An architectural model for the bladderwort *Utricularia gibba* (Lentibulariaceae)

Author(s): Theresa A. Chormanski and Jennifer H. Richards
Source: The Journal of the Torrey Botanical Society, 139(2):137-148. 2012.
Published By: Torrey Botanical Society
DOI: http://dx.doi.org/10.3159/TORREY-D-11-00088.1
URL: http://www.bioone.org/doi/full/10.3159/TORREY-D-11-00088.1

Journal of the Torrey Botanical Society 139(2), 2012, pp. 137–148

An architectural model for the bladderwort *Utricularia gibba* (Lentibulariaceae)[1]

Theresa A. Chormanski[2]
Biology/Health & Wellness, Miami Dade College, 11011 SW 104th Street, Miami, FL 33176

Jennifer H. Richards
Dept. of Biological Sciences, Florida International University, 11200 SW 8th Street, Miami, FL 33199

Chormanski, T. A. (Biology/Health & Wellness, Miami Dade College, 11011 SW 104th Street, Miami, FL 33176) and J. H. Richards (Dept. of Biological Sciences, Florida International University, 11200 SW 8th Street, Miami, FL 33199). J. Torrey Bot. Soc. 139: 137–148. 2012.—Members of the aquatic plant genus *Utricularia* have extremely modified vegetative morphology, which includes absence of roots and lack of a clear distinction between leaves and stems. In this study, the vegetative morphology, anatomy and growth pattern of *Utricularia gibba* (L.), a common species in the southern Florida Everglades, was analyzed to determine general developmental patterns and establish a basic model for growth of this species. Specimens were collected from different habitats in southern Florida during both the wet and dry seasons. Light and scanning electron microscopy were used to quantify anatomical and morphological characteristics. *Utricularia gibba* has a shoot apical meristem that produces repeating developmental units, each of which consists of a stolon bearing dichotomously branched leaf-like structure that supports the traps. These structures may subtend one or more axillary buds that can grow out to form additional stolons or inflorescences. The inflorescence produces aerial flowers, but also forms a modified stolon at its base that is covered with unique glandular papillae. Re-iteration of this model through branching creates the floating and benthic mats found in this species. The basic model can be expanded to other species of section Utricularia but not to all species of the genus.

Key words: Carnivorous plants, Everglades, morphology, *Utricularia gibba*.

The carnivorous plant family Lentibulariaceae contains 42% of all carnivorous plant species in three genera, *Pinguicula, Genlisea*, and *Utricularia* (Albert et al. 1992, Jobson et al. 2003, Müller et al. 2004, Müller et al. 2006). *Utricularia*, the largest and most evolutionarily derived genus of the family, has over 200 species (Taylor 1989, Müller et al. 2006). These species have unusual vegetative morphology that includes the production of unique suction traps. These small globose traps actively expel water, creating an internal lumen with negative pressure. When external hairs on the trap door are triggered by small aquatic organisms, the door bulges inward, causing the trap walls to curve inward, and creating a negative pressure that sucks in the prey (Lloyd 1929, Lloyd 1942, Taylor 1989, Joyeux et al. 2011, Vincent and Marmottant 2011). Recent molecular work has shown that *Utricularia* species have very high rates of molecular evolution (Jobson and Albert 2002, Jobson et al. 2004, Müller and Borsch 2005, Müller et al. 2006, Ibarra-Laclette et al. 2011a), and a 88.3 Mbp genome that is one of the smallest plant genomes described to date (Greilhuber et al. 2006). The high rates of molecular evolution likely contributed to the evolution of the COX gene, potentially allowing for the greater respiratory rate in the active bladders (Laakkonen et al. 2006). Genomic work has shown *Utricularia*, *U. gibba* in particular, to be an ideal candidate for use as a model organism to study the relationship between genomic mutations and physiological manifestations, highlighting the need for detailed morphological and anatomical work (Ibarra-Laclette et al. 2011a, Ibarra-Laclette et al. 2011b).

Besides exhibiting the unusual characteristic of carnivory, species of the genus *Utricularia* are unique because they have lost the typical angiosperm root, stem, and leaf organography, are highly plastic in their growth patterns, and display a range of vegetative morphological characters that have unknown homology (Lloyd 1942, Rutishauser and Sattler 1989, Richards 2001, Rutishauser and Isler 2001). Most angiosperms have clearly defined stems, roots, and leaves that have a predicable positional relationship to each other and are distinguishable by unique identifying characteristics.

[1] This work was funded by Florida Fish and Wildlife Commission, Nongame Wildlife Grants Program (NG06-003).

[2] Author for correspondence. E-mail: tchorman@mdc.edu

Received for publication December 12, 2011, and in revised form April 3, 2012.

