# Unveiling the Karyotypes of New England *Utricularia* Species

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Carnivorous plants, described as "the most wonderful plants in the world" by Darwin [1], have always attracted enormous attention, as their existence seems to challenge the conventional notion of plants being immobile producers consumed by animals. Among all the carnivorous plants, *Utricularia* (Lentibulariaceae) famously stands out as "a favorite of Darwin's" [1]. *Utricularia* is the largest (*ca.* 220 listed species), most diverse, and widespread genus of carnivorous plants, containing both terrestrial and aquatic species, and occupying temperate and tropical habitats throughout the world [2].

There are a number of unique features of *Utricularia* that made it not only "a favorite of Darwin's" [1], but also of many botanists, evolutionary biologists, engineers, and architects. For instance, all species of the genus completely lack roots, which challenged the conventional body plans of angiosperms [3]; and *Utricularia* traps are morphologically complex and produce the fastest known plant movement when they close their trap doors [4, 5]. Moreover, the genome of one species, *U. gibba*, which was revealed in 2013, shows astonishingly, that although at least three rounds of whole genome duplication occurred since the time it diverged from the common ancestor with tomato and grape, it possesses one of the smallest genomes among land plants, with an extremely high gene turn-over rate and a dramatic reduction of the amount of non-coding DNA [6, 7]. These remarkable data on *U. gibba* show that genome evolution is a particularly interesting aspect of *Utricularia* biology, and worthy of further study.

Nonetheless, there is surprisingly little known about the genomes of other *Utricularia* species; even knowledge about the most fundamental genomic information, such as karyotype, is very scarce. So far, the chromosome numbers of only 15 of the more than 200 *Utricularia* species have been reported [8], most of which were from studies conducted between 1952–1990. Only two studies have illustrated the karyotypes of three species, which both suffer from poor quality [8, 9]. The general lack of karyotypic data for *Utricularia* is due to four major challenges: 1) difficulty of accessing specimens from the genus; 2) difficulty of obtaining suitable tissue for karyotype analysis; 3) the optimal length of pretreatment is unknown; 4) chromosomes of diminutive sizes may be difficult to stain with standard dyes.

With the support of the Les Mehrhoff Botanical Research Fund from the New England Botanical Club (NEBC) and collaboration with members from the New England Carnivorous Plant Society (NECPS), we were able to take the initiative to fill in our limited understanding of cytogenetics of this enigmatic plant genus. The goals of this project are to establish a chromosome preparation protocol for *Utricularia* and to characterize the karyotypes of the *Utricularia* species in the New England region.

## Preliminary results

From June to Sept., 2017, we collected vegetative tissues and flower buds of eight identified *Utricularia* species in New England regions from various bogs, kettle ponds, and river banks [e.g. Fig. 1A]: *U. geminiscapa*, *U. purpurea*, *U. inflata*, *U. resupinata*, *U. gibba*, *U. cornuta*, and *U. radiata*. In addition, we also collected tissues from a suspected hybrid between *U. gibba* and *U. geminiscapa*. We were unable obtain suitable materials for six New England species (*U. vulgaris, U. minor*, *U. intermedia*, *U. striata*, *U. ochroleuca*, and *U. subulata*) for a

number of reasons, including populations of some species that did not flower this season, or we were unable to locate populations of some species. Nonetheless, we were able to collect *U. macrorhiza*, which is considered as *U. vulgaris* var. *americana*, and obtained good quality karyotype. It will thus be interesting to examine the similarity of karyotypes between *U. macrorhiza* and *U. vulgaris* in future studies, which will be important for determining the unsettling question of whether or not *U. macrorhiza* is a variety of *U. vulgaris.*

In order to examine the best material for chromosome preparation, we tested a variety of tissues from different species, and concluded that the anther is the most, and possibly the only, ideal option. The stolons (i.e. air shoots) of *Utricularia* contains the meristematic tips, in which cell division is supposed to occur frequently, but it is very difficult to collect stolons of the right stages as they are submerged in water or soil. Turions, which are overwintering vegetative buds enclosing a modified shoot apex produced by a number of species, are not suitable for chromosome preparation either, because cell division has arrested by the time a turion is visible. Moreover, although metaphase chromosomes have been observed from carpel squashing, both the pre-treatment solution for accumulating metaphase chromosomes and the enzyme mix solution for digesting cell walls appeared to be difficult to penetrate the thick carpel and ovary walls, resulting in chromosome spreads of low quality (e.g. chromosomes overlap with each other and thus not countable).

Among all the species examined, anthers from flower buds between 2 mm and 5 mm in length appeared to have the largest number of dividing cells. The best pre-treatment method to accumulate metaphase chromosomes was shown to be 8 h in 0.002M 8-hydroxyquinoline at 4°C followed by 8 h in water at 4°C. Longer treatments with 8-hydroxyquinoline led to over-condensation of chromosomes, making chromosomes of small sizes difficult to be distinguished from other small particles stained by DAPI on the slides. Pre-treatment in water at 4°C for 4h to over-night has been shown to be an efficient way to accumulate the number of dividing cells in many angiosperm species [e.g. 10, 11], but this method gave very poor results for *Utricularia*. This is not entirely surprising considering that most aquatic *Utricularia* species constantly experience low temperature water over-night during certain seasons, but the growth and development do not seem to be disturbed.

