ABSTRACT

TRAP MORPHOLOGY IN THE CARNIVOROUS PLANT GENUS *UTRICULARIA*: EFFECTS OF HABITAT ON TRAP MORPHOLOGY

Bladderworts (genus *Utricularia*) are the second most diverse genus of carnivorous plants, with over 274 known species. Bladderworts inhabit a wide range of geographic locations and habitats and have evolved a highly derived and variable morphology to match. All but one species have motile traps, and these traps can make up a majority of the plant's biomass, representing a considerable investment by the plant into carnivory. Traps are expensive organs, and (re)setting them to prepare them for prey capture is an expensive process in *Utricularia*. We therefore propose that bladderworts developed adaptations to increase the traps' effectiveness, such as structures that increase encounter probability with profitable prey, increase capture probability, or decrease damage, fouling, kleptoparatisism, or misfiring. Previous studies have shown that structures around the trap entrance affect capture success. We hypothesize that trap morphology will vary according to habitat. We will define morphological traits to assess morphological variation between species as a function of habitat. We will describe the structures at the trap entrance along with entrance position, shape and size of the trap, and attachment point of the trap to the rest of the plant. To better understand how *Utricularia* have adapted across terrestrial and aquatic habitats, we will categorize morphological data of the plants against ecological covariate data. This study should facilitate future studies into how bladderworts have optimized their traps to overcome obstacles to predation in their respective environments.

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TRAP MORPHOLOGY IN THE CARNIVOROUS PLANT GENUS *UTRICULARIA*: EFFECTS OF HABITAT ON TRAP MORPHOLOGY

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TABLE OF CONTENTS

Page

Page

LIST OF TABLES

Page

LIST OF FIGURES

Page

Page

INTRODUCTION

Trap Types in Carnivorous Plant Traps and Their Interactions with Animals

Carnivorous plants have long been the subject of study because they allow scientists to address fundamental biological questions, such as how these plants catch their prey, which morphologies enhance prey capture, and what is the evolutionary origin of these specialized plant organs that trap prey (Cresswell, 1993; Ellison & Adamec 2018). Previous studies have demonstrated that carnivorous plants are suitable model organisms to study predator-prey interactions, including form-function relations (Cresswell, 1993; Englund & Harms, 2001; Gordon & Pacheco, 2007; Harms, 1999; Harms & Johansson, 2000; Lester & Harmsen, 2002).

There are two main types of traps, motile and non-motile traps (Poppinga et al., 2021). The most iconic non-motile traps are the pitcher of most pitcher plant species; the most iconic motile traps are the Venus flytraps. Recent studies suggest that many carnivorous plants use motion to capture, retain, and/or kill their prey (Poppinga et al. 2021). For example, the Venus fly trap uses a snap trap mechanism to capture and crush its prey. Some species combine non-motile and motile mechanisms; for example, the sundew species *Drosera glanduligera*, which combines motile 'catapulting' snap tentacles with non-motile sticky 'flypaper' secretions to capture prey (Poppinga et al., 2012). Some species have more than one capture mechanism or use a combination of techniques that use different capture principles, such as the pitcher plant *Nepenthes rafflesiana*, which uses two different anti-adhesion mechanisms (wax-crystal lined walls plus hydrophilic peristome), or the pitcher plant *Nepenthes gracilis*, which combines anti-adhesion waxes with a rain-drop activated lid (Bauer & Federle, 2009; Bauer et al., 2011; Bauer, Paulin, et al., 2015).

While in some families motion is used only in a few species, such as a rain-drop activated pitcher plant *Nepenthes gracilis* within the family Nepenthaceae (Bauer et al., 2012; Bauer, Paulin, et al., 2015), other families are dominated by species with motile traps, such as the family Droseraceae, which contains two genera with motile traps (*Dionaea* and *Aldrovanda*), and Lentibulariaceae, which contains one genus with motile traps (*Utricularia*). Among these genera with motile traps, *Utricularia* is the most specious with more than 274 species (Cheng et al., 2021; Jobson et al., 2018).

Motile traps vary greatly in the speed of their motion, with motions taking several hours to less than a millisecond to complete (Poppinga et al., 2021). The slowest movements on the time scale of minutes to hours, such as the opening of Venus flytraps (*Dionaea*), are powered by growth (Stuhlman, 1948). Slow movements on the time scale of seconds to minutes are powered by hydraulics, such as in the 'flypaper' leaves of motile sundews (*Drosera*) and butterworts (*Pinguicula*) (Poppinga et al., 2018). Fast movements that take a few seconds are powered by combinations of hydraulics and elastic energy storage, such as the closing of Venus flytraps, which is powered by hydraulics and elastic energy stored in the form of a snap-buckle mechanism (Volkov et al. 2008). Ultrafast movements that take just milliseconds to complete are powered by elastic energy and often employ structures with buckling instabilities, such as the traps of bladderworts (*Utricularia*), and waterwheel plants (*Aldrovanda*) (Vincent et al., 2011; Westermeier et al., 2018).

Traps have adaptations that allow them to attract, capture, retain, and digest prey (Poppinga et al., 2021). Examples of adaptations to attract prey are the pitcher plants' producing volatile compounds to mimic fruits or flowers, and the antennae of bladderwort traps that funnel prey to the trap entrance (Bauer et al., 2008; Jürgens et al., 2009; Meyers & Strickler, 1979), but there is evidence that carnivorous plants do not use so-called aggressive mimicry that imitate complete sets of signals, such as scent plus

shape plus color (Foot et al., 2014; Jürgens et al., 2009). Traps have also been shown to use the social behavior of their prey to attract prey and increase their capture success, such as pitcher plants with hydrophilic peristomes. These plants prey on ants and use intermittent wetting to increase the likelihood of scout ants escape the trap only to recruit a large number of ants to the trap (Bauer, Federle et al., 2015). Traps may also attract prey by providing food, such as pitcher plants providing nectar (Bauer et al., 2008) and bladderworts growing algae on their traps and stolon for prey items to graze (Meyers & Strickler, 1978).

Many carnivorous plant species invest a substantial amount of their biomass in traps (Friday, 1992; Osunkoya et al., 2007). Plant organs associated with carnivory often have a reduced photosynthesis and high respiration rates (Adamec, 2006; Knight, 1992; Méndez & Karlsson, 1999). Motile traps incur additional costs associated with setting and resetting the traps (Adamec, 2006; Poppinga et al., 2018). Many species have a large number of traps per plants, in some case hundreds of traps per plant (Friday, 1992). Yet capture success varies widely among the traps within a plant (Cresswell, 1991).

Carnivorous structures are expensive to build and to operate, and it is therefore likely that traps have evolved defenses against misfiring or fouling of the traps. For example, the traps of the Venus flytrap require a series of mechanical stimuli for the traps to initiate a capture event (Böhm et al., 2016). Traps also ensure that they remain operational by preventing damage or fouling (Gibson, 1991; Hsu et al., 2015). The high cost of building and maintaining traps might also drive prey selectivity, in particular selectivity for large prey and the evolution of large traps (Gibson, 1991). However, the evidence for size selectivity is mixed (Harms, 1999; Hutchens & Luken, 2009). Many carnivorous plants are generalists, catching a wide range of prey types (Cresswell, 1991; Harms, 1999; Gordon & Pacheco, 2007; Guiral & Rougier, 2007; Hutchens & Luken,

2009), but some species specialize on a particular prey type or exclude a particular prey type, especially pollinators (Jürgens et al., 2012; Youngsteadt et al., 2018).

Carnivorous plants interact with animals along a spectrum ranging from mutualism over commensalism to parasitism (Antor & García 1995, Anderson & Midgley 2003, Fleischmann et al., 2016). So far, we have addressed one end of this spectrum, carnivorous plants preying on animals. At the other end of the spectrum are animals preying on carnivorous plants either through herbivory or kleptoparatisism (Fleischmann et al., 2016; Zamora & Gomez, 1996). Kleptoparasitism is a serious problem mainly for the non-motile traps, such as the pitchers of pitcher plants, who can lose up to a quarter of their prey items to lizards and slugs removing captured prey from the pitchers (Zamora, 1995). Some kleptoparasitic species specialize on parasitizing a carnivorous plants, such as a syrphid larva parasitizing sundews (Fleischmann et al., 2016). In *Utricularia*, there is speculation that the algae caught in their traps might be kleptoparasites (Płachno et al., 2015). An example for mutualism are two species from the carnivorous plant genus *Roridula* who are inhabited by two species of capsid bugs (*Pameridea*) – the bugs feed on caught insect prey while the plant takes up nutrients from the bug feces (Anderson & Midgley, 2003; Ellis & Midgley, 1996). Another example for mutualism is protection provided by ants against herbivores (weevil) in a pitcher plant (Merbach et al., 2007). There is also evidence that traps maintain microbiomes that aid in prey digestion and provide nutrients to the carnivorous plants (Chan et al., 2016; Sirová et al., 2018).

To summarize, the traps of carnivorous plants have adaptations that attract, capture, and retain prey. Many of these adaptations have structural components, such as the antennae of *Utricularia* that funnel prey toward the trap opening. Scientists have proposed that these structures help improve capture success by increasing encounter probability and by selecting for profitable prey (Darwin, 1875; Meyers & Strickler,

1979), yet the evidence that the observed morphologies indeed achieve those performance goals is mixed (Hutchens & Luken, 2009; Meyers & Strickler, 1979).

Trap Morphology in *Utricularia*

The traps of the genus *Utricularia* have been studied extensively, both their morphology (Friday, 1991; Westermeier et al., 2017) and their mechanics (Poppinga et al., 2016, 2017; Vincent et al., 2011), with many studies focused on aquatic species (*U. gibba*, *U. vulgaris*, *U. australis*).

A typical trap comprises a bladder lumen that is sealed by a trap door (**Figure 1**). The trap door can be positioned close to the outside (*U. australis*) or recessed within a hood or vestibulum (*U. gibba*). The door attaches to the trap body by a hinge on one side and presses against a velum or threshold on the other side. The trap door has several trigger hairs on the outside, which can protrude beyond the trap hood and are typically arranged in a close cluster opposite the hinged edge of the door (*U. australis*).

Species differ widely in the morphology of the traps' entrance, which has been used to define trap types (Westermeier et al., 2017). The main differences are (1) the shape and position of the door, (2) the shape of the door opening (varying from round to slit-shaped), and (3) structures surrounding the entrance. Aside from the trigger hairs inserting on the door, the entrance of the trap can be surrounded by a wide range of bristles and hairs, such as antennae and bristles (*U. australis*), radiating rows of stipitate glands (*U. livida*) or spurs (tusk-like projections) (*U. graminifolia*) (Taylor, 1989) (**Figure 2**).

