An intimate view of the leaf surface of *Pinguicula warijia*

Martín Mata-Rosas • Instituto de Ecología, A.C., Red Manejo Biotecnológico de Recursos • Carretera antigua a Coatepec 351 • El Haya • Xalapa • Ver. México 91073 • martin.mata@inecol.mx
Greta Rosas-Saito • Instituto de Ecología, A.C., Red Estudios Moleculares Avanzados • Carretera antigua a Coatepec 351 • El Haya • Xalapa • Ver. México 91073 • greta.rosas@inecol.mx
Sofía Gámez-Landaverde • Instituto de Ecología, A.C., Red Manejo Biotecnológico de Recursos • Carretera antigua a Coatepec 351 • El Haya • Xalapa • Ver. México 91073 • sgam.landa@gmail.com

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Abstract: Detail of the structure of the glands of *P. warijia*, a recently rediscovered and described endemic species of Mexico, is shown with the help of photomicrographs taken with scanning electron microscopes. Its three types of glands can be observed in detail: stalked, sessile, and hydathodes, as well as the architecture of the stomata.

Carnivorous plants have various adaptations to benefit from the capture and digestion of prey. In the genus *Pinguicula*, the most notable modification of their leaves is the presence of multiple glands on the leaf surface that gives them a glistening appearance. *Pinguicula* leaves bear two types of glands on the upper surfaces, stalked and sessile (Zamudio-Ruiz 2006). The stalked glands carry permanent mucilaginous droplets giving the characteristic greasy feel. The generic name is derived from the Latin pinguis-fatty or greasy to the touch. The gland secretions can trap and digest prey. The glands then absorb the products of digestion (Heslop-Harrison 2004). Previous studies have described in detail the anatomy of leaves and glands of some *Pinguicula* species (Heslop-Harrison & Knox 1971; Heslop-Harrison 1970, 2004; Vintéjoux & Shoar-Ghafari 2000). The species observed all have a structural similarity. In the present study, we show the architecture of the leaves of *P. warijia* from photomicrographs taken with scanning electron microscopes (SEM).

Pinguicula warijia (Fig. 1), was rediscovered in 2022 and described by Zamudio *et al*. (2023). It is endemic to the Sierra Obscura in the state of Chihuahua in a small area located on the walls of the Arroyo Babarocos Canyon. It thrives on calcium carbonate concretions accumulated on igneous rocks with continuous water runoff in an oak-pine forest and deciduous tropical forest at an altitude of 1590 m (Zamudio *et al*. 2023).

Materials and Methods

We collected leaves from plant specimens of *P. warijia* grown in a greenhouse, cut them into 1 cm pieces, and fixed them with 2.5% glutaraldehyde at 4°C for 24 h. The pieces were processed as described by Hermida-Montero *et al*. (2019). The samples were washed with Sorenson phosphate buffer three times for 1 min in each wash. Then, they were dehydrated in a graded series of ethanol,

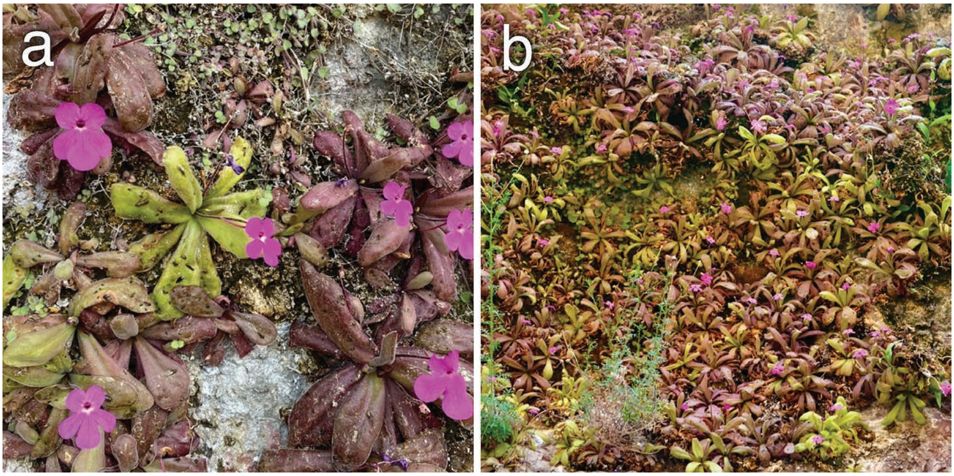

Figure 1: *Pinguicula warijia* is the most recently described species in Mexico, (a) the specimens may display different tones, from green to reddish, (b) it is possible to find many individuals in a very small area.

