

CONTRIBUTIONS TO THE KNOWLEDGE OF THE MORPHO-ANATOMY OF THE CARNIVOROUS PLANTS *Darlingtonia californica* AND *Cephalotus follicularis*

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Abstract

This paper focuses on the morpho-anatomical characteristics of the carnivorous plants Darlingtonia californica and Cephalotus follicularis, which, although not native species, provide valuable information for multiple scientific and practical fields. The analysis includes both the morphological examination of the vegetative organs and the anatomical structure revealed through transverse sections taken from different regions of these organs. Although they originate from completely different families and continents, these plant species have evolved traps that are functionally similar but structurally distinct—both internally and externally—as adaptations to extreme environments. Their study contributes to understanding the relationship between form and function, ecological and physiological adaptations, and the development of conservation strategies. It was also examined the current literature regarding their importance and methods of propagation, particularly since Darlingtonia californica is known for its richness in compounds with potential pharmaceutical and medical applications, while also having a restricted natural habitat. In this context, future research aims to develop conservation methods, cultivation techniques adapted to local climate conditions, and even micropropagation approaches to support the maintenance of existing populations.

Key words: carnivorous plants, *Cephalotus follicularis*, *Darlingtonia californica*, trap structures.

INTRODUCTION

Carnivorous plants live in nutrient-poor environments, which is why they have developed complex food capture mechanisms (leaves modified into traps, special digestive secretions, and even rapid movements to catch prey) that allow them to survive and develop in these habitats.

Darlingtonia californica (cobra lily) lives in damp swamps, cold springs in California and Oregon, USA, while *Cephalotus follicularis* (Australian pitcher plant) lives on the southwest coast of Australia, with sandy soils, humid environments.

Darlingtonia californica has been listed as an endangered plant by the Smithsonian Institution since 1975 (Family Lists of Candidate Endangered and Threatened Plant Species in the Continental United States); it is on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species/ the IUCN Red List or Red Data Book (Schnell et al., 2000).

It was discovered by botanist William D. Brackenridge in 1841 and described by John Torrey in 1853. *D. californica* is a carnivorous plant that is difficult to grow and propagate outside its natural range.

The roots develop in a cooler environment than the rest of the plant.

However, some authors have demonstrated that these plants are able to grow on nutrient media rich in mineral composition, at a temperature of $24 \pm 1^\circ\text{C}$ and do not require low positive temperatures for the root system (Erst et al., 2022).

Morphological and anatomical studies confirm the high level of ecological adaptation of this species to extreme conditions.

It is known that if the geochemical situation meets the requirements of plants, then the influence of the genetic factor is reflected mainly in the elementary chemical composition. At the same time, a genotypic program for the absorption of chemical elements is carried out and qualitative and quantitative regulations for the saturation of tissues with ions are maintained

(Ilyin & Yudanov, 1989; Ilyin & Syso, 2001 cited by Erst et al., 2022).

Brittnacher (2014) published methods for cultivating this plant, including its cumbersome propagation by seeds, and concluded that the best way to propagate is through stolons.

Lyons (2023) has successfully grown *D. californica* by maintaining an optimal root zone temperature (~10°C) using semi-hydroponic floodplain culture, incorporating a chiller that cools the water to the desired temperature as it circulates through the floodplain system. There is a chance that the method can be tested so that it can be successfully grown in more varied climates.

Although initially thought to not produce digestive enzymes, decomposition of captured insects relies on symbiotic bacteria and protozoa; later studies (Koller-Peroutka et al., 2019) have shown that *Darlingtonia* does possess proteases.

Meindl & Mesler (2011) demonstrated that *D. californica* is melitophilous, observing that the bee *Andrena nigrihirta* pollinated its flowers at five sites in northern California, while other regular floral visitors included thrips and several species of spiders.

Miclea (2022) conducted a review aimed at providing further support for the knowledge that secondary metabolites of plants from species belonging to the Sarraceniaceae family are useful in the fight against various diseases and emphasizes the need for further investigations into their biochemistry and the production of pharmaceutically and commercially valuable compounds from their tissues. The Sarraceniaceae family of the order Ericales is a family of carnivorous plants native to the American continent. Currently, the three genera that make up this family are *Darlingtonia*, *Heliophora* and *Sarracenia*.

