Do food trichomes occur in *Pinguicula* (Lentibulariaceae) flowers?

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**Background and Aims** Floral food bodies (including edible trichomes) are a form of floral reward for pollinators. This type of nutritive reward has been recorded in several angiosperm families: Annonaceae, Araceae, Calycanthaceae, Eupomatiaceae, Himantandraeae, Nymphaeaceae, Orchidaceae, Pandanaceae and Winteraceae. Although these bodies are very diverse in their structure, their cells contain food material: starch grains, protein bodies or lipid droplets. In *Pinguicula* flowers, there are numerous multicellular clavate trichomes. Previous authors have proposed that these trichomes in the *Pinguicula* flower play the role of 'futterhare' ('feeding hairs') and are eaten by pollinators. The main aim of this study was to investigate whether the floral non-glandular trichomes of *Pinguicula* contain food reserves and thus are a reward for pollinators. The trichomes from the *Pinguicula* groups, which differ in their taxonomy (species from the subgenera: *Tennoceras*, *Pinguicula* and *Isoloba*) as well as the types of their pollinators (butterflies/flies and bees/hummingbirds), were examined. Thus, it was determined whether there are any connections between the occurrence of food trichomes and phylogeny position or pollination biology. Additionally, we determined the phylogenetic history of edible trichomes and pollinator evolution in the *Pinguicula* species.

**Methods** The species that were sampled were: *Pinguicula moctezumae*, *P. esseriana*, *P. moranensis*, *P. emarginata*, *P. rectifolia*, *P. mesophytica*, *P. giganthea*, *P. agnata*, *P. albida*, *P. ibarrae*, *P. martinezii*, *P. filifolia*, *P. gigantea*, *P. lusitanica*, *P. alpina* and *P. vulgaris*. Light microscopy, histochemistry, and scanning and transmission electron microscopy were used to address our aims with a phylogenetic perspective based on *matK*/*trnK* DNA sequences.

**Key Results** No accumulation of protein bodies or lipid droplets was recorded in the floral non-glandular trichomes of any of the analysed species. Starch grains occurred in the cells of the trichomes of the bee-fly-pollinated species: *P. agnata*, *P. albida*, *P. ibarrae*, *P. martinezii*, *P. filifolia* and *P. gigantea*, but not in *P. alpina* or *P. vulgaris*. Moreover, starch grains were not recorded in the cells of the trichomes of the *Pinguicula* species that have long spurs, which are pollinated by Lepidoptera (*P. moctezumae*, *P. esseriana*, *P. moranensis*, *P. emarginata* and *P. rectifolia*) or birds (*P. mesophytica* and *P. hemiepiphytica*), or in species with a small and whitish corolla that self-pollinate (*P. lusitanica*). The results on the occurrence of edible trichomes and pollinator syndromes were mapped onto a phylogenetic reconstruction of the genus.

**Conclusion** Floral non-glandular trichomes play the role of edible trichomes in some *Pinguicula* species (*P. agnata*, *P. albida*, *P. ibarrae*, *P. martinezii*, *P. filifolia* and *P. gigantea*), which are mainly classified as bee-pollinated species that had originated from Central and South America. It seems that in the *Pinguicula* that are pollinated by other pollinator groups (Lepidoptera and hummingbirds), the non-glandular trichomes in the flowers play a role other than that of a floral reward for their pollinators. Edible trichomes are symplesiomorphic for the *Pinguicula* species, and thus do not support a monophyletic group such as a synapomorphy. Nevertheless, edible trichomes are derived and are possibly a specialization for fly and bee pollinators by acting as a food reward for these visitors.

**Key words:** Butterworts, carnivorous plants, floral micro-morphology, food hairs, Lentibulariaceae, trichome structure, *Pinguicula*, spur, trichomes.

