose + glucose) nectar in *U. multifida* and *U. tenella* as well as in *U. violacea*. In both *U. multifida* and *U. tenella*, there were trichomes that blocked the entrance into the throat and spur. Because these trichomes were rich in chromoplasts and contained lipid droplets, they may form an additional visual attractant. Bearing in mind the phylogenetic hypothesis for the genus, we suggest that an early ancestor of *Utricularia* had a nectariferous spur flower with a lower lip that formed a wide landing platform for bee pollinators.

Keywords Australian bladderwort · Bee pollination · Carnivorous plant · Floral micromorphology · HPLC · Lentibulariaceae · Nectary structure · Nectar composition · *Polypompholyx* · Pleiochasia · Spur · Trichomes

Introduction

The Lentibulariaceae comprise three monophyletic genera of carnivorous plants: *Pinguicula* L., *Genlisea* A.St.-Hil., and *Utricularia* L. (Juniper et al. 1989; Jobson et al. 2003). According to Silva et al. (2018), the last common ancestor of the *Genlisea-Utricularia* clade was a South American lineage that arose 39 million years ago (Mya). The genus

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**Material and methods**

**Plant material**

Flowers of *Utricularia multifida* R.Br., *U. tenella* R.Br. (sect. *Polypondophylyx*), and *U. violacea* R.Br. (sect. *Pleiochasia*) were collected from the Alison Baird Reserve (Yule Brook) in Western Australia by HL, FJN, and GRC. The flowers were fixed in a mixture of 2.5% (v/v) or 5% (v/v) glutaraldehyde.
with 2.5% (v/v) formaldehyde in a 0.05-M cacodylate buffer (pH 7.2; Sigma) or 70% (v/v) ethanol and sent to Poland for the morphological and histochemical studies (Jagiellonian University, Kraków). Additional plant material (U. tenella flowers in ethanol) was provided by the National Herbarium of Victoria, Melbourne, Australia.

Floral structure and histochemical investigations

The distribution of the secretory glandular trichomes was determined by examining whole flowers (corollas) using a Nikon SZ100 stereoscopic microscope (Nikon Instruments Europe B.V., City, Country). The floral parts, namely the spurs, were examined using light microscopy and scanning electron microscopy. Fixed material was washed three times in a 0.1-M sodium cacodylate buffer and post-fixed in a 1% (w/v) osmium tetroxide solution at room temperature for 1.5 h. Dehydration was performed using a graded ethanol series, and infiltration and embedding using an epoxy embedding medium kit (Fluka). Semi-thin sections (0.9–1.0 μm) were prepared for light microscopy and stained for the general histology using aqueous methylene blue/azure II (MB/AII) for 1–2 min (Humphrey and Pittman 1974) and examined with an Olympus BX60 light microscope (Tokyo, Japan).

The hand sections were immersed in water and analysed using bright field and fluorescence microscopy. The material was tested for lipids, starch, and mucilage, using a saturated ethanol solution of Sudan III, an aqueous IKI (iodine-potassium iodide) solution, and a ruthenium red solution, respectively. The autofluorescence of the cuticle was observed under UV light, and the structure of the cuticle was studied on sections that had been stained with auramine O (Gahan 1984). For the scanning electron microscopy, the representative floral parts were fixed (as above), dehydrated, and subjected to critical drying point using liquid CO₂. Then, they were sputter-coated with gold and examined at an accelerating voltage of 20 kV using a Hitachi S-4700 scanning electron microscope (Hitachi, Tokyo, Japan) at the Institute of Geological Sciences, Jagiellonian University in Kraków.

Ultrastructure analysis

For the transmission electron microscopy (TEM), the flowers were fixed in a mixture of 2.5% (v/v) or 5% (v/v) glutaraldehyde with 2.5% (v/v) formaldehyde in a 0.05-M cacodylate buffer (pH 7.2; Sigma), washed three times in a 0.1-M sodium cacodylate buffer, and post-fixed in a 1% (w/v) osmium tetroxide solution at room temperature for 1.5 h. Dehydration using a graded ethanol series and infiltration and embedding using an epoxy embedding medium kit (Fluka) were followed. After polymerisation at 60 °C, sections for TEM were cut at 70 nm using a Leica ultracut UCT ultramicrotome, stained with uranyl acetate and lead citrate (Reynolds, 1963), and examined using a Hitachi H500 transmission electron microscope (Hitachi, Tokyo, Japan) at an accelerating voltage of 75 kV.