They are rich in compounds with potential pharmaceutical and medical uses. They belong to several classes such as flavonoids, with flavonol glycosides being the most abundant, monoterpenes, triterpenes, sesquiterpenes, fatty acids, alkaloids and others. Some of them are well characterized in terms of chemical properties and biological activity and have wide commercial applications.

Plants belonging to the Sarraceniaceae family have a limited natural habitat (*Darlingtonia*

californica, *Heliophora* sp.) or are endangered (*S. alabamensis*, *S. jonesii*, *S. oreophila*) due to habitat destruction or excessive collection.

Cephalotus foliularis is the only genus and species in the Cephalotaceae family (Conran, 2004). It is one of the most desirable carnivorous plants in culture, prized by collectors, cultivated and sold worldwide.

Recently, the genome of *C. foliularis* has been sequenced, and analysis of carnivorous vs. non-carnivorous leaves has identified genetic changes associated with carnivory (Fukushima et al., 2017). This shows how the plant has reused stress-related genes for capture, digestion and absorption functions. The anatomy of the trap and the differentiation of the leaves (carnivorous vs. non-carnivorous) is a subject of interest.

This plant is listed as vulnerable by the IUCN (The International Union for Conservation of Nature), due to population decline (over-collection), highlighting the need for a better understanding of its ecology.

The endemic, insectivorous, monotypic family Cephalotaceae of Western Australia has been allied with Nepenthaceae, Sarraceniaceae, Saxifragaceae and Crassulaceae. Recent studies show a close relationship with the latter two families. An examination of the flavonoids in *C. foliularis* showed that the predominant flavonol profile more closely resembles the flavonoid profile of Saxifragaceae than that of Crassulaceae and supports morphological features that suggest it is most closely related to that family (Nicholls et al., 1985).

Pavlovic (2011) performed simultaneous comparative measurements of gas exchange and chlorophyll fluorescence in the two distinct leaf shapes. Stomatal density, an important component of gas exchange, and chlorophyll concentration were also determined. The trap had a lower net photosynthetic rate compared to the non-carnivorous leaf, while the respiration rate was not significantly different. This was in accordance with the lower stomatal density and chlorophyll concentration in the trap, the results obtained being consistent with the hypothesis that changing the leaf shape in carnivorous plants to make it a better trap generally makes it less efficient at photosynthesis.

Concerning the present work, it aims to pursue the morpho-anatomical description of the

carnivorous plants *Darlingtonia californica* and *Cephalotus follicularis* and highlight their importance for subsequently finding the best methods of multiplication.

MATERIALS AND METHODS

For the morphoanatomical study, fresh material belonging to the carnivorous plants *Darlingtonia californica* (Ericales: Sarraceniaceae) and *Cephalotus follicularis* (Oxalidales: Cephalotaceae) purchased online (https://heldros.com/en_US/basket) was used. They were morphologically described and anatomically analyzed by performing numerous cross-sections through the vegetative organ (Andrei, 2003), using the Motic Panthera S binocular microscope model available at the Faculty of Agriculture, Biology program within USAMV Bucharest. Also, for *Darlingtonia californica*, the stomatal index was determined to evaluate the plant's adaptation to the environment. The stomatal index represents the percentage value of the ratio between the number of stomata to the total number of epidermal cells, including stomata, per unit area (Ciulei et al., 1995).

RESULTS AND DISCUSSIONS

I. Morphoanatomy of the carnivorous plant *Darlingtonia californica*

The leaves of *Darlingtonia californica* (Figure 1) are tubular and contain a digestive fluid formed by water (absorbed from the soil and even resulting from the condensation created inside the urn), mucilage (produced by epidermal glands), products of digestion and symbiotic microorganisms that help with decomposition. This fluid prevents insects from getting out, facilitates the digestion of prey and possibly the absorption of nutrients.



Figure 1. *Darlingtonia californica*, general aspect

The upper part (Figure 2), dilated, has the shape of a cobra's head, with translucent areas similar to luminous windows (to confuse prey) and also in this area of the leaf there is a circular opening, preceded by a formation like a bifurcated appendix ("fishtail appendage") through which insects can enter.