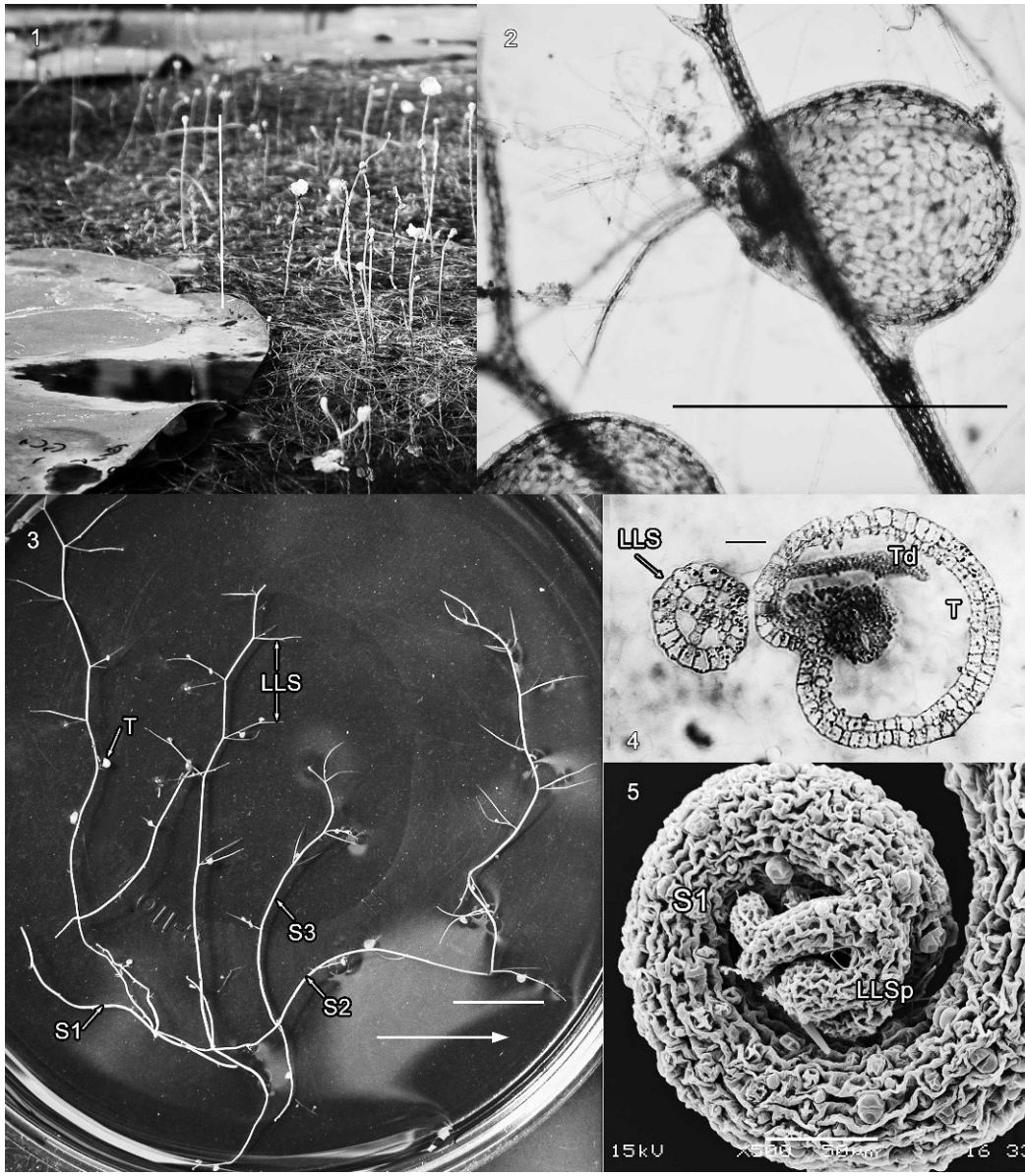
Utricularia species lack identifiable roots at any ontogenetic stage, and the identity of the other two organs types is not always obvious (Lloyd 1942, Rutishauser and Sattler 1989, Rutishauser and Isler 2001). Descriptions of shoot morphology range from a traditional approach that labels part of the plant as leaf and part as stem (Taylor 1989), to one that labels the plant as either wholly leaf-like (Goebel 1891, Kumazawa 1967), or wholly stem-like (Godfrey and Wooten 1981). This lack of consensus on the morphological classification and homology of the vegetative organs extends to the traps. The traps have generally been interpreted as parts of leaves, representing leaf divisions (Goebel 1891, Lloyd 1942, Taylor 1989). Traps appear in diverse positions on the plant body throughout the genus, including stolons, leaves, tips of leaves, and rhizoids, suggesting that trap homology is still uncertain (Taylor 1989). Regardless of their homology, the traps remain one of the most complex structures found on these plants (Lloyd 1942, Adamec 2011, Joyeux et al. 2011).

The vegetative body of *Utricularia* species is primarily composed of long, highly branched, horizontally-growing stems, referred to as stolons (Taylor 1989). Other flattened photosynthetic structures, normally referred to as leaves, arise from the primary stolon. Axillary buds or extra-axillary buds are seen in some species (Goebel 1891, Rutishauser and Sattler 1989). Additional vegetative structures, referred to as rhizoids and air shoots, are often found on *Utricularia* plants. The term rhizoid has been used to describe structures of uncertain homology that arise from the stolon, leaves, or inflorescence base and that appear to help anchor the plant in the soil (Goebel 1891, Taylor 1989). Air shoots are filamentous, floating, lateral structures with scales (typically referred to as "bract-like organs" [Taylor 1989]) at the tip; air shoots may become stolons as a result of apical growth and elongation (Lloyd 1942, Taylor 1989). In native environments, some species form dense, floating mats, commonly seen in aquatic *Utricularia* species, including *U. gibba*, or a complex matrix of plant segments can exist within the substrate in benthic or terrestrial species.

Utricularia's nonconformity to typical root, stem and leaf characteristics followed by most angiosperms, coupled with its carnivorous ability, highlight questions about how the *Utricularia* plant body is constructed. Prior work has aimed to reconcile structure in various species with "normal" plant morphology. In this study, we asked a different question: What is the basic architecture of the plant; i.e., what are the rules of construction and patterns of growth followed by *Utricularia* species? The aim of this study was to create an architectural model for *U. gibba* L., a common Everglades *Utricularia* species, through analysis of basic morphology and anatomy, as well as to quantify the range of variation on that model through analysis of phenotypic variations among field collected plants.

TERMINOLOGY. In order to avoid bias as to the homology of structures in *Utricularia* plants, we have used neutral terminology to describe these structures (Fig. 19). The largest horizontally growing stem on a plant is referred to as the primary stolon (S1). Branches from the primary stolon are referred to as secondary stolons (S2), branches from the secondary stolons are referred to as tertiary stolons (S3), and so forth. Branches arising from the base of the inflorescence are referred to as modified stolons (SM); these branches have been referred to as rhizoids by some authors (Lloyd 1942, Taylor 1989). Two classes of lateral structures arise from the different types of stolons: photosynthetic leaf-like structures (LLSs), which are terete to flat, usually determinate, and photosynthetic; and inflorescence appendages (IAs), which are claw-like lateral structures found on modified stolons at the base of the inflorescence. The region of the stolon where the LLSs attach is designated a node. The small organism-trapping bladders, which have elaborate doors and glandular hairs, are called traps (T). Inflorescence (INF) refers to the upright flowering stem.

Materials and Methods. SPECIES DESCRIPTION. *Utricularia gibba* is a small floating, suspended, or attached aquatic species (Fig. 1) abundant throughout the tropics, Europe, temperate North and South America, Asia, and Australia (Taylor 1989). Taylor (1989) and Jobson et al. (2003) placed this species in section *Utricularia*, a large section of suspended or affixed aquatics characterized morphologically by long stolons, two and four armed



FIGS. 1–5. *Utricularia gibba*. **1.** *U. gibba* as a floating aquatic plant; flowering. **2.** Trap on a LLS. **3.** One plant of *U. gibba* showing a dying S1 with multiple S2 and S3 growing out from the axils of the LLSs. All stolons show circinnate vernation, dichotomously branched LLS, and traps. **4.** Cross-section of a trap and LLS. **5.** Circinnate apex of S1 with emerging LLS primordium. Fig. 1, bar = 6 cm; Fig. 2, bar = 1 mm; Fig. 3, bar = 1 cm. Fig. 4, bar = 0.05 mm. *Figure abbreviations*: LLS, leaf-like structure; LLSp, leaf-like structure primordium; S1, primary stolon; S2, secondary stolon; S3, tertiary stolon; T, trap; Td, trap door; arrow, direction of growth of S1.

glands in their traps, circinnate vernation, and traps located only on their "leaves" (Figs. 2, 4, Taylor 1989). Historically, *U. gibba* was identified as *U. biflora*, *U. pumila, U. fibrosa,* and *U. gibba* proper; Taylor (1989) considered these to represent the same species and reduced them to the single species, *U. gibba*, but he acknowledged significant morphological variation within the species.