starting with gradual dehydration in ethanol from 30, 40, 50, 60, 70, 80 to 90% for 40 min in each solution. The last step was performed in 100% ethanol for 30 min thrice. The dehydrated samples were dried in a Quorum K850 critical point dryer and placed in aluminum specimens on double adhesive carbon conductive tape, and finally coated with gold in a Quorum Q150R S coater for 1 min (Bozzola & Russell 1992). The images were obtained and studied using a FEI Quanta 250 FEG scanning electron microscope (FEI Co., Brno, Czech Republic).

From eight images obtained with the SEM, a count of pedunculated and sessile glands and stomata was performed using Image-J software. Finally, we calculated the average and standard deviation of the two types of glands.

Results

Figure 2 shows the adaxial section of the leaf of *P. warijia*, where it is possible to observe the two types of glands, stalked and sessile, which are characteristic of all *Pinguicula* species. The sessile and stalked glands which are modified trichomes of epidermal origin and are distributed nearly evenly over most of the lamina (Heslop-Harrison 1970; Heslop-Harrison & Knox 1971).

Despite the stalked glands being less numerous than the sessile in all *Pinguicula* species, on *P. warijia*, 27 and 189 per mm^2, respectively (Table 1), they are still the most obvious to the naked eye. Stalked glands are specialized for insect capture through the secretion of translucent secretion droplets with adhesive properties (Heslop-Harrison 2004). According to the description of Heslop-Harrison and Knox (1971), a typical stalked gland consists of a large basal cell, replacing an epidermal cell, a stalk cell, and a columellar cell supporting the glandular head. The basal cell is thicker walled than adjacent epidermal cells, giving the mechanical strength required to support the secretion droplet carried on the glandular head (Fig. 3a, b).

In *P. warijia*, as described by Heslop-Harrison (2004) for *P. vulgaris*, each gland head consists of up to 16 secretory cells radiating from and supported on a single “endodermal” cell (Fig. 3b, c).