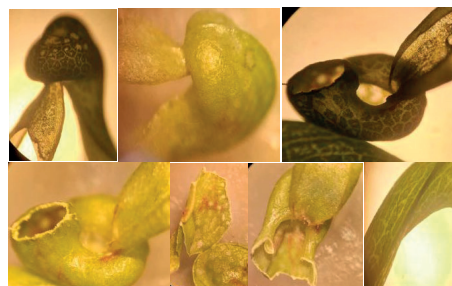


Figure 2. *D. californica*, upper part

The reddish areas observed in the head area are due to anthocyanin pigments in the cells (role in attracting insects), and the nectar glands, which secrete sweet nectar and attract insects, are present in the entrance area (in the external epidermis) but also on the bifurcated appendages.

Along the length of the tube there are downward-facing hairs that prevent insects from exiting the trap (Figure 3). At the bottom of the trap there are symbiotic microorganisms that ensure the decomposition of insects. The plant does not have digestive glands and does not produce digestive enzymes.

For a better understanding of the morphology of these leaves, we fragmented the traps into different areas: "tube base" – i.e. the small-diameter tube area, "under the head" – i.e. the large-diameter tube area, bifurcated appendage – the fin-shaped appendages in the head area and "head" – the dilated part of the tube.

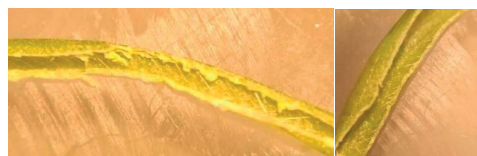


Figure 3. *D. californica*, downward-facing hairs

The cross-sections, both at the "base" and in the "under the head" areas and bifurcated appendix, show two epidermises (external protected by the cuticle and internal), modified mesophyll (with

photosynthetic parenchyma but also weakly pigmented cells), mechanical tissue (collenchyma and sclerenchyma) and conducting tissue (phloem and xylem). In the upper and middle area of the tube, elongated cells transformed into sharp bristles (Figure 4) similar to a saw with teeth facing downwards, for directing the insect (mechanical adaptation), are differentiated in the lower epidermis.

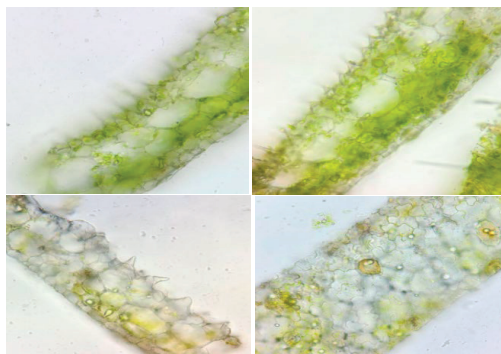


Figure 4. *D. californica*, cross sections: “under the cap” area (top) and “fishtail appendage” area (bottom)

At the base of the urn (Figure 5) the epidermal bristles are small, rounded with a thin external wall, which, possibly, reduce direct contact with cold water.

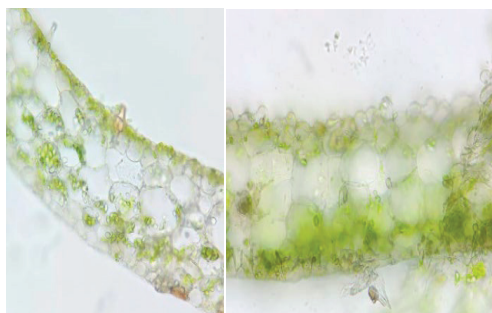


Figure 5. *D. californica*, cross sections: “tube base” area

The “head” area has a specialized internal structure, adapted to both photosynthesis and insect capture. The epidermal cells arranged like a puzzle are transformed, towards the inside of the trap, into short, unicellular, rigid mechanical hairs (Figure 6, arrow), oriented downwards, with a role in retaining the prey. The presence of stomata was observed especially on the external face (Figure 6).

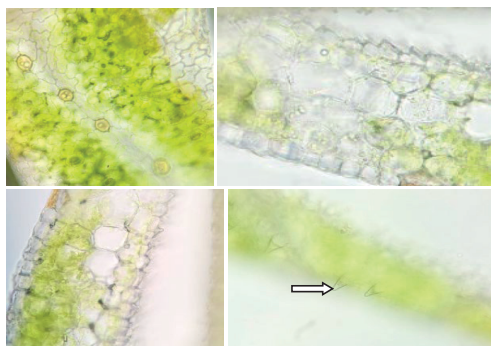


Figure 6. *D. californica*, cross sections: “head” area

The assimilatory tissue is rich in chloroplasts, but in the translucent areas the epidermis is thinner and the cells are poorly pigmented, thus creating the illusion of a window through which insects can exit. Nectar glands are present in the entrance area (in the external epidermis) but also on the bifurcated appendages.