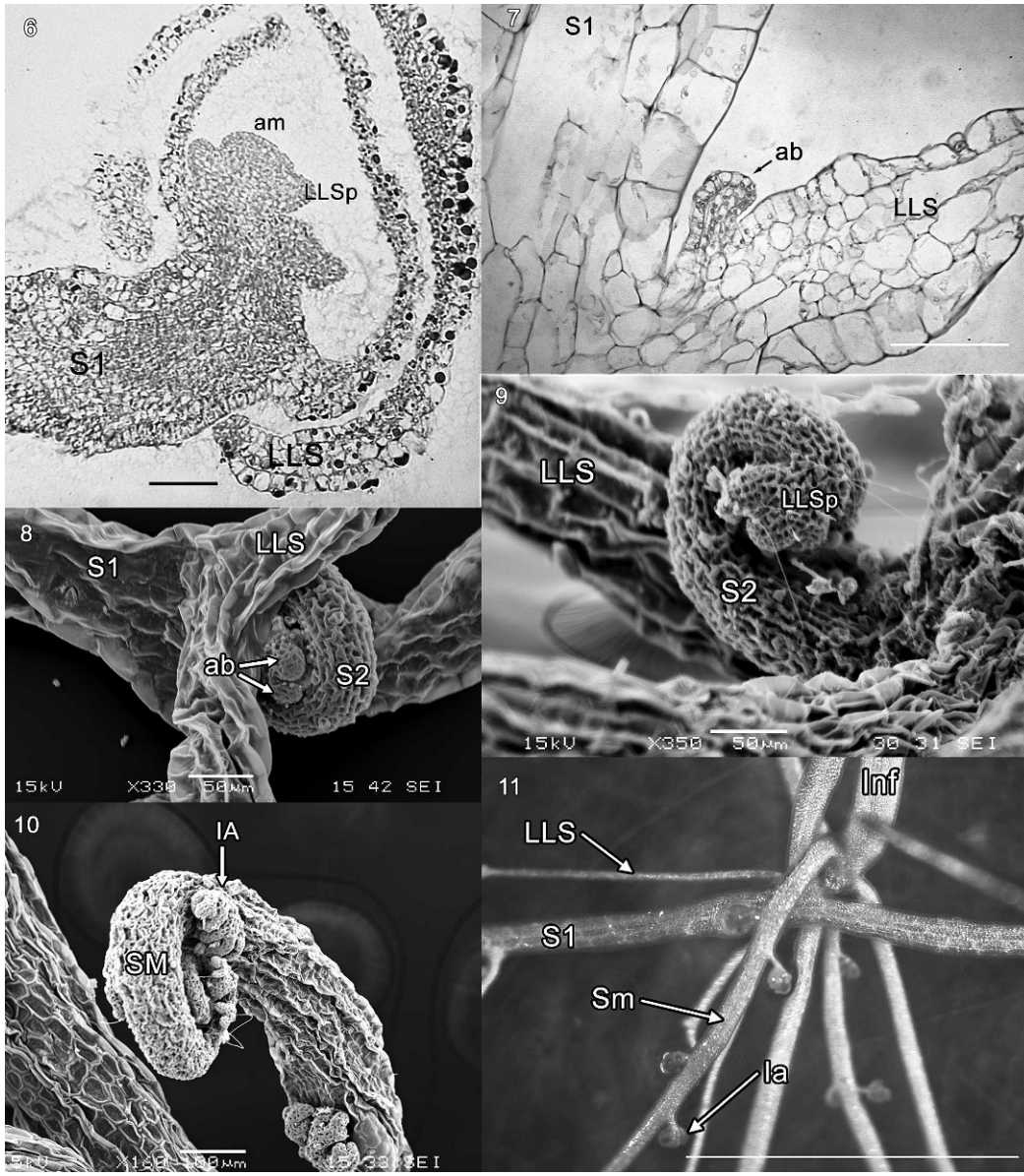
PLANT MORPHOLOGY AND GROWTH PATTERN. In order to create a morphological model for

Utricularia gibba, 10 plants were collected from three field sites in southern Florida during the dry season (January to April 2006) and again during the wet season (July to August 2006). The sites were Singeltary (SING) near Florida City (UTM 17R 0550213, 2847738); Henington Pond (HP) on the Florida International University campus (UTM 17R 0562313, 2849027); and a Wildlife and Environmental Area (WEA) adjacent to Everglades National Park (UTM 17R 0543841, 2800748). Morphological observations were made by mapping the presence and relative position of structures on these plants. Quantitative variation was determined by measuring plant segment length, internode length, and LLS length, and noting the number and position of branches or axillary buds. Segment length was determined by gently pulling apart the sample to find the longest intact primary stolon with a growing apex and a dying distal end. Differences between seasons and among sites in quantitative characters were compared using a two sample t-test assuming unequal variances. Differences between sites within a wet or dry season were calculated using Welch's ANOVA and a Bonferroni test for multiple comparisons.

PLANT NUTRIENT ANALYSIS. Percent carbon, nitrogen, and phosphorus were determined for each site by conducting nutrient analysis on plants with traps and plants with traps removed. Analyses were done by the FIU Southeast Environmental Research Center (SERC) Nutrient Analysis Laboratory using standard methods. The large amount of aerenchyma tissue present in the plants significantly reduced the amount of material available for analysis, so individual plant segments from each site were combined into a single sample for each site.

