Botanical Sciences 102 (2): 1-15. 2024
DOI: 10.17129/botsci.3437

Received: November 7, 2023, Accepted: December 21, 2023
On line first: March 5, 2024

Ecology / Ecología

Comparison of the microbiome and mycobiome in tissues of the tropical carnivorous epiphytic herb *Utricularia jamesoniana* Oliv. (Lentibulariaceae)

Valeria Naranjo-Aguilar[1], Rebeca Mora-Castro[1,2,3*], Jessica Morera-Huertas[1], Rafael H. Acuña-Castillo[1,4], Keilor Rojas-Jimenez[1*]

[1] Escuela de Biología, Universidad de Costa Rica, San Pedro de Montes de Oca, Costa Rica.
[2] Centro de Investigación en Biología Celular y Molecular, Universidad de Costa Rica, San Pedro de Montes de Oca, Costa Rica.
[3] Centro de Investigación en Ciencia e Ingeniería de Materiales and Escuela de Física, Universidad de Costa Rica, San Pedro de Montes de Oca, Costa Rica.
[4] Herbario Luis A. Fournier Origgi, Centro de Investigación en Biodiversidad y Ecología Tropical (CIBET), Universidad de Costa Rica, San Pedro de Montes de Oca, Costa Rica.
*Authors for correspondence: keilor.rojas@ucr.ac.cr; rebeca.mora@ucr.ac.cr

Abstract
Background: *Utricularia jamesoniana*, a small epiphytic plant found in wet tropical forests, stands out for its carnivorous habit, intricate trap system, and small but beautiful and complex flowers. This species remains relatively understudied despite its wide geographical distribution and curious adaptations.
Questions: Our aim is to elucidate the composition of bacterial and fungal communities inhabiting both the bladders and leaves of *Utricularia jamesoniana*, facilitating the comprehension of the physiology and ecological dynamics of this carnivorous species.
Methods: In this study, we employed 16S rRNA and ITS sequencing to compare the prokaryotic and fungal communities within leaves and traps of *U. jamesoniana*.
Results: The analysis of amplicon sequence variants (ASVs) unveiled notable differences in community composition depending on the plant tissue and type of microorganism. Prokaryotic communities predominantly comprised Proteobacteria and Actinobacteriota, featuring genera such as *Acidocella*, *Bradyrhizobium*, *Ferritrophicum*, and *Ferrovum*. Fungal communities were dominated by Ascomycota and Basidiomycota, encompassing representatives of Dothideomycetes, Sordariomycetes, Eurotiomycetes, and Agaricomycetes, as well as ASVs related to Mycosphaerellaceae, *Colletotrichum*, *Aspergillus*, and *Thanatephorus*. We determined that the prokaryotic diversity was higher in the bladders with respect to the leaves. Fungal communities, in turn, were more diverse in leaves than in bladders.
Conclusions: This study sheds light on the microbial communities associated with this carnivorous epiphyte and provides valuable insights into the intricate relationships between the plant and its microbial inhabitants across different tissues.
Keywords: bladderworts, carnivorous plants, bacteria, fungi, microbial diversity

Resumen
Antecedentes: *Utricularia jamesoniana* es una pequeña planta epífita que se encuentra en bosques tropicales húmedos. Destaca por su hábito carnívoro, su intrincado sistema de trampas y sus pequeñas pero complejas flores. A pesar de su amplia distribución geográfica y sus curiosas adaptaciones, esta especie sigue estando relativamente poco estudiada.
Objetivo: Nuestro objetivo es dilucidar la composición de las comunidades bacterianas y fúngicas asociadas tanto en los utrículos como en las hojas de *Utricularia jamesoniana*, facilitando la comprensión de la fisiología y la dinámica ecológica de esta especie carnívora.
Métodos: Empleamos la secuenciación de los marcadores 16S ARNr e ITS para comparar las comunidades procariotas y fúngicas dentro de hojas y trampas de *U. jamesoniana.*
Resultados: El análisis de las variantes de secuencia de amplicones (ASVs) reveló notables diferencias en la composición de la comunidad dependiendo del tejido vegetal y del tipo de microorganismo. Las comunidades procariotas estaban compuestas predominantemente por Proteobacteria y Actinobacteriota, incluyendo los géneros *Acidocella, Bradyrhizobium, Ferritrophicum* y *Ferrovum*. Las comunidades fúngicas estaban dominadas por Ascomycota y Basidiomycota, con representantes de Dothideomycetes, Sordariomycetes, Eurotiomycetes y Agaricomycetes; así como ASVs relacionados con Mycosphaerellaceae, *Colletotrichum*, *Aspergillus* y *Thanatephorus*. La diversidad procariota es más alta en los utrículos con respecto a las hojas, pero las comunidades fúngicas fueron más diversas en las hojas que en los utrículos
Conclusiones: Este estudio arroja luz sobre las comunidades microbianas asociadas a esta epífita carnívora y proporciona valiosos conocimientos sobre las intrincadas relaciones entre la planta y los habitantes microbianos asociados a sus diferentes tejidos.
Palabras clave: utrículos, plantas carnívoras, bacterias, hongos, diversidad microbiana

Lentibulariaceae (Lamiales) is a family of carnivorous herbs composed of three genera: *Genlisea* A. St.-Hil., *Pinguicula* L., and *Utricularia* L. Among these, *Utricularia* is the most diverse, with terrestrial, epiphytic, lithophytic, rheophytic, and aquatic species. These plants are commonly referred to as "bladderworts" due to their complex traps, which are shaped like bladders. With approximately 250 species, *Utricularia* and similar species-rich *Drosera* L. (Droseraceae) are recognized as the most diverse genera of carnivorous plants (Fleischmann 2015, Rutishauser 2016). *Utricularia* is also the most widely distributed carnivorous plant genus, with a subcosmopolitan distribution encompassing every continent except Antarctica. The centers of diversity for *Utricularia* are Australia and the Neotropics (Henning *et al*. 2021, Taylor 1989).