The stomatal index was determined to be moderate (9.37%), indicating that the plant adopts a balanced physiological strategy compatible with its natural environment (cold, humid areas with nutrient-poor soils), a controlled gas exchange.

II. Morphoanatomy of the carnivorous plant *Cephalotus follicularis*

Cephalotus follicularis (Figure 7) is a small herbaceous plant. It has fibrous, thin roots in the soil, later short, thickened underground rhizomes (underground stems) appear, on which the two types of leaves are formed.



Figure 7. *Cephalotus follicularis*, general aspect

Unlike most carnivorous plants that develop a single type of leaf, *Cephalotus* has two very

different types of leaves: non-carnivorous leaves and pitcher-shaped trap leaves.

The non-carnivorous leaves (Figure 8), the first to appear, are simple, hairy, with an entire margin, bright green on the upper surface and dark purple on the dorsal side.



Figure 8. *C. follicularis* - non-carnivorous leaves

In cross-section they present (Figure 9) an internal structure typical of a dorsiventral leaf specialized exclusively for photosynthesis, as follows: upper epidermis formed by a layer of cells, covered by cuticle, without stomata; mesophyll with two rows of assimilatory/palisade tissue arranged towards the upper epidermis formed by elongated cells, rich in chloroplasts; lacunar tissue formed by cells with intercellular spaces; conducting fascicles surrounded by a fascicular sheath, with the wood arranged towards the upper epidermis and the free towards the lower epidermis; lower epidermis formed by a layer of cells and numerous stomata.

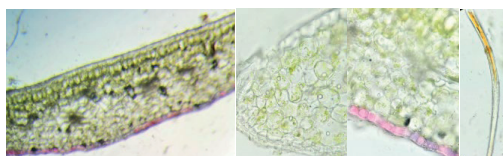


Figure 9. *C. follicularis*, cross-sections through non-carnivorous leaves

Numerous hairs are present on the leaf edge (Figure 10).

The trap leaves (Figure 10) are small, green with red-violet areas (more intense on the upper and inner side) and play a role in capturing and digesting insects.

The traps (small, 2-5 cm) are short, have on the outside three longitudinal, hairy ridges (Figure 11) that guide insects towards the mouth and a fourth, slightly highlighted one.

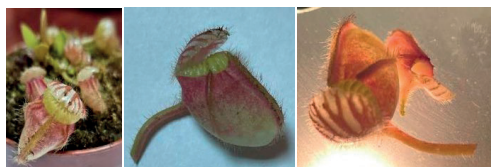


Figure 10. *C. follicularis*, pitfalls

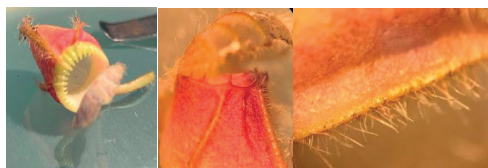


Figure 11. *C. follicularis*, hairy growths

They have a granular, rough, pigmented texture in order to attract insects.

There are three distinct macroscopic areas belonging to the urn of the carnivorous plant: the operculum or lid, the edge of the opening (peristome) and the urn with the waxy area and the digestive area.

Each of these areas has a specific function, with corresponding morpho-physiological characteristics.

The cap or operculum is attached a short distance above the peristome and covers the opening of the urn, preventing rain from filling the urn excessively, but still allowing light to enter. It is hairy (Figure 12, arrow), stiff, with white veins, in places with anthocyanin papillae. It has translucent areas to confuse insects.

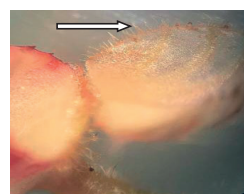


Figure 12. *Cephalotus follicularis*, lid (operculum)

The cap has polygonal epidermal cells, arranged like a puzzle, and downward-oriented bristles to guide prey to the opening of the trap (Figure 13).

