

# HISTO-ANATOMICAL ASPECTS OF THE *NEPENTHES MAXIMA* REINW. EX NESS METAMORPHOSED LEAF

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In this paper the histo-anatomical aspects of the metamorphosed leaf of *Nepenthes maxima* were underlined. The pitchers are highly specialized organs for the attraction and capture of insects and absorption of nutrients from them. The basal region of the trap had large multicellular glands that developed from single epidermal cells. These glands were closely associated with underlying vascular traces and provided a mechanism for supplying fluid to closed immature pitchers. We especially examine the glandular cells and their developing stages during the ontogenesis.

*Key words:* *Nepenthes maxima*, pitcher, digestive glands, cuticle.

## INTRODUCTION

Tropical Pitcher Plants of the genus *Nepenthes* are among the most ornate and bizarre plants in the world – their carnivorous nature and peculiar shape have attracted the attention of botanists and horticulturalists since the Victorian Age. The genus *Nepenthes* is widespread in the Malaysian Region with outlying species occurring west to Madagascar and north to India. To date nearly 90 species are recognized, displaying a great diversity of forms.

Carnivore is an answer to the lack of nutrients. While plants get their energy from sunlight, air and water, they still need nitrogen and phosphor to grow. Usually these can be found in the soil but in some places they are so rare that it is hard for plants to grow. Some plants have opened up another source: living animals. With sticky leaves, quick closing traps or slippery pitchers they catch insects and digest them to get the precious nutrients.

All *Nepenthes* grow in extreme habitats poor of nutrients. Peat swamp and mountain rainforests or degraded, eroded areas are typical habitats for pitcher plants. Some are even pioneers growing on pure sand where the tropical forest has been destroyed. When soils are rich they are not strong in concurrence, and other plants are growing faster, *Nepenthes* use a passive method of attraction and entrapment (8, 13). Specifically, the traps are modified epiascidiolate leaves, in which the adaxial surface curls around and fuses to form the inner wall of the pitcher tube (8). The lip of the *Nepenthes* pitcher, a ridged double-edged collar called the peristome, contains nectar (5, 9). The nectar is particularly attractive to ants (Kato, 1993). Insects lose their footing while foraging for nectar and slip down the steep walls of the pitcher. They are trapped at the base in a fluid that has been reported to contain proteases and chitinases (1) that are presumably secreted by the plant although bacterial activity, or a combination of the two, is a possibility.

## MATERIAL AND METHODS

The studied material was grown in the greenhouses of Botanical Garden of University "Al. I. Cuza" Iași. The pitchers were collected in different stages of development, fixed in FAA (formic aldehyde: acetic acid: ethylic alcohol 5: 5: 90) and conserved in ethylic alcohol 70%. The sections (at the base, middle and top of the pitchers) were made with free hand using a razor blade and colored with red-ruthen and methyl-blue and mounted in Canada balsam. The photos were taken after the obtained permanent slides using a Novex (Holland) microscope and a Minolta photo camera.

## RESULTS AND DISCUSSIONS

The leaf of *Nepenthes* is formed from three parts: a basal one, breadth, and assimilator, a tendril and a pitcher. Each pitcher initiated as a tiny, flattened end of a tendril. The youngest pitchers were dark-brown in color but turned green during elongation. Mature pitchers developed a red color beneath the opening to above the bulbous base.

*The leaf basis:* shows, in transverse section, a bifacial dorsiventral structure. The upper epidermis is formed by small cells, tangentially elongated, and covered by a thick cuticle. A thinner cuticle than upper epidermis covers the lower epidermis. The stomata are present only in lower epidermis therefore the lamina is hypostomatic.

Under the upper epidermis, 3–4 layers of large, isodiametrical cells (aquiferous cells) could be observed. The palisade parenchyma is located under this aquiferous tissue and is formed by 2–3 layers of short cells. The spongy parenchyma is thick, formed by rounded cells with small aeriferous spaces between them. Isolate sclereids, with spiral thickness and calcium oxalate druses could be observed in the mesophyll (photo 1).