Nectar collection and analysis

For each species (U. multifida, U. tenella, and U. violacea), the flowers from ten individual plants were collected in the field. Each flower was immediately stored on ice for transport to the laboratory. As it was not possible to extract free nectar using glass capillary tubes, washing flower spur in accordance with Morrant et al. (2009), who recommends flower wash for nectar collection from flowers with low nectar volumes in the field, was undertaken. The spur was removed from each flower with a scalpel blade under a magnifying glass. The spur was then placed in an Eppendorf tube and 100 μL Milli-Q water was added. Samples were vortex mixed before being centrifuged for 5 min; this was repeated three times. Later, the samples were analysed using high-performance liquid chromatography (HPLC) as in Plachno et al. (2019b).

Results

Utricularia multifida (Figs. 1, 2, 3, and 4)

The corolla of U. multifida was pink with a yellow palate (Fig. 1a–b). It was bilabiate and spurred. The lower lip of the corolla was trilobate and flat and formed a wide landing platform for pollinators (Fig. 1a, c). Flat, “jigsaw-puzzle”-shaped cells covered the entire flat surface of the lower lip of the corolla (Fig. 1d). The palate had clearly visible parallel ridges; this part of the palate was covered by papillae with cuticular striations (on the ridges) or papillose cells with cuticular striations (Fig. 1e–f). The most prominent character of the palate was the occurrence of multi-celled trichomes (Figs. 1e and 2a–c), which had specific shapes (like an inflated balloon with constrictions, Fig. 2a). These trichomes blocked the entrance to the throat. The cells of these trichomes had many chromoplasts and contained some lipid droplets (Fig. 2c), which were also revealed by the Sudan staining. These trichomes did not have protein bodies or starch. These trichomes also occurred in the throat and in the basal part of the spur (Fig. 2b, d).

The cylindrical spur was directed downwards and parallel to the lower lip. Both the external and internal epidermis of the spur had small capitate glandular trichomes (Figs. 2b, d, e and 3a). The capitate glandular trichomes from the external spur surface consisted of a stalk cell, a pedestal cell and a four-celled head (Figs. 2e and 3c). In the transverse section, the wall of the spur was composed of several cell layers: the internal epidermis, layers of parenchyma cells and the outer epidermis (Fig. 3a–b). There were collateral vascular bundles...
in the ground parenchyma (Fig. 3a–c) and each contained both xylem and phloem elements. The internal epidermis formed papillae, which were unicellular with cuticular striations on their surface (Fig. 3a–f). The papillae from the basal part of the spur were slightly different from those of the apical part of the spur when the cuticular striations were developing. The papillae from the apical part of the spur had cuticular striations on the entire external cell surface (Fig. 3e–f) and the cuticular striations of the neighbouring cells were fused (Fig. 3e). There were no nectary stomata. Nectar trichomes occurred on the internal side of the spur on its apical part (Figs. 2d and 3a, f). Each nectar spur trichome was composed of a single basal cell that formed a unicellular stalk, a pedestal cell (barrier cell), and a multi-celled head (Fig. 3f). The basal cell had prominent cuticular striations on its surface (Figs. 3f and 4a). There were numerous plasmodesmata in the transverse walls between the stalk cell and the pedestal cell (Fig. 4a). The pedestal cell had a thick radial wall, which was impregnated with cutin (Fig. 4a). The cytoplasm of the pedestal cell contained a nucleus and the usual organelles (mitochondria, plastids, and profiles of the endoplasmic reticulum; Fig. 4a).

In addition, there were also lipid droplets. The reticulate cell wall ingrowths occurred on the transverse wall and partially on the lateral wall of the pedestal cell (Fig. 4b). The terminal (head) cells had large vacuoles (Fig. 4b–d). Mitochondria and plastids were numerous (Fig. 4c–f). The plastids were cup-shaped and contained numerous small lipid globules (Fig. 4c and f). These organelles were associated with the rough endoplasmic reticulum. Only few dictyosomes were of a observable (Fig. 4c). Lipid droplets were visible in the cytoplasm (Fig. 4f). The small multivesicular bodies in the thin layer of cytoplasm between the plasmalemma and vacuole were observed (Fig. 4d). There were small cell wall ingrowths only on the inner surface of the outer wall (Fig. 4d). The cuticle of terminal cells was very thick. Amorphous globules occurred in the cutinised layer of the cell wall (Fig. 4e). The cuticle frequently became distended and separated from the cell walls and formed a subcuticular space (Fig. 4e). We observed some lipid or cutin material between the cuticle and the cell wall (Fig. 4e). Hexose-dominated nectar was detected in the flower spurs (fructose 54 ± 1.2%, glucose 46 ± 1.2%; sugar concentration, fructose 8.1 ± 3.0 μg flower⁻¹, glucose 6.7 ± 2.4 μg flower⁻¹).

