Structure and development of the pitchers from the carnivorous plant *Nepenthes alata* (Nepenthaceae)[1]

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The pitchers of the tropical carnivorous plant *Nepenthes alata* are highly specialized organs for the attraction and capture of insects and absorption of nutrients from them. This study examined the structure and development of these pitchers, with particular focus on the nectaries and digestive glands. Immature pitchers developed at the tips of tendrils and were tightly sealed by a lid structure that opened during the end of pitcher elongation. Opened pitchers exposed a ridged peristome containing large nectaries. Like other members of the genus, a thick coating of epicuticular waxy scales covered the upper one-third of the pitcher. Scattered within this zone were cells resembling a stomatal complex with a protruding ridge. Cross sections showed that this ridge was formed by asymmetric divisions of the epidermal cells and lacked an underlying pore. 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.

Key words: carnivory; epicuticular wax; glands; nectaries; *Nepenthaceae*; *Nepenthes*; pitcher plant; SEM.

Carnivorous plants have unique structural specializations for the procurement of insect-derived nutrients. As carefully examined by Darwin (1875), carnivorous plants attract and trap insects, then breakdown and absorb the by-products (reviewed in Juniper, Robins, and Joel, 1989). Surprisingly different trap morphologies have evolved in carnivorous plants for the capture of insects. These different forms include adhesive traps, suction traps, snap traps, and pitchers. Phylogenetic analysis of nucleotide sequences from the plastid *rbcL* gene indicates that carnivory and stereotyped trap forms arose independently in six angiosperm lineages (Albert, Williams, and Chase, 1992).

In contrast to the spectacular and well-known snap-traps of the Venus flytrap, pitcher plants use a passive method of attraction and entrapment (Slack, 1980; Juniper, Robins, and Joel, 1989). Specifically, the traps are modified epiascidiate leaves, in which the adaxial surface curls around and fuses to form the inner wall of the pitcher tube (Juniper, Robins, and Joel, 1989). The lip of the *Nepenthes* pitcher, a ridged double-edged collar called the peristome, contains nectaries (Hooker, 1859; Lloyd, 1942). The nectar is particularly attractive to ants (Kato, 1993). The upper pitcher region is frequently lined with an exfoliating epicuticular wax (Phillipps and Lamb, 1996) that creates a surface slippery to arthropods (Lloyd, 1942). 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 (Vines, 1901; Amagase, 1969, 1972a, b; Tokés, 1974) that are presumably secreted by the plant (Fig. 8.17C in Juniper, Robins, and Joel, 1989), although bacterial activity, or a combination of the two, is a possibility (Prankevicius and Cameron, 1991; Lennon, 1995).

While the *Nepenthes* trap structure has been superficially examined in early works (e.g., Hooker, 1859; Troll, 1932; Arber, 1941; Lloyd, 1942), morphological data on the development of the pitcher, especially on the secretory and absorptive glands, are lacking and are the focus of this study. Multicellular digestive glands of carnivorous plants are of considerable interest as model systems for plant development and membrane transport. The glands of *Nepenthes* may serve as pathways for short-distance transport between the pitcher fluid and the underlying wall tissue (Lüttge, 1965, 1971). These glands have been reported to secrete a diverse group of molecules, including proteolytic enzymes, organic acids, Cl^- and Na^+ ions, and water, and to absorb ions and low molecular mass solutes associated with the breakdown of insect prey (see Lüttge, 1971, for review). Many plant glands have the singular function of secretion, such as salt glands (Thomson, 1975), hydathodes, and nectaries (Fahn, 1979). However, the nectaries of some species can both secrete and reabsorb at least the sugar components of nectar (Pedersen, LeFevre, and Wiebe, 1958; Bieleski and Redgwell, 1980; Burquez and Corbet, 1991). Thus, glands that involve both secretion and uptake, while not unique in carnivorous plants, are remarkably more complex than single-function glands (Lüttge, 1971).
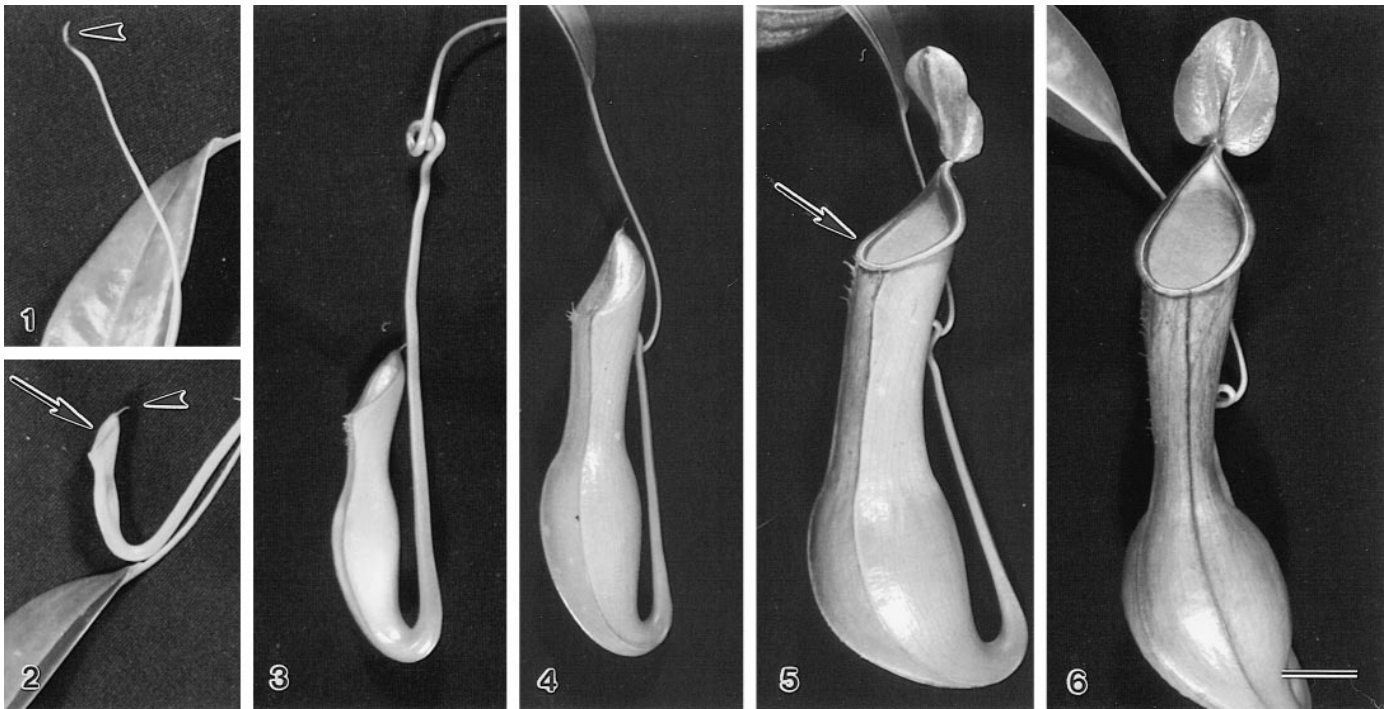
MATERIALS AND METHODS

Pitcher plants (*Nepenthes alata* Blanco) were grown in the greenhouses of Connecticut College (22°–28°C) in sphagnum moss without fertilizer. Pitchers were observed several times per week for 10 wk and measured from the hinge of the lid to the rounded junction of the pitcher and petiole. Tissue from immature (closed, 1–6 cm) and mature (open, 12–15 cm) pitchers was fixed overnight in 2.5% glutaraldehyde in 0.05

Figs. 1–6.   Pitcher development in *Nepenthes alata*. Bar = 5 mm. **1.** Swollen tip of the tendril has a clearly defined shape of a pitcher (arrowhead). **2.** Slightly larger pitcher. The terminal spur has formed (arrowhead) next to the lid structure (arrow). **3.** The tightly closed lid is apparent but the developing peristome does not protrude. **4.** Swollen lid structure on elongating, closed pitcher. The peristome is still not visible. **5.** Pitcher with recently opened lid and protruding peristome (arrow). **6.** Mature pitcher with elongated neck region and protracted lid.
  Figure Abbreviations: L, lid; N, nectary; P, peristome; T, trichomes; VB, vascular bundle; W, wax-covered cells.

mol/L sodium phosphate buffer, pH 7.2, washed in buffer, then dehydrated in an ethanol series to 95%. Samples were embedded in JB-4 methacrylate resin (Polysciences, Warrington, Pennsylvania), and sections were stained with 0.05% toluidine blue in benzoate buffer (pH 4.4). In addition, autofluorescence of the cuticle and suberized substances was examined on unstained hand and plastic sections by epifluorescence microscopy with an Olympus (Lake Success, New York) UV filter pack.

Tissue for scanning electron microscopy (SEM) was freshly collected from immature and mature pitchers, affixed to aluminum supports with carbon tape, and quickly examined uncoated and fully hydrated in a LEO 435 variable pressure SEM (LEO Electron Microscopy, Thorn-

wood, New York) operated at 80–250 Pa. In this mode, back-scattered electrons were used to create the image. Alternatively, tissue was fixed as for light microscopy and postfixed with 1% OsO_4 in buffer overnight at 4°C. The tissue was dehydrated with an ethanol series, critical point dried in CO_2, sputter-coated with gold-palladium, and examined under high vacuum (10^{-5} Pa) using a secondary electron detector.

RESULTS

Pitcher growth trends—Each pitcher initiated as a tiny, flattened end of a tendril (Fig. 1). In contrast to the subtending green petiole, the youngest pitchers were dark brown in color (Figs. 1–2) but turned green during elongation (Figs. 3–5). Mature pitchers developed a red color beneath the opening to above the bulbous base (Fig. 6). The area that eventually formed the pitcher opening was smooth and tightly sealed with a flap-like lid (Figs. 2–4). As the pitcher increased in size a short spur formed at the apex of the closed lid (Fig. 2). During the elongation phase of development (6–8 wk; Figs. 3–5) the pitcher partially filled with fluid, averaging 12 mL at maturity. Opened pitchers had a ridged structure containing nectaries, collectively called the peristome, that curled over both inner and outer surfaces (Figs. 5–6).

Regular measurements showed the pitchers of *N. alata* had a uniform rate of growth ($1.47 \times 10^{-2} \pm 1 \times 10^{-4}$ cm/h) from initiation to the point of lid opening (Fig. 7). At this point pitcher growth was limited (Fig. 6). There were occasional outliers to this growth average. While these pitchers were similar in the relationship of lid opening to the growth phase, their average rate of growth was approximately half the previously observed rate. These
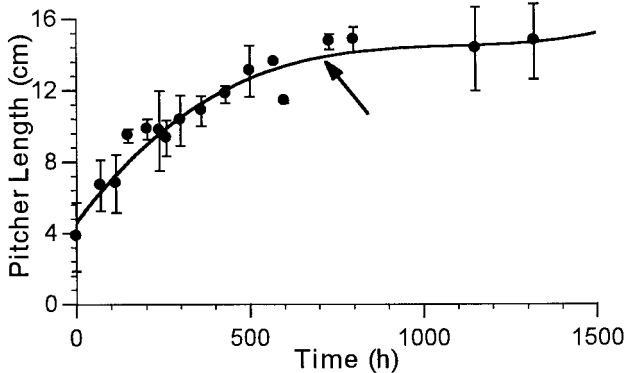