Project Proposal

Introduction

Utricularia L. is the largest carnivorous plant genus; with c. 240 species distributed worldwide (Silva et al. 2018). These species bear tiny bladder-shaped traps in their submerged or subterranean stolons and leaves (Lloyd et al. 1942). The three different types of trichomes in the inner surface of the traps play significant roles in prey capture and digestion (Adamec 2011) and their shape have high taxonomic value in sectional and interspecific levels (Taylor 1989; Reifenrath et al. 2006; Westermeier et al. 2017).

In addition to the traps, the pollen grains have also been highlighted as important in species delimitation in the family. Palynological features such as shape, apertures, endoapertures and exin attributes have been used in characterization of Lentibulariaceae species (Erdtman 1952; Huynh 1968; Lobreau-Callen et al. 1999; Rodondi et al. 2010; Beretta et al. 2014; Cruz et al. 2018). In a recent taxonomic research, Guedes et al. (2019) were able to highlight the ultrastructure of traps and pollen grains to support species delimitation and propose a taxonomic reestablishment.

In all phylogenetic studies presented so far, only three to six currently accepted species of *U.* sect. *Setiscapella* were sampled, the results are quite controversial, presenting placement divergences when sequences of *U.* sect. *Avesicaria* and *U.* sect. *Steyermarkia* are included in the analyses (Jobson & Albert 2002; Jobson et al. 2003; Westermeier et al. 2017; Jobson et al. 2018; Silva et al. 2018). Besides, *U. flaccida* A.DC. has always emerged phylogenetically distant to other members of *U.* sect. *Setiscapella*, in clades with low support, with only a few plastid markers used. When, sequences of *U.* sect. *Steyermarkia* are included, *U. flaccida* is placed phylogenetically closer to its members (Westermeier et al. 2017). Despite the amount of phylogenetic studies performed for the genus, there are still important gaps regarding most of the Neotropical species, often undersampled, compromising the phylogenetic inferences in sectional levels (Jobson et al. 2018).

In Brazil, there are eight out of the nine species currently accepted in *U.* sect. *Setiscapella* (*U. flaccida*, *U. nervosa* Weber ex Benj., *U. nigrescens* Sylvén, *U. physoceras* P.Taylor, *U. pusilla* Vahl, *U. subulata* L., *U. trichophylla* Spruce ex Oliv. and *U. triloba* Benj.), and the remaining is African (*U. stanfieldii* P.Taylor). In Brazil, there are also the two species of *U.* sect. *Avesicaria* (*U. neottioides* A.St.-Hil. & Girard and *U. oliveriana* Steyerm.), whilst the two species of *U.* sect. *Steyermarkia* (*U. aureomaculata* Steyerm. and *U. steyermarkii* P.Taylor) are endemic do Venezuela (Taylor 1989).

Members of *U.* sect. *Setiscapella* and *U.* sect. *Steyermarkia* are morphologically very similar in general corolla and trap shapes, especially *U. flaccida* and the Venezuelans *U. aureomaculata* and *U. steyermarkii*, which present further similarities such as lithophytic life form and minute punctuations in the adaxial surface of the leaves. Moreover, some species present great phenotypic plasticity, sympatry and population extremes observed (e.g. *U. pusilla*, *U. subulata* and *U. triloba*), which makes identification difficult, even under the eyes of experts. Taylor (1989) segregated these two sections based on seed (oblique-ellipsoids with elongated testa cells in *Setiscapella* and globose with isodiametric testa cells in *Steyermarkia*) and leaf (1-nerved in *Setiscapella* and multinerved in *Steyermarkia*) morphology.

Palynological studies are occasional, Lobreau-Callen et al. (1999) described nine types of pollen grains to the genus, mainly based on aperture number and exin attributes. Cruz et al. (2018) characterized pollen grains of Western Cuban Lentibulariaceae, the authors categorized three types of pollen for the seven *Utricularia* species sampled and presented a pollen identification key. According to Jobson et al. (2018), the exin attributes, with few exceptions, is more associated with phylogeny rather than habitat specialization. The aperture number is a character with extreme interspecific, and sometimes even intraspecific, variations, however, it might reflect in a cryptic taxonomic diversity rather than intraspecific variation (Baleeiro in press).

Among the main reasons why most researchers still use Taylor's (1989) infrageneric classification are the high level of incongruities in phylogenetic proposals so far and the lack of consensus in proposing a new classification system. In this context, new studies can contribute to the resolution of systematic issues still observed in the genus. Such as the understanding of the relationship of *U. flaccida* with the congeners, the delimitation of widely distributed and highly plastic species (*U. pusilla, U. subulata* and *U. triloba,* for instance), as well as the relationship of species of other phylogenetically close sections. Thus, the multidisciplinary approach of the integrative taxonomy, combining different scientific tools (classic morphology, Scanning Electron Microscopy, palynology and molecular phylogeny) can help to better understand the biology, ecological implications and evolutionary history of the group in study.

Objectives

To perform Scanning Electron Microscopy (SEM) analyses to investigate the ultrastructure of external and internal trap glands and pollen grains of the *U.* sect. *Setiscapella, U.* sect. *Steyermarkia* and *U.* sect. *Avesicaria* members. In order to combine macro and micro morphological data of these structures with molecular data to answer the following questions: 1. Is there a cryptic taxonomic diversity underneath some binomials of *U.* sect. *Setiscapella*? 2. Besides macromorpholgy, are there micromorphological similarities between members of *U.* sect. *Setiscapella* and *U.* sect. *Steyermarkia*? 3. Does *U.* sect. *Setiscapella* in its current circumscription correspond to a natural group? 4. Should *U. flaccida* still be placed in *U.* sect. *Setiscapella*? 5. Do trap and pollen micromorphology have phylogenetic signal?

Material and Methods

Field expeditions, collecting samples and herbarium visits – The IAPT Grant will provide support to collect samples of eight species from the field at previously known populations from the Brazilian state of Pará and from Ptari-Tepui and Ayuan-Tepui in Venezuela. The key species to include in this research are the Venezuelans and the one endemic to Pará (*U. physoceras*), with very few collections made and few or no sequences in previous phylogenetic analyses. This grant will also provide support to visit two important herbarium collections, Museu Paraense Emílio Goeldi and Central University of Venezuela herbarium, allowing the analyses of valuable specimens for the taxonomic review in the present research.

SEM analysis – The IAPT Grant will also provide support to perform SEM analyses in previous and newly collected material. The aim is to investigate ultrastructural characters of the inner and outer surface of the traps (n=3) and pollen grains (n=3 anthers) from three individuals from at least two or three different populations of 12 species, totalizing 216 to 324 traps and anthers sampled, giving scope to cover possible intraspecific variations. Mature anthers will be fixed in FAA70 and later carefully dissected in Alcohol 70%, dried in ethanolic series in critical point (Bal-Tec CPD 030), and coated in gold (Bal-Tec SCD 050). The traps will be cut in longitudinal axis and fixed in glutaraldehyde + formaldehyde + sodium cacodylate solution, later washed three times in 0.1 M sodium Cacodylate and fixed in 1% osmium tetroxide solution for 1.5 h. Then, dried in ethanolic series in critical point (Bal-Tec CPD 030), and coated in gold (Bal-Tec SCD 050) (Plachno et al. 2019). The stubs will be observed in Hitachi TM3000 microscope at Universidade Estadual Paulista, Campus Jaboticabal. The data matrix generated will be incorporated to the cladograms in the phylogenetic analysis.

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