The midvein is prominent at the abaxial face of the leaf. The vascular bundles are numerous, disposed in circle. The xylem has different orientation (both from upper and from lower epidermis). One vascular bundle is formed from phloem (sieve tubes and companion cells) and xylem (vessels and parenchyma cells); a sclerenchyma sheath surrounds it. Sometimes, this sheath is common for two or more vascular bundle of different sizes (someone only with phloem).

*The tendril:* in the cross section has triangular shape, with rounded angles. Small cells, covered by a thick cuticle, form the epidermis. Their structure is similarly with that of the basal part of the leaf in the midvein region. The schlerenchyma is very well developed in concordance with the sustaining role of the tendril (photo 2).

Near the pitcher the tendril's shape become circular; the vascular bundles (each surrounded by a schlerenchyma sheath) are disposed in three rings.

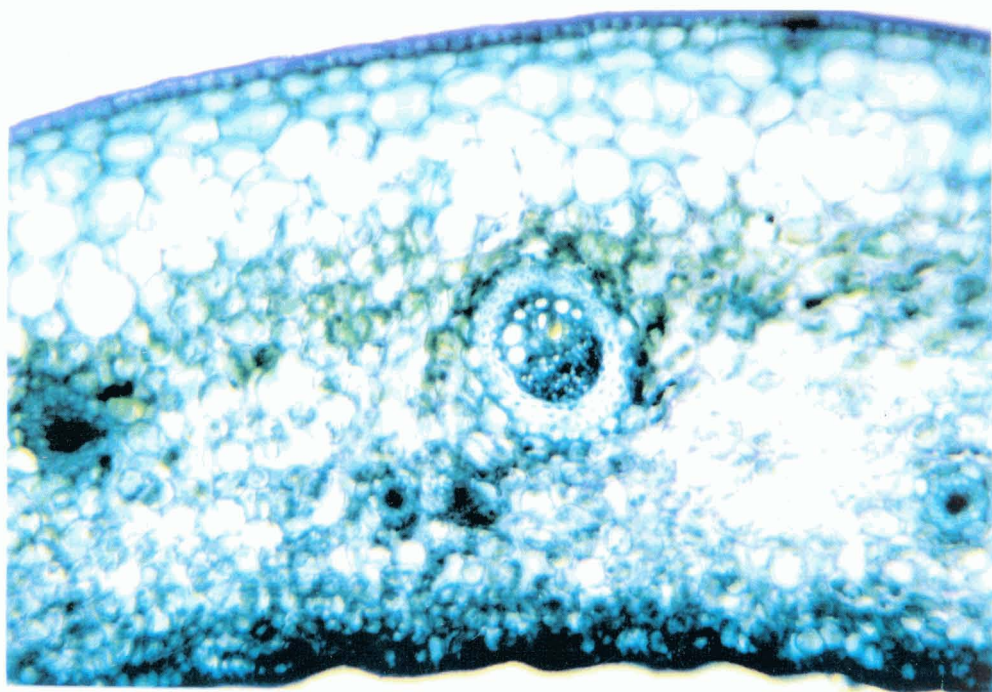


Photo 1 – Cross section from the leaf basis ( $\times 100$ ).