Utricularia has become a focus of increasing attention from the scientific community because the homologies of the organs that constitute their structure, compared to more typical plants, remain unclear (Rutishauser 2016). Nonetheless, their vegetative structures are highly diverse and perform functions of rhizoids, rhizomes, stolons or pseudobulbs, and photosynthetic organs that resemble leaves (for practicality purposes, we will call these structures simply leaves, even if their homology has been subject to discussion). The stolons typically emerge from the base of the inflorescences and are robust in aquatic species but delicate and hidden in the substrate in terrestrial and epiphytic species. Furthermore, the genus includes some angiosperms with the smallest known genomes (Ibarra-Laclette *et al*. 2013).

Although most species of *Utricularia* are aquatic or inhabit wet soil, some members have evolved as epiphytes, particularly in tropical wet forests. Among these, several species of the Neotropical section Orchidioides, which includes 17 accepted species, are known to have such a habit (Henning *et al*. 2021). *Utricularia jamesoniana* Oliv. (Figure 1) is one of species in this monophyletic section and can be found from Mexico to Bolivia and northern Brazil, including Hispaniola and the Lesser Antilles (Gomes Rodrigues *et al*. 2017, Silva *et al*. 2018). It generally grows on tree trunks and branches, associated with bryophytes in humid to pluvial, frequently cloudy forests, including forest edges, secondary growth, and pastures (Crow 2007, Valdés 2008).

The bladders (or traps) have been reported in all *Utricularia* species (Miranda *et al*. 2021). They are seemingly foliar structures whose function is small prey capture through the secretion of enzymes and are considered to be the fastest carnivorous trap system in plants, although they have mainly been studied in aquatic species (Adamec *et al*. 2010, Westermeier *et al*. 2017). A mature trap is triggered by elastically loading the walls of the bladder as water is removed from the trap lumen, causing the walls to bend inward and generate a lower-than-ambient internal pressure. When prey touches the trigger hairs at the opening, the movable gate or valve bends inward, and the prey is drawn within a few milliseconds due to lower pressure within the bladder (Ortega Ardila & Romero Salgado 2016, Peroutka *et al*. 2008).

In general, solid objects such as prey experience three forces that propel the prey toward the trap: pressure, viscous drag, and acceleration reaction. The prey is subsequently dissolved and digested by specialized cells. Traps can be ready to capture new prey again in 10-15 minutes, but water evacuation continues longer. It is worth noting, however, that the bladders of epiphytic species can differ significantly from those found in aquatic species, which may result in differences in their trapping mechanism (Deban *et al*. 2020, Givnish *et al*. 2018, Singh *et al*. 2020).

Numerous investigations have been conducted on the prey spectra of mainly aquatic *Utricularia* (Sanabria-Aranda *et al*. 2006, Walker 2004). However, less attention has been paid to exploring prey-capturing abilities in terrestrial species, in which their ability to catch prey has also been confirmed (Jobson & Morris 2001, Moseley 1884). The reported diet types include micro-crustaceans, nematodes, rotifers, insect larvae, tadpoles, and fish larvae (Martens & Grabow 2011, Płachno *et al*. 2006, Sirová *et al*. 2003).

Several different clades of microorganisms that inhabit the traps have been documented, including bacteria, ciliates, algae, and other protozoans. These microorganisms play crucial roles in the conversion of decaying matter. Certain species are considered commensals, while others are recognized as parasites, further adding to the complexity of the bladder ecosystem (Adamec 2011, Alkhalaf *et al*. 2009, Ibarra-Laclette *et al*. 2013, Peroutka *et al*. 2008, Płachno *et al*. 2006, Sirová *et al*. 2018b). Furthermore, the microbial communities in the bladders can vary depending on the anatomical structure of the trap as well as the stage of development of the plant (Friday 1989, Płachno *et al*. 2012, Sirová *et al*. 2018a).