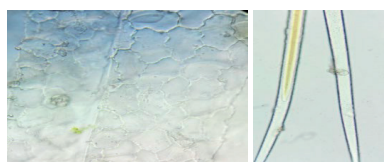


Figure 13. *C. follicularis*, cross-sections through the cap

In cross-section, the cap shows: upper epidermis with large cells, covered with a highly developed cuticle and few stomata, undifferentiated mesophyll clearly in assimilatory (reduced) and lacunar (large-celled) tissue, with few chloroplasts located in the subepidermal cells, lower epidermis formed by a layer of cells with a thin cuticle (Figure 14) and poorly developed and reduced number of conducting fascicles.

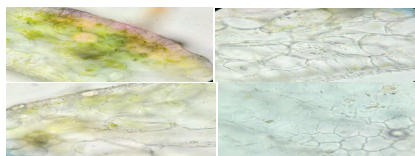


Figure 14. *C. follicularis*, cross section through the lid (operculum)

The peristome (upper edge) has the shape of a glossy, yellowish-white collar, slippery, lobed (large lobes in the shape of teeth arranged radially). The area below the teeth (Figure 15) with a spongy appearance is a nectar secretor that attracts insects, being formed by epidermal nectariferous glands with small, dense secretory cells, protected by a thin cuticle for easy release of nectar.

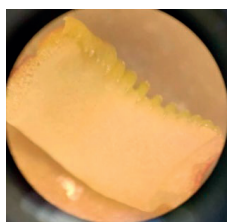


Figure 15. *C. follicularis*, peristome below the teeth

The lobes (Figure 16) curve over the edge of the urn (cause insects to slide inside) and end in a sharp tip facing downwards (prevent insects from exiting). The lateral and frontal lobes are well developed and those arranged posteriorly in the area where the lid covers the urn are small, short, crowded.



Figure 16. *C. follicularis*, the lobes

The urn has two areas: slippery and digestive. The upper part of the interior of the urn is smooth, slippery, formed by specialized cells that have a smooth, waxy cuticle to prevent insects from climbing. The mesophyll is reduced, without chloroplasts.

The lower part is glandular - the digestive zone (Figure 17), has a moist surface and epidermal cells with thin cell walls; here too there are golden-yellow digestive glands, which secrete enzymes and absorb products resulting from digestion.

Subepidermally, there is a homogeneous, compact tissue, formed by weakly pigmented cells with thin walls (Figure 18).

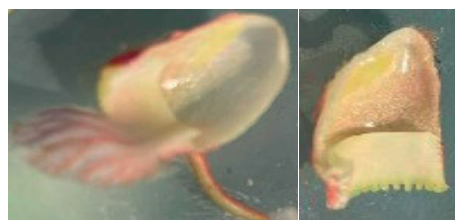


Figure 17. *C. follicularis*, internal surface of the trap

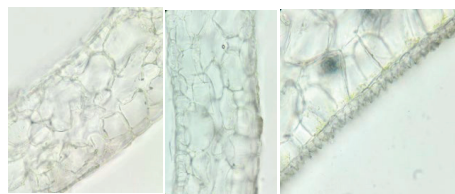


Figure 18. *C. follicularis*, cross section through the urn

CONCLUSIONS

The study of cross-sections made at the level of trap leaves highlighted morpho-anatomical peculiarities that reflect the high degree of specialization of carnivorous plants.

Darlingtonia californica has unique, tubular leaves (hollow tubes), with a widened upper part and a narrowed base, resembling a cobra's head. *Cephalotus follicularis*, unlike *Darlingtonia californica*, has two very different types of leaves: non-carnivorous leaves and carnivorous trap leaves.

The results obtained emphasize the importance of morpho-anatomical studies in understanding the adaptive mechanisms of carnivorous plants and provide useful data for future research on the relationship between anatomical structure,

digestive function and ecological strategies of these species.

The interest in carnivorous plants and substances with chemopreventive and therapeutic potential involves additional research on the chemical composition of the genus *Darlingtonia*, knowing the richness in compounds with potential pharmaceutical and medical uses.

Knowledge of these plants is of great importance, given that the manufacture of products intended for commercialization requires considerable quantities of plant material necessary for the pharmaceutical industry, and in vivo propagation methods or micropropagation can be a lifesaver in this regard.

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