Whereas the entrance region is highly variable between species, the trap body is more uniform. The main difference between species is size, size range, and the position of the stalk relative to the trap entrance. The traps are attached to the stolon via the stalk, this stalk can insert at the trap entrance, or anywhere along the trap (**Figure 3**) (Poppinga

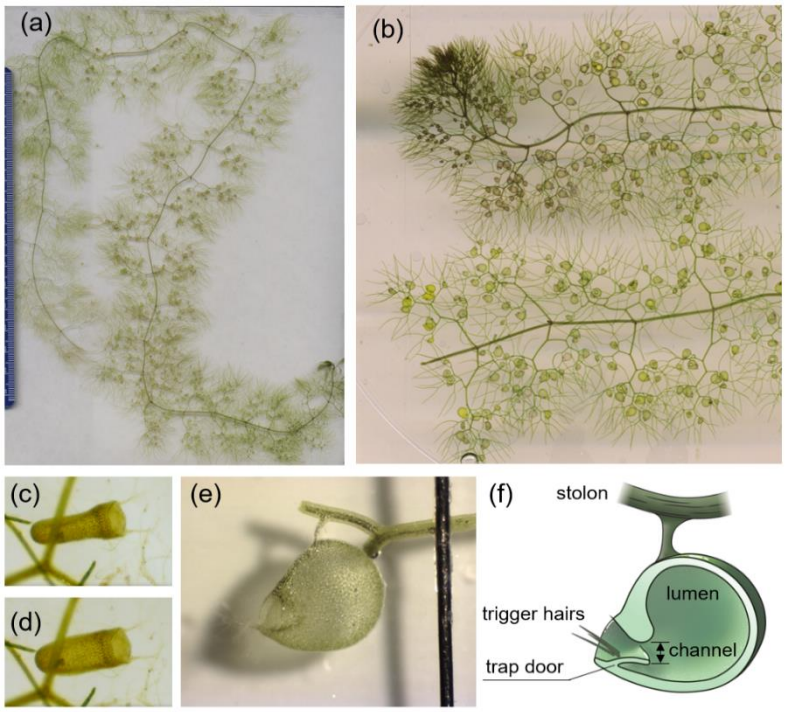


Figure 1. Typical bladderwort morphology exemplified by *Utricularia australis*.
(A) Complete plant. (B) Detailed view of the growing end of the plant with immature bladders (top) and a middle section with mature bladders (bottom). (C) Ventral view of a loaded bladder. (D) Ventral view of an unloaded bladder soon after being triggered. (E) Lateral view of a bladder. (F) Drawing of a bladder indicating trigger hairs, trap door, bladder lumen and bladder channel.

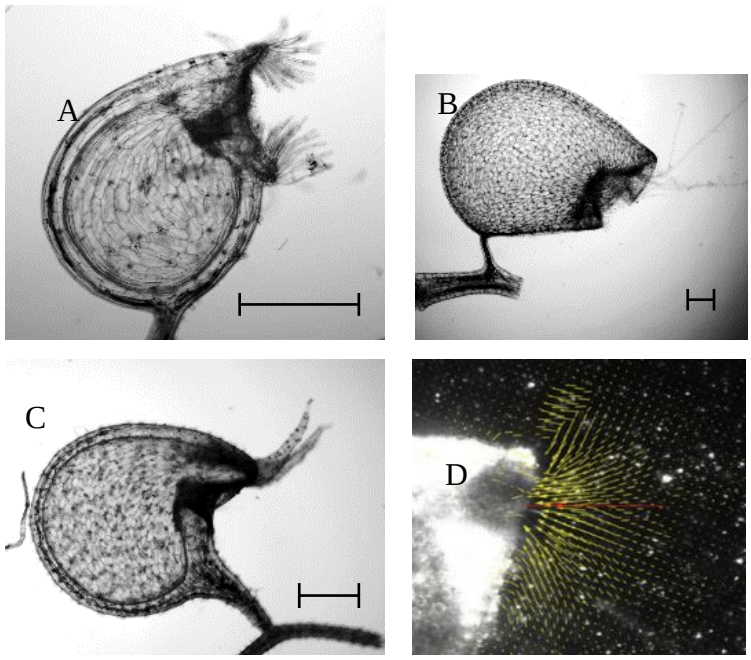


Figure 2. Examples of traps and their entrance structures.
(A) *Utricularia livida* – radiating rows of stipitate glands, (B) *U. australis* - antennae, (C) *U. graminifolia* - spurs. (D) Suction flow of a trap (*U. gibba*). Scale bar 0.2 mm.

et al., 2016). Traps also vary in shape, such as the ratio of major to minor axis, the uniformity of curvature in the lateral view, and the size of the trap entrance relative to the trap body.

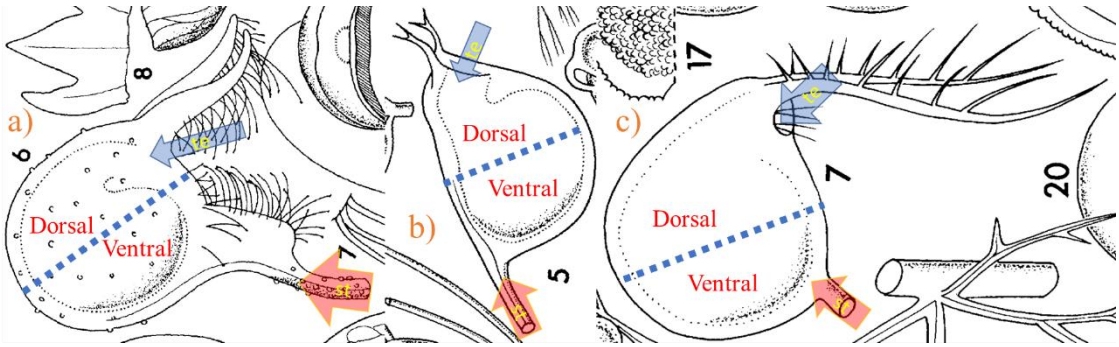


Figure 3. Lateral view of different trap types, indicating the position of the trap entrance (labeled 'te' with a blue arrow) and of the stalk (labeled 'st' with a red arrow).
a) Basal position (*U. amethystina*). b) Terminal position (*U. neottioides*). c) Lateral position (*U. minor*). The ventral and dorsal trap parts are indicated.

Several hypotheses have been proposed, and sometimes been tested, about the form-function relations of the traps' morphological features, in particular the appendages (Reifenrath et al., 2006). Clusters of hair-like bristles are one such set of appendages, and these may increase the rate of prey capture, or as pilot data in the Müller lab suggests they may reduce the fouling of the trap by dirt participles (Meyers & Strickler, 1979, Reifenrath et al., 2006). Yet there are terrestrial species that lack these bristles entirely, suggesting they are not essential for prey capture and may serve some other purpose (Reifenrath et al., 2006). However, there are a few similarities between traps from similar habitats. Epiphytic traps are the most uniform, having basal stalk attachments and recurved, usually branching, appendages (Reifenrath et al., 2006). The inward curve of these appendages is thought to prevent the desiccation of the trap (Reifenrath et al., 2006). Terrestrial traps appear to be smaller in size than aquatic traps (Reifenrath et al., 2006; Westermeier et al., 2017). Aquatic traps have antennae near the entrance that guide prey in like a funnel (Reifenrath et al., 2006).

Trap Behavior in *Utricularia*

In all but one *Utricularia* species these traps are motile, capturing prey by sucking prey items into the bladder lumen (Poppinga et al., 2016). The traps of *Utricularia* activate with imperceptible speed and this is possible because the traps in the genus conserve a specific "lentiform" shape, which enables them to suction-feed (Poppinga et al., 2016; Singh et al., 2011). The steps of the prey capture process have been described in detail in several aquatic species (**Figure 4**) (Poppinga et al., 2016, 2017; Singh et al., 2011). Traps are set by removing water from the trap lumen, which generates a sub-ambient pressure of 10 to 17 kPa and elastically loads the trap walls (Sasago & Sibaoka, 1985; Singh et al., 2011; Sydenham & Findlay, 1973). Prey capture events are triggered when prey touches the trigger hairs on the door, causing the door to snap-buckle inward and opening the trap (Vincent et al., 2011). As the door opens, prey is sucked into the trap by a suction flow powered by the sub-ambient pressure in the trap. The trap then door closes, trapping prey inside the trap lumen (Poppinga et al., 2016, 2017). Each step of this capture sequence takes just a few milliseconds (Poppinga et al., 2017). Traps then reset by removing water from the lumen, and can be triggered again with 10 to 15 minutes (Adamec, 2012; Sydenham & Findlay, 1973).

Taxonomy of *Utricularia*

One of the most specious family of carnivorous plants is the family of Lentibulariaceae, which contains the genera *Utricularia*, *Genlisea*, and *Pinguicula*. Of these two genera, the genus *Utricularia* is the most specious with more than 274 species (Cheng et al., 2021; Rutishauser, 2016; Taylor, 1989). *Utricularia* is a monophyletic genus with three sub genera, *Utricularia*, *Bivalvaria*, and *Polypompholyx* (Jobson et al., 2018; Silva et al., 2018). A molecular phylogeny is available for 78 the described species (Westermeier et al., 2017) (**Figure 5**).

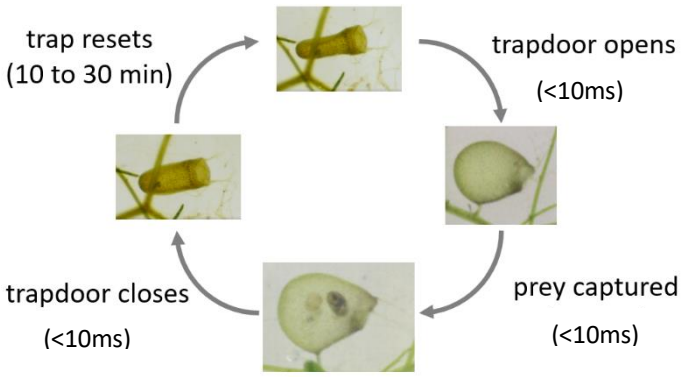


Figure 4. Summary of prey capture cycle in *U. australis* (starting at the top, clockwise) . When the trap is set, it is laterally compressed (top image, ventral view of the trap). When prey touches the trigger hairs on the trap door, the door opens within 10 ms (right image, lateral view), prey is sucked into the trap within 10 ms (bottom image), and the trap door then closes within 10 ms, leaving the trap looking inflated in the ventral view (left image). The trap then reloads within 10 to 30 minutes.

Within the Lentibulariaceae, *Utricularia* is also the most rapidly diversifying genus (Jobson et al., 2018). This rapid diversification has been linked to *Utricularia*'s "extreme morphological flexibility" apparent in its morphological and habitat diversity (Jobson & Albert, 2002). *Utricularia* has a "highly modified bauplan, including its suction traps", and its lack of roots, and unique physiological adaptations linked to prey capture (Ibarra-Laclette et al., 2013; Jobson et al., 2004, 2018). These suction traps are modified cup-shaped leaves that evolved from ancestors that had flat leaves (Whitewood, et al., 2020).