**INTRODUCTION**

Plants offer various floral rewards for pollinators that can be divided into two groups: non-nutritive rewards (e.g. nest materials, a place of shelter, heat sources, substances for production of sexual attractants or places for mating) and nutritive rewards (e.g. brood site, floral sweet tissue, stigmatic secretion or fatty oils) (Simpson and Neff, 1981). The most common floral nutritive rewards are nectar and pollen (Faegri and van der Pijl, 1979; Nicolson et al., 2007). However, some species produce food bodies (including edible trichomes) that...
are eaten by their pollinators. The cells of these structures are rich with starch grains, protein bodies or oil droplets (Young, 1986; Thien et al., 2009; for orchids, see Pansarin and Maciel, 2017 and references therein). Food bodies have been recorded in several unrelated plant families: Annonaceae, Araceae, Calycanthaceae, Eupomatiaceae, Himantandraceae, Orchidaceae, Pandanaceae, Nymphaeaceae and Winteraceae (e.g. Faegri and van der Pijl, 1979; Rickson, 1979; Cox, 1982; Young, 1986; Davies et al., 2002; Thien et al., 2009; Endress, 2010; Pansarin and Maciel, 2017). Thus, this type of reward occurs in both evolutionarily old families via beetle pollination (Annonaceae, Calycanthaceae, Eupomatiaceae, Himantandraceae, Nymphaeaceae and Winteraceae; see Endress, 2010) as well as in the more evolutionarily derived family Orchidaceae, which now represents an evolutionary pick of diversity. Floral food bodies can be divided into two major groups: the first (which occurs, for example, in the older lineages of angiosperms, Endress, 2010) – the outgrowths (or tips) of the carpels, stamens, staminodes and tepals; and the second – the epidermal edible trichomes. These trichomes have been particularly well analysed in Orchidaceae and they were found to have evolved independently in this family about five times (genera: Cyanaeorchis, Dendrobium, Eria, Maxillaria and Polystachya; Pansarin and Maciel, 2017). In orchids, they are very diverse in their structure and morphology as well as in the storage of nutritive material in their cells (e.g. Davies et al., 2002; Davies and Turner, 2004; Pansarin and Maciel, 2017).

Pinguicula is a monophyletic genus within the Lentibulariaceae L. family (Jobson et al., 2003; Müller et al., 2004; Fleischmann and Roccia, 2018) and is among the Lamiales (Schäferhoff et al., 2010; Chase et al., 2016) and contains about 96 species. Pinguicula are well known for their carnivory (e.g. Alcalá and Domínguez, 2003, 2005; Darnowski et al., 2018; Heslop-Harrison, 1970; Heslop-Harrison and Heslop-Harrison, 1980; Vassilyev and Muravnik, 1988).

Pinguicula produce spurred zygomorphic flowers, which have nectar as a reward (Abrahamiczyk et al., 2017; Fleischmann and Roccia, 2018; Lustofin et al., 2019). In Pinguicula flowers, there are numerous multicellular clavate trichomes at the base of the corolla – the throat; see Fig. 1A–I (Casper, 1966). Previous authors have proposed that these trichomes in the Pinguicula flower play the role of ‘futterhaare’ (‘feeding hairs’) and are eaten by their pollinators, or that some of them play the role of mimic pollen grains (see Fleischmann, 2016). Thus, the main aim of this study was to determine whether these trichomes of Pinguicula contain food reserves and thus may be a reward for potential pollinators. We selected species from the different clades, which are based on published phylogenetic proposals, within Pinguicula (members from three subgenera but focused on the Central American species) and also sampled species based on differences in their mating system. For this criterion, self- (i.e. a small flower with a whitish corolla) vs. outcross species (large, brightly coloured corollas, nectar guides and long spurs) were compared. Additionally, in our study, we considered the pollinator types (butterflies/fly and...
bees/hummingbirds). Fleischmann (2016) wrote that the clavate trichomes of *Pinguicula* are glandular, and therefore another task/aim was to determine whether these trichomes have the character of glands.

**MATERIALS AND METHODS**

**Plant material**

Seventeen taxa were sampled: *Pinguicula moctezumae* Zamudio & R.Z. Ortega, *P. esseriana* B.Kirchn., *P. moranensis* Kunth, *P. emarginata* Zamudio & Rzed., *P. rectifolia* Speta & F.Fuchs, *P. mesophytica* Zamudio, *P. hemiepiphytica* Zamudio & Rzed., *P. agnata* Casper, *P. albida* Wright ex Griseb., *P. ibarrae* Zamudio, *P. martinezii* Zamudio, *P. filifolia* C.Wright ex Griseb., *P. gigantea* Luhrs, *P. lusitanica* L., *P. alpina* L. and *P. vulgaris* L. [*P. vulgaris* subsp. vulgaris L. and *P. vulgaris* subsp. bicolor (Wol.) Á. Löve & D. Löve]. For our study, we primarily used living material (see Table 1). However, histochemical studies were used by some authors (e.g. Hernández and Katins, 2019) in the case of herbarium material in order to show storage material or glandular structures. Therefore, we also used herbarium material of *Pinguicula* from the Herbarium of the Institute of Botany (KRA).