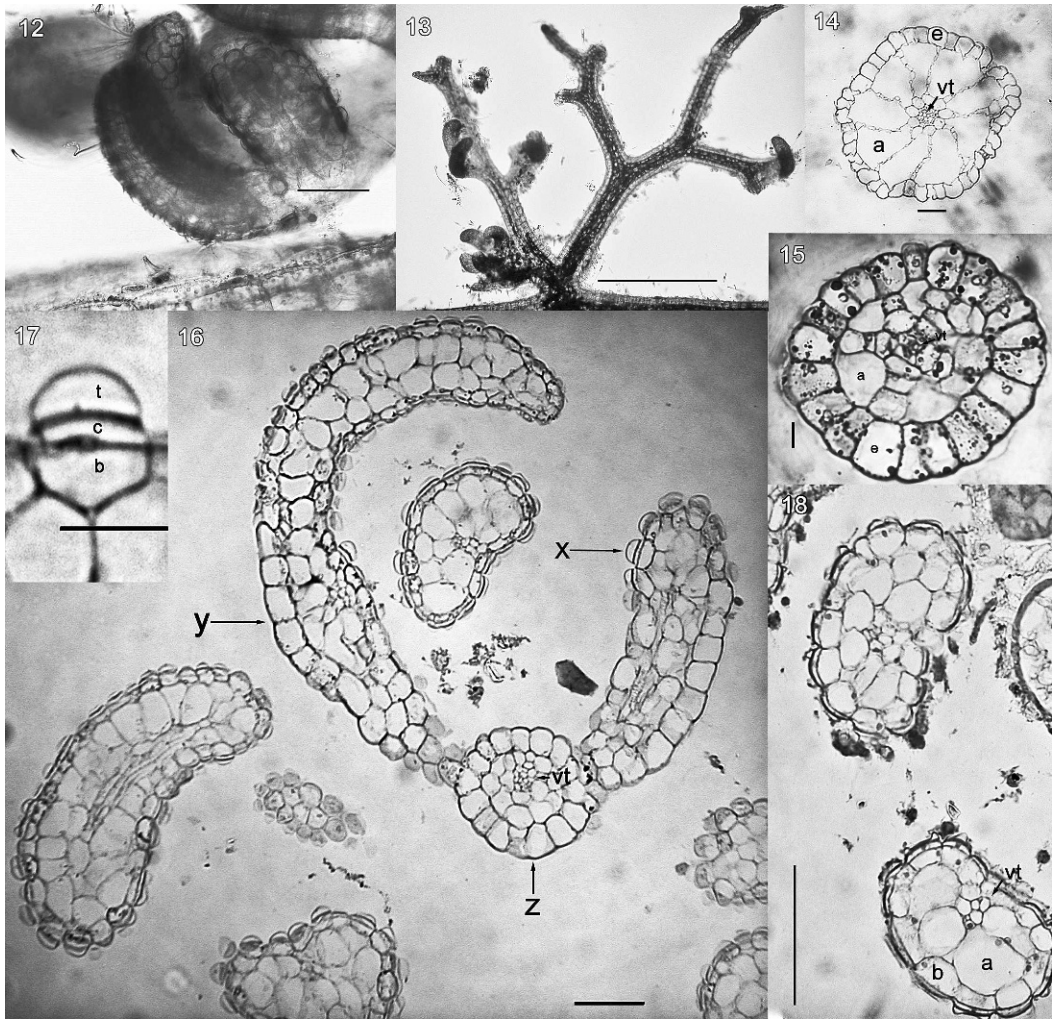
ANATOMICAL ANALYSIS. Light microscopy of paraplast-embedded and stained tissue and scanning electron microscopy were used to study anatomical characteristics. Plants for these analyses were sampled from the Singeltary property and the Wildlife and Environmental Conservation Area. Plants were dissected into segments and fixed in Craf III for 24 hours, washed in distilled water, and dehydrated with tertiary butyl alcohol (TBA) (Berlyn and Miksche 1976). The dehydrated segments were embedded in paraplast, sectioned with a microtome at 5μm and stained with hematoxylin-safranin to observe general anatomical characteristics. Fresh material was stained with Sudan IV to identify suberin, with toluidine blue to observe general characteristics, with phloroglucinol and HCl to identify lignin, and with neutral red to determine vascular connections (Berlyn and Miksche 1976). For examination with scanning electron microscopy additional plant segments were dehydrated in a graded series of ethyl alcohol, transferred to 100% acetone, then to hexamethyldisilazane (HMDS) and allowed to air dry, following the protocol of E. Capitano, Y. Bootwala, and J. Prince (U. of Miami, Miami, FL) (Nation 1983, Araujo et al. 2003). Dried material was sputter-coated with Au/Pd and examined using a JEOL JSM 5900LV scanning electron microscope (Peabody, MA) at the FIU Center for Analytical Electron Microscopy.

Results. PLANT MORPHOLOGY AND GROWTH PATTERN. *Utricularia gibba* plants grew as individual filaments, as branching plants embedded in the benthos or as entwined mats floating on the water surface. The individual filaments were found as suspended fragments floating below the water surface, whereas mats ramified throughout moist soils and benthic periphyton (algal) mats or formed large colonies floating at the water surface (Fig. 1). Each individual filament, whether growing singly (Fig. 3) or as part of a mat, consisted of a primary stolon (S1) that grew monopodially from a terminal, circinnate apex (Fig. 5), while more basal, older parts of the stolon broke off or died. No roots, root-like structures, rhizoids or rhizoid-like structures were found on *U. gibba*. Any additional structures were produced laterally on the primary stolon. Lateral leaf-like structures (LLSs) occurred singly and were arranged spirally along the S1 (Figs. 3, 6). LLSs were lateral organs that were determinate and could subtend axillary buds (Fig. 7). The LLSs were rounded, filamentous and less that 1 mm in diameter with a single basal region that attached to the S1, but each LLS then branched dichotomously distal to the region of attachment (Figs. 3, 8). The LLSs observed in this study branched dichotomously up to four times but ultimately became determinate, producing an elongated, needle-like, vacuolated tip. Traps arose laterally on the LLS segments. Axillary buds occurred in some, but not all, LLS axils (Fig. 7). Buds were found in the axils of



FIGS. 6–11. *Utricularia gibba*. **6.** Longitudinal section through the apex with emerging leaf primordia. **7.** Longitudinal section of a LLS with ab. **8.** Multiple axillary buds in the axil of a LLS. **9.** Secondary stolon in the axil of a LLS. **10.** Circinnate apex of SM with emerging IA. **11.** Inflorescences in the axil of a LLS. Modified stolons form from the inflorescence tissue and bear lateral inflorescence appendages. Figs. 6, 7, bar = 0.05 mm; Fig. 11, bar = 0.5 cm. *Figure abbreviations*: ab, axillary bud, am, apical meristem; IA, inflorescence appendage; INF, inflorescence; LLS, leaf-like structure; LLSp, leaf-like structure primordium; S1, primary stolon; S2, secondary stolon; S3, tertiary stolon; SM, modified stolon; T, trap; arrow, direction of growth of S1.

15–45% of all LLS nodes on the plant segments measured (Table 1). Axillary buds tended to be found associated with every second or third LLS, but this pattern was not consistent within a single plant or between plants, and portions of a plant segment could branch from axils of all LLSs. More than one bud could be found in the axil of a LLS, so accessory or supernumerary buds occurred (Fig. 8).



FIGS. 12–18. *Utricularia gibba*. **12.** IA stained with Sudan IV. **13.** Transitional lateral structure found along a SM. **14.** Transverse section through a S1. **15.** Transverse section through a LLS. **16.** Section through the IA with parts of the IA in longitudinal section (y) and others in transverse section (z). Glandular projections line the edges of the IAs (x). **17.** A three-celled glandular projection of an IA. Each projection is composed of a terminal cell, a central cell, and a basal cell. **18.** Cross-section of several IA stained with safranin. Figs. 12, 14, 16, 18 bar = 0.05 mm; Fig. 13, bar = 1 mm; Fig. 15, bar = 0.01 mm; Fig. 17, bar = 0.005 mm. *Figure abbreviations*: a, aerenchyma, e, epidermis, IA, inflorescence appendage; vt, vascular tissue; y, longitudinal section; z, transverse section.