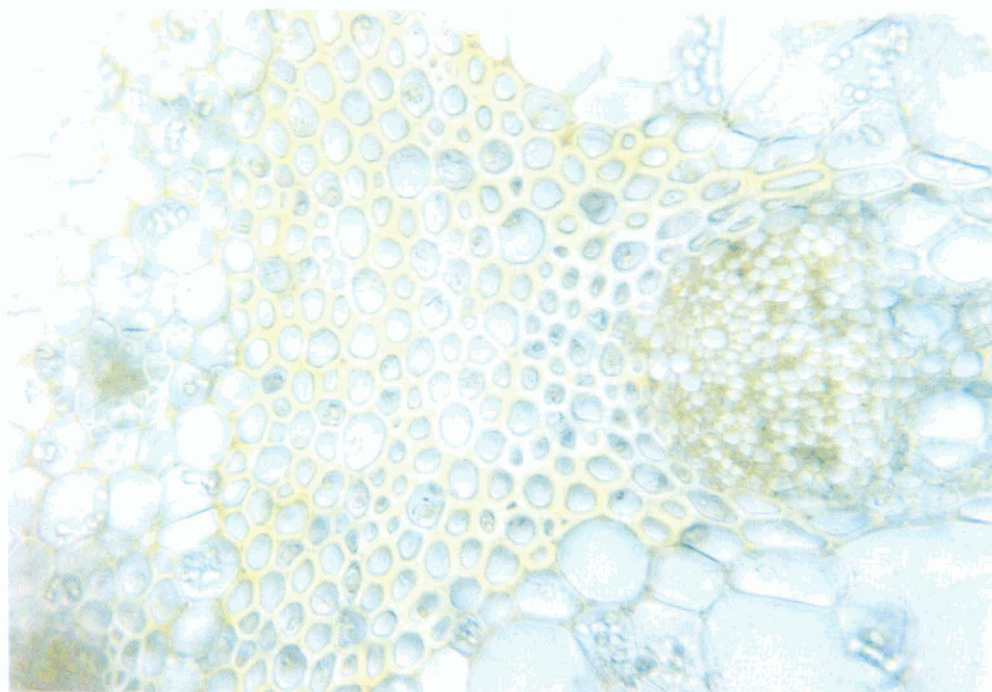


Photo 2 – Cross section from the tendril ( $\times 200$ ).

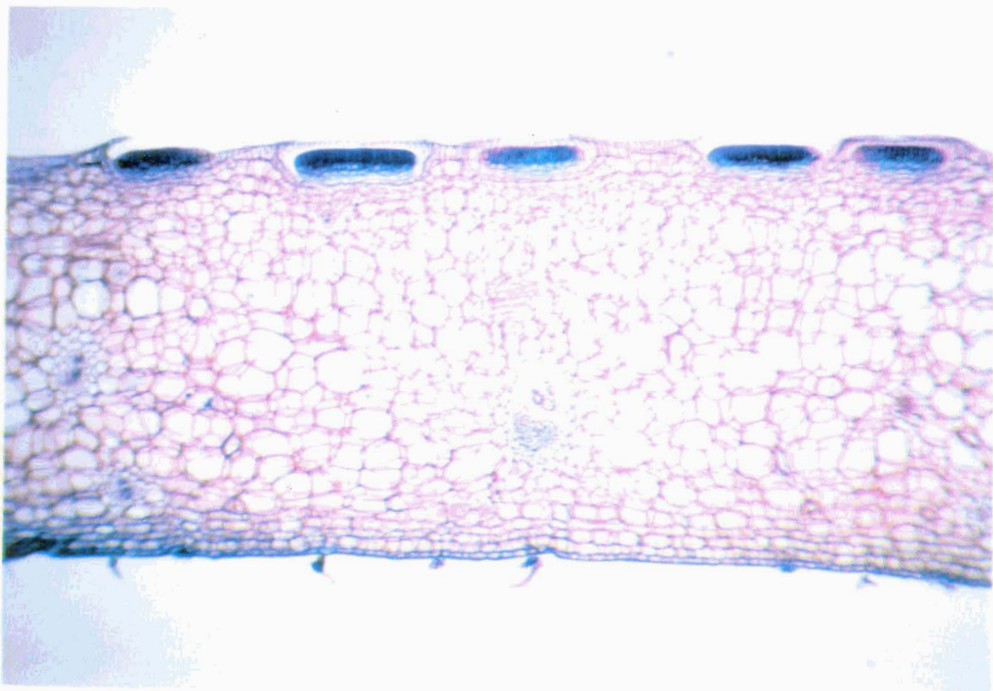


Photo 3 – Cross section from the basis of the mature pitcher ( $\times 100$ ).

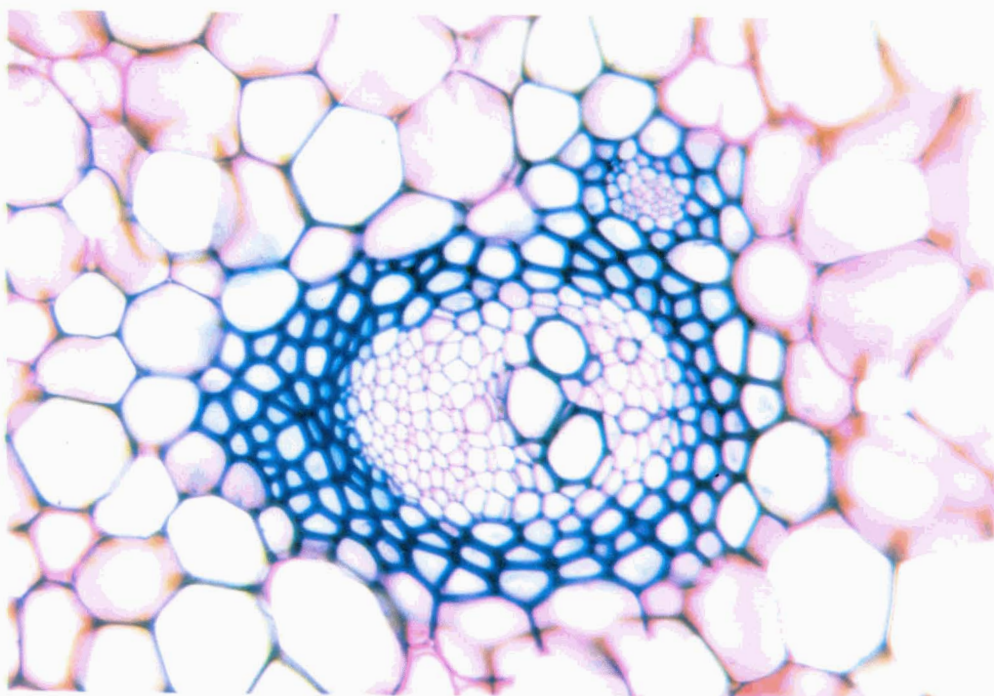


Photo 4 – Cross section from the basis of the mature pitcher; detail ( $\times 200$ ).

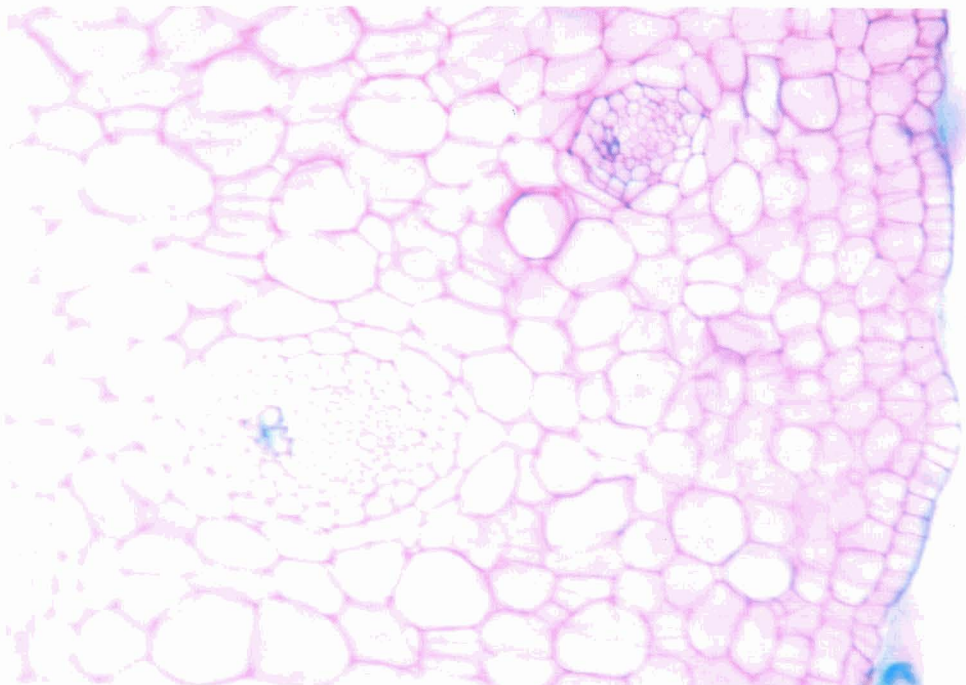


Photo 5 – Cross section from the young pitcher ( $\times 200$ ).

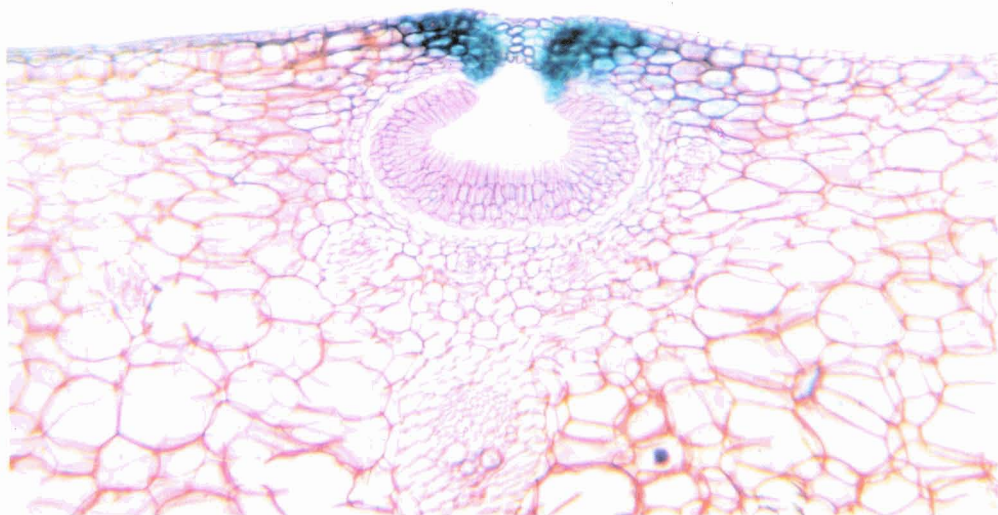


Photo 6 – Cross section from the middle zone of the mature pitcher ( $\times 200$ ).  
Development of the digestive glands.

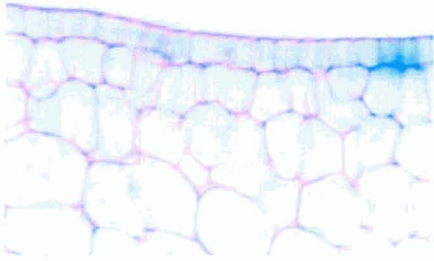


Photo 7 – Cross section from the top of the young pitcher ( $\times 200$ ).



Photo 8 – Cross section from the basis of the young pitcher ( $\times 200$ ).



Photo 9 – Cross section from the top of the mature pitcher ( $\times 200$ ).

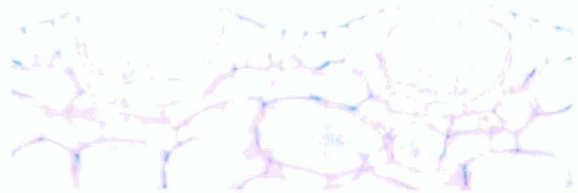


Photo 10 – Cross section from the top of the mature pitcher ( $\times 200$ ).

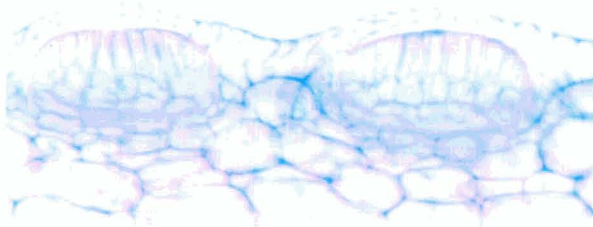


Photo 11 – Cross section from the middle zone of the mature pitcher ( $\times 200$ ).



Photo 12 – longitudinal section from the middle zone of the mature pitcher ( $\times 200$ ).



Photo 13 – Cross section from the basis of the mature pitcher (unstained section)( $\times 200$ ).



Photo 14 – Cross section from the basis of the mature pitcher (stained section)( $\times 200$ ).

*The pitchers* are highly modified leaves adapted to effectively attract, capture, and kill arthropods, then break down and absorb the nutrients there from. The structural features involved in carnivore, which were the focus of this study, included extrafloral nectar located at the apex of the pitcher, ridged cells covered by a dense layer of epicuticular waxy scales, a fluid reservoir, and large multicellular glands.

In transection the pitcher wall present a typical leaf structure (photo 3). Small cells covered by a thin cuticle form the external (lower) epidermis. Here and there stomata and pluricellular tector hairs could be observed. The mesophyll is thick, homogenous, formed by small cells at the external zone and big cells in the internal zone. A lot of calcium oxalate druses could be observed especially in this external zone.

The organization of the vascular bundles (photo 4), in a ring with the xylem oriented toward the pitcher lumen, indicated that the interior of the pitcher is the adaxial surface, typical of an episcidiate leaf. This supports Arber's (1941) assertion that the pitchers of the genus evolved through the enfolding of a leaf with the adaxial surface to the inside of the pitcher.

The superior part of the pitcher presents some peculiarities: the external (lower) epidermis has numerousness tector hairs; the thickness of the assimilatory parenchyma is reduced; schlerenchymatic sheath missing around the vascular bundles; some little fascicle contain only phloem; the internal (upper) epidermis is covered by a thin cuticle; the digestive glands are in incipient stage of development (they are formed by two cells layers and almost entirely covered by an epidermic fold).

At the basis of the young pitcher tector hairs from the external epidermis are very dense; the assimilatory parenchyma is thick, with a lot of idioblasts; in the xylem of vascular bundle the tracheogenesis process is visible.

In young pitcher a mechanical sheath (formed from cells with thin and ungnified wall) surrounds the vascular bundles (photo 5).

The extrafloral nectar in the peristome was a large teardrop-shaped gland that extended far back into the rim. Similar peristome nectar has also been reported in *N. rafflesiana* (12), *N. alata* (11) and *N. maxima* (8). There has been speculation that the nectar may interconnect to form a continuous glandular ring around the pitcher rim (8). However, we observed that parenchyma cells interspersed with vascular bundles separated the nectarines.

On the external part at the young and at the mature pitcher appears another type of nectariferous glands. In transactions they are massive, pluricellular structures, composed by three cell layers, which delimitate a point shaped opening (photo 6). These glands have the role to attract the prey (insects) towards the pitcher and in their length as far as the peristome.

*The digestive glands* (photos 7–14): In the smallest immature pitchers examined the epidermis had small cells that resembled meristemoids (photo 7). Larger traps had clusters of 4 to 5 cells wide that had divided once periclinally. This gave the appearance of placing the epidermis below a single row of protoglandular cells (photo 8). The gland become bigger and acquires the typical shape (photos 9, 10). The basal cells layers increase in number from 1 to 2 at young glands (photos 11–12). The mature glands consisted of three cell layers above a depression of cells continuous with the epidermis (photos 13–14).

The outermost layer had small columnar-shaped cells, while immediately underneath were densely cytoplasmic rectangular cells. The third cell layer of the gland had cubical cells that rested on a row of small vacuolated cells.

In transection the digestive glands appear covered by an epidermic layer (photo 11). In longitudinal section we can observe an epidermic fold (photo 12); the placement and orientation of this fold may prevent insects from using the glands or epidermal cavity as footholds for escape.

The gland cells generally had thicker cell walls as compared to the surrounding cells.

The digestive glands were closely associated with vascular bundles (14), and terminal ends of tracheids ended right beneath the gland bases.

## CONCLUSIONS

The leaf of *Nepenthes maxima* is profoundly metamorphosed in concordance to a particular type of nutrition of this species.

A tendril with a very well developed sclerenchymatic tissue sustains the pitcher.

The pitcher evolved through the enfolding of a leaf with the adaxial surface to the inside of the pitcher.

The extrafloral nectarines are located also in the peristome and at the external surface of the pitcher; they have the role to attract the prey through the peristome.

The digestive glands appear in on the internal part of the pitcher before these attend maturity. They are numerous in the basal zone of the mature pitchers. In this zone the cuticle is very thick and protect the plant tissues against autodigestion.

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