Utricularia tenella (Fig. 5)

The corolla of U. violacea was blue-violet with a yellow palate with dark violet marks (Fig. 6a framed part). It was bilabi ate and spurred. The lower lip of the corolla formed a wide landing platform for pollinators (Fig. 6a). The palate had clearly visible protrusions; this part of the palate was covered by papillae with cuticular striations (Fig. 6b). The inner part of the palate was glabrous (Fig. 6c). Papillose cells covered the entire flat surface of the lower lip of the corolla (Fig. 6d). Transverse sections showed that the wall of the spur was composed of several cell layers: the internal epidermis, layers of parenchyma cells, and the outer epidermis. Within the spur were multicellular, capitate, sessile-glandular trichomes, and papillae (Fig. 6e–f). Each nectar spur trichome was composed of two basal cells, a pedestal cell (barrier cell), and a multicelled head (Fig. 6f). The terminal (head) cells were transfer cells; there were cell wall ingrowths on the inner surface of the outer wall and on the inner walls between the terminal cells (not shown). The thick cuticle frequently became distended and separated from the cell walls of the head cells to form a subcuticular space (Fig. 6f). Hexose-dominated nectar was detected in the flower spurs (fructose 58 ± 0.3%, glucose 42 ± 0.3%; sugar concentration, fructose 20.5 ± 1.5 μg flower⁻¹, glucose 14.7 ± 1.2 μg flower⁻¹).

Discussion

We show that all three examined species, Utricularia multifida, U. tenella, and U. violacea, had hexose-dominated (fructose + glucose) nectar, and this suggests that they are pollinated by similar pollinators. However,
Abrahamczyk et al. (2017) have shown that low nectar sucrose proportion (i.e. hexose-dominated nectar) may indicate generalist-pollinated plants. The U. multifida flowers were visited by European honeybees, which are
probably also pollinators of the other species. Carow (Fig. 22.2a in Cross et al. 2018) also observed a halictid bee visiting a *U. multifida* flower. To date, the nectar of only one other species from the subgenus *Polypompholyx*
has been examined—*U. menziesii* (Plachno et al. 2019b), which is probably bird-pollinated (its flowers are visited by a bird—the Western spinebill, *Acanthorhynchus superciliosus*, Lambers et al. 2014; Lowrie 2013). *Utricularia menziesii* also has hexose-dominated nectar; the occurrence of this kind of nectar does not exclude...
insects as additional visitors of the flowers of *U. menziesii*, but such an observation is lacking. Unfortunately, other data about the sugar composition in *Utricularia* nectar are limited to only three South American species: *U. alpina* Jacq., *U. reniformis* A.St.Hil., and *U. nephrophylla* Benj. (Abrahamczyk et al. 2017), which
are classified as pollinated by bees and wasps. These species belong to sect. *Orchidioides*, subgenus *Utricularia* (Rodrigues et al. 2017; Silva et al. 2018). According to Abrahamczyk et al. (2017), although both *U. nephrophylla*
and *U. reniformis* have hexose-dominated nectar, only sucrose was detected in the nectar of *U. alpina*. Among these species, pollinators are only known for *U. reniformis* – *Xylocopa* sp. and *Bombus* sp. (Clivati et al. 2014). Hobbahn et al. (2006) examined nectar production in *P. purpurascens* and *U. reticulata* (both from section Oligocista, subgenus *Bivalvaria*). Nectar was detected in *Genlisea* spurs (Fleischmann 2012), and it is produced by small capititate trichomes in *Genlisea violacea* spurs (Aranguren et al. 2018). *Genlisea violacea* nectar is mainly composed of fructose and glucose, which is similar to assessed *Utricularia* species, and its quantities are stable during the day (Aranguren 2016). Abrahamczyk et al. (2017) provided data about the nectar in five species of *Pinguicula*. Species that are classified as pollinated by butterflies, *Pinguicula macrophylla* Kunth, and *Pinguicula moctezumae* Zamudio & R.Z. Ortega, have fructose-dominated nectar, whereas species that are classified as pollinated by bees and wasps have sucrose-dominated nectar (*Pinguicula gigantea* Luhrs) or hexose-dominated nectar (*Pinguicula leptoceras* Rehb.). *Pinguicula alpina* L., which is pollinated by flies, have sucrose-dominated nectar.