Utricularia gibba produced two types of branches: secondary stolons (S2s) and modified stolons (SMs). Secondary stolons (S2) arose in the axils of the LLSs of the S1 and reiterated the architecture of the S1, having a circinnate tip with the shoot apical meristem and producing LLSs (Fig. 9). Multiple S2s could arise in a LLS axil from accessory axillary buds. Higher order branching occurred from buds in the axils of the LLSs borne on the S2; successive branching created the thick floating mats found in the field or the ramifying benthic form, or the S2s (and higher order branches) broke off to produce clones of the parent plant.

Modified stolons resembled the primary and secondary stolons morphologically in that they had circinnate vernation and apical meristems (Fig. 10), but they deviated from the S1/S2 structure by their position and by the types of lateral structures that they produced. SMs were located at the base of

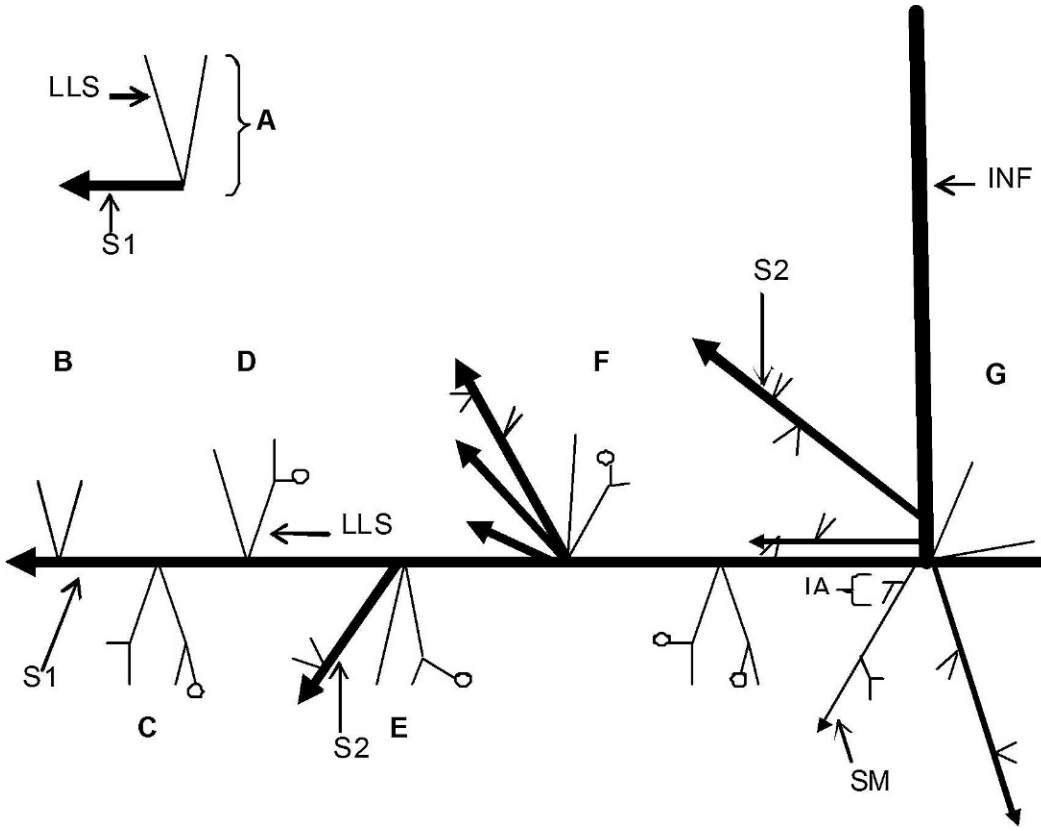


FIG. 19. Model of *Utricularia gibba*. A represents the simplest unit of *U. gibba* plants. Multiples of these simple units combine together to form a single plant segment. A-G represent the possible modifications of unit A on *U. gibba* plants. The arrow points indicate the postion and direction of growth. The basic unit of the plant (A) consists of a primary stolon and one LLS branched dichotomously once. Modifications include: additional dichotomous branching of the terminal ends of the LLS (C and D), the presence and outgrowth of an axillary bud (E), the presence and outgrowth of extra-axillary buds (F), and the presence of an inflorescence (G). Two types of branches arise off of the inflorescence peduncle, one, the secondary stolon (S2), a reiteration of the primary stolon (S1) and the second, the modified stolon (SM), a stolon with unique lateral organs referred to as inflorescence appendages (IA).

the inflorescence and arose from inflorescence tissue instead of from the axil of a LLS (Fig. 11). Instead of bearing only LLSs, they produced inflorescence appendages (IA), which were unique multifid structures born at the distal end of the SM. The tips of the IA were covered with epidermal papillae that readily absorbed stains when exposed to toluidine blue, neutral red, and Sudan IV (Fig. 12). Although the structural characteristics of the IAs differed from the LLSs, the IAs occurred in the same position on the modified stolons that the LLSs occupied on the S1 or S2. Inflorescence appendages (IA) formed proximally on the SMs, while LLSs were found on distal nodes of the SMs. Transitional structures combining the dichotomous branching of the LLSs and the rounded, glandular tips of the IAs were occasionally seen on the SMs (Fig. 13).

Inflorescences (INF) were produced on the free-floating mats or on plants embedded in moist soils or periphyton mats. Each INF grew orthotropically from the nodal position in the axil of a LLS and projected above the substrate or water (Fig. 1). One or two inflorescences could be produced in the same LLS axil. Inflorescence height ranged from 4.5–9.4 cm with a mean value of 5.9 $\pm$ 2.1 cm (n = 15). Flower number per inflorescence ranged from 1–2 flowers per inflorescence with a median value of 1 flower per inflorescence. The inflorescence was a raceme, producing flowers first at the base of the inflorescence when two flowers are present.

Table 1. Variation among sites and between seasons in morphology of *Utricularia gibba*. Data are given as means ± SD. HP = Henington Pond; In L = length of internodes along primary stolon; LLS L. = length of LLS; % Buds = percent of nodes with axillary buds along primary stolon; S1 L = length of primary stolon; SING = Singeltary; WEA = Wildlife and Environmental Area. Site values with different superscripts are significantly different.