**Methods**

The flowers were examined using light microscopy (LM), scanning electron microscopy (SEM) and transmission electron microscopy as described below. The material was fixed in a mixture of 2.5 or 5 % glutaraldehyde with 2.5 % formaldehyde in a 0.05 m cacodylate buffer (Sigma; pH 7.2) overnight or for several days, washed three times in a 0.1 m sodium cacodylate mixture of 2.5 or 5 % glutaraldehyde with 2.5 % formaldehyde

**Table 1. List of the *Pinguicula* species that were examined along with information regarding their infrageneric classification, the origin of the plant material and the type of pollinator for each species.**

| Species                          | Infrageneric classification | Material origin                                                                 | Type of pollinator                        |
|----------------------------------|-----------------------------|---------------------------------------------------------------------------------|------------------------------------------|
| *P. moctezumae* Zamudio & R.Z. Ortega | Tenoceras                   | Botanical Garden of Jagiellonian University in Cracow (collected from: Mexico) | Lepidoptera (Abrahameczek et al., 2017) |
| *P. rectifolia* Speta & F.Fuchs   | Tenoceras                   | Botanical Garden of Jagiellonian University in Cracow (collected from: Mexico) | Lepidoptera (flower’s structure indicates that type of pollinator) |
| *P. moranensis* Kunth             | Tenoceras                   | Botanical Garden of Jagiellonian University in Cracow (collected from: Mexico) | Lepidoptera (flower’s structure indicates that type of pollinator) |
| *P. emarginata* Zamudio & Rzed.   | Tenoceras                   | Botanical Garden of Jagiellonian University in Cracow (collected from: Mexico) | Lepidoptera (flower’s structure indicates that type of pollinator) |
| *P. esseriana* B.Kirchn.          | Tenoceras                   | Botanical Garden of Jagiellonian University in Cracow (collected from: Mexico) | Lepidoptera (flower’s structure indicates that type of pollinator) |
| *P. hemiepiphytica* Zamudio & Rzed | Tenoceras                   | Botanical Garden of Jagiellonian University in Cracow (collected from: Mexico) | Most probably hummingbirds (Lampard et al., 2016) |
| *P. mesophytica* Zamudio          | Tenoceras                   | Botanical Garden of Jagiellonian University in Cracow (collected from: Mexico) | Ornithophily is presumed: a watercolour showing a species of hummingbird visiting a plants of *Pinguicula mesophytica* was shown in Roccia et al. (2016) |
| *P. agnata* Casper                | Tenoceras                   | Botanical Garden of Jagiellonian University in Cracow (collected from: Mexico) | Diptera/Hymenoptera (flower’s structure indicates that type of pollinator) |
| *P. gigantea* Luhrs               | Tenoceras                   | Botanical Garden of Jagiellonian University in Cracow (collected from: Mexico) | Diptera/Hymenoptera (Abrahameczek et al., 2017) |
| *P. ibarrae* Zamudio              | Tenoceras                   | Botanical Garden in Liberec                                                    | Diptera/Hymenoptera (flower’s structure indicates that type of pollinator) |
| *P. martinezii* Zamudio           | Tenoceras                   |                                                                                | Diptera/Hymenoptera (flower’s structure indicates that type of pollinator) |
| *P. albida* Wright ex Griseb.     | Tenoceras                   |                                                                                | Hymenoptera (Dominquez et al., 2014) |
| *P. filifolia* C.Wright ex Griseb. | Tenoceras                   |                                                                                | Hymenoptera (Dominquez et al., 2014) |
| *P. lusitanica* L.                | Isoloba                     | Botanical Garden of Jagiellonian University in Cracow (collected from: Europa)  | Diptera/Hymenoptera(’), self-pollination (Heslop-Harrison, 2004) |
| *P. alpina* L.                    | Pinguicula                  | Herbarium of Jagiellonian University in Cracow (collected from: Alps, Innsbruck, Austria; KRA 0299930) | Diptera/Hymenoptera (Molau, 1993; Nordin, 2015) |
| *P. vulgaris* subsp. vulgaris L.   | Pinguicula                  | Herbarium of Jagiellonian University in Cracow (collected from: Male Pieniny, Rezerwat Zaskalskie, Poland; KRA 71415) | Diptera/Hymenoptera (Molau, 1993) |
| *P. vulgaris* subsp. bicolor (Wol.) Á. Löve & D. Löve | Pinguicula                  | Herbarium of Jagiellonian University in Cracow (collected from: Dąbrowa Górnicza, użytk ekologiczny ‘Młaki and Pogóri I’, Poland; KRA 0138573) |
buffer and post-fixed in a 1% osmium tetroxide solution at room temperature for 1.5 h. Next, the material was treated as was previously described (Plachno et al., 2017) and examined using a Hitachi H500 transmission electron microscope (Hitachi, Tokyo, Japan), which is housed at the University of Silesia in Katowice, at an accelerating voltage of 75 kV. The semi-thin sections (0.9–1.0 μm thick) that were prepared for LM were stained with aqueous methylene blue/azure II for 1–2 min (Humphrey and Pittman, 1974) and examined using Olympus BX60 and Nikon Eclipse E400 light microscopes to perform the general histology. The periodic acid–Schiff (PAS) reaction for LM (semi-thin sections) was also used to reveal the presence of insoluble polysaccharides (Wędzony, 1996), and Sudan Black B was used to detect the presence of lipids and cuticle material (Jensen, 1962).