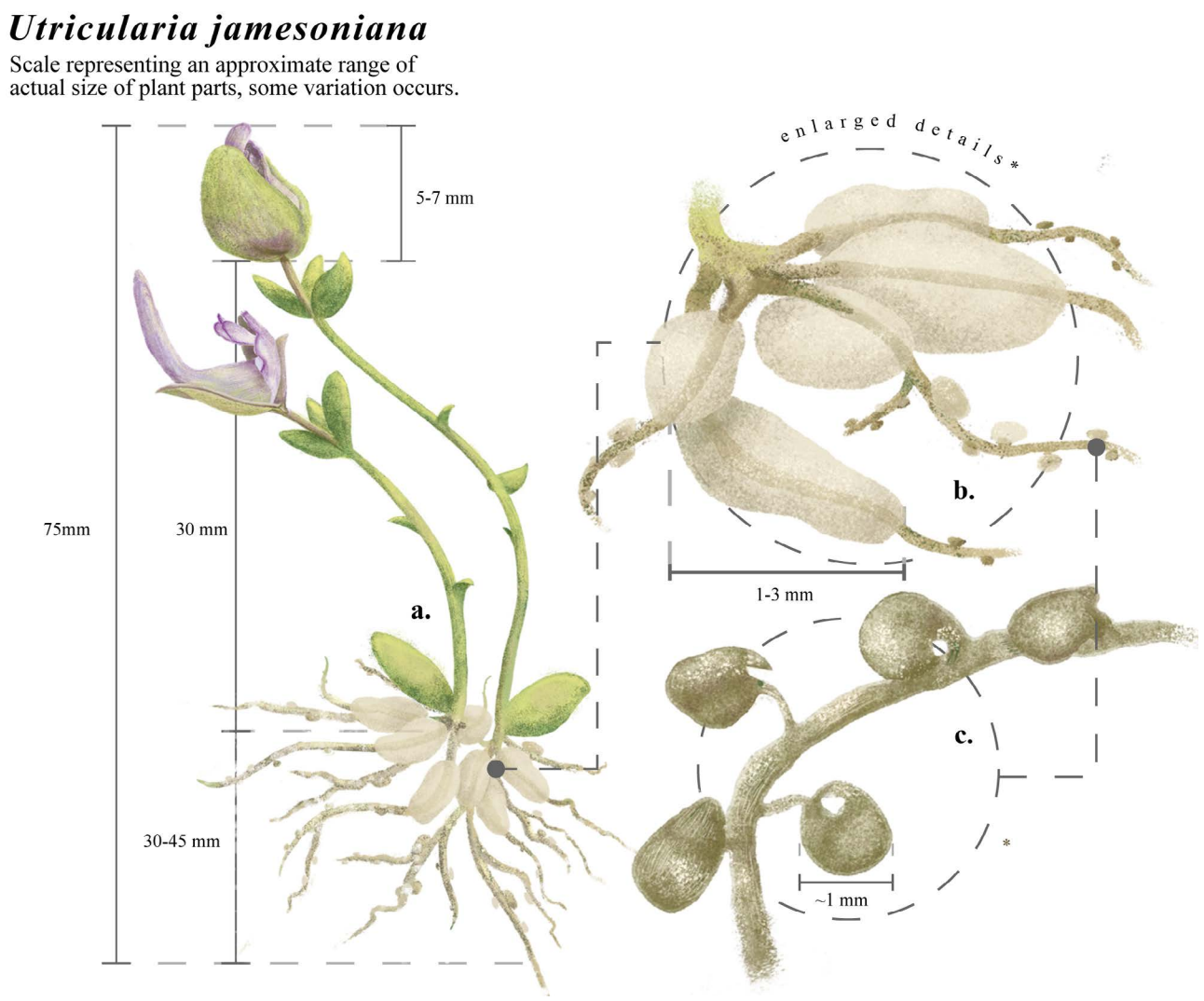

Figure 1. *Utricularia jamesoniana.* A. habit, B. stolons with tubers, C. traps. Measurements refer to the approximate sizes of different structures. In this work, the green tissues illustrated in panel A are considered leaves, excluding flowers. Structures depicted in panel C but without the supporting pseudo-roots were considered traps. Drawing was prepared by Victor Adrian Mora Mora.

Information on this species of epiphytic carnivorous plant is limited, except for some taxonomic treatments providing basic descriptions of the morphology, ecology, and distribution (Crow 2007, Guedes *et al*. 2023, Oliver 1860, Taylor 1989). To our knowledge, no studies have explored the prokaryotic microbiota and mycobiome in tissues of *Utricularia jamesoniana*, including its traps. In this work, we provide the first characterization of the bacterial and fungal communities associated with the bladders and leaves of *Utricularia jamesoniana*, which contribute to a better understanding of the physiology and ecology of this terrestrial carnivorous species.

Materials and methods

Sample collection. In July 2022, we collected representative *Utricularia jamesoniana* plants from the Robert and Catherine Wilson Botanical Garden, part of Las Cruces Biological Station (LCBS) owned by the Organization for Tropical Studies (OTS). LCBS is in southern Costa Rica in the Coto Brus county of Puntarenas Province, ca. 5 km away in a straight line from the Panamanian border and 4 km in a straight line to the SW of the town of San Vito,

coordinates 8° 47’ 9.54” N 82° 57’ 37.49” W (Figure 2). The Garden is at about 1,200 m on the Fila Cruces, part of the Fila Costeña Sur, located south of the Coto Brus Valley. The Garden is in the Premontane Rain Forest Life zone, according to Tosi (1969), and is surrounded by young and mature secondary forests. The *Utricularia jamesoniana* specimens we collected were not planted; instead, they were found naturally growing as epiphytes (or facultatively epilithic) among dense bryophyte mats on a cultivated *Socratea exorrhiza* (Mart.) H.Wendl. palm. The mat containing three *U. jamesoniana* specimens, separated by a few centimeters, was carefully collected onto a polyethylene bag. Then, in order to keep the specimens as intact as possible, the entire mat was then transported to the laboratory for processing on the same day. Once in the laboratory, with gloves and under aseptic conditions, two specimens were separated for this study and the third was used as a voucher for the herbarium. The voucher was adequately preserved in 70 % ethanol solution in the spirit collection of the USJ herbarium (Acuña *et al. 3183*). The plant collection was carried out with the respective permits of the authorities (collection License ACLAP-077-2021) and the official permission of the Biodiversity Commission of the Universidad de Costa Rica (CBio-73-2022, resolution # 371). To augment the sample size, extensive efforts were undertaken to procure additional plant specimens, both from the original collection site and alternative locations. Regrettably, it was not possible to find more specimens. In consideration of the preservation of *Utricularia jamesoniana* populations, we opted to conduct the endosymbiont characterization exclusively with the two extant samples at our disposal.

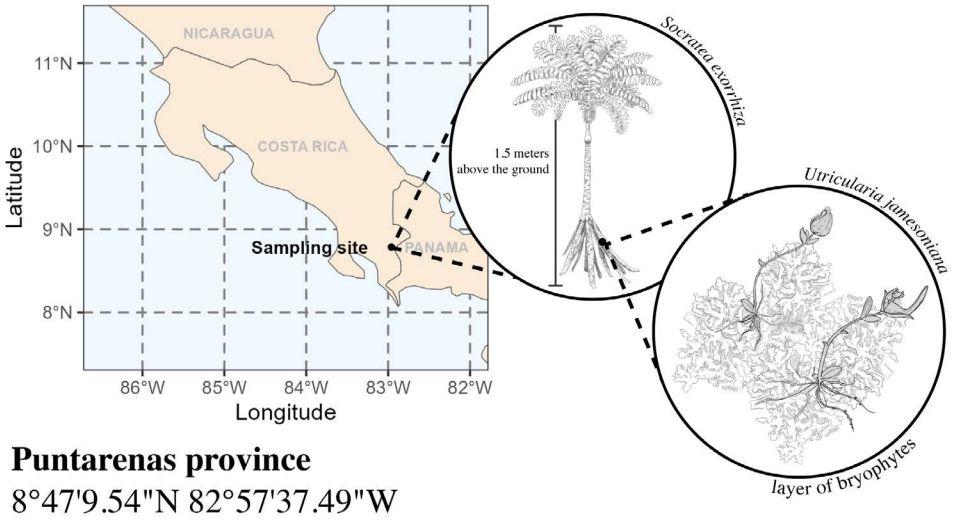

Figure 2. The geographical location of sampling point in Las Cruces Biological Station in southern Costa Rica. *Utricularia jamesoniana* was found growing as an epiphyte among dense bryophyte mats. The collected plants grew on the mossy stilt roots of mature, cultivated *Socratea exorrhiza* palm.