	Dry season	Wet season	P^1
S1 L (mm)			
HP	112 ± 17^a	128 ± 48^a	
SING	69 ± 13^b	261 ± 97^c	
WEA	67 ± 34^b	95 ± 23^{bc}	
Total	83 ± 31^x	161 ± 96^y	< 0.001
In L (mm)			
HP	6 ± 0.7^a	5 ± 0.5^a	
mSING	5 ± 1.2^b	7 ± 0.9^c	
WEA	3 ± 0.6^d	5 ± 1.3^b	
Total	4.7 ± 1.4^x	5.5 ± 1.3^y	0.001
LLS L (mm)			
HP	4 ± 2^a	4 ± 2^a	
SING	6 ± 2^b	5 ± 3^b	
WEA	2 ± 2^c	3 ± 1^c	
Total	4 ± 2	4 ± 2	0.273
% Buds			
HP	20 ± 8	15 ± 5	
SING	33 ± 8	35 ± 15	
WEA	42 ± 11	36 ± 17	
Total	32 ± 13	29 ± 16	0.795

[1] Significance in ANOVA comparing differences between seasons.

ANATOMICAL CHARACTERISTICS. The S1 and SM of *Utricularia gibba* were both radially symmetrical in cross-section and composed of epidermal cells containing chloroplasts surrounding a region of aerenchyma and a central vascular bundle (Fig. 14). The aerenchyma had radiating partitions that were a single cell wide in cross-section and gave the cross-section the overall appearance of a wheel with spokes. Cross-sections of the LLSs were similar to the S1s in that they were radially symmetrical in cross-section, had epidermal cells with chloroplasts surrounding aerenchyma and a central vascular bundle (Fig. 15).

The IAs differed anatomically from both the LLSs and the stolons. Within each IA, the aerenchyma was not as regularly or radially organized as in the stolons and LLSs; the IAs had a dorsiventral symmetry associated with the curled ends of the structure. The IAs also had a distinctive epidermal layer with densely aggregated epidermal papillae that projected from the epidermis (Fig. 16). This layer was unique to the ends of the IAs and was not found on any other part of the plant. Each epidermal papilla on the IA was composed of three different cells: a basal cell continuous with the epidermis; a flattened central cell; and a hemispherical terminal cell (Fig. 17). The central cell had thickened walls. Sections stained in hematoxylin and safranin showed these papillae as a band of cells whose walls stained more darkly with safranin than the surrounding cells (Fig. 18). These walls were suberized, as they stained red with Sudan IV (Fig. 12).

PHENOTYPIC VARIATION IN QUANTITATIVE CHARACTERS. Plants in the field-collected samples varied significantly among sites and between seasons in segment length and internode length per segment, and there were significant site × season interactions (Table 1). The LLS length and percent of nodes with axillary buds did not differ significantly between seasons; LLS differed among sites but the percent of nodes with axillary buds did not, and there were no significant interactions (Table 1).

PLANT NUTRIENT ANALYSIS. Tissue carbon (C), nitrogen (N) and phosphorus (P), of *Utricularia gibba* was within the normal ranges for flowering plants (Table 2). Similarity in nutrient levels for *U. gibba* plants with and without traps suggested that the presence of traps did not influence results of these analyses. Total differences in the two types of samples were minimal, and when differences between samples with traps and without traps were compared by site, the sample with

Table 2. Percent carbon, nitrogen, and phosphorus for *Utricularia gibba* with and without traps at each collection location. NES = not enough sample was obtained for analysis.

	U. gibba + traps			*U. gibba* (−) traps		
Nutrient	HP	SING	WEA	HP	SING	WEA
Carbon	41.5	41.4	39.7	NES	41.3	40.0
Nitrogen	2.3	1.8	1.8	NES	1.9	1.7
Phosphorus	NES	0.19	0.12	0.12	0.16	NES

traps was greater at one site, but less at the other site for both C and N, so the effect traps had on nutrient levels was not consistent among sites (Table 2).

Discussion. In addition to their unique, carnivorous mode of nutrient acquisition, *Utricularia* species are often recognized for their deviations from typical plant morphology and for our inability to label their plant organs definitively as roots, shoots, or leaves (Goebel 1891, Arber 1920, Rutishauser and Sattler 1989, Taylor 1989, Sattler and Rutishauser 1990, Richards 2001, Rutishauser and Isler 2001). We have shown here, however, that *U. gibba* does have a predictable pattern of construction or architecture (Fig. 19). The vegetative portion of *U. gibba* is composed of different types of stolons (S1, S2, and SM), dichotomously branched, leaf-like structures (LLS), multifid inflorescence appendages (IA), and traps. Both types of lateral appendages, the LLSs and the IAs, branch dichotomously some number of times until reaching a point of determinancy. The basic architectural unit of *U. gibba* is the primary stolon with one LLS branched dichotomously into two segments (Fig. 19A); the LLS may but does not always have one or more buds located in the LLS axil. All other structures are modifications of this basic unit (Fig. 19B–G). Morphological modifications include an increase in the number of dichotomous branches of the LLS (Fig. 19C, D), limited to four dichotomous divisions in our field-collected plants. Secondary stolons (S2) form in the axil of a LLS (Fig. 19E), and either one or multiple secondary stolons (S2) can be found (Fig. 19E, F). In our material, 30% of LLS had the potential to form secondary stolons. Secondary stolons reiterate the structure of the primary stolon. Inflorescences are also formed at the nodal position in the axil of a LLS (Fig. 19G), and multiple inflorescences and S2s can form in a single axil. Like other members of section *Utricularia*, the traps of *U. gibba* are formed only on the LLSs (Taylor 1989).

Utricularia gibba also produces a second type of stolon, which we call modified stolons (SMs), formed around the base of the inflorescence. The function of these stolons is unknown, but they may provide either structural or physiological support for the upright inflorescence. The SMs bear the unusual inflorescence appendages (IAs). The occurrence and position of intermediate structures between the appendages and LLSs suggest that IAs are homologous to the LLSs. The presence of these appendages was noted by Goebel (1891) and Taylor (1989), but their structure has not previously been described. An unusual finding was the cellular structure of the IAs, which bear epidermal papillae composed of three different cells, possibly an ectopic expression of one type of the trap glands (Fig. 17). Similar cells are found widely distributed around the inside of the trap, with a suggested function of expelling and/or absorbing water (Fineran 1985). The specific function of this dense cover of glands on the outside of the multifid IAs is unknown, but the observed characteristics suggest two possibilities. Quick and selective absorption of dyes observed in living plants suggests that their function may be absorption of nutrients or water from the surrounding medium, paralleling the function of true roots. These glands also have a layer of suberized tissue surrounding the central cells, identified by Sudan IV staining. This layer is similar to the casparian strip around the endodermis of a typical root and therefore may serve as a barrier to flow, forcing water to cross a membrane, which would allow the plant to select solutes. Alternately, or in addition, the plant may be receiving adequate nutrition from carnivory or from solutes in the surrounding medium, and the purpose of the glands may be to absorb water, which would be pulled up the inflorescence by transpiration, increasing hydraulic support and keeping the inflorescence upright. This could provide a means to support the growth of the relatively large inflorescence on the proportionately small plants.