Species within *Utricularia* and its sister genus *Genlisea* have the smallest angiosperm genome (Fleischmann et al., 2014; Ibarra-Laclette et al., 2013). These small genomes are the result of multiple whole-genome-duplication and reduction events, leading to genomes with fewer than 100 Mbp (Carretero-Paulet et al., 2015; Fleischmann et al., 2014; Ibarra-Laclette et al., 2013,). This high genome turnover and size reduction in *Utricularia* has been linked to their prey capture mechanism, in particular the possibility of high oxidative stress, possibly caused by the traps' high metabolism (Ibarra-Laclette et al., 2011).

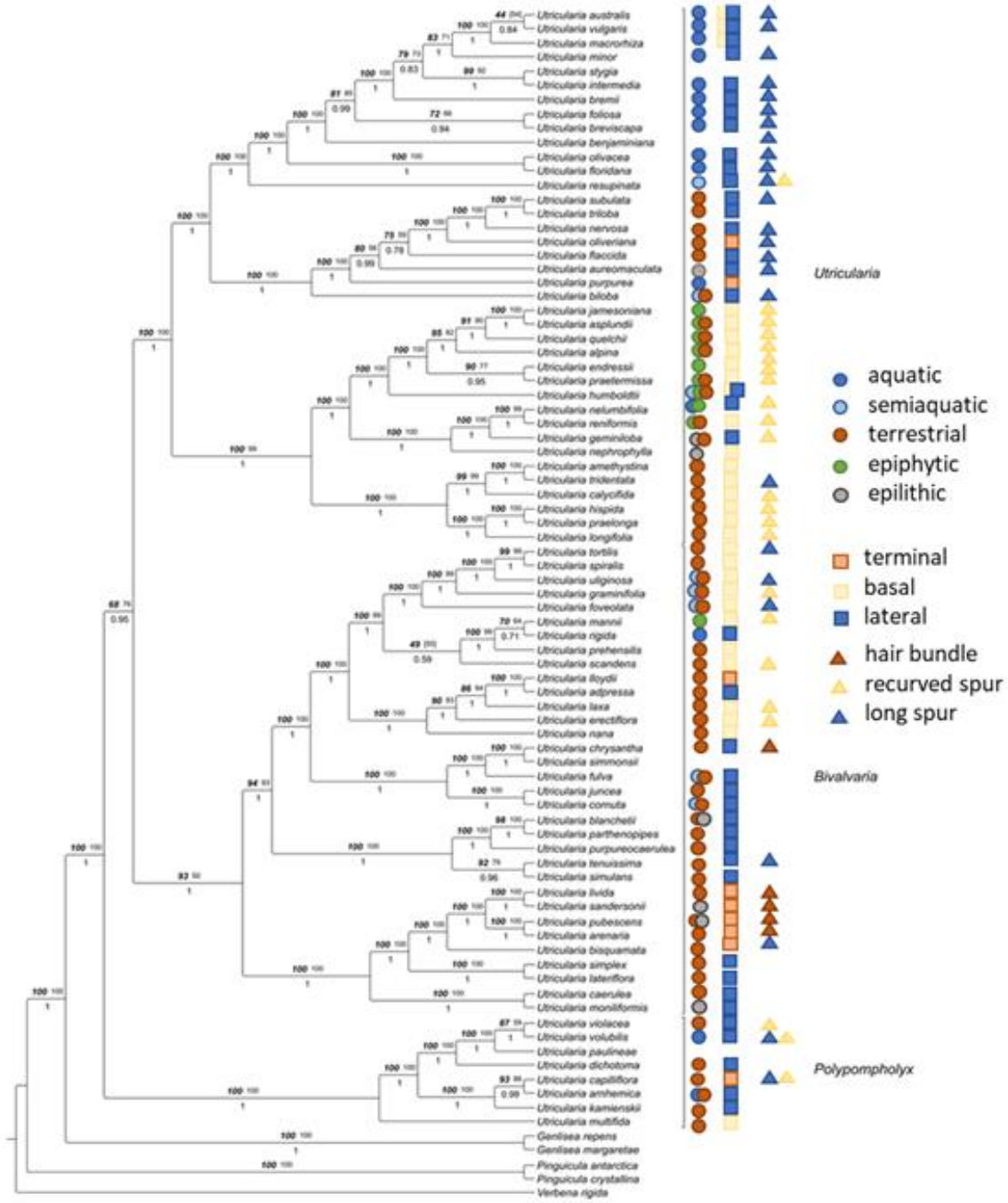


Figure 5. Molecular phylogeny of the genus *Utricularia*, modified from (Westermeier et al., 2017).

Distribution and Habitat of *Utricularia*

Bladderworts' geographic range is global; it occurs on all continents but is absent on the poles and on most oceanic islands (Jobson et al., 2018). Based on molecular clock estimates, the genus likely evolved 40 million years ago (mya) in South America and spread from there via North America 12 mya and the Atlantic 4.7 mya to the rest of the world (Ibarra-Laclette et al., 2013; Silva et al., 2018) (**Figure 6**).

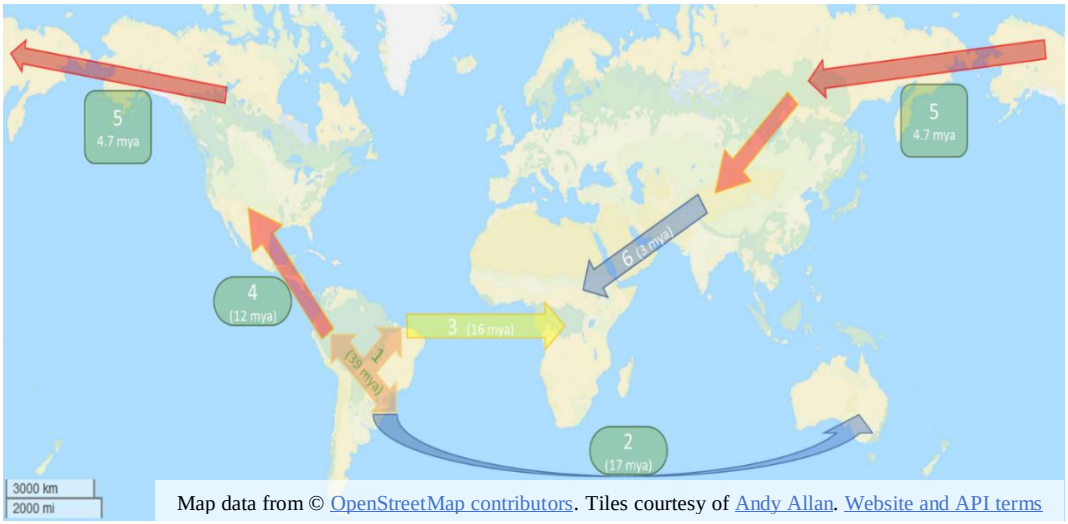


Figure 6. Inferred dispersal routes of *Utricularia* lineages, with their respective possible ages (Silva et al., 2018). Based on data from © OpenStreetMap contributors.

The vast majority of *Utricularia* species are terrestrial, with roughly 20% of the species being aquatic and another few species being epiphytic or rheophytic (Silva et al., 2018; see **Figure 5** for similar trend).

Aims of This Study

This project has the following aims.

Aim 1: characterize trap morphology for at least 150 bladderwort species.

Aim 2: examine the effects of habitat on trap morphology.

Within Aim 2, I will address the following hypotheses:

Hypothesis 1: Aquatic species have antennae (long spurs) near their trap entrance to funnel prey toward the entrance.

Rationale: Previous studies on an aquatic species (*U. vulgaris*) have shown that antennae help funnel prey toward the trap entrance (Meyers & Strickler, 1979).

Hypothesis 2: Terrestrial species have clusters of long bristles around the entrance to reduce fouling, such as dirt particles being sucked into the traps during prey capture.

Rationale: Pilot experiments in the Müller lab on a terrestrial species (*U. livida*) have shown that small particles deposited in front of the entrance are not sucked into the trap.

Hypothesis 3: aquatic species have larger traps than non-aquatic species.

Rationale: Work in the Müller lab suggests that increasing size increases hydrodynamic effectiveness. Aquatic species are less spatially constrained by their environment than terrestrial, epiphytic, and epilithic traps, allowing them to catch larger prey (Reifenrath et al., 2006; Westermeier et al., 2017).

Hypothesis 4: epiphytic species have curved spurs to retain a bubble of water at the entrance.

Rationale: Reifenrath has suggested these traps use their spurs to retain water in front of the trap (Reifenrath et al., 2006).

Hypothesis 5: aquatic species have lateral stalk attachment to allow recoil, which moves the trap mouth towards the prey during a capture event.

Rationale: Pilot experiments in the Müller lab on aquatic species have shown considerable recoil in traps with laterally attached stalks.

Hypothesis 6: suction feeding by creating a sub-ambient pressure in the trap lumen is a shared characteristic across bladderwort habitats and this uniformity of mechanism might result in a uniformity of shape.

Rationale: Previous studies found that most if not all bladderwort traps operate by active suction (Poppinga et al., 2020). This shared mechanism suggests that the shape of the trap body might be conserved across habitats because suction feeding is powered by the same conserved mechanism in all habitats: generating sub-ambient pressure in the trap lumen by storing elastic energy in the trap walls (Singh et al., 2011).

METHODS

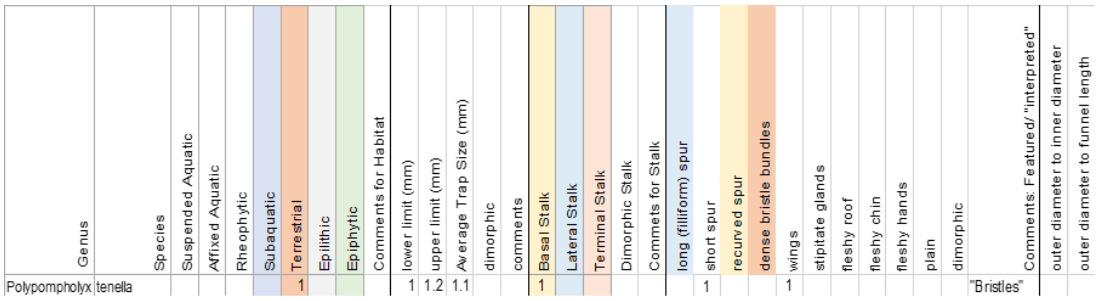
Collecting Data on Trap Morphology

Aim 1 of the study was to characterize the trap morphology of at least 150 *Utricularia* species. To achieve Aim 1, we used the written descriptions and drawings of bladderwort traps to characterize trap morphology for as many species as we can find drawings and descriptions for. Following scientific precedent (Carrier et al., 2002), we used a scientific monograph (Taylor, 1989) as our main source for those drawings to ensure that most illustrations were done by the same author, which reduces variation in the data caused by the artist, and is an established methodology (Panagiotopoulou et al., 2016). Taylor (1989) is the most exhaustive compilation of *Utricularia* specimens, including drawings and information about 214 species. The drawings typically provide a lateral view of the trap and, if relevant, document multiple morphs or provide additional (frontal) views. All drawings contain a magnification factor in the figure legend for each figure element.

