IAPT 2020 - RESEARCH GRANT PROPOSAL

**General Information:**

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**List of relevant publications:**

* **Lustofin K**, Świątek P, Miranda VFO, Płachno BJ. 2020. Flower nectar trichome structure of carnivorous plants from the genus butterworts Pinguicula L. (Lentibulariaceae). *Protoplasma* 257: 245-259. Doi: 10.1007/s00709-019-01433-8 **(IF 2,633)**
* Pawlicki P, Hejmej A, Miłoń A, **Lustofin K**, Płachno BJ, Tworzydło W, Górowska-Wójtowicz E, Pawlicka B, Kotula-Balak M, Bilińska B. 2019. Telocytes in the mouse testicular interstitium. Implications of G-protein coupled estrogen receptor (GPER) and estrogen related receptor (ERR) in regulation of Mouse testicular interstitial cells. *Protoplasma* 256: 393-408. Doi: 10.1007/s00709-018-1305-2 **(IF 2,633)**.
* **Lustofin K.** 2020. Szkodliwy wpływ flutamidu na układ rozrodczy ssaków*. Wszechświat* 120(1-3):39-46.

**Project Details:**

**Title of proposed project:**

Development of non-glandular and glandular trichomes within flowers of selected *Pinguicula* species.

**Description of the project in less than 50 words:**

The aim of this project is to show anatomy and micromorphology at each developmental stage of non-glandular and glandular trichomes located within flowers of *P. agnata*, *P. moranensis* and *P. vulgaris*. We would like to compare trichomes development processes of these species, in order to establish differences and similarities.

**Total budget request:** 1.810,00 US$

**Project Proposal**

**Introduction:**

*Pinguicula* genus consist of 96 known carnivorous plant species, what makes it second-largest genus of Lentibulariaceae family. Except of *Pinguicula* to Lentibulariaceae family we can also include both *Utricularia* and *Genlisea* genera (Fleischmann and Roccia, 2018). The first genus that evolved was *Pinguicula* and has most common features with ancestor of this family (Ibarra-Laclette et al., 2013). *Pinguicula* is cosmopolitan genus present in almost all continents except of Australia and Antarctica, however center of biodiversity of this genus is located in Central America (Roccia et al. 2016).

 Among *Pinguicula* species we can distinguish the three subgenera (xxxx) and also groups of species, which differ in terms of the type of pollinator. The majority of *Pinguicula* species are pollinated by insects. Within this group of plants we can further differentiate between species pollinated by Diptera and/or Hymenoptera and species pollinated by Lepidoptera. There also exist ornitophilous species, which are most probably pollinated by the hummingbirds (Lampard et al. 2016; Villegas and Alcalá, 2018).

 Lentibulariaceae species have flowers with bilateral symmetry, which are bilobed. These flowers contain elongated spur responsible for nectar production (Fleischmann et al., 2010; Villegas and Alcalá, 2018; Aranguren et al., 2018; Płachno et al. 2018; Lustofin et al., 2019). *Pinguicula* species display a wide range of corolla sizes, colors, as well as dependently on the type of pollinator spur length and shape (Lampard et al., 2016; Roccia et al., 2016). A common feature of *Pinguicula* flowers is presence of various types of non-glandular trichomes, which are located on basal parts of corolla lobes and inside the throat (Fig. 1). Owing to diverse micromorphology of these trichomes, their structure are used as a taxonomical trait to recognize species (Casper, 1966). Also Inside spur are located numerous glandular trichomes which form nectary (Lustofin et al. 2019). Additionally in our preliminary study which concerns anatomy and micromorphology of *Pinguicula* mature flowers we observed numerous glandular trichomes located on basal part of corolla lobes, as well as on generative organs (Fig. 1).

Figure 1. Micromorphology of *P. agnata* mature flower. Pistil with glandular trichomes on the outer surface of ovary (A, B) and different types of non-glandular trichomes located at the entrance to the throat (C) and inside the throat (D).

 Nevertheless there is lack of knowledge about development processes of *Pinguicula* flowers. It seems to be very interesting to examine and compare ontogeny of chosen flowers, in order to test if there exist any differences between closely related species during these process.