The architectural model presented here applied to plants collected from different habitats and in different seasons. Plants varied morphological characteristics quantitatively with habitat and season but the variation was based on this basic model. Quantitative analysis of the vegetative organs during the wet and dry seasons showed increased plant segment lengths and internode lengths, both characters related to growth of the stolons, but no change in the LLS lengths or percent of axillary buds in wet season compared to the dry season (Table 1). Differences in stolons could result from the differences in the habitat between the two seasons. In the wet season,

Utricularia gibba was collected as a floating or attached aquatic, whereas with the dry season's lower water levels, *U. gibba* was collected as a benthic species growing in periphyton mats or in muddy soils. Plant and internode length and leaf length also varied significantly among sites. These results suggest potential environmental differences. Nutrient analysis of plant tissues did not reveal large differences in nutrient levels among sites or between plants with traps and plants without traps (Table 2). Hydrologic differences among sites may be more important in determining morphological variations in this species.

This general model for growth of *Utricularia gibba* may apply, with modifications, to a large number of species in the genus, in particular the members of section Utricularia (Taylor 1989), which includes well-studied species such as *U. vulgaris*, *U. macrorhiza*, and *U. foliosa*. Species in this section have a primary stolon that bears lateral appendages with an alternate phyllotaxis (Taylor 1989). In each of the species in this section, the lateral appendages branch dichotomously some number of times, but ultimately become determinate. The range of the number of dichotomous branching in the LLS may be characteristic of each species, and studies comparing this could provide additional taxonomic information. Traps are born on the lateral appendages but not in a terminal position. Within the section, branching tends to occur in the axil of the lateral appendages and the primary stolon (Rutishauser and Sattler 1989, Taylor 1989), but not all lateral appendages subtend axillary buds. This model, which may apply to section Utricularia, is modified in other sections of the genus. For example, section Vesiculina, which contains *U. purpurea*, has whorled lateral appendages and traps are terminal (Taylor 1989, Richards 2001). Similarly, *U. cornuta* (section Stomoisia) and *U. subulata* (section Setiscapella) are architecturally different from *U. gibba*, as well as from each other (Chormanski 2007).

In *Utricularia gibba*, as in other members of section Utricularia, the LLSs ultimately become determinate organs. Molecular studies of angiosperms with divergent or odd morphology and studies of *Arabidopsis* mutants indicate that determinancy in plant development is controlled by the interaction between *ARP* and *KNOX* genes (Jackson et al. 1994, Byrne et al. 2003, Harrison et al. 2005). Expression of *ARP* represses the expression of *KNOX*, a class of regulatory genes that has been identified as responsible for indeterminate apical growth (Jackson et al. 1994). Thus, *ARP* contributes to determinacy of leaf growth (Lincoln et al. 1994, Hofer et al. 2001). Applying this information to the model for *U. gibba*, and the other species of *Utricularia*, we predict that *KNOX* genes would be expressed in the stolons while *ARP* would be expressed in the LLS. Uncovering the patterns of expression of these groups of genes in *Utricularia* species could provide insight into the regulatory controls on organ determinancy, since throughout the genus identification of determinate organs is not always obvious from observations of plant morphology, and organs which appear determinate often have the ability to return to active growth (Chormanski, pers. obs.). Goebel (1891) hypothesized that the unusual morphology of *Utricularia* was a result of release from nutritional constraints provided by carnivory. The aquatic habit, as well as carnivory, provides a release from normal vascular plant ecological and developmental constraints. A modern equivalent of this release from constraints has been found in the yogurt bacteria *Lactobacillus bulgaricus* (= *L. delbrueckii* ssp. *bulgaricus*), which has undergone recent and relatively rapid genome reduction and modification associated with adaptation to the lactose-rich yogurt habitat (van de Guchte et al. 2006). The extremely small genome size (Greilhuber et al. 2006) and rapid genome evolution rates (Jobson and Albert 2002, Jobson et al. 2004, Müller and Borsch 2005, Müller et al. 2006) found in *Utricularia* species may reflect a similar release from one set of developmental constraints and adaptation to a new set.

We have shown that *Utricularia gibba* follows structural rules that are different from but related to those of more typical plants. The unique characteristics of *Utricularia* as a genus; no true roots, the complex traps (Lloyd 1942, Sydenham and Findlay 1973, Adamec 2011), extremely modified vegetative morphology (Arber 1920, Rutishauser and Sattler 1989, Taylor 1989, Sattler and Rutishauser 1990), relaxed developmental constraints (Müller et al. 2006), and high species diversity (Albert et al. 1992), suggest that molecular genetic work on members of this genus could provide insight into fundamental aspects of

more typical plant morphology. The genus provides alternative developmental architectures that phylogenetic studies confirm are derived from more typically structured plants, but it also has an unmapped set of variations on that architecture that may or may not be associated with variation in habitat (floating, benthic, terrestrial) or in nutrition and trap function. Previous phylogenetic studies of carnivorous plants have emphasized the divergence and evolutionary significance of the different methods of trapping prey and their corresponding structures (Albert et al. 1992, Jobson and Albert 2002, Jobson et al. 2003, Jobson et al. 2004, Müller et al. 2004, Müller et al. 2005). These studies suggest that the genus *Utricularia* is the most highly derived genus in the Lentibulariaceae. Understanding what developmental patterns are followed in different species of the genus, as done here for *U. gibba*, is a necessary prerequisite to discover the developmental genetic alterations that led to the establishment of these atypical features, as well as the divergent morphological patterns.

Literature Cited

Adamec, L. 2011. Functional characteristics of traps of aquatic carnivorous *Utricularia* species. Aquat. Bot. 95: 226–233.

Albert, V. A., S. E. Williams, and M. W. Chase. 1992. Carnivorous plants: phylogeny and structural evolution. Science 257: 1521–1525.

Arber, A. 1920. Water Plants: A study of aquatic angiosperms. Cambridge University Press, Cambridge, UK. 436 p.

Araujo, J. C., F. C. Teran, R. A. Oliveira, E. A. A. Nour, A. P. Montenegro, J. R. Campos, and R. F. Vazoller. 2003. Comparison of hexamethyldisilazane and critical point drying treatments for SEM analysis of anaerobic biofilms and granular sludge. J. Electron Microsc. 52: 429–433.

Berlyn, G. P. and J. P. Miksche. 1976. Botanical microtechnique and cytochemistry. The Iowa State University Press, Ames, IA. 326 p.

Byrne, M. E., C. A. Kidner, and R. A. Martienssen. 2003. Plant stem cells: divergent pathways and common themes in shoots and roots. Curr. Opin. Genetics Dev. 13: 551–557.

Chormanski, T. 2007. Morphology and anatomy of three common Everglades *Utricularia* species; *U. gibba*, *U. cornuta*, and *U. subulata*. MS thesis. Florida International University, Miami, FL.

Fineran, B. A. 1985. Glandular trichomes in *Utricularia*: a review of their structure and function. Isr. J. Bot. 34: 295–330.