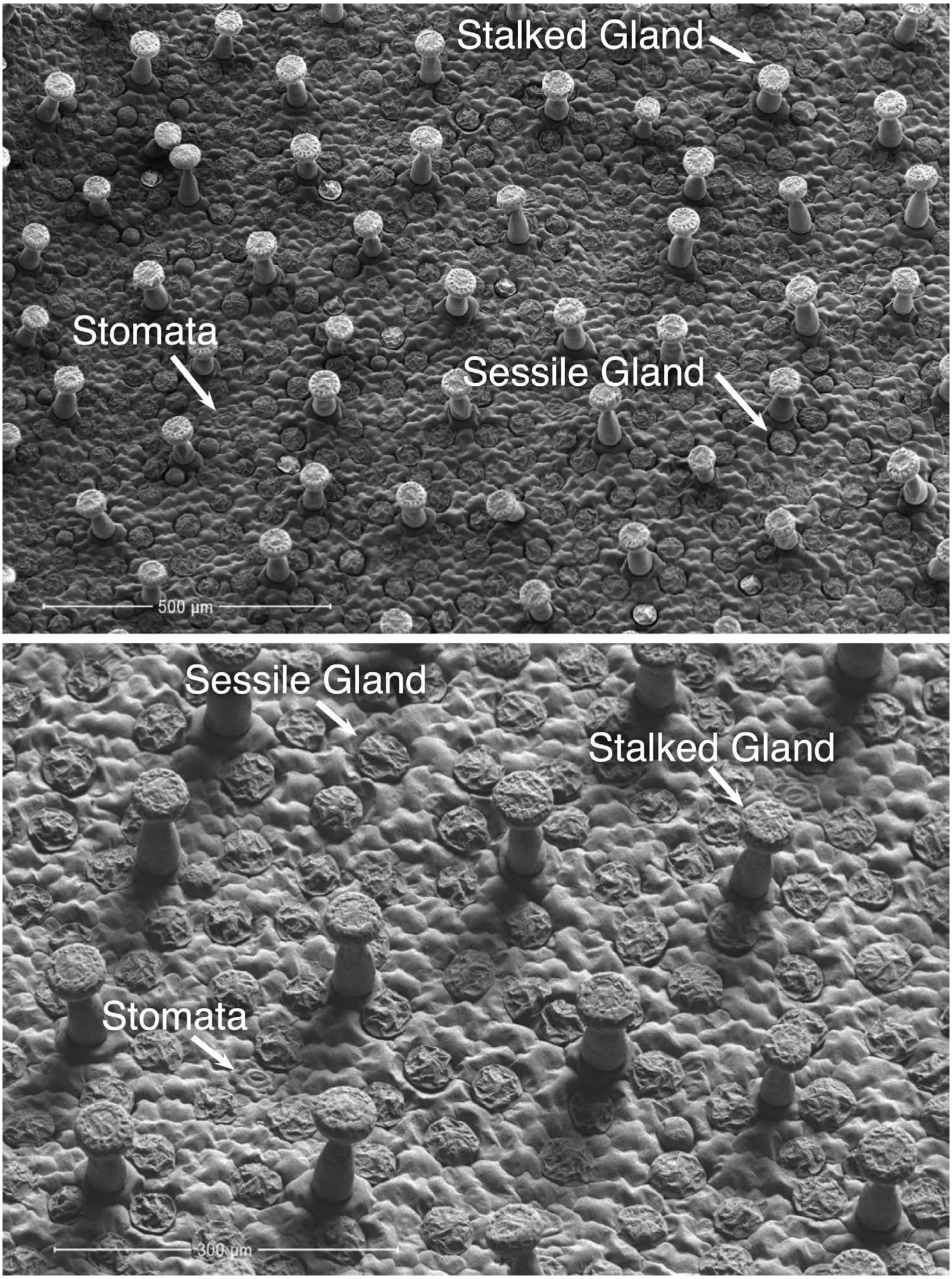

Figure 2: Adaxial views of the leaf of *Pinguicula warijia*. Both stalked and sessile glands and some stomata are visible.

Table 1. The number of glands (stalked and sessile) and stomata on the upper side of *Pinguicula warijia* leaves per mm^2.

Stalked glands per mm^2	Sessile glands per mm^2	Stomata per mm^2
26.9±5.7	189.2±32.6	8.1±1.8