Types of Data Recorded

All the categorical data recorded for this study were copied, verbatim, from a digitized edition of the taxonomic monograph mentioned (Taylor, 1989) based on a protocol from another study using a monograph (Panagiotopoulou et al., 2016). Habitat and morphological information (**Table 1**) along with length-based trap-specific data (**Figure 2**) were developed from drawings and text in Taylor (1989) or Poppinga et al. (2016). Information about morphological traits was complemented by information on habitat and geographic distribution to build the database required to address the research questions of this project. We collected all data in Excel, building a spreadsheet as exemplified below for one species' habitat and morphological data (**Table 1**).

Table 1. Example of Habitat and Morphological Categoric Data Collected for Each Trap



Genus	Species	Suspended Aquatic	Affixed Aquatic	Rheophytic	Subaquatic	Terrestrial	Epilithic	Epiphytic	Comments for Habitat	lower limit (mm)	upper limit (mm)	Average Trap Size (mm)	dimorphic	comments	Basal Stalk	Lateral Stalk	Terminal Stalk	Dimorphic Stalk	Commets for Stalk	long (filiform) spur	short spur	recurved spur	dense bristle bundles	wings	stipitate glands	fleshy roof	fleshy chin	fleshy hands	plain	dimorphic	Comments: Featured/ "interpreted"	outer diameter to inner diameter	outer diameter to funnel length
Polypompholyx	tenella					1				1	1.2	1.1			1						1			1							"Bristles"		

These traits above were tabulated in columns grouped by habitat, trap size features, stalk attachment style, trap appendage morphology and trap funnel measurements. A comprehensive guide of these traits and their definitions is included (**Table 2**).

We also constructed a spreadsheet of raw morphological data gathered from the text (Taylor, 1989) and as a result of our image processing method (**Table 2**). These morphological traits are tabulated thusly (**Table 3**):

Habitat and geographic range information was cross referenced with more recent sources (Jobson et al., 2018; Silva et al., 2018). To minimize human error from having a single data entry person while recording categoric data (**Table 2**), we collected the data in a group of four members from the Müller lab. We decided to record all traits verbatim from Taylor, only changing “fimbriate appendages” to “fleshy-hands.” We calculated a consensus of categoric traits for each trap (**Table 2**).

In addition to recording data from the book, we also needed to record data of the dimensions of the book because each image in the book is drawn to scale. Using our trap measurements and magnification information in the book we calculated the dimensions of each trap in units of millimeters (mm). Found in the document properties of this PDF formatted text, each page is 156 by 234 mm. This approach was more precise than our follow-up measurement of a page from a physical paperback copy of the book using a 12-

Table 2. Explanation of Habitat and Morphological Categoric Data Collected for Each Trap

Column	Groups	Header	Description
A	Habitat	Genus	Copied from Taylor, 1989 - binary presence/absence
B		Species	Copied from Taylor, 1989 - binary presence
C		Suspended Aquatic	Copied from Taylor, 1989 - binary presence - lacks roots and is suspended in water
D		Affixed Aquatic	Copied from Taylor, 1989 - binary presence - has true roots and grows to the water's surface
E		Rheophytic	Copied from Taylor, 1989 - binary presence - grows in rivers
F		Subaquatic	Copied from Taylor, 1989 - binary presence - has true roots and grows below the water's surface
G		Terrestrial	Copied from Taylor, 1989 - binary presence - grows in soil, ancestral clade
H		Epilithic	Copied from Taylor, 1989 - binary presence - attaches directly to rock surface
I		Epiphytic	Copied from Taylor, 1989 - binary presence - attaches to other plants
J		Comments for Habitat	Used to denote a more nuanced habitat designation found in Taylor, 1989
K	Trap	lower limit (mm)	Copied verbatim from Taylor, 1989
L		upper limit (mm)	Copied verbatim from Taylor, 1989
M		Average Trap Size (mm)	Calculated from the upper and lower limits
N		Dimorphic	Copied verbatim from Taylor, 1997, states if there is a dimorphic variation in trap size
O		Comments	Denote if a trap has a polymorphic size variant
P	Stalk	Basal Stalk	Copied from Taylor, 1989 - binary presence - stalk faces same direction as mouth
Q		Lateral Stalk	Copied from Taylor, 1989 - binary presence - stalk is on the other side of the trap
R		Terminal Stalk	Copied from Taylor, 1989 - binary presence - stalk is opposite the mouth
S		Dimorphic Stalk	Copied from Taylor, 1989 - binary presence
T		Comments for Stalk	Used to denote a more nuanced stalk designation found in Taylor, 1989
U	Mouthpart	long (filiform) spur	Copied from Taylor, 1989 - binary presence - long and slender, may be branching
V		short spur	Copied from Taylor, 1989 - binary presence - pointed and conical
W		recurved spur	Copied from Taylor, 1989 - binary presence - curved like rams' horns
X		dense bristle bundles	Copied from Taylor, 1989 - binary presence - bristles like a brush
Y		Wings	Copied from Taylor, 1989 - binary presence - flat, flappy appendages
Z		stipitate glands	Copied from Taylor, 1989 - binary presence - finger-like glands
AA		fleshy roof	Copied from Taylor, 1989 - binary presence - large roof over the mouth
AB		fleshy chin	Copied from Taylor, 1989 - binary presence - thick chin-like region between the mouth and stalk
AC		fleshy hands	Copied from Taylor, 1989 - binary presence - similar to wings but more pointed
AD		Plain	Copied from Taylor, 1989 - binary presence - lack appendages
AE		Dimorphic	Copied from Taylor, 1989 - binary presence
AF		Comments: Featured/ "interpreted"	Mouthparts not fully represented by any of the above categories, either copied from Taylor, 1989 verbatim or "interpreted" by us
AG	Funnel	outer diameter to inner diameter	The unitless ratio of the outer trap diameter to its inner diameter
AH		outer diameter to funnel length	The unitless ratio of the outer trap diameter to its mouth funnel length

Table 3. Explanation of Trap Morphology Length-Based Data Measured for Each Trap

Column	Header	Description
A	ROI #	252 total traced trap bodies, this column indicates which trace the data came from as they were recorded in chronological order
B	Label	Lists the name of the stack, followed by the name of specific traced ROI area, then the name of the image within the stack, all three separated by colons (:)
C	Area	pixel area of the traced trap bodies
D	X	centroid X, center of ellipse
E	Y	centroid y, center of ellipse
F	Perim.	The length of the outside boundary of the selection
G	BX	Coordinate of the upper left corner of the bounding rectangle
H	BY	Coordinate of the upper left corner of the bounding rectangle
I	Width	Width of the bounding rectangle, the smallest rectangle enclosing the selection
J	Height	Height of the bounding rectangle, the smallest rectangle enclosing the selection
K	Major	major elliptically fit axis
L	Minor	minor elliptically fit axis
M	Angle	the angle between the primary axis and a line parallel to the x-axis of the image
N	Circ.	Circularity: 4π*area/perimeter^2. A value of 1.0 indicates a perfect circle. As the value approaches 0.0, it indicates an increasingly elongated shape.
O	Feret	longest distance between any two points along the selection boundary, also known as maximum caliper
P	Skew	Skewness: the third order moment about the mean
Q	Kurt	Kurtosis: the fourth order moment about the mean
R	Slice	209 total slices
S	FeretX	starting X coordinate of the Feret's diameter
T	FeretY	starting Y coordinate of the Feret's diameter
U	FeretAngle	the angle between the Feret's diameter and a line parallel to the x-axis of the image
V	MinFeret	minimum caliper diameter
W	AR	(aspect ratio): major_axis/minor_axis
X	Round	Roundness: 4*area/(π*major_axis^2), or the inverse of the aspect ratio
Y	Solidity	area/convex area

inch ruler (Taylor, 1989). Morphological data were collected by digitizing the drawings from Taylor (**Figure 7**) with the image processing software ImageJ (Taylor, 1989). All length-based traits were expressed in absolute units by measuring trap size in mm within ImageJ, explained in-depth later in this section. All images were saved as raw data in TIFF format individually and collectively as a TIFF formatted slideshow, so that scaling could be applied equally to all images for the for the data processing step before data analysis.

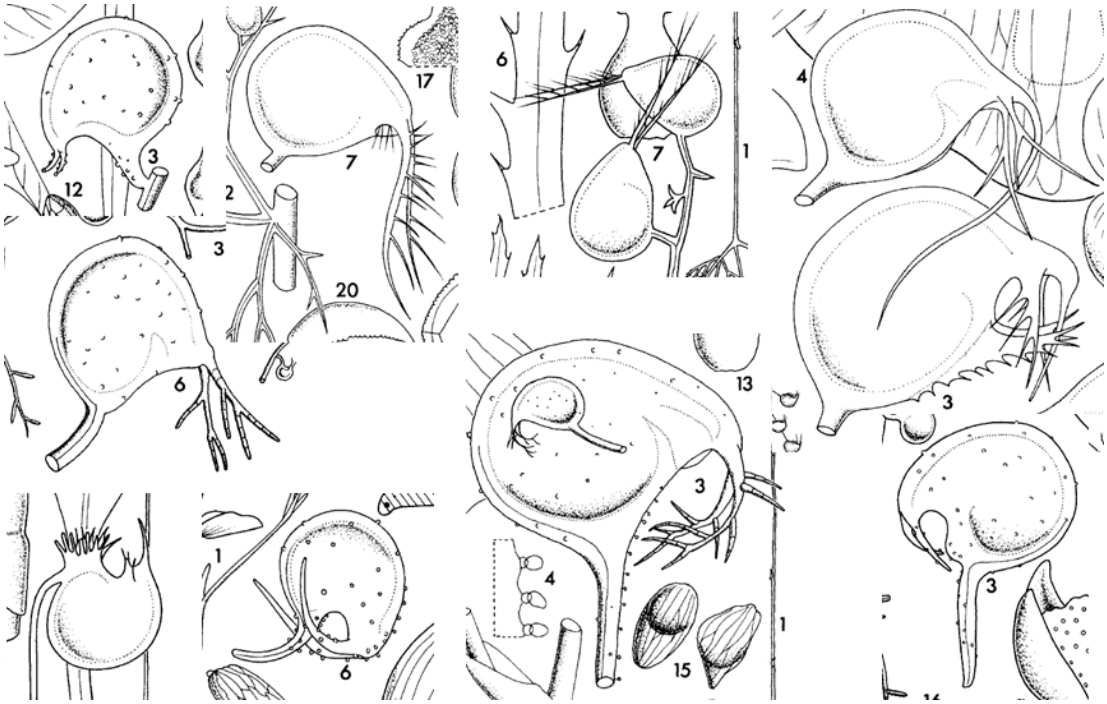


Figure 7. Example unprocessed drawings from Taylor (Taylor, 1989).