Greilhuber, J., T. Borsch, K. Müller, A. Worberg, S. Porembski, and W. Barthlott. 2006. Smallest angiosperm genomes found in Lentibulariaceae with chromosomes of bacterial size. Plant Biol. 8: 770–777.

Godfrey, R. K. and J. W. Wooten. 1981. Aquatic and Wetland Plants of the Southeastern United States: dicotyledons. The University of Georgia Press, Athens, GA. 933 p.

Goebel, K. I. E. 1891. Morphologische und biologische Studien V. *Utricularia*. Ann. Jard. Buitenz. 9: 41–119.

Harrison, C. J., S. B. Corley, E. C. Moylan, D. L. Alexander, R. W. Scotland, and J. A. Langdale. 2005. Independent recruitment of a conserved developmental mechanism during leaf evolution. Nature 434: 509–514.

Hofer, J. M., C. W. Gourlay, A. Michael, and T. H. Ellis. 2001. Expression of a class 1 Knotted1-like homeobox gene is down-regulated in pea compound leaf primordial. Plant Mol. Biol. 45: 387–398.

Ibarra-Laclette, E., V. A. Albert, C. A. Pérez-Torres, F. Zamudio-Hernández, M. Ortega-Estrada, A. Herrera-Estrella, and L. Herrera-Estrella. 2011a. Transcriptomics and molecular evolutionary rate analysis of the bladderwort (*Utricularia*), a carnivorous plant with a minimal genome. BMC Plant Biol 11: 101 p. DOI: 10.1186/1471-2229-11-101

Ibarra-Laclette, E., V. A. Albert, A. Herrera-Estrella, and L. Herrera-Estrella. 2011b. Is GC bias in the nuclear genome of the carnivorous plant *Utricularia* driven by ROS-based mutation and biased gene conversion? Plant Signal. Behav. 6: 1631–1634.

Jackson, D., B. Veit, and S. Hake. 1994. Expression of maize Knotted1 related homeobox genes in the shoot apical meristem predicts patterns of morphogenesis in the vegetative shoot. Development 120: 404–413.

Jobson, R. W. and V. A. Albert. 2002. Molecular rates parallel diversification contrasts between carnivorous plant sister lineages. Cladistics 18: 127–136.

Jobson, R. W., R. Nielson, L. Laakkonen, M. Wikstrom, and V. A. Albert. 2004. Adaptive evolution of cytochrome c oxidase: Infrastructure for a carnivorous plant radiation. Proc. Natl. Acad. Sci. USA 101: 18064–18068.

Jobson, R. W., J. Playford, K. M. Cameron, and V. A. Albert. 2003. Molecular phylogenetics of Lentibulariaceae inferred from plastid rpS16 intron and trnL-F DNA sequences: Implications for character evolution and biogeography. Syst. Bot. 28: 157–171.

Joyeux, M., O. Vincent, and P. Marmottant. 2011. Mechanical model of the underwater trap of *Utricularia*. Phys. Rev. E 83: 1–12.

Kumazawa, M. 1967. An experimental study on the seedling of *Utricularia pilosa* Makino. Phytomorphology 17: 524–528.

Laakkonen, L., R. W. Jobson, and V. A. Albert. 2006. A new model for the evolution of carnivory in the bladderwort plant (*Utricularia*). Adaptive changes in cytochrome c oxidase (COX) provide respiratory power. Plant Biol. 8: 758–764.

Lincoln, C., J. Long, J. Yamaguchi, K. Serikawa, and S. A. Hake. 1994. Knotted1-like homeobox gene in *Arabidopsis* is expressed in the vegetative meristem and dramatically alters leaf morphology when overexpressed in transgenic plants. Plant Cell 6: 1859–1876.

LLOYD, F. E. 1929. The mechanism of the water tight door of the *Utricularia* trap. Plant Physiol. 4: 87–102.

LLOYD, F. E. 1942. The Carnivorous Plants. Chronica Botanica, Vol. 9. Ronald Press, New York, NY. 352 p.

MÜLLER, K., T. BORSCH, L. LEGENDRE, S. PORembski, AND W. BARTHLOTT. 2006. Recent progress in understanding the evolution of carnivorous Lentibulariaceae. Plant Biol. 8: 748–757.

MÜLLER, K., T. BORSCH, L. LEGENDRE, S. PORembski, I. THEISEN, AND W. BARTHLOTT. 2004. Evolution of carnivory in Lentibulariaceae and the Lamiales. Plant Biol. 6: 477–490.

MÜLLER, K. AND T. BORSCH. 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.

NATION, J. L. 1983. A new method using hexamethyldisilazane for preparation of soft insect tissues for scanning electron microscopy. Stain Technol. 58: 347–351.

RICHARDS, J. H. 2001. Bladder function in *Utricularia purpurea* (Lentibulariaceae): is carnivory important? Am. J. Bot. 88: 170–176.

RUTISHAUSER, R. AND B. ISLER. 2001. Developmental genetics and morphological evolution of flowering plants, especially bladderworts (*Utricularia*): Fuzzy Arberian morphology complements classical morphology. Ann. Bot. 88: 1173–1202.

RUTISHAUSER, R. AND R. SATTLER. 1989. Complementarity and heuristic value of contrasting models in structural botany III. Case study of shoot-like 'leaves' and leaf-like 'shoots' in *Utricularia macrorhiza* and *U. purpurea* (Lentibulariaceae). Bot. Jahrb. Syst. 111: 121–137.

SATTLER, R. AND R. RUTISHAUSER. 1990. Structural and dynamic descriptions of the development of *Utricularia foliosa* and *U. australis.* Can. J. Bot. 68: 1989–2003.

SYDENHAM, P. H. AND G. P. FINDLAY. 1973. The rapid movement of the bladder of *Utricularia* sp. Aust. J. Biol. Sci. 26: 1115–1126.

TAYLOR, P. 1989. The genus *Utricularia*. Kew Bulletin Series XIV. 1-724. Her Majesty's Stationery Office, London, UK. 724 p.

VINCENT, O. AND P. MARMOTTANT. 2011. Carnivorous *Utricularia* The buckling scenario. Plant Signal. Behav. 6: 1752–1754.

VAN DE GUCHTE, M., S. PENAUD, C. GRIMALDI, V. BARBE, K. BRYSON, P. NICOLAS, C. ROBERT, S. OZTAS, S. MANGENOT, A. COULOUX, V. LOUX, R. DERVYN, R. BOSSY, A. BOLOTIN, J. M. BATTO, T. WALUNAS, J. F. GIBRAT, P. BESSIÈRES, J. WEISSENBACH, S. D. EHRLICH, AND E. MAGUIN. 2006. The complete genome sequence of *Lactobacillus bulgaricus* reveals extensive and ongoing reductive evolution. Proc. Natl. Acad. Sci. USA 103: 9274–9279.