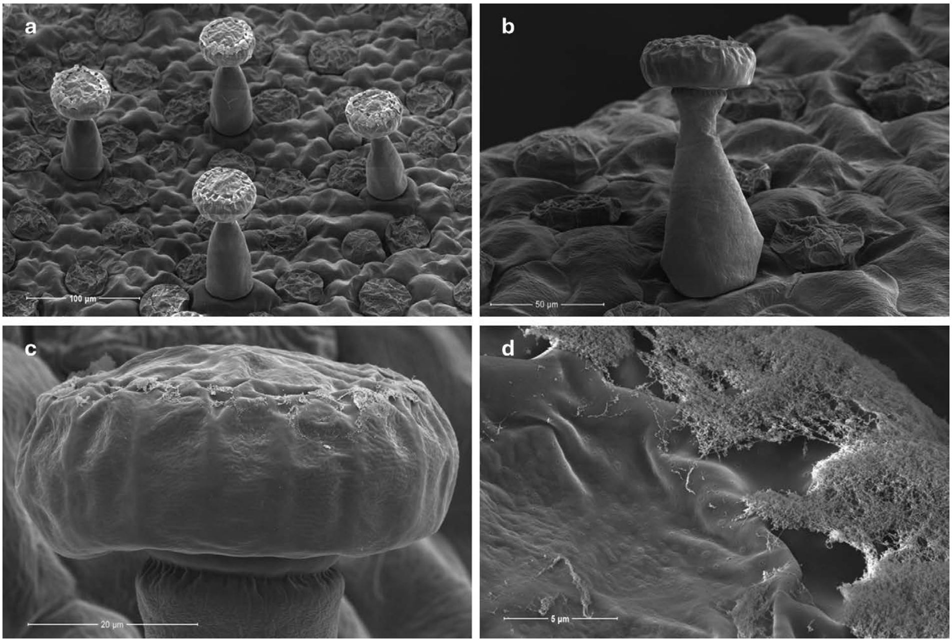

Figure 3: Detail of stalked gland: (a) a group of four stalked glands, (b) the head of the stalked gland is supported by a large basal cell, (c) the glandular head consists of 16 secretory cells in a radial formation, (d) multiple pores are distributed on the surface of the glandular head and remains of the secretion are visible on the periphery of the gland.

Heslop-Harrison and Knox (1971) mentioned that the analyses of the contents of the secretion are not available for *Pinguicula*; however, they mentioned that Schnepf (1963) reported *Drosophyllum*, which secretion is quite similar to that of *Pinguicula*, that secretion is a polysaccharide which yielded galactose, arabinose, xylose, and rhamnose on hydrolysis; ascorbic acid and rather unusually gluconic acid were also found to be present (Fig. 3d).

At the level of the leaf blade, numerous sessile glands can be seen, surrounded by epidermal cells and stomata; in our study, we found 8.1 stomata per mm^2 in the adaxial surface of *P. warijia* leaf (Fig. 4a), unlike Heslop-Harrison (2004), reports for *P. vulgaris* about 40 stomata per mm^2 in both surface of the leaf. The head of the glands is made up of eight cells (Fig. 4b), and the epidermal cells surrounding the sessile gland are slightly depressed (Fig. 4a, b); Heslop-Harrison (2004) indicated that the sessile glands consist of a basal cell, sunken slightly below the level of the epidermis, and a columellar cell supporting the capital of 2–8 cells and that the depression of the epidermal cells allows the head cells will be immersed very readily in fluid droplets formed on the leaf surface.

In the unstimulated leaf, the sessile glands do not secrete the quantity of fluid found on the stalked glands. However, they retain a tiny droplet in a central position.

Numerous hydathodes and stomata are observed on the abaxial surface of the leaf (Fig 4c, d). These glands secrete excess water from the plant (guttation) and have also been reported to secrete phosphatases (Nedoma *et al*. 2003; Płachno *et al.* 2006).

Heslop-Harrison (2004) mentioned that *Pinguicula* have active traps because the leaf margins enroll laterally upon stimulation by insect prey, this is possible due to the cells of the epidermis having a zigzag-shaped cell wall that allows the blade to bend when stimulated by prey without causing damage (Fig. 5a). Also, Heslop-Harrison stated that the cuticle on the outer wall of the epidermis is very thin and can also accommodate to the bending, and the stomata are supported on raised arches of epidermal cells (Fig. 5b), a further adaptation to curling without tearing.

We show and describe the morphology of *P. warijia* leaf, but how do the plants digest their prey? As already mentioned, the two types of glands they have, the pedicellate and the sessile, have specific functions that allow them to trap and digest prey and then absorb the products of digestion (Heslop-Harrison & Knox 1971; Heslop-Harrison 1978; Heslop-Harrison & Heslop-Harrison 1980, 1981). The main function of the pedicle glands is to secrete a sticky mucilage (trapping mucilage) that attracts and traps prey. It is mainly made up of polysaccharides and also produces phosphatases, although to a lesser degree than sessile glands (Płachno *et al*. 2006).