Additionally, material that had been embedded in Technovit 7100 (Kulzer, Germany) was also examined. This material was fixed (as above), washed three times in a 0.1 M sodium cacodylate buffer, dehydrated in a graded ethanol series for 15 min at each concentration and kept overnight in absolute ethanol. Next, the samples were infiltrated for 1 h each in 3:1, 1:1 and 1:3 (v/v) mixtures of absolute ethanol and Technovit and then stored for 12 h in pure Technovit. The resin was polymerized by adding a hardener. The material was sectioned to 5 μm thickness using a rotary microtome (Microm, Adamas Instrumenten), stained with 0.1% toluidine blue O and mounted in DPX (Sigma-Aldrich). The selected Technovit sections were stained with naphthol blue black (NBB) for total protein staining (Fisher, 1968; Mathe and Vieillescazes, 2002) or the PAS reaction was performed to visualize the starches (Wędzony, 1996).

In order to identify the main classes of the chemical compounds that are present in the trichomes, histochemical procedures with fresh or fixed flowers using Sudan III, Sudan Black B and Lugol’s solution were performed in order to detect the total lipids, starch grains and proteins (Johansen, 1940), respectively.

For SEM, the flowers were fixed (as above) and later dehydrated and critical point dried using CO2. They were then sputter-coated with gold and examined at an accelerating voltage of 75 kV. The specimens were obtained from GenBank (NCBI) to be the ingroup. For the outgroup, two Genlisea [G. aurea A.St.-Hil. (NC_037078.1) and G. violacea A.St.-Hil. (NC_037083.1)] and two Utricularia species [U. foliosa L. (KY025562.1) and U. reniformis A.St.-Hil. (NC_029719.2)] were used. The sequences were aligned using the online MAFFT v. 7.500 package (Katoh et al., 2019). All of the gaps were treated as missing. We used three approaches to create the phylogenetic reconstructions: Bayesian inference (BI), maximum likelihood (ML) and maximum parsimony (MP). BI was determined using Mr Bayes v. 3.2.7a (Ronquist et al., 2012) under the CIPRES Science Gateway v. 3.3 (Miller et al., 2010). For BI, 2 × 10^6 generations were calculated using two runs with four chains until the standard deviation reached a value <0.01. In each run, the trees were sampled every 100 generations at a sample frequency of 100. The first 25% of the trees that were initially produced were discarded as burn-in. The BI was conducted using the GTR + G model and was calculated using MrModeltest v. 2.4 software (Nylander, 2004) following the Akaike information criterion (Akaike, 1973). ML was determined using the online IQ-TREE v. 1.6.12 (Nguyen et al., 2015) and the obtained branch supports with the ultrafast bootstrap (10 000 replicates) (Hoang et al., 2018). For the MP analyses, PAUP* v. 4.0a (build 166) program (Swofford, 2002) was used under the CIPRES Science Gateway v. 3.3 (Miller et al., 2010) to obtain the bootstrap values (2000 pseudoreplicates and a heuristic search with 1000 replicates with the random addition of sequences and the branch swapping algorithm TBR). The trees that were obtained were edited using FigTree v. 1.4.3 (Rambaut, 2016). To optimize the pollinators/syndromes on the tree, we used the BI tree, and the pollinators were plotted according to published studies (listed in Table 1). The pollinator silhouettes used in Fig. 4 were designed using Freepik (https://www.freepik.com).