Molecular analyses. Plant samples were washed thoroughly in running water, and healthy and physically undamaged tissues (leaves and bladders) were selected and sterilized on the surface with 70 % ethanol and aseptically cut. The DNA was extracted from approximately 100 mg of the plant tissues using a DNA isolation kit (PowerSoil, Qiagen, USA) following the manufacturer’s instructions. PCR, library preparation, and sequencing were performed by Novogene (Novogene Bioinformatics Technology Co., Ltd, CA, USA). Briefly, this process included the prokaryotic amplicon library of the V4 hypervariable region of the 16S rRNA gene using universal primers 515F and 806R (Caporaso *et al*. 2011). The eukaryotic amplicon library was generated by amplifying the ITS2 region using primers ITS3-2024F (5’ GCATCGATGAAGAACGCAGC) and ITS4-2409R (5’ TCCTCCGCTTATTGATATGC). PCR amplification of targeted regions was performed using specific primers connecting with barcodes. The PCR conditions for both markers consisted of 95 °C for 3 min initial denaturation followed by 35 cycles at 95 °C for 45 s, 53 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 5 min. The PCR products with the proper size were selected by 1 % agarose gel electrophoresis. Each sample’s exact amount of PCR products was pooled, end-repaired, A-tailed,

and further ligated with Illumina adapters. Libraries were sequenced on a paired-end Illumina Novaseq-6000 platform to generate 250bp paired-end raw reads.

Biofinformatic analyses. We used the DADA2 version 1.21 to process the Illumina-sequenced paired-end fastq files and to generate a table of amplicon sequence variants (ASVs), which are higher-resolution analogs of the traditional OTUs (Callahan *et al*. 2016). Briefly, we removed primers and adapters, inspected the quality profiles of the reads, filtered and trimmed sequences with a quality score < 30, estimated error rates, modeled and corrected amplicon errors, and inferred the sequence variants. Then, we merged the forward and reverse reads to obtain the full denoised sequences, removed chimeras, and constructed the ASV table. We assigned taxonomy to the ASVs with the function *assignTaxonomy*, of DADA2. For the prokaryotic assignment, we used as input the SILVA reference database version 138.1 (Quast *et al*. 2013). We carried out a second taxonomic assignment of the prokaryotic ASVs using the tool IDTAXA of DECIPHER (Murali *et al*. 2018) with the same version of the SILVA database and using the RDP database version 18 (rdp.cme.msu.edu). For the fungal taxonomic assignment, we used the UNITE ITS database version 8.3 (https://doi.org/10.15156/BIO/1280049). We carried out a second taxonomic assignment of the fungal ASVs using the tool IDTAXA of DECIPHER with the same version of UNITE as a reference and a confidence threshold > 60 %, and additionally, a third classification using the Classifier tool (Wang *et al*. 2007) implemented in the Ribosomal Database Project (rdp.cme.msu.edu) using as reference the Warcup Fungal ITS trainset V2 database (Deshpande *et al*. 2015). We verified and manually curated the consistency between the taxonomic assignments of the different programs for prokaryotes and fungi. In cases of discrepancies, a comparison with the BLAST tool of NCBI Genbank was applied. Sequence data were deposited at the NCBI Sequence Read Archive under accession number PRJNA1005337 (https://www.ncbi.nlm.nih.gov/sra/PRJNA1005337). In the 16S rRNA prokaryotic dataset, we removed sequences assigned to Chloroplast and Eukarya. This process generated 518,491 sequences from the four samples. The average number of sequences per sample was 129,623 (ranging from 111,131 to 149,377). In the ITS2 eukaryotic dataset, we removed sequences assigned to vertebrates and plants. This process generated 283,135 sequences from the samples. The average number of sequences per sample was 70,784 (ranging from 57,195 to 94,867).

Statistical analyses. The statistical analyses and visualization of results were performed with the R statistical program (R Core Team 2023) and the R studio interface. Package Vegan v. 2.6-4 (Oksanen *et al*. 2022) was used to calculate alpha diversity estimators and non-metric multidimensional scaling analyses (NMDS). Data tables with the amplicon sequence variants (ASV) abundances were normalized into relative abundances and converted into a Bray-Curtis similarity matrix. To determine significant differences between the bacterial and fungal community composition according to the plant tissue, we used the non-parametric multivariate analysis of variance (Permanova) with *adonis2* function and 999 permutations. To estimate differences in the relative proportion of microorganisms and the diversity indices, we used the non-parametric Kruskal-Wallis test, while pairwise comparisons were performed using Wilcox and Dunn's tests (with Bonferroni adjustment). The map of the sampling point was generated with ggplot2 and sf libraries using the world database.

Results

In this work, we compared the prokaryotic and fungal communities in the leaves and bladders of the carnivorous epiphytic plant *Utricularia jamesoniana*. The prokaryotic community of the samples was composed of 2,258 amplicon sequence variants (ASVs), according to the analysis of sequences of the V4 region of the 16S rRNA gene. All the prokaryotic sequences were assigned to 44 phyla and 87 classes. Proteobacteria were the most abundant, representing 66 % of all sequences and 37.5 % of the ASVs. Actinobacteriota represented 11.3 % of the sequences and 9.3 % of the ASVs, and Acidobacteriota represented 3.9 % of the sequences and 5.6 % of the ASVs. Other abundant groups were Firmicutes, Verrucomicrobiota, Planctomycetota, and Cyanobacteria. Archaea represented only 0.28 % of the sequences and 0.56 % of the ASVs.