It should be added that Abrahamczyk et al. (2017) presented evidence that nectar sucrose proportion is an adaptation in nectar evolution to pollinator group in asterids, but these authors also suggested that adaptation to pollinators is not a sufficient explanation on its own. We showed that species from *Utricularia* early evolutionary lineage (subgenus *Polypompholyx*) had hexose-dominated nectar, but occurrence of sucrose-rich nectar in species from advanced evolutionary lineage (*U. alpina*, sect. Orchidioideae, subgenus *Utricularia*; Abrahamczyk et al. 2017) may indicate that sugar composition is not “phylogenetically constrained” (phylogenetic conservatism in sugar composition) in the case of *Utricularia*.

According to Lang (1901), the edge of the *U. multifida* throat is surrounded by a wreath of peculiarly shaped trichomes. He described this as “hairs are very rich in plasma and the outer walls of their cells are only weakly cutinized; the transverse walls between the individual cells are extremely delicate; they are not cutinized.” We found that this type of trichomes also occurs in *U. tenella*. It is possible that they block the entrance into the throat and nectariferous spur to visiting insects that do not fit their pollination syndrome (illegitimate visitors); this may protect them from having their nectar stolen. Because these trichomes were yellow and rich in chromoplasts, we suggest that they form an additional visual attractant for bees. Because we wanted to determine whether they could be food trichomes (an alternative food reward for pollinators), we checked for the occurrence of starch, protein bodies, and lipid droplets. Because the cells of these trichomes did not accumulate starch and protein bodies and contained only some lipid droplets, we have no evidence that they play the role of food trichomes and suggest that they are a visual and tactile guide for their pollinators. The occurrence of lipid droplets is relatively rare in edible trichomes and has only been recorded in some species of Orchidaceae, e.g. in *Cyaneaorchis arundinae* (Pansarin and Maciel 2017) and *Maxillaria* (Davies et al. 2000). Lang (1901) was thought that these trichomes were undoubtedly used for insect pollination; however, he also proposed that they might prevent the penetration of rain. Jachula et al. (2018) proposed that the non-glandular trichomes of the *Linaria vulgaris* palate are involved in protecting against airborne fungal propagules or dust particles; therefore, they may also play a similar role here. However, *U. violacea*, which grows together with *U. multifida* and *U. tenella*, does not have these trichomes, whereas other species from the subgenus *Polypompholyx* (*U. menziesii*, *U. uniflora*, and *U. dichotoma*) exhibit many unicellular trichomes at the palate and throat (Plachno et al. 2016, 2019b). The histochemical tests indicated that these trichomes did not produce mucilage or proteins, but that they did have chromoplasts. Plachno et al. (2019b) proposed that these trichomes are a tactile signal. In *Genlisea violacea*, there are non-glandular trichomes on the palate or throat (Aranguren et al. 2018). However, these trichomes are different from the trichomes of *U. multifida* and *U. tenella* which have thick cuticular striations (well visible under UV light in *Genlisea*, see Fig. 4 in Aranguren et al. 2018) and a different cell shape. In *Pinguicula* flowers, there are numerous non-glandular multicellular trichomes at the palate and in the throat. These trichomes are useful taxonomic characters and aid in species identification (Casper 1966). According to Fleischmann (2016), these trichomes are considered “feeding hairs,” and in some *Pinguicula* species, these trichomes (a cluster of yellow trichomes on the palate) mimic a stamen or pollen. Examined species did not have glandular trichomes at the palates, in contrast to some species of *Utricularia* (Plachno et al. 2017b) and *Genlisea* (Plachno et al. 2018a).