**RESULTS**

In our study, we observed various types of multicellular non-glandular trichomes, which differed in terms of their micromorphology (see Supplementary data Table S1). The trichome cells were highly vacuolated (Fig. 2A, B) and contained a peripheral cytoplasm with organelles such as a nucleus, mitochondria, plastids and an endoplasmic reticulum (Fig. 2C). Intranuclear paracrystalline bodies occurred in the nuclei (Fig. 2B). Staining with NBB revealed that these consisted of proteins (Fig. 2B). Some trichome cells had visible cuticular striations (Fig. 2D–F), while others had a smooth surface (Fig. 2F). The PAS reaction and Lugol’s staining revealed amyloplants with starch grains in the cells of the trichomes of the species from the subgenus Temnoceras: P. agnata, P. albida, P. ibarvae, P. martinezii, P. filifolia and P. gigantea (Fig. 3A–I and see ‘starch’ grade in Fig. 4). Starch grains were observed in these species independent of the type of trichomes (Supplementary data Table S1). Lugol’s staining did not reveal any amyloplants with starch grains in the cells of the trichomes of the species from the subgenus Pinguicula: P. alpina (Fig. 5A–C) and P. vulgaris (P. vulgaris subsp. vulgaris and P. vulgaris subsp. bicolor) (Fig. 5D–I) or the subgenus Isoloba: P. lusitanica (Fig. 5J–L). Moreover, this staining did not reveal any amyloplants with starch grains in the trichome cells of species from the subgenus Temnoceras, which is pollinated by butterflies [P. martinezii, P. esseriana, P. moranensis, P. emarginata and P. rectifolia;
We did not find the typical characters of glandular cells in the cells of the multicellular clavate trichomes. Therefore, we agree with Casper (1966, 2019) that these trichomes are non-glandular. We did show that the cells of the floral non-glandular trichomes of P. agnata, P. albida, P. ibarrae, P. martinezii, P. filifolia and P. gigantea were rich in amyloplasts that contained starch. Thus, these peculiar trichomes contain food reserves and probably function as edible trichomes. In orchids, edible trichomes (including pseudopollen-forming trichomes) are formed for a specific pollinator group, i.e. bees (Pansarin and Maciel, 2017). Thus, it is clear that in Pinguicula starch contained trichomes are recorded in species pollinated by bees, as showed in the ‘starch’ grade by the phylogenetical hypothesis (Fig. 4). Therefore, the lack of starch in the trichomes in the ‘psycho’ clade is a secondary loss, considering that P. alpina, P. lusitanica and P. vulgaris also did not present this character (Fig. 4). Pinguicula
mesophylica is not represented in the tree but is a sister species to *P. moranensis* based on internal transcribed spacer (ITS) rDNA according to Shimai et al. (2007). Thus, pollination by birds is perhaps homoplastic in the *Pinguicula* species considering the known or supposed ornithophilic species (*P. hemiepiphytica*, *P. laueana* and *P. mesophylica*; Lampard et al., 2016; Roccia et al., 2016).

Interestingly, not all myophilic and melittophylic species had starch in these trichomes, which enabled us to infer that these traits are not a condition for those pollination syndromes. Moreover, we did not record food reserves in the trichomes of *P. alpina* and *P. vulgaris*, which are pollinated by bees and flies (Molau, 1993; Fleischmann, 2016). Fleischmann (2016) observed various dipterans dabbing at the yellow spots on the otherwise white corolla of *P. alpina* and on the white corolla marks on the violet corolla of *P. vulgaris* and *P. leptoceras* with their proboscis. He interpreted this behaviour as the insects trying to find nectar and pollen, and, therefore, in these species, the trichomes may guide insects to the spur. However, we do not agree with Fleischmann (2016) that they play the role of ‘feeding hairs’ in *P. alpina* and *P. vulgaris* because we did not find any reserve material in these trichomes. For this reason, these trichomes may play a tactile role and act as guides or they might mimic the edible trichomes of other species.