The fungal community was composed of 1,620 amplicon sequence variants, according to the analysis of sequences of the ITS region. All the fungal sequences were assigned to 10 phyla and 32 classes. Ascomycota was the most abundant phylum comprising 79,6 % of the sequences and 78.9 % of the ASVs, while Basidiomycota represented 20.2 % of the sequences and 19.3 % of the ASVs. Other less abundant fungal phyla included Rozellomycota and Chytridiomycota.

The fungal classes with the highest relative abundance were Dothideomycetes, Sordariomycetes, Eurotiomycetes, and Agaricomycetes. Within Dothideomycetes, the most abundant ASVs were unclassified members of Mycosphaerellaceae and Cladosporiaceae, while *Colletotrichum, Aspergillus,* and *Thanatephorus* were the most abundant genera within Sordariomycetes, Eurotiomycetes, and Agaricomycetes, respectively.

The bacterial classes with the highest relative abundance were Alphaproteobacteria and Gammaproteobacteria. The most abundant genera within Alphaproteobacteria were *Acidocella*, *Bradyrhizobium*, and *Rhizobium.* The most abundant genera within Gammaproteobacteria were *Ferritrophicum*, *Ferrovum*, and *Chromobacterium*. In general, we observed differences in the composition of some particular groups of the prokaryotic communities between the leaves and traps (Figure 3A).

We also observed variations in the composition of the fungal communities within samples and between tissues. Notably, we detected a higher proportion of Lecanoromycetes, Leotiomycetes, Orbiliomycetes, and Sordariomycetes in the bladder, while Dothideomycetes and Eurotiomycetes were more abundant in the leaves. We detected 25 and 30 classes of fungi in the bladder and leaf tissues, respectively. Chytridiomycetes were detected only in the bladder, where they have been found actively growing in aquatic *Utricularia* species (Sirová *et al*. 2018b). Conversely, some classes were only present in the leaves, including Agaricostilbomycetes, Saccharomycetes, and Rhizophydiomycetes (Figure 3B).

We observed notable differences in the alpha diversity indices of prokaryotic communities in the tissues of *Utricularia jamesoniana*. The richness of prokaryotic ASVs has higher in the bladders with respect to the leaves, with mean richness values of 1,174 and 356, respectively. On the other hand, the mean value of the Shannon diversity index in the traps was also higher than that of the leaves, with mean values of 6.0 and 4.3, respectively (Figure 4). We also observed differences in the indices of fungal communities. However, the mycobiome was more diverse in the leaves than in the traps. The richness of fungal ASVs has higher in the leaves with respect to the traps, with mean richness values of 652 and 433, respectively. On the other hand, the mean value of the Shannon diversity index in the leaves was also higher than that of the traps, with mean values of 4.2 and 3.7, respectively (Figure 4).

The NMDS analysis of the bacterial communities showed that the trap samples clustered together, and their community composition separated from the plant leaves (Figure 5A). However, the Permanova showed no significant differences in the structure of prokaryotic communities between tissues (Permanova > 0,05). The analysis of the fungal communities showed that the composition in both bladders and leaves tissues in one of the plants clustered together, whereas they separated in the second plant (Figure 5B). The Permanova showed no significant differences in the structure of prokaryotic communities between tissues (Permanova > 0.05).

The heat map shows the variations in the relative abundances of the sample's most abundant genera of prokaryotes. In general, most genera presented relative abundances of less than 5 %. However, some of the most abundant genera in the bladder were *Bradyrhizobium*, *Ferritrophicum*, *Acidocella*, *Methylocapsa*, and *Mycobacterium*, while in the leaves, they were *Acidocella*, *Ferrovum*, *Methylocapsa*, *Rhizobium*, and *Gallionella*. *Acidocella* and *Methylocapsa* (Figure 6A).

Our results confirm the previously observed differences in fungal diversity within and between samples regarding the most prevalent fungal genera across the tissue samples (Figure 6B). In particular, we identified several fungal genera that were highly abundant in traps, including *Thanatephorus*, *Clavatospora*, and *Phaeothecoidiella*. In contrast, leaves' most prevalent fungal genera were an unclassified member of the Mycosphaerellaceae, *Aspergillus*, and *Phaeococcomyces.*

Discussion

We elucidate the distinctive differences in prokaryotic and fungal communities between the traps of *Utricularia jamesoniana* and its leaves. The specialized bladders or traps constitute a singular ecological niche that harbors a