The floral nectaries in the analysed species are not much different from those of previously investigated *Utricularia* species in their anatomy and micromorphology (Clivati et al. 2014; Plachno et al. 2016, 2017a, 2018b, 2019b). The spur is the organ in which nectar is produced and stored. The nectar is produced by small capititate trichomes, which have a similar architecture across the genus (Plachno et al. 2018b). The only difference is the presence of two basal cells in the trichomes of *U. violacea*. Glandular capititate trichomes with two basal cells were recorded in *Byblis* (Lloyd, 1942), which is a genus that was previously considered to be related to Lentibulariaceae, but which was reclassified into Lamiaceae (APG IV 2016; Schäferhoff et al. 2010). The ultrastructure of the nectary trichomes in *Utricularia multifida* is very similar to that of species from section *Utricularia* (Plachno et al. 2018b); this is evidence for a conservative construction of the nectary cells in this genus. The cup-shaped plastids that have been recorded in
the glandular trichome cells of *U. multifida* are typical for the secretory cells of nectary trichomes (Plachno et al. 2018b) and palate trichomes in *Utricularia* (Plachno et al. 2017b). The presence of lipid droplets in the cytoplasm in the nectary trichome cells of *U. multifida* suggests that it may enrich the nectar with lipids. This was suggested for the nectaries of various plant species from other families (Machado et al. 2017). The low densities of dictyosomes that were observed in the glandular trichome cells of *U. multifida* indicate that nectar secretion occurs via an eccrine mode. The presence of cell wall ingrowths and numerous mitochondria (Gunning and Pate 1969) in the glandular trichome cells also supports this. This mode of secretion was previously proposed by Plachno et al. (2018b) for the nectary trichomes of *Utricularia* species from section *Utricularia*. Vassilyev (2010) proposed that sugars cross the plasma membrane via an active transport, which characterises an eccrine secretion. Other authors also favour an eccrine mode of the secretion of nectar, e.g. Lüttge and Schnepf (1976), Nepi (2007), and Paiva (2012). Based on evidence available from phylogenetic studies of this genus (e.g. Jobson and Albert 2002; Jobson et al. 2017, 2018; Müller and Borsch 2005; Silva et al. 2018) and recent studies on their floral and nectary structure (e.g. Clivati et al. 2014; Lowrie 2013; Plachno et al. 2016, 2017a, 2018b, 2019b; Taylor 1989, and our data), we suggest that an ancient ancestor of *Utricularia* had a nectariferous spur flower with a lower lip that formed a wide landing platform for its bee pollinators.

**Conclusions**

*Utricularia multifida*, *U. tenella*, and *U. violacea* exhibit traits indicative of the bee pollination syndrome (melittophily): closed, zygomorphic flowers with vivid colours, and hexose-dominated (fructose + glucose) nectar inside the spur of the corolla. However, the occurrence of hexose-dominated nectar may also indicate a broader spectrum of pollinators. Both *U. multifida* and *U. tenella* have trichomes that block the entrance into the throat and the nectariferous spur to visiting insects that do not fit their pollination syndrome. Because these trichomes are rich in chromoplasts and have a specific shape, we suggest that they are additional visual and tactile attractants for pollinators.

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**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflicts of interest.

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**References**

Abrahamczyk S, Kessler M, Hanley D, Karger DN, Müller MPJ, Knauer AC, Keller F, Schwerdtfeger M, Humphreys AM (2017) Pollinator adaptation and the evolution of floral nectar sugar composition. *J Evol Biol* 30(1):112–127. https://doi.org/10.1111/jeb.12991

APG IV (2016) An update of the angiosperm phylogeny group classification for the orders and families of flowering plants: APG IV. *Bot J Linn Soc* 181(1):1–20

Aranguren Y (2016) Genética molecular de *Genlisea violacea* A.St.-Hil. e *Genlisea aurea* A.St.-Hil. (Lentibulariaceae). PhD thesis, São Paulo State University, School of Agricultural and Veterinarian Sciences, Jaboticabal

Aranguren Y, Plachno BJ, Stipczyńska M, Miranda VFO (2018) Reproductive biology and pollination of the carnivorous Genlisea violacea (Lentibulariaceae). *Plant Biol* 20(3):591–601. https://doi.org/10.1111/plb.12683

Casper SJ (1966) Monographie der Gattung Pinguiscula L. *Bibliotheca Botanica* 127–128:1–209

Clivati D, Cordeiro GD, Plachno BJ, Miranda VFO (2014) Reproductive biology and pollination of *Utricularia reniformis* A.St.-Hil. (Lentibulariaceae). *Plant Biol* 16(3):677–682