Image Capture and Processing

We measured the outline of each trap using ImageJ to create regions of interest (ROIs) within each trap (**Figure 8g**). We did this by first going sequentially down the list of plant species found in Taylor (Taylor, 1989), and recording an image of each trap as it appeared on the page using the Windows Snipping Tool and pasting the result into ImageJ. It is important to note that no matter the magnification used to view the Taylor

(Taylor, 1989) PDF, it always displays the same number of pixels. To save storage space, only images of measurable traps were cropped, not whole pages. This cropping method meant that the species of a trap needed to be verified after processing, as the name was not cropped along with the trap from that species. The magnification factors of each trap image also needed to be verified for this reason. These separate images were each saved in TIFF format in ImageJ, then the best 205 of these images were combined into a TIFF formatted slideshow, or stack. With the ROI manager open in ImageJ, ROIs were captured as soon as they were traced for each image. Multiple ROIs were captured for each image in cases where the traps of a given species could be found as one of several morphs. ROIs were automatically marked using the Wand tool from the base version of ImageJ. This tool draws a contentious outline, one pixel thick, along the inside of any line ≥ four more pixels wide.

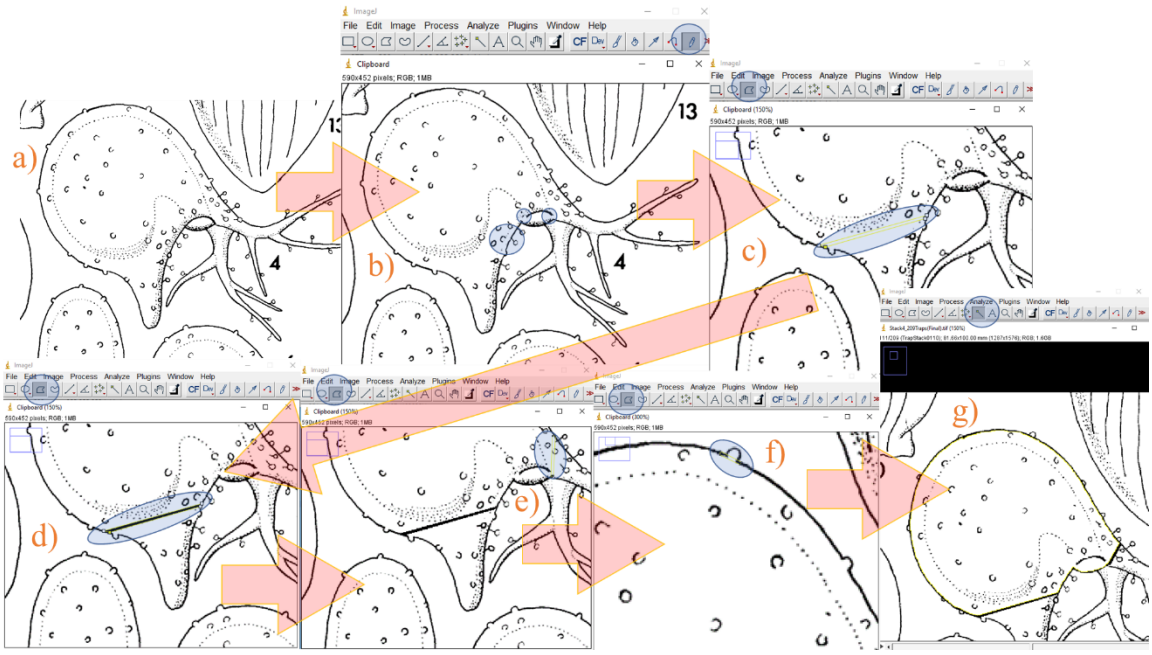


Figure 8. Image capture workflow for *U. foveolata*.
a) the raw starting image as cropped from Taylor, 1989, b) deleting features interrupting the outline such as mouth shape and shading in this case with the Draw tool, c),d), and e) using the Polygon Selection tool to complete the outline of the shape by deleting the white foreground and exposing the black background, f) using the Polygon Selection tool to remove bumps along the trap surface from consideration for the outline, g) fully processed image with outline selected via the Wand tool.

Our goal was to mark the most elastic outline of the inside of a trap (**Figure 8g**), so in post-capture processing we removed the stalk attachments and all appendages from the outline of the trap body using a 5 pixel wide brush (**Figure 8b**). This brush was the Draw tool, and we used it to draw a single point in white where the Wand tool would otherwise continue the outline to include either the stalk or appendages of a trap. We connected all outlines to make an unbroken perimeter. Using the Polygon Selections tool we clicked in two places from one part of an implied, or dotted, outline to the other side in two places and deleted the white foreground (**Figure 8 c - e**) to complete the perimeter of trap bodies. These polygon sections were made to curve gradually to follow the implied curvature of the unobstructed trap body. The Polygon Selections tool was also used to smooth over bumps on the trap surface (**Figure 8f**).

The Results command of the ROI Manager in ImageJ was used to prepare a table (**Table 3**) of derived results calculated from the dimensions of the perimeter we drew with the Wand tool for each trap and morphological variant. Before any data were exported to Excel, we set the scale conversion of all the images in our stack based on the length of a page of Taylor (Taylor, 1989). Because the PDF version of Taylor (Taylor, 1989) had larger margins when we attempted to copy and paste it into ImageJ, we instead cropped the pages with the 10 largest traps so that the whole page was showing. We pasted these pages into images J, where we used the Straight Freehand Line tool to draw a single line down the length of each image and record each as an ROI. We then calculated the average of the length of these 10 lines, to get 3686.903 pixels with a standard deviation of 4.255 pixels, which was set to 234 mm in length. In other words, 1 mm corresponds to ~15.76 pixels. This scaling factor (1 mm per 15.76 px) was used to calculate the dimensions of each trap in millimeter (mm) units, as they appeared on each page for our normalized trap measurements. These raw data were exported to Excel in a CSV format, where the length data for each trap were divided by the magnification factor

of each trap to get their real-world dimensions. Duplicate rows were copied for categorical morphological data in species with multiple ROI variants.

Copies of all the data collected up to this step were made across five sheets in Excel for minor changes to better prepare the data for analysis. In the first sheet of raw data, a column was added to mark whether an ROI was a variant. The next sheet divided the raw data measured from ImageJ by the scaling factor of the trap image as recorded from Taylor (1989). The third sheet is where we aligned all data with the correct species. The forth sheet is where we produced diagnostic charts of each variable to identify outliers, and to check if any variable had a discernibly normal distribution. The last of the five sheets is where we duplicated the raw data from the sheet containing the habitat and binary mouthpart features data to match and align with the data collected from variant ROIs. The method used in the fifth data processing sheet has implications for our data analysis that will be discussed further in this report.

Statistical Tests and Rationale

Aim 2 of this project is to examine the effect of habitat on trap morphology. To achieve Aim 2, we formulated the following statistical hypotheses that allowed us to test our scientific hypotheses.

Hypothesis 1: Aquatic species have antennae to funnel prey toward the entrance.

Statistical hypothesis 1: aquatic species are more likely to have antennae than non-aquatic species.

Test: Chi-squared habitat vs mouthparts

Rationale: Previous studies on an aquatic species (*U. vulgaris*) have shown that antennae help funnel prey toward the trap entrance (Meyers & Strickler, 1979).

Hypothesis 2: Terrestrial species have clusters of long bristles around the entrance that prevent dirt particles from being inhaled

Statistical hypothesis 2: terrestrial species are more likely to have bristle bundles than non-terrestrial species.

Test: Chi-squared habitat vs mouthparts

Rationale: Pilot experiments in the Müller lab on a terrestrial species (*U. livida*) have shown that small particles deposited in front of the entrance are not sucked into the trap.

Hypothesis 3: aquatic species have larger traps than non-aquatic species

Statistical hypothesis 3: Aquatic species are more likely to have larger traps on average than aquatic species.

Test: Wilcoxon Signed Rank test, habitat vs trap size

Rationale: Work in the Müller lab suggests that increasing size increases hydrodynamic effectiveness.

Hypothesis 4: epiphytic species have curved spurs to retain a bubble of water at the entrance

Statistical hypothesis 4: epiphytic species are more likely to have recurved spurs than non-epiphytic species.

Test: Chi-squared habitat vs mouthparts

Rationale: Reifenrath suggests these traps use their spurs to retain water in front of the trap (Reifenrath et al., 2006).

Hypothesis 5: aquatic species have lateral stalk attachment to allow recoil, which moves the trap mouth towards the prey during a capture event

Statistical hypothesis 5: aquatic species are more likely to have a laterally attached stalk than non-aquatic species.

Test: Chi-squared habitat vs stalks

Rationale: Pilot experiments in the Müller lab on aquatic species have shown considerable recoil in traps with laterally attached stalks.

Hypothesis 6: suction feeding by creating a sub-ambient pressure in the trap lumen is a shared characteristic across habitats and this uniformity of mechanism might result in a uniformity of shape.

Statistical hypothesis 6: There should be no significant difference between roundness, solidity, and circularity between species from different habitats.

Rationale: Previous studies found that most if not all bladderwort traps operate by active suction (Poppinga et al., 2020). The shape of the trap body is conserved across all habitats because species in all habitats power suction feeding by the same mechanism, generating subambient pressure in the trap lumen by storing elastic energy in the trap walls (Singh et al., 2011)

Test: (Q) Friedman Test species vs unitless shape data

Test: (W) Kendall's Coefficient of Concordance

Test: (r) Spearman Correlation Coefficient

Test: (Q) Friedman Test species vs Aspect Ratio, Circularity, Roundness, and Solidity

Test: (W) Kendall's Coefficient of Concordance

Test: (r) Spearman Correlation Coefficient

Test(s): Wilcoxon Signed Rank test: Roundness, Solidity, and Circularity vs Each Other in Pairs, Then Each One vs Habitat – six (6) tests total

Data Analysis

For hypotheses 1, 2, 4, and 5 we made separate and new columns to better account for the habitats and morphological features of each trap. A species was classified as “Aquatic” if it was either suspended aquatic, affixed aquatic, rheophytic, or subaquatic. A species was classified as “Terrestrial” if it was already categorized as such or if it was epilithic or epiphytic. We made four new columns, one for each hypothesis, to

count negative instances of the test condition. These columns were non-lateral for stalks that were not lateral, along with non-antennae, non-bristle bundles, and non-spurs for species with mouthparts that were not those being focused on for that hypothesis. These new binary columns along with those already recorded were used to calculate binary data in additional columns used in each test for each hypothesis (**Table 4**).

These new columns tabulate the results of a logical IF statement, so that if there is a 1 in each of the columns specified, the ones that feed the new column raw data (Group/Description of **Table 4**), then the new column will also contain a 1, if not a 0. The bottom of each group of four columns is where their totals are summed for each Chi-squared test. The total counts of the four variables are then summed separately such that there is a total for all instances of the habitat in question, a total for the other habitat(s), a total for all species with the trait, and a total for all species lacking the trait. These four totals are then used to calculate four expected totals for each of the four original column totals. We put these 8 totals as input variables for the Excel function CHISQ.TEST and Excel returns a p-value (**Table 4**) for each Chi-squared test.