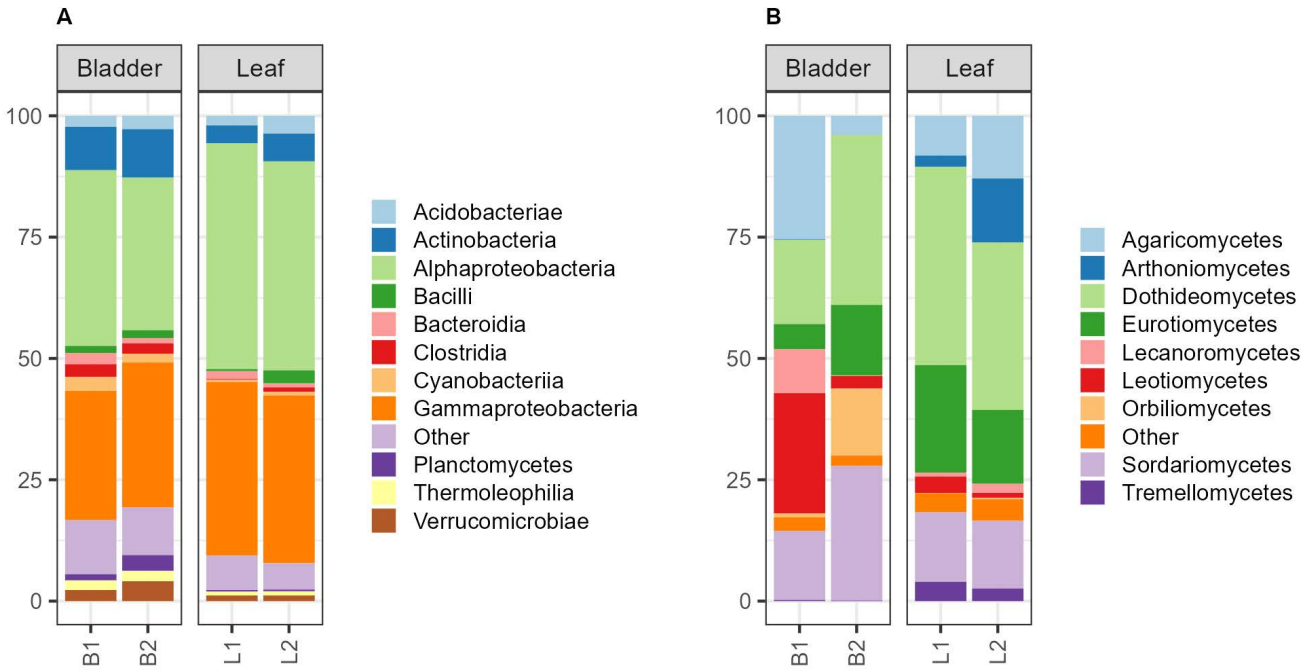

Figure 3. Relative abundance of the main taxonomic groups in bladders and leaves of *Utricularia jamesoniana.* A corresponds to the main prokaryotic groups, and B to the main fungal groups.

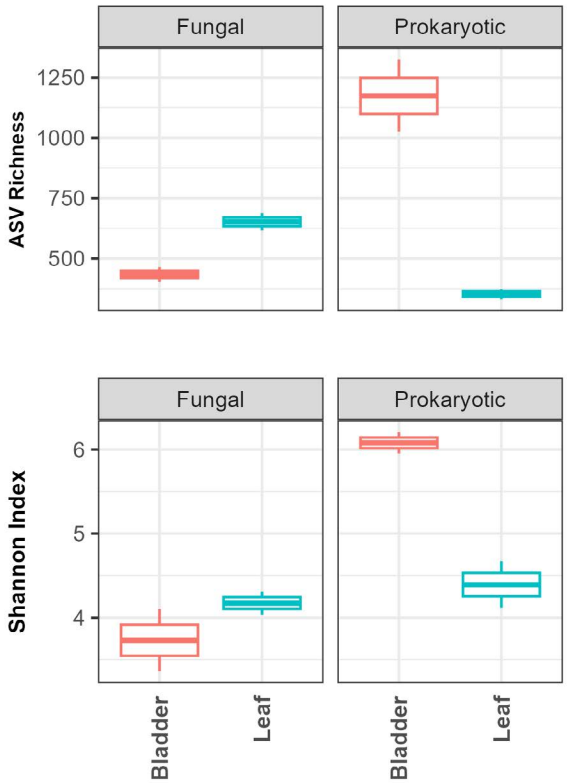

Figure 4. Alpha diversity values of prokaryotic and fungal communities in the bladder and leaves of *Utricularia jamesoniana*. The upper panel represents the richness of Amplicon Sequence Variants (ASVs), while the panel below shows the values of the Shannon Index.

multitude of microbial communities. These communities potentially exert a significant influence on plant fitness by participating in prey digestion and facilitating environmental adaptation (Caravieri *et al*. 2014, Sirová *et al*. 2018a). Notably, the resident microbiome within these traps may undergo modifications due to the introduction of microbial communities from captured prey, as well as the assimilation of metabolizable compounds derived from the prey. It is pertinent to recognize that each prey item might harbor its own distinct microbial diversity, as well as inherent chemical characteristics, thereby introducing an additional layer of complexity to the trap's microbiome composition.

Rhizobial bacteria have been reported as one of the most abundant groups in some aquatic *Utricularia* species, while *Acidocella* and *Bradyrhizobium* have also been reported in the traps of different carnivorous plants (Grothjan & Young 2022, Sickel *et al*. 2019). The predominance of *Bradyrhizobium*, a nitrogen-fixing bacterium (Zahran 1999), was particularly conspicuous within the traps. Nevertheless, uncertainty persists regarding the nitrogen-fixing capability of this species within a non-root nodule, such as the bladder. On the other hand, it is plausible that *Bradyrhizobium* might serve additional functions, such as metabolizing different carbon sources (VanInsberghe *et al*. 2015).

Furthermore, the traps exhibited a notable abundance of metal-oxidizing bacteria such as *Acidocella*, *Ferritrophicum*, *Ferrovum*, and *Chromobacterium* which suggests the occurrence of these processes within this plant tissue (Johnson *et al*. 2014, Okamoto *et al*. 2017, Weiss *et al*. 2007). However, only *Chromobacterium* has been documented previously as a constituent of the microbiome associated with *Utricularia*, but in aquatic species (Grothjan & Young 2022). This assemblage of microbial species suggests an environment conducive to stimulating microbial metabolism, leading to the release and recycling of nutrients (Sirová *et al*. 2003, 2018a).

The differences in the relative abundances of some bacterial and fungal groups between the trap samples may be potentially related to both variations in the preys captured over time and the current stage of prey decomposition. In addition, the interior of the trap is completely sealed and maintains an anoxic environment (Adamec 2007) and hosting a complex microbial food web responsible for a significant proportion of enzymatic activity associated with prey digestion (Caravieri *et al*. 2014, Sirová *et al*. 2009). The preservation of anoxic conditions within the traps potentially serves as the mechanism for incapacitating captured prey (Adamec 2007).

Given this context, it stands to reason that the microbial community dwelling within these traps has adapted to this unique environment, which experiences intermittent spikes of elevated oxygen concentration subsequent to firing events, followed by periods of anoxia (Adamec 2007). These fluctuating conditions create a niche favoring the coexistence of organisms that exhibit heightened tolerance to anoxia (Richards 2001, Sirová *et al*. 2003), consequently contributing to prey degradation (Caravieri *et al*. 2014). These conditions could also be responsible for the changes in the composition of the fungal communities in this tissue and the lower values of diversity observed.