Cross AT, Davis AR, Fleischmann A, Horner JD, Järgens A, Merritt DJ, Murza GL, Turner SR (2018) Reproductive biology and pollinator-prey conflicts. In: Ellison AM, Adamec L (eds) Carnivorous plants: physiology, ecology, and evolution. Oxford University Press, Oxford, pp 294–313. https://doi.org/10.1093/oso/9780198779841.003.0022

Davies KL, Winters C, Turner MP (2000) Pseudopollen: its structure and development in Maxillaria (Orchidaceae). *An Bot* 85:895–899

Fleischmann A (2012) Monograph of the genus Genlisea. Redfern Natural History, Poole, 727 pp

Fleischmann A (2016) Pinguiscula flowers with pollen imitations close at night—some observations on butterwort flower biology. *CPN* 45:84–92

Gahan PB (1984) Plant histochemistry and cytochemistry: an introduction. Academic Press, London

Gunning BES, Pate JS (1969) Transfer cells, "plant cells with wall ingrowths" specialized in relation to short distance transport of solutes—their occurrence, structure, and development. *Protoplasma* 68:107–133

Hobbhahn N, Kühmeister H, Porembski S (2006) Pollination biology of mass flowering terrestrial *Utricularia* species (Lentibulariaceae) in the Indian Western Ghats. *Plant Biol* 8:791–804