While we are still in progress of analyzing the karyotypes of all the species we collected, we have already observed a number of interesting features. First of all, the interphase nuclei stained by DAPI revealed large variation in the heterochromatic chromocenters between different species. Some species displayed a nearly homogenous staining without conspicuous heterochromatin clusters (e.g. Fig. 1B), while some others showed very distinct clustering (e.g. Fig. 1C). The heavily-stained heterochromatin is generally compact and transcriptionally silent, and it has been shown that heterochromatic marks are often distributed more uniformly because of higher density of mobile elements to be silenced [12, 13]. However, species with one of the smallest genome sequenced in plants, *U. gibba*, displayed a heterchromatin clustering similar to *U. purpurea* (Fig. 1B), which appeared to be much more homogenous than many other *Utricularia* species investigated in this study. Therefore, it will be interesting to analyze the genome sizes and sequences of those which showed distinct heterochromatin clustering. Secondly, chromosomes of all the examined species appeared to be diminutive, in general less than 1 μm in length. The chromosome sizes of an individual are relatively uniform, and no exceptional size variation has been observed (e.g. Fig. 1C-E). However, we observed a large variation in the chromosome numbers, and preliminary analysis revealed a range of $n = 18$ for *U. cornuta* (Fig. 1D) to $n = 54$ for *U. inflata* (Fig. 1E). Moreover, it is surprising to find that almost

all the species included in the current study have shown a distinct chromosome number, including those that are phylogenetically close to each other. After we confirmed their karyotypes, we will map the chromosome numbers onto the most recent phylogeny of *Utricularia*. We are hopeful that our results will provide invaluable information for further phylogeny-based analysis of karyotype and genome evolution. More importantly, as all the slides of the current study can be stored for a long period of time and can be subsequently used for florescent *in situ* hybridization, this means that we have already laid the foundation for the further investigation of the molecular-level chromosome properties of this genus.

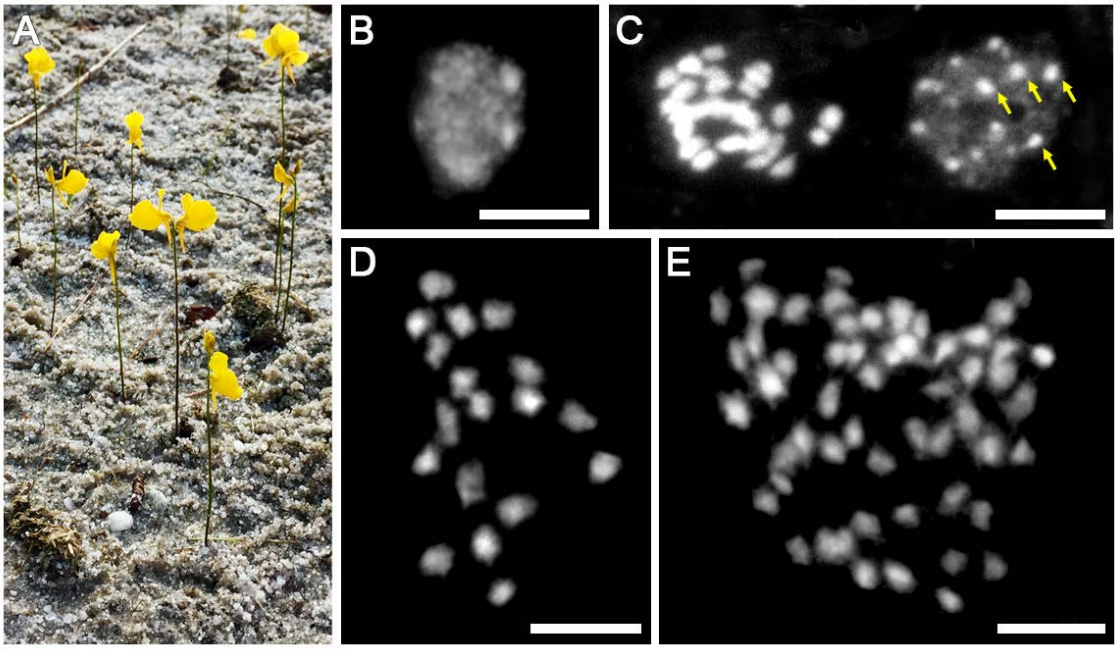


**Figure 1. Unveiling the karyotypes of New England *Utricularia* species.** A) *U. inflata* population found by the bank of the Three Cornered Pond (MA) as an example of the habitats of the New England *Utricularia* species. B) Interphase nuclei of *U. purpurea* stained by DAPI. C) A metaphase cell with condensed chromosomes (left) and an interphase nuclei (right) of *U. radiate*, both stained by DAPI. Yellow arrows indicate some of the clustered heterochromatin as examples. D) Karyotype of *U. cornuta* showing a chromosome number of *n = 18*. E) Karyotype of *U. inflata* showing a chromosome number of *n = 54*. Scale bar in B-E is 5 µm.

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