Proteobacteria emerged as the predominant inhabitants within the *Utricularia jamesoniana* tissues. The prevalence and roles of this taxonomic group are intricately linked to the microenvironment provided by the plant tissue, its genetic composition, physiological attributes, as well as the biotic and abiotic factors inherent in the surrounding environment (Afzal *et al*. 2019, Brader *et al*. 2017, Hardoim *et al*. 2015). Prior microbiome investigations associated with other carnivorous plants have consistently pinpointed Proteobacteria as the most abundant phylum (Alcaraz *et al*. 2016, Chan 2019, Sickel *et al*. 2019).

Taxonomic parallels with earlier studies conducted on other aquatic *Utricularia* indicate the prevalence of *Rhizobium* as one of the most abundant genera within certain species. Likewise, *Acidocella* and *Bradyrhizobium* have been noted in the traps of diverse carnivorous plants (Grothjan & Young 2022, Sickel *et al*. 2019). Among Gammaproteobacteria, *Chromobacterium* has also been described as part of the microbiome across several *Utricularia* species (Grothjan & Young 2022). To date, the majority of research efforts have focused on aquatic *Utricularia* species, including *U. gibba* L., *U. australis* R.Br., and *U. vulgaris* L. (Alcaraz *et al*. 2016, Sirová *et al*. 2018b) characterized by their larger size and accelerated growth rates. Notably, this study represents a pioneering investigation into the composition of the microbiome and mycobiome associated with an epiphytic species such as *Utricularia jamesoniana*.

One of the most revealing results of this study was the elevated levels of prokaryotic species richness and Shannon diversity detected within the traps, constituting a digestive ecosystem (Adamec 2007, Sirová *et al*. 2018a, b) where

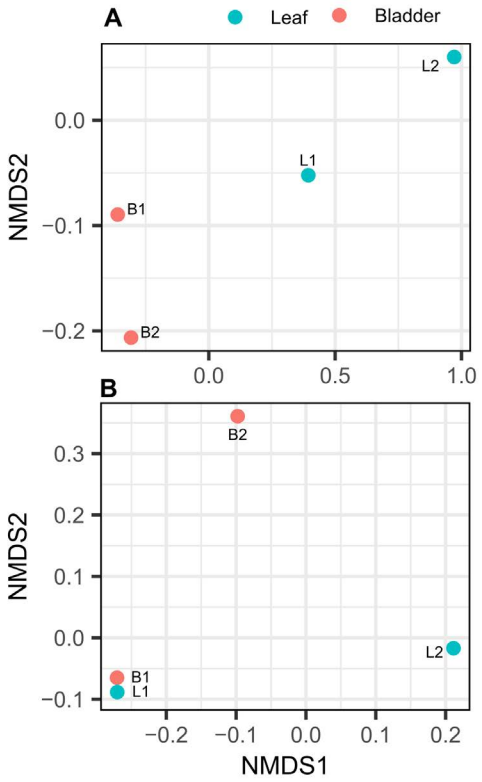

Figure 5. NDMS analysis of the microbial communities in bladders and leaves of *Utricularia jamesoniana.* A corresponds to the prokaryotic groups, and B to the fungal groups.

microorganisms face challenging living conditions typical of a digestive ecosystem. This intriguing observation prompts speculation that the increased richness and diversity of communities within the traps stem from the dynamic nature of this environment. In contrast, the conditions within the leaves can be more stable.

The diversity of the endophytic fungal community within the traps exhibited lower values compared to that observed in the leaves. This trend stands in contrast to the patterns observed within bacterial communities. Notably prevalent within this tissue were the genera *Thanatephorus*, *Leohumicola*, *Clavatospora*, *Orbilia*, and *Phaeothecoidiella*. However, the distinctive attributes and mechanisms underlying these genera's apparent compatibility within the host traps fluctuating anaerobic microenvironment demand further exploration. Particularly intriguing is the prevalence of *Thanatephorus*, known as the teleomorph of *Rhizoctonia*, a recognized plant pathogen (Anderson 1982, Donk 1956), which was present even when no disease symptoms were observed on the plants examined.

In general, the phylum Ascomycota predominated in the tissues of *Utricularia jamesoniana*, with higher abundances of Dothideomycetes, Sordariomycetes, Eurotiomycetes, a pattern consistent with earlier findings in other carnivorous plants belonging to the same botanical family (Rueda-Almazán *et al*. 2021). Particularly noteworthy is that a substantial proportion, approximately 45 %, of the fungal genera were present in both the traps and leaves. This observation underscores the notion that certain endophytic fungi can colonize diverse tissues within the same plant organism effectively (Behie *et al*. 2015, Rueda-Almazán *et al*. 2021, Wu *et al*. 2013).

The interactions between plants and endophytes have been recognized for their myriad benefits to plants, particularly species that thrive in challenging or nutrient-deficient environments, as is the case with carnivorous plants (Ellison & Adamec 2018, Jobson *et al*. 2018, Rueda-Almazán *et al*. 2021). However, it is crucial to highlight that not all identified fungi necessarily qualify as pure endophytes, as some could originate from external and potentially serve as nutrient sources (Sirová *et al*. 2018b).