Hypothesis 3 (H3 in **Table** 4) was not tested using a Chi-Squared test, instead we used a Wilcoxon Signed-Rank test. For this test we separated the average sizes of aquatic and terrestrial traps into different column. We then calculated the differences and made another column to determine if those differences were positive or negative. An additional column was created to record the absolute value of the differences and another column was created after that to rank all the differences from largest to smallest. In cases of ties in difference ranks, the average of two ties was calculated, but not the average of several ties greater than two – those cases were calculated the same as if there had been only two ties. There were several ties with no difference because that species had both aquatic and terrestrial traps of the same size. All of these ranks were given a sign in the final column of the test so that their differences could be recognized and calculated as either negative

Table 4. Hypotheses 1-5 Data Analysis Columns

Column	Group/Description	Header
AM	**Chi-Squared Tests**	H1, H2 & H4
AN	Habitat	Combined Aquatic
AO		Epiphytic
AP		ALL Non-Epiphytic
AQ		Combined Terrestrial w/Epiphytic
AR	Mouthparts	long (filiform) spur
AS		short spur
AT		recurved spur
AU		dense bristle bundles
AV		Lateral Stalk
AW	Non-Mouthparts (opposite AV)	Non-Lateral
AX	opposite AR	Non-Antennae
AY	opposite AU	Non-Bristle Bundles
AZ	opposite AT	Non-Recurved Spurs
BA	Hypothesis 1(AN+AR)	Aquatic + Antennae
BB	(AQ+AR)	Terrestrial + Antennae
BC	(AN+AX)	Aquatic + Non-Antennae
BD	(AQ+AX)	Terrestrial + Non-Antennae
BE	Hypothesis 2 (AQ+AU)	Terrestrial + Bristle Bundles
BF	(AN+AU)	Aquatic + Bristle Bundles
BG	(AQ+AY)	Terrestrial + non-Bristle-Bundles
BH	(AN+AY)	Aquatic + non-Bristle-Bundles
BI	Hypothesis 4 (AO+AT)	Epiphytic + Recurved Spurs
BJ	(AP+AT)	Non-Epiphytic + Recurved Spurs
BK	(AO+AZ)	Epiphytic + non-Recurved-Spurs
BL	(AP+AZ)	Non-Epiphytic + non-Recurved-Spurs
BM	Hypothesis 5	H5
BN	(AN+AV)	Aquatic + Lateral Stalk
BO	(AQ+AV)	Terrestrial + Lateral Stalk
BP	(AQ+AW)	Aquatic + non-Lateral Stalk
BQ	(AQ+AW)	Terrestrial + non-Lateral Stalk
BR	**Wilcoxon Signed-Rank Test**	H3
BS	Trap measurements (Table 2, column M)	Aquatic Average Size
BT	(Table 2, column M)	Terrestrial Average Size
BU	BS-BT	Differences
BV	Sign of BU	Positive (and Zero) or Negative
BW	|BU|	|Differences|
BX	Rank of each difference	Rank
BY	BV+BX	Signed Rank
BZ	Calculates for p-value	Calculating the statistic
CA	Describes BZ	Parameters

or positive differences in terms of aquatic versus terrestrial traps. The statistics for a Wilcoxon signed-rank test are calculated when an alpha value is designated, 0.05 in this case, the number of tails or test variables must also be included, and the count of all species considered. We then calculate the T value by picking the lowest absolute value from the two separate totals of all the negative ranks and all the positive ranks. The mean is calculated as the count multiplied by the count plus 1 all divided by 4. The variance and the mean multiplied by the count times 2, all divided by 6. The standard deviation is the square root of the variance. We then calculate our Z-score from the difference in absolute value between the mean and our chosen T value and divide that by the standard deviation. Before we can calculate our p-value using the function in Excel we need to calculate the critical T value, beyond which our result will be statistically significant. The mean plus the standard deviation all multiplied by the result of the Excel function NORM.S.INV and the alpha value divided by 2 gets our critical T value, any T score below this value is statically significant. Finally, we calculate the p-value to better understand the probability of our resulting T value. In this case the p-value is 2 times 1 minus the Z score after it goes through the Excel function NORM.S.DIST.

The two Friedman tests for the Q value we set up used eight columns calculated from the unitless shape data we collected on the traps of *Utricularia* (**Table 3**) For the first test we calculated eight columns for the variables of Angle, Circularity, Skew, Kurt, Feret Angle, Aspect Ratio, Roundness, and Solidity. Our calculated columns ranked each valve within each column, ties were averaged as if two identical values existed. We then made 8 additional columns to rank each rank across the each columns, row by row, and ties were averaged as above. The total values of each column were summed. The grand total of all values was summed. The sum of squares was calculated using Excel's built-in function SUMSQ across the range of column sums. The Q value was calculated by dividing 12, a constant, by the number of rows times columns all times the number of

rows minus 1 multiplied by the sum of squares minus 3 times the number of rows times the number of columns minus 1. We used the Excel function CHI.DIST.SQ with the Q value and row count minus 1 as arguments. We also did two follow-up tests using the same values from the Friedman test. Dividing the Q value by the columns times the rows minus 1 yields the W value or Kendall's Coefficient of Concordance. A low W value means that the columns are all measuring the same thing, trap shape. We used the W value to calculate the r value or the Spearman Correlation Coefficient. We did this by multiplying the number of rows by the W value minus 1 all divided by the number of rows minus 1. The second Friedman test only used four columns, these were Aspect Ratio, Circularity, Roundness, and Solidity. Calculations done on these columns were the same as for the first test explained above.

We then performed six Wilcoxon Signed-Rank tests, with the same calculations as mentioned above for Hypothesis 3, there were only two notable differences in how these tests were set up. Firstly these tests compared differences in the ranks of the variables as calculated from the first Friedman test, we did this to control for the fact that each variable was recorded across a different scale and using ranks put them all on the same scale, from 1 to 205. The second difference was in the variables we used, they were different the test for hypothesis 3. The first three tests were for pairs of unitless data across all species based on data from the Circularity, Roundness, and Solidity columns. So, we tested Circularity versus Roundness, Roundness versus Solidity, and Solidity versus Circularity. We then did three tests for each of the three variables but this time aquatic versus terrestrial. Finally, we made six columns, three each for aquatic and terrestrial species to find the median and mean values for Circularity, Roundness, and Solidity. At the bottom of these columns the Excel INDEX function was used with the MATCH function as an argument to find the name of each species that most closely matched each calculated value. A similar Excel formula was performed to also find the

exact ROI names for those species. These ROIs were retrieved and cropped in the Results section below (**Figure 15**).

RESULTS

Results for Aim 1

In total we analyzed the 205 species in genus *Utricularia*, meeting our stated Aim 1 of recording data for at least 150 species. This included 205 total ROIs captured to record those data measured by us in ImageJ. Most species are terrestrial, which is the ancestral condition (**Figure 9**).

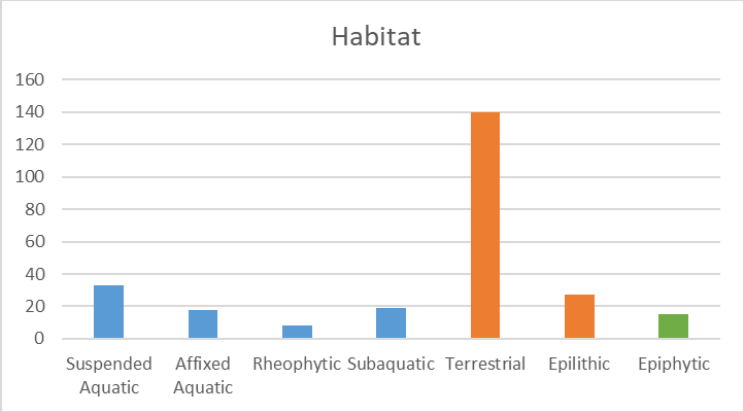


Figure 9. Counts of species in *Utricularia* grouped by habitat

Trap size ranges widely. The specific-specific bottom-bracket values range from 0.15 to 4.0 mm and the top-bracket values from 0.15 to 12 mm (**Figure 10**).

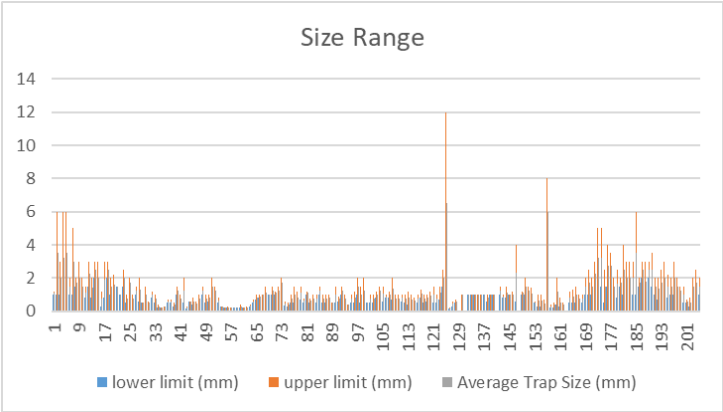


Figure 10. Lower limit, upper limit, and average size of traps (mm) among *Utricularia*, plotted over image number, which represents the order in which species are listed in Taylor and which roughly corresponds to a sequence from more ancestral to more derived species. There are 205 images representing 205 species.

The most common type of stalk attachment is ‘lateral’. Interestingly, more species have traps with a basal than a terminal stalk attachment (**Figure 11**). There are also a few species with a dimorphism in their stalk attachment.

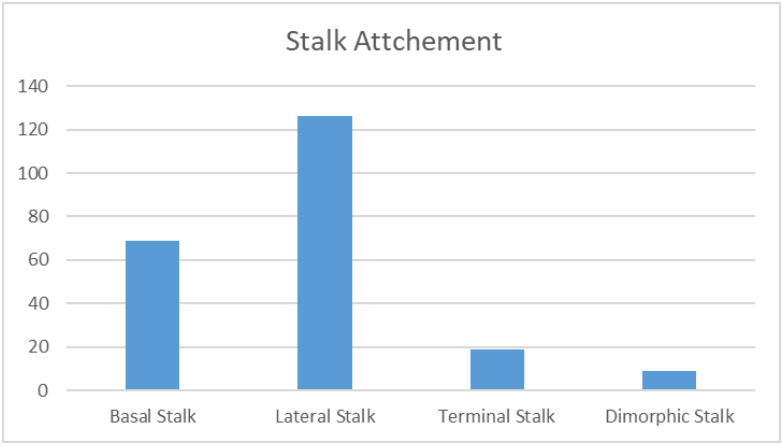


Figure 11. Number of occurrences of trap dimorphism and type of stalk attachment types in genus *Utricularia*.

We found that of the 205 species for which we have data on mouth morphology, 102 had antennae, 11 had bristle bundles, and 47 had curved spurs; 129 had a lateral, 74 a basal, and 19 a terminal stalk attachment. Long filiform spurs, along with the two other types of spurs and fleshy roofs are the most common types of appendages (**Figure 12**).