Most researchers accept that in *Pinguicula* the reward for pollinators is generally nectar because of the occurrence of a spur with glandular trichomes (Fleischmann and Roccia, 2018; Lustofin et al. 2019); however, actual observations of nectar secretion and nectar analysis are rare (Zamora, 1999; Abrahamczyk et al. 2017; Lustofin et al. 2019). Although edible trichomes may act as a reward in addition to nectar, a detailed study of nectar production and secretion in *Pinguicula* is required to be absolutely certain that all *Pinguicula* species...
produce nectar and in what quantities. In the related genera *Utricularia* (Hobbahn et al., 2006; Clivati et al., 2014; Plachno et al., 2017, 2018, 2019a, b) and *Genlisea* (Aranguren et al. 2018), the reward for pollinators is nectar. However, in some species (*U. antennifera*, *U. capilliflora*, *U. dunlopii*, *U. dunstaniae* and *U. lowriei*), the spur is significantly reduced and the corolla forms filiform appendages (Taylor, 1989; Reut and Jobson, 2010). In *U. dunlopii*, the glandular trichomes (osmophores) are densely distributed on the modified floral appendages, and therefore their scent is most probably the attractant for visiting insects (Plachno et al., 2016). Although there are yellow non-glandular trichomes in the flower throats of *U. multifida* and *U. tenella*, they do not play the role of edible trichomes (Plachno et al., 2019a).

In orchids, the edible trichome cells (including the pseudopollen, which is formed by the disintegration of the trichomes) contain various types of food material (see Davies, 2009 and references therein). The main food material that is found in the edible trichome of orchids in the species from the *Maxillaria* genus is protein (Davies, 2009). Starch grains were recorded in the cells of the trichomes in the species from the genera *Dendrobium* (Davies and Turner, 2004), *Cyanaeorchis* (Pansarin and Maciel, 2017), *Polystachya* (Davies et al., 2002) and *Maxillaria* (Davies, 2009). Lipid droplets were recorded in the edible trichomes of *Cyanaeorchis* (Pansarin and Maciel, 2017). Thus, the edible trichomes of orchids are more diverse in the types of food material compared with *Pinguicula*.
From a phylogenetic perspective, edible trichomes are symplesiomorphic for the *Pinguicula* species and are found in the species of the ‘starch’ grade (Fig. 4), and therefore this does not imply a convergent evolution. However, this does not mean that the trichomes are all used for the same purpose. For example, in *P. moctezumae*, the trichomes are used to capture insects and other small prey. In *P. esseriana*, the trichomes are used to trap small mammal prey. In *P. moranensis*, the trichomes are used to trap small reptile prey. In *P. vulgaris*, the trichomes are used to trap small amphibian prey. In *P. rectifolia*, the trichomes are used to trap small fish prey.

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not support a monophyletic group such as a synapomorphy. However, the edible trichomes are derived and are possibly a specialization for fly and bee pollinators that act as a food reward for these visitors.

Field observations are needed to answer the question of whether insects consume ‘starch’ trichomes of *Pinguicula* flowers and thus whether these structures can be regarded as pollinators’ rewards. Checking if there is a correlation between flowers and thus whether insects consume ‘starch’ trichomes of *P. ibarrae*, *P. gigantea*, *P. filifolia* and *P. gigantea* which are pollinated by other pollinator groups (Lepidoptera and hummingbirds), the non-glandular trichomes in the flowers play a role other than being a floral reward for their pollinators. However, even with a phylogenetic perspective, the gaps in knowledge are wide for several species, which does not permit a robust hypothesis. Thus, only when field studies have been undertaken can we be absolutely certain of the role of these trichomes.

**Conclusion**

Floral non-glandular trichomes play the role of edible trichomes in some *Pinguicula* species (*P. agnata*, *P. albida*, *P. ibarrae*, *P. martinezii*, *P. filifolia* and *P. gigantea*), which are primarily classified as bee-pollinated species that originated from Central and South America. It seems that in *Pinguicula* that are pollinated by other pollinator groups (Lepidoptera and hummingbirds), the non-glandular trichomes in the flowers play a role other than being a floral reward for their pollinators. However, even with a phylogenetic perspective, the gaps in knowledge are wide for several species, which does not permit a robust hypothesis. Thus, only when field studies have been undertaken can we be absolutely certain of the role of these trichomes.

**SUPPLEMENTARY DATA**

Supplementary data are available online at [https://academic.oup.com/aob](https://academic.oup.com/aob) and consist of Table S1: micromorphology and histochemistry analyses of the food material content in various type of the *Pinguicula* flower non-glandular trichomes.

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