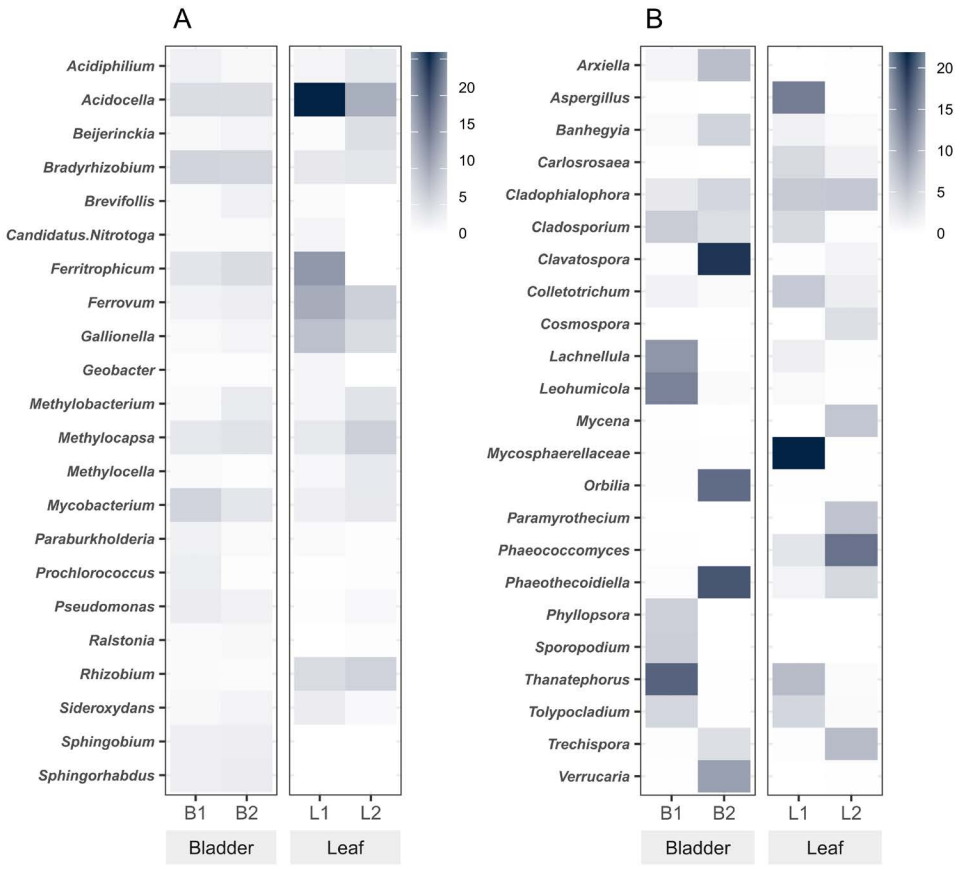

Figure 6. Heatmap of the most abundant genera in the bladder and leaves tissues of *Utricularia jamesoniana.* A corresponds to the prokaryotic genera determined according to the relative abundance of 16S rRNA gene sequences, and B to the fungal genera determined according to the relative abundance of ITS sequences.

In the present investigation, we identified distinct groups of genuine endophytes, such as *Aspergillus*, *Cladosporium*, *Colletotrichum*, and Mycosphaerellacea. These groups have previously been isolated from both leaves and traps in other carnivorous plant species (Glenn & Bodri 2012, Lee *et al*. 2014, Naseem & Kayang 2018, Quilliam & Jones 2010, Rueda-Almazán *et al*. 2021). However, some genera were exclusive to either the traps or leaves. *Pseudochaetosphaeronema*, *Periconia*, *Remotididymella*, and *Phragmotaenium* have also been described for the first time in an epiphytic carnivorous plant species. These findings underscore the distinctive biodiversity ecosystem hosted by *Utricularia jamesoniana*, justifying comprehensive further investigation.

Finally, the exploration of plant-microbial interactions serves as a window into the intricate web of ecological relationships spanning diverse lineages and their evolutionary trajectories. The dynamic composition of these microbial communities can fluctuate in response to a spectrum of biotic and abiotic factors that shape these interactions, where microorganisms undertake an array of functions, encompassing enzymatic activities such as prey digestion, fortification against pathogens, facilitation of growth, and protection (Hung & Rutgers 2016, Lee *et al*. 2014, Rueda-Almazán *et al*. 2021). Examining the microbiome and mycobiome of carnivorous plants provides a captivating model that advances our understanding of plant ecology, evolution, and the intricate interplay between plants and their microbial inhabitants.

We contribute novel insights that enhance the comprehension of the microbiological community associated with *Utricularia jamesoniana*, shedding light on previously undocumented groups present within plant leaves and traps. For future investigations, it is imperative to incorporate a larger sample size to facilitate robust statistical analyses

while exercising caution to prevent any adverse impact on the vulnerable populations of this delicate species. Furthermore, it is necessary to explore in more detail the physiological and biochemical nuances characterizing the traps. This entails discerning the intrinsic microbiome residing within the traps and disentangling it from the influx of nutrient-rich biological material. Additionally, there is a need to meticulously document any discernible shifts in microbial populations triggered by the plant's climatic seasonality and phenological state.

This study offers insight into the intricate microbial and fungal communities associated with traps and leaves of *Utricularia jamesoniana*. The distinctive biodiversity composition suggests plausible ecological connections between the plant tissues and the microbiome. These interactions and their potential entanglement with the fungal communities hold promise for illuminating the intricate host-endophyte dynamics. A more exhaustive microbiome characterization can unveil the plant's dietary preferences, nutrient cycling potential, and the spectrum of associated microorganisms. The description of several previously unreported fungal groups highlights the specific and underexplored nature of this microbial and fungal habitat. We emphasize the need for further investigation to unravel the microbiome and mycobiome of this epiphytic carnivorous plant, its intricate relations, and interaction dynamics.

Supplementary material

The datasets presented in this study can be found in the NCBI Sequence Read Archive under accession PRJNA1005337: https://www.ncbi.nlm.nih.gov/sra/PRJNA1005337.

Acknowledgements

We thank Víctor Adrián Mora Mora for the design and drawing of Figure 1.

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Associate editor: Eduardo Ruiz Sánchez

Author contributions:

VNA, conception and design of the study, investigation and data analysis; RMC, conception and design, supervised the project; JMH, investigation and data analysis; RAC, investigation and data analysis; KRJ conception and design, investigation and data analysis, wrote the first draft of the manuscript.

Supporting Agencies: This project was partially supported by Vicerrectoría de Investigación, University of Costa Rica (project C2-234).

Conflicts of interest: The authors declare that there is no conflict of interest, financial or personal, in the information, presentation of data and results of this article.