The transformation of organic phosphorus compounds (Po) to soluble inorganic phosphorus (Pi) is called mineralization and is carried out by a group of enzymes known as phosphatases. It has

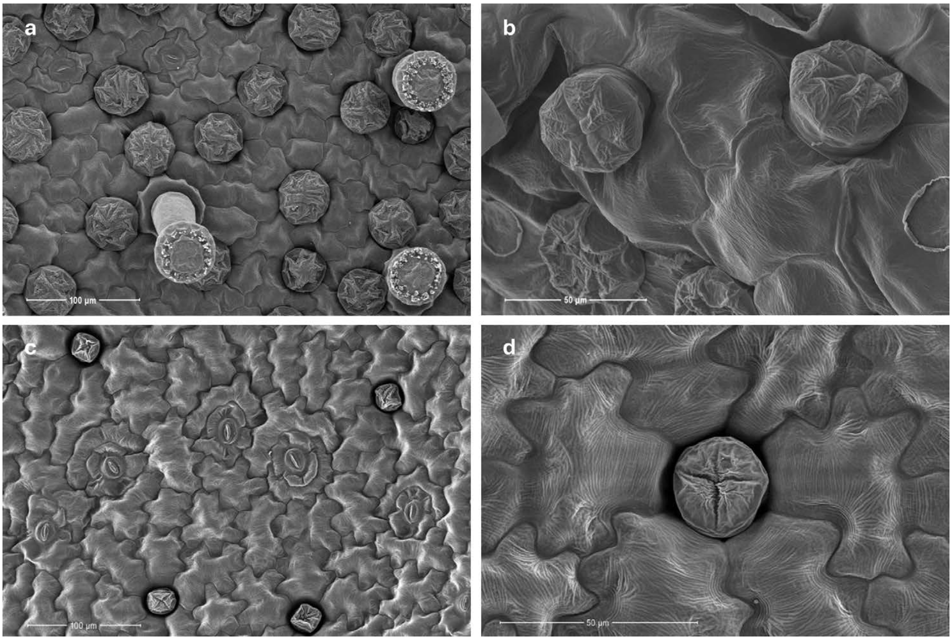

Figure 4: (a) The multiple sessile glands are surrounded by epidermal cells and stomata, (b) the sessile glands are made up of eight cells, (c) the abaxial leaf surface has multiple stomata and hydathodes, and the cells of the epidermis have a zigzag-shaped cell wall that allows the blade to bend without causing damage, (d) the primary function of hydathodes is to exude excess water from the plant.

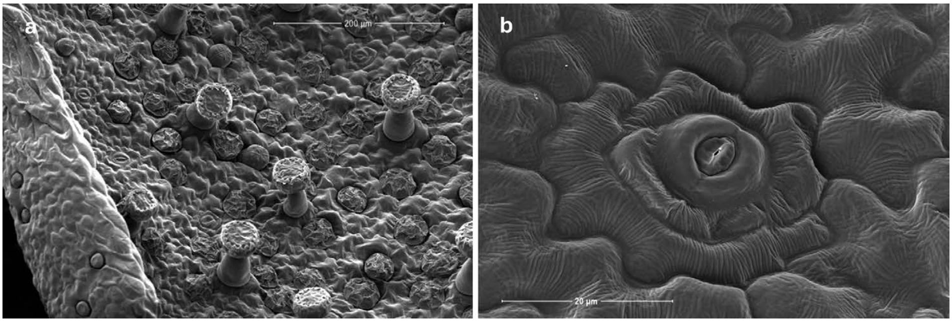

Figure 5: (a) Leaf margins can enroll laterally upon stimulation by insect prey due to the epidermis cells zigzag-shaped cell wall, which allows the blade to bend when stimulated by prey without causing damage, (b) the stomata and surrounding cells are also adapted to curling without tearing.

been recorded that the sessile glands have an intensive phosphatase activity (Płachno *et al*. 2006) and *Pinguicula*, as many other carnivorous plant species, must possess other enzyme classes that contribute to degrading the body of insects. For this purpose, they can utilize a wide range of prey-derived small and large molecules (Freund *et al*. 2022).

Heslop-Harrison and Heslop-Harrison (1981) mention that the enzymes are secreted up to 10 minutes after they were stimulated, that the secretion can continue for up to two hours, and that the glands only function once; that is, they cannot synthesize and store the enzymes again.

All these modifications presented by *Pinguicula* species confirm that the capture and digestion of prey are important for the development of plants; it has been mentioned that "Animals are an additional source of N, P, S, K, and Mg for carnivorous plants; some can even take up more than 50% of their N and P from prey" (Lollar *et al*.,1971; Friday & Quarmby 1994; Adamec 1997; Płachno *et al.* 2006).

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