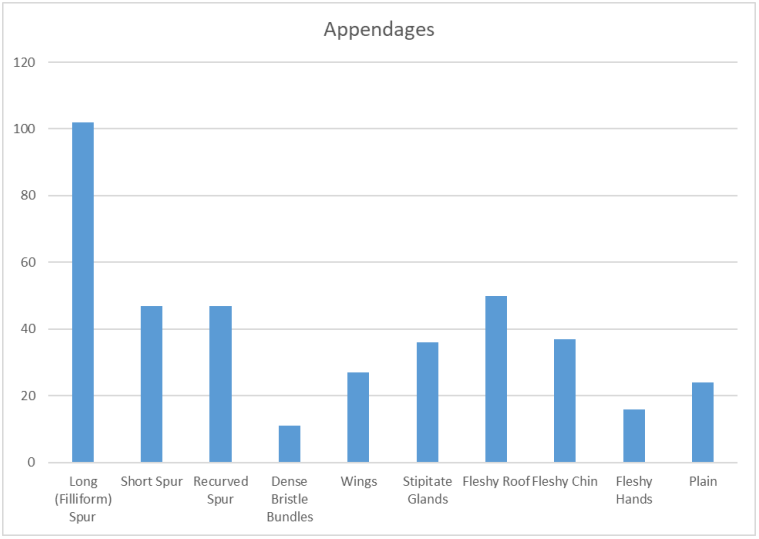


Figure 12. Number of occurrences of morphological features of the traps recorded in this study in the genus *Utricularia*, such as appendages near the trap entrance.

We also found that larger traps generally have larger mouths with longer mouth-funnel lengths. In general funnels appear to get shorter and wider over evolutionary time (**Figure 13**).

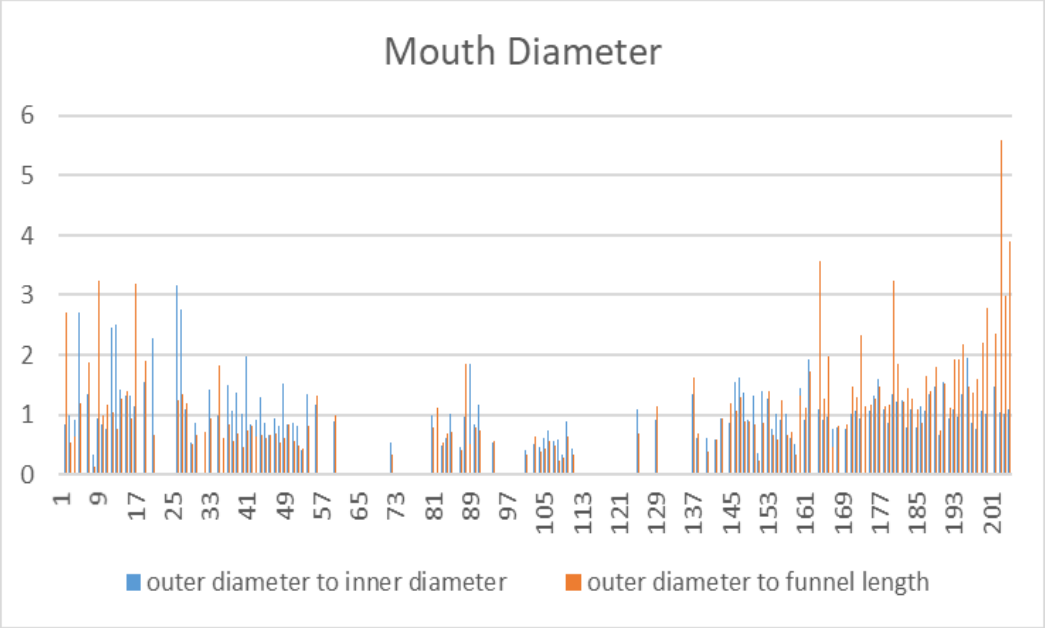


Figure 13. Ratio of exterior trap mouth diameter to the interior mouth diameter graphed alongside the funnel length of the trap, plotted over image number, which represents the order in which species are listed in Taylor and which roughly corresponds to a sequence from more ancestral to more derived species. There are 205 images representing 205 species.

We determined values for eight shape characteristics—Angle, Circularity, Skew, Kurt, Feret Angle, Aspect Ratio, Roundness, and Solidity—plus two length characteristics, major and minor axis. We found that angle ranges from 1.61 to 179 degrees, circularity ranges from 0.573 and 0.887, skew ranges -10.1 to -1.48, kurtosis ranges 0.641 to 109, Feret Angle ranges 1.14 to 177 degrees, aspect ratio ranges 1.01 to 2.83, roundness ranges 0.420 to 0.987, and solidity ranges 0.867 to 0.992 (**Figure 14**).

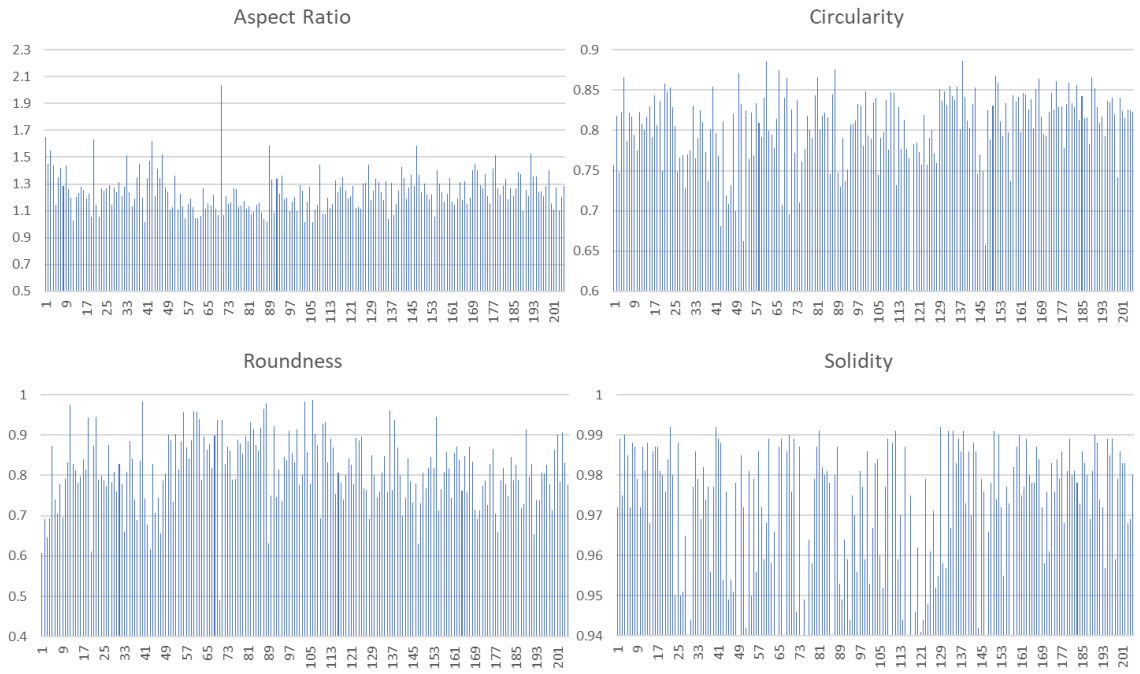


Figure 14. Aspect Ratio, Circularity, Roundness, and Solidity histograms for *Utricularia*.

Results for Aim 2

We tested our six hypotheses with 17 statistical tests, 12 of which were for hypothesis 6. These tests, the variables included for testing, the test statistics calculated, the critical values used to test the statistics, our alpha values for each test, the p-value of each test if applicable, along with the significance of the p-value, the statistical result, and logical conclusion are tabulated thusly (**Table 5**).

Hypothesis 1 is accepted: aquatic species are more likely to have long filiform antennae. Hypothesis 2 is rejected: terrestrial species are not more likely to have bristles than aquatic species. Hypothesis 3 is accepted: aquatic species have larger traps than terrestrial species. Hypothesis 4 is accepted: Epiphytic species are more likely to have recurved spurs. Hypothesis 5 is accepted: Aquatic species have lateral stalks. We will next examine and summarize the 12 tests used for hypothesis 6.

Table 5. Results of Statistical Analysis

Conclusion	Result	Significance?	p-value	Alpha	Critical Value	Statistic	Variables/ Question(s) Tested	Test(s)	Hypothesis
Aquatic species are more likely to have antennae	Reject Null	Yes	0.000000	0.05	N/A	N/A	Aq vs Te w&w/o antennae	Chi-Squared	1
Terrestrial species are not more likely to have bristle bundles	Fail to Reject Null	No	0.445086	0.05	N/A	N/A	Te vs Aq w&w/o bristles	Chi-Squared	2
Aquatic species are more likely to be larger	Reject Null	Yes	0.014759	0.05	14354	13560.5	Aq vs Te avg. trap size	Wilcoxon Signed-Rank	3
Epiphytic species are more likely to have recurved spurs	Reject Null	Yes	0.000005	0.05	N/A	N/A	Ep vs Non-Ep w&w/o recurved spurs	Chi-Squared	4
Aquatic species are more liekly to have lateral stalks	Reject Null	Yes	0.000886	0.05	N/A	N/A	Aq vs Te w&w/o lateral stalk	Chi-Squared	5
There is a difference in the shape of the traps	Fail to Reject Null	No	1.000000	0.05	2.1927	1.89083	All unitless shape data	(Q) Friedman Test	6
All the unitless variables are measuring trap shape - the same thing	No Concordance	N/A	N/A	0.05	0.0015	0.00105	Are all the unitless variables all measuring trap shape?	(W) Kendall's Coefficient of Concordance	
The results of each coloumn are not strongly correlated, so these variables are independent	No Correlation	N/A	N/A	0.05	-0.003	-0.0028	Are the all the unitless variables correlated?	(r) Spearman Correlation Coefficient	
There is a difference in the shape of the traps	Fail to Reject Null	No	1.000000	0.05	3.4785	0.98721	AR, Circ., Round, Solidity	(Q) Friedman Test	
the unitless variables of AR,Cric., Round, and Solidity are measuring trap shape - the same	No Concordance	N/A	N/A	0.05	0.0057	0.00128	Are all the unitless variables all measuring trap shape?	(W) Kendall's Coefficient of Concordance	
The results of each coloumn are not strongly correlated, so these variables are independent	No Correlation	N/A	N/A	0.05	0.0008	-0.0026	Are the all the unitless variables correlated?	(r) Spearman Correlation Coefficient	
There is agreement between each metric, so they are both measuring similar shape data	Fail to Reject Null	No	0.796320	0.05	8890.7	10338	Circ. vs Round	Wilcoxon Signed-Rank	
There is NO agreement between each metric, so they are both measuring different shape data	Reject Null	Yes	0.000000	0.05	8890.7	-9572	Round vs Solidity	Wilcoxon Signed-Rank	
There is NO agreement between each metric, so they are both measuring different shape data	Reject Null	Yes	0.000000	0.05	8890.7	-9426.5	Circ. vs Solidity	Wilcoxon Signed-Rank	
Aquatic and Terrestrial traps are shapped differently	Reject Null	Yes	0.000006	0.05	8890.7	6700.5	Aq vs Te w&w/o Circularity	Wilcoxon Signed-Rank	
Aquatic and Terrestrial traps are shapped differently	Reject Null	Yes	0.000000	0.05	8890.7	-9163.5	Aq vs Te w&w/o Roundness	Wilcoxon Signed-Rank	
Aquatic and Terrestrial traps are shapped differently	Reject Null	Yes	0.000003	0.05	6581.5	-9163.5	Aq vs Te w&w/o Solidity	Wilcoxon Signed-Rank	

The first six tests of hypotheses 6 all had the same results. The first Friedman test (Q) showed that there was a difference in the shape of the traps across the eight variables tested. This result, like that for hypothesis 2 was interesting because we had reason to believe that all traps had a certain shape in common (Singh et al., 2011). This Friedman test (Q) did not narrow down where that difference was, so we pursued increasingly more specific tests to find where the difference was. We next calculated Kendall's Coefficient of Concordance (W) and it showed that all variables in the Friedman test were measuring the same thing, trap shape. After that we calculated the Spearman Correlation Coefficient (r) for our entered data and it showed that the results of each column were not strongly correlated, thus independent for each variable. We reduced our second test to only four input variables, but the results were the same – the traps of *Utricularia* do not have the same shape, the data agrees across all columns and the columns are independent. We moved on to consider if Circularity, Roundness, and Solidity were in agreement with what they had measured, so we paired each to have three Wilcoxon Signed-Rank tests that tested if each metric agreed. Circularity and Roundness agreed, wherever the difference in shape was, these two measured that same difference. The difference in shape found with Roundness had a 0 p-value that agree with the nearly 0 p-value found for Circularity. For our final round of three Wilcoxon Signed-Rank tests we assumed the difference in trap shaped came from habitat and tested aquatic versus terrestrial across the three variables mentioned above. There was a significant difference between aquatic and terrestrial traps in all three variables. Solidity, although potentially measuring shape differently than the other two, still had a nearly 0 p-value for similar shape in terrestrial and aquatic traps, so the trend for two distinct shapes in aquatic and terrestrial traps remained. Although it was hard to tell what that difference could be, so we decided to visually compare the difference based on the means and medians of aquatic and terrestrial

traps across these three traits. We found 12 ideal species that most closely fit these means and medians respectively (**Table 6** and **Figure 15**).

Table 6. Summary of Closest Ideal Match Species

	Aquatic Circulairty	Terrestrial Circularity	Aqautic Roundness	Terrestrial Roundness	Aquatic Solidity	Terrestrial Solidity
Group Median	0.8195	0.8055	0.7955	0.8305	0.978	0.9755
Closest Match Median Species	reniformis	singeriana	arnhemica	graminifolia	bisquamata	tubulata

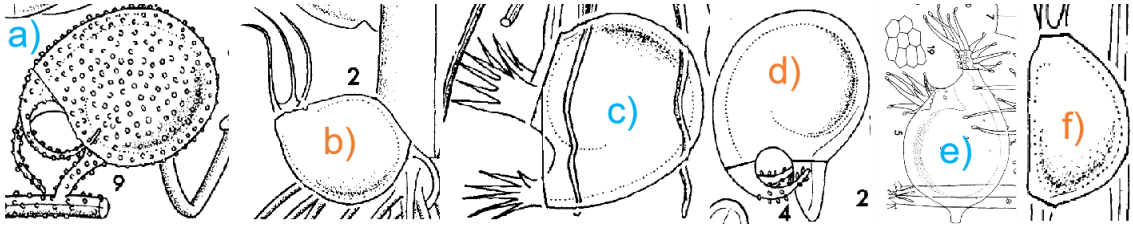


Figure 15. Processed images of a) *U. reniformis*, b) *U. singeriana*, c) *U. amhemica*, d) *U.graminifolia*, e) *U. bisquamata*, f) *U. tubulata* Blue are aquatic, orange are terrestrial. The two on the left are ideal in circularity, the two in the middle are ideal in roundness, and the two on the right are ideal in solidity.

These six species, three terrestrial and three aquatic, have trap shapes that most closely match the medians (**Figure 15**) calculated from the circularity (two traps on left), roundness (middle two traps), and solidity (two traps on right) of all species, but any trends are not immediately apparent.

DISCUSSION AND CONCLUSION

Discussion for Aim 1

Whenever organisms that share an evolutionary history are analyzed for the physical traits that they share, a phylogenetic correction is done to control for statistical errors caused by a lack of independence in the data initially sampled. A previous study suggests that a vast majority of aquatic *Utricularia* species are monophyletic, sharing a terrestrial ancestor within genus *Utricularia* (Silva et al., 2018). This monophyletic origin makes it difficult for us to distinguish between the role of habitat versus ancestry in shaping morphological features shared among aquatic species. Yet executing similar statistical tests with the proper phylogenetic corrections should be a major aim of any future study based on the data we have collected here.

Several *Utricularia* species have dimorphic traps, which may have an impact on our conclusions, depending on how multiple variants are coded in the analysis. The data of the variants of traps not recorded from ROIs were copied so that all variants had habitat, trap size, mouthpart, and stalk attachment data to be used in our analyses. This is an important distinction with implications for future studies. Variant data were copied to complete our analyses, and those copied data may not actually reflect the true aspects of each variant. Also, some ROIs variants may have actually been repeated measurements of the same trap but from a different perspective. We suspect this inconsistency because variants were recorded for some species that the text did not specify as having variants. Recording unspecified variants was not a common occurrence, but it may have had a statistical effect. The data was copied, rather than matched more methodically to each variant, to control for uncertainty in knowing exactly which categoric variable should and should not be the same for each variant of a species. In other words, although Taylor would specify which species had trap variants, Taylor would not specify under which

conditions those variants would be expressed, so we assumed all the same variables were present (Taylor, 1989). For example, assuming a species had two variants with unique mouthparts for each variant and inhabited two separate habitats, we would record the habitat and mouthpart data for the first variant because that is what the book shows, but then we would copy all that data for the second variant. The implication here is that we may not be representing all these features accurately for species that have variants, in terms of which variant has which features and in which habitat. For this reason, we omitted all variants and non-lateral images from consideration for our final data analysis. A more careful future study might overcome the design limitation imposed by polymorphic variants.

Interestingly, there are more traps with a basal than a terminal stalk attachments. If we assume the perimeter of a trap has a certain number of attachment points going all the way around its surface, then it is most likely that those points are lateral. In other words, the basal attachment region is so small, it is unlikely that the trap would be basal by chance, therefore the basal attachment style is ancestral (**Figure 11**, p. 30).

Aim 1 Conclusion

We collected data for 205 species in genus *Utricularia* across 205 total ROIs in 65 columns of data, totaling 13325 data points. Our data represents 74.8% of *Utricularia* species cataloged as of 2021 (Cheng et al., 2021). The trends in morphological traits our data reveal come without phylogenic correction, and should cautiously be interpreted because they lack this critical step. All of the data we recorded can be explored in the Excel file "Utricularia_Data_12_7_21_Final.xlsx" stored in the Google Drive of the Müller research group.

Discussion for Aim 2

Most of the hypotheses tested in this study were supported by our data. Our data supported hypothesis 1, which stated that aquatic traps are more likely to have filiform antennae, most likely to funnel zooplankton prey into their mouth. Also supported was hypothesis 3 (aquatic species have larger traps than terrestrial species) because larger size increases the hydrodynamic effectiveness of traps, and because aquatic species are less spatially constrained by their environment than terrestrials, thus allowing them to catch more prey. We saw from the result of hypothesis 4 that epiphytic traps may have recurved spurs, and this is most likely for water retention in front of their mouths. We saw that in hypothesis 5, aquatic traps usually had laterally stalks, this is most likely to allow more recoil while the trap activates so that the mouth of the trap moves towards the prey to help catch prey.

The hypotheses that were not supported by our data were hypotheses 2 and 6. The result for hypothesis 2 is surprising considering the contrary evidence present in our review of the literature and comes with a few caveats (Reifenrath et al., 2006). This could be because bristles are not a common mouthpart, occurring in fewer than 20 of more than 200 species we examined. Furthermore, phylogeny might explain the result for hypothesis 2 since bristle bundles are a relatively rare trait only seen in a small group of closely related species (Westermeier et al., 2017; **Figure 5**, p. 10). The results of hypothesis 6 show that upon visual inspection the difference between aquatic and terrestrial shape is not clear, and this has important implications for future studies.

When deciding which traits to examine with statistical tests we implicitly assumed that some habitats are more likely to correlate with particular trap structures. These assumptions were informed by several studies, cited above where our hypotheses are explained in detail, but in one case were unfounded. Hypotheses 2 showed that despite other researchers similarly hypothesizing that terrestrial species may have specific

utility for dense bristle bundles (Meyers & Strickler, 1979; Reifenrath et al., 2006), the data actually showed there is no trend in terrestrial species having a higher than excepted chance of exhibiting this trait. It is also important to consider that all the drawings examined in this study are 2-dimensional representations of mostly transparent 3-dimensional traps. Some of these transparent features are difficult to render in 2 dimensions so while processing these images, we had to make some assumptions about how to interpret these shapes. Overall, we tried to make the mostly elastic shapes possible, preferably without sharp lines. It is possible that Taylor drew these traps in a way that both may emphasize unique features and may exaggerate their shape (Taylor, 1989). The detail provided within each drawing certainly varied, with some traps drawn in finer details than others.

Overall, phylogeny might play an important role in explaining the correlations between habitat and form that we found in this study. Traits like having a lateral stalk attachment, dense bristle bundles, or recurved spurs may be due to sharing a common ancestor rather than sharing the same habitat. Future studies should perform a phylogenetically informed test of the hypotheses tested in this study.

Aim 2 Conclusion

The features found in traps may be caused by the habitats those species are found in. Aquatic traps have filiform antennae to funnel prey into their mouth. Aquatic traps are larger because they are less constrained from being larger under water than on land. Epiphytic traps have recurved spurs to keep a bubble of water in front of their mouth to not dry out. Aquatic traps take advantage of their lateral stalk to give themselves recoil so that they lunge toward prey when they activate. Aquatic and terrestrial traps both have separate and optimal shapes to activate in their respective habitats. Future work finding out which categoric trap features, whether they be mouthparts, stalk attachments, trap

size or trap funnel dimensions, are expressed in which habitats and if differences exist across genus *Utricularia* is worthy of further study. Designing a studying to better understand the specific features expressed in each variant is a potential future use for the data we have collected. Also, five traps had their data entirely truncated for the analysis portion of this study because their images did not appear in Taylor (Taylor, 1989). These five traps warrant further study on that basis alone, because their shapes are unknown. The two improvements above may be incorporated into a future study that runs similar statistical tests as the ones discussed throughout our present study, with the additional improvement of having a phylogenic correction for those tests. More correlations can be tested this way. A separate study might test some of the trends found in our study experimentally with live traps and prey, if applicable. Experimenting with live plants could mean removing the appendages of traps and recording the effect that would have on prey capture success. Live experiments could also show how successful plants from different habitats are at catching similar prey, to control for habitat.

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