**Objective and hypotheses:**

 The research goal of this project would be analyses of anatomy and micromorphology of flower buds, including all types of non-glandular and glandular trichomes. Subsequently I would like to compare development processes of flowers for selected species, which belongs to different subgenus: Pinguicula (*P. vulgaris*), Temnoceras (*P. agnate* and *P. moranensis*) and differ in terms of pollinator: Hymenoptera/Diptera (*P. vulgaris, P. agnate*), Lepidoptera (*P. moranensis*) . **We have hypothesis** that structure of flower buds, including non-glandular and glandular trichomes on each development stage differ dependently on the type of pollinator and subgenus.

**Material and methods:**

 We will used cultivated plant material from Liberec Botanic Garden (Dr. Miloslav Studnička, Liberec, Czech Republic) Prague Botanic Garden (Czech Republic) and the Botanical Garden of the Jagiellonian University in Cracow. We would like to examine at least 5 flower buds at each development stage on each species. Development stage will be divided into 3 phases (beginning of flower bud development phase, middle bud development phase and before anthesis bud development phase), basing on the length of the buds.

 For micromorphology analysis plant material will be fixed, dehydrated in a graded ethanol series, subjected to critical-point drying using liquid CO2 and subsequently sputter-coated with gold. Micromorphology analysis will be evaluated by scanning electrone microscope. In order to examine anatomy of flower buds, previous fixed and dehydrated in a graded ethanol series plant material will be embedded in resin Technovit 7100 and subsequently sectioned with a histological knife on rotary microtome into semi-thin sections (3-5µm). Sections will be stained with 0.1% toluidine blue O. Such prepared material will be examined using a light microscope.

**Itemized budget:**

Reagents for fixation and preparation of flower buds material; SEM analysis; resin necessary for anatomy analysis; Transport to Liberec Botanic Garden, Prague Botanic Garden for plant material; costs: 1.810,00 US$

**Literature citations:**

* Aranguren Y, Płachno BJ, Stpiczyńska M, Miranda VFO (2018) Reproductive biology and pollination of the carnivorous *Genlisea violaceae* (Lentibulariaceae). Plant Biol, 20(3):591-601.
* Casper SJ (1966) Monographie der Gattung Pinguicula L. Bibliotheca Botanica, 127-128:1-209.
* Fleischmann A, Roccia A (2018) Systematics and evolution of Lentibulariaceae: I. *Pinguicula*. In: Ellison AM, Adamec L (eds) Carnivorous plants: physiology, ecology and evolution. Oxford University Press, pp 70–80.
* Fleischmann A, Schäferhoff B, Heubl G, Rivadavia F, Barthlott W, Müller KF (2010) Phylogenetics and character evolution in the carnivorous plant genus *Genlisea* A. St.-Hil. (Lentibulariaceae). Mol Phylogenet Evol, 56(2):768–783.
* Ibarra-Laclette E, Lyons E, Hernández-Guzmán G, Pérez-Torres CA, Carretero-Paulet L, Chang T-H, Lan T, Welch AJ, Abraham Juárez MJ, Simpson J, Fernández-Cortés A, Arteaga- Vázquez M, Góngora-Castillo E, Acevedo-Hernández G, Schuster SC, Himmelbauer H, Minoche AE, Xu S, Lynch M, Oropeza-Aburto A, Cervantes-Pérez SA, de Jesús O-EM, Cervantes-Luevano JI, Michael TP, Mockler T, Bryant D, Herrera-Estrella A, Albert VA, Herrera-Estrella L. 2013. Architecture and evolution of a minute plant genome. *Nature*, 498:94–98.
* Lampard S, Gluch O, Robinson A, *et al.* (2016) *Pinguicula* of Latin America. Poole, UK: Redfern Natural History.
* Lustofin K, Świątek P, Miranda VFO, Płachno BJ (2019) Flower nectar trichome structure of carnivorous plants from the genus butterworts *Pinguicula* L. (Lentibulariaceae). Protoplasma, 257:245-259.
* Płachno BJ, Stpiczyńska M, Świątek P, Lambers H, Miranda VFO, Nge FJ, Stolarczyk P, Cawthray GR (2018) Floral micromorphology of the bird-pollinated carnivorous plant species *Utricularia menziesii* R.Br. (Lentibulariaceae). Ann Bot, 123(1):213–220.
* Roccia A, Gluch O, Lampard S, Robinson A, Fleischmann A,McPherson S, Legendre L, Partrat E, Temple P (2016) *Pinguicula* of the temperate north. Redfern, Dorset, pp 350.
* Villegas SG, Alcalá RE (2018) Reproductive ecology of the carnivorous plant *Pinguicula moranensis* (Lentibulariaceae). Plant Biol, 20(2): 205–212.