Humphrey C, Pittman G (1974) A simple methylene blue-azure II-basic fuchsin for epoxy-embedded tissue sections. Stain Technol 49:9–14
Jachula J, Konarska A, Denisow B (2018) Micromorphological and histochemical attributes of flowers and floral reward in Linaria vulgaris (Plantaginaceae). Protoplasma 255:1763–1776
Jobson RW, Albert VA (2002) Molecular rates parallel diversification contrasts between carnivorous plant sister lineages. Cladistics 18:127–136
Jobson RW, Playford J, Cameron KM, Albert VA (2003) Molecular phylogenetics of lentibulariaceae inferred from plastid rps16 intron and tranL-F DNA sequences: implications for character evolution and biogeography. Syst Bot 28:157–171
Jobson RW, Balseiro PC, Reut MS (2017) Molecular phylogeny of subgenus Polypompholyx (Utricularia; Lentibulariaceae) based on three plastid markers: diversification and proposal for a new section. Aust Syst Bot 30:259–278
Jobson RW, Balseiro PC, Guisande C (2018) Systematics and evolution of Lentibulariaceae: III. Utricularia. In: Ellisson AM, Adamiec L (eds) Carnivorous plants: physiology, ecology, and evolution. Oxford University Press, Oxford, pp 89–104
Juniper BE, Robins RJ, Joel DM (eds) (1989) The carnivorous plants. Academic Press, London
Lammers H, Shane MW, Laliberté E, Swarts ND, Teste FP, Zemunik G (2017) Floral ultrastructure of two Brazilian aquatic-epiphytic bladdersworts: Utricularia cornigera Studnička and U. nelumbifolia Gardner (Lentibulariaceae). Protoplasma 254:353–366
Machado SR, Souza CV, Guimarães E (2017) A reduced, yet functional, nectary structure and ultrastructure. In: Nicolson SW, Lloyd FE (1932) The range of structural and functional variety in the traps of Utricularia. In: Ellinor G, Müller KF (2010) Towards resolving Lamiales relationships: plastidial and nuclear DNA sequences. Mol Phylogen Evol 118:103(3):533–542
Müller K, Borsch T (2005) Phylogenetics of Utricularia (Lentibulariaceae) and molecular evolution of the trnK intron in a lineage with high substitutional rates. Plant Syst Evol 250:39–67
Nepi M (2007) Nectary structure and ultrastructure. In: Nicolson SW, Nepi M, Pacini E (eds) Nectaries and nectar. Springer, Rotterdam, pp 244–277
Nepi M (2007) Nectary structure and ultrastructure. In: Nicolson SW, Nepi M, Pacini E (eds) Nectaries and nectar. Springer, Rotterdam, pp 244–277. https://doi.org/10.1007/978-1-4020-5937-7_3
Paiva EA (2012) Anatomy, ultrastructure, and secretory activity of the floral nectaries in Swietenia macrophylla (Meliaceae). Am J Bot 99:1910–1917
Pansarin ER, Maciel A (2017) Evolution of pollination systems involving edible trichomes in orchids. Aob PLANTS 9:px033. https://doi.org/10.1093/aobpla/plx033
Plachno B, Stępińska M, Świątek P, Davies KL (2016) Floral micro-morphology of the Australian carnivorous bladderwort Utricularia dunlopii, a putative pseudocopulatory species. Protoplasma 253:1463–1473
Plachno B, Stępińska M, Davies KL, Świątek P, Miranda VFO (2017a) Floral ultrastructure of two Brazilian aquatic-epiphytic bladdersworts: Utricularia cornigera Studnička and U. nelumbifolia Gardner (Lentibulariaceae). Protoplasma 254:353–366
Plachno B, Stępińska M, Krajewski Ł, Świątek P, Adamiec L, Miranda VFO (2017b) Flower palate structure of the aquatic bladdersworts Utricularia brevii Heer and U. minor L, from section Utricularia (Lentibulariaceae). Protoplasma 254:2007–2015
Plachno B, Świątek P, Stępińska M, Miranda VFO (2018a) Flower palate ultrastructure of the carnivorous plant Genlisea hispidula Stapf with remarks on the structure and function of the palate in the subgenus Genlisea (Lentibulariaceae). Protoplasma 255(4):1139–1114
Plachno B, Stępińska M, Adamiec L, Miranda VFO, Świątek P (2018b) Nectar trichome structure of aquatic bladdersworts from the section Utricularia (Lentibulariaceae) with observation of flower visitors and pollinators. Protoplasma 255:1053–1064
Plachno B, Świątek P, Adamiec L, Carvalho S, Miranda VFO (2019a) The trap architecture of Utricularia multifida and Utricularia westonii (subg. Polypompholyx) (Lentibulariaceae). Front Plant Sci 10:336. https://doi.org/10.1038/s41399-2019-00036
Plachno B, Stępińska M, Świątek P, Lambers H, Miranda VFO, Nge FJ, Stolarczyk P, Crawthray GR (2019b) Floral micromorphology of the bird-pollinated carnivorous plant species Utricularia menglesi R.Br. (Lentibulariaceae). Ann Bot 123:213–220. https://doi.org/10.1093/aob/mcy163
Reifenrath K, Theisen I, Schnitzler J, Porembski S, Barthlott W (2006) Trap architecture in carnivorous Utricularia (Lentibulariaceae). Flora 201:597–605
Reut MS, Jobson RW (2010) A phylogenetic study of subgenus Polypompholyx: a parallel radiation of Utricularia (Lentibulariaceae) throughout Australasia. Aust Syst Bot 23:152–161
Reynolds ES (1963) The use of lead citrate at high pH as an electronopaque stain for electron microscopy. J Cell Biol 17:208–212
Rodrigues FG, Marulanda NF, Silva SR, Plachno BJ, Adamiec L, Miranda VFO (2017) Phylogeny of the ‘orchid-like’ bladdersworts (gen. Utricularia sect. Orchidioides and Iperuia: Lentibulariaceae) with remarks on the stolon-tuber system. Ann Bot 120:709–723. https://doi.org/10.1093/aob/mcx056
Schäferhoff B, Fleischmann A, Fischer E, Albach DC, Borsch T, Heubl G, Müller KF (2010) Towards resolving Lamiales relationships: insights from rapidly evolving chloroplast sequences. BMC Evol Biol 10:352
Silva SR, Gibson R, Adamiec L, Domínguez Y, Miranda VFO (2018) Molecular phylogeny of bladdersworts: a wide approach of Utricularia (Lentibulariaceae) species relationships based on six plastidial and nuclear DNA sequences. Mol Phylogen Evol 118:244–264. https://doi.org/10.1016/j.ympev.2017.10.010
Taylor P (1986) New Taxa in Utricularia (Lentibulariaceae). Kew Bull 41(1):1–18
Taylor P (1989) The genus Utricularia — a taxonomic monograph. Kew Bull Addit Ser XIV. HMSO, Kew, London, 14,724 pp
Vassilyev AE (2010) On the mechanisms of nectar secretion: revisited. Ann Bot 105:349–354
Westemeier AS, Fleischmann A, Müller K, Schäferhoff B, Rubach C, Speck T, Poppinga S (2017) Trap diversity and character evolution in carnivorous bladdersworts (Utricularia, Lentibulariaceae). Sci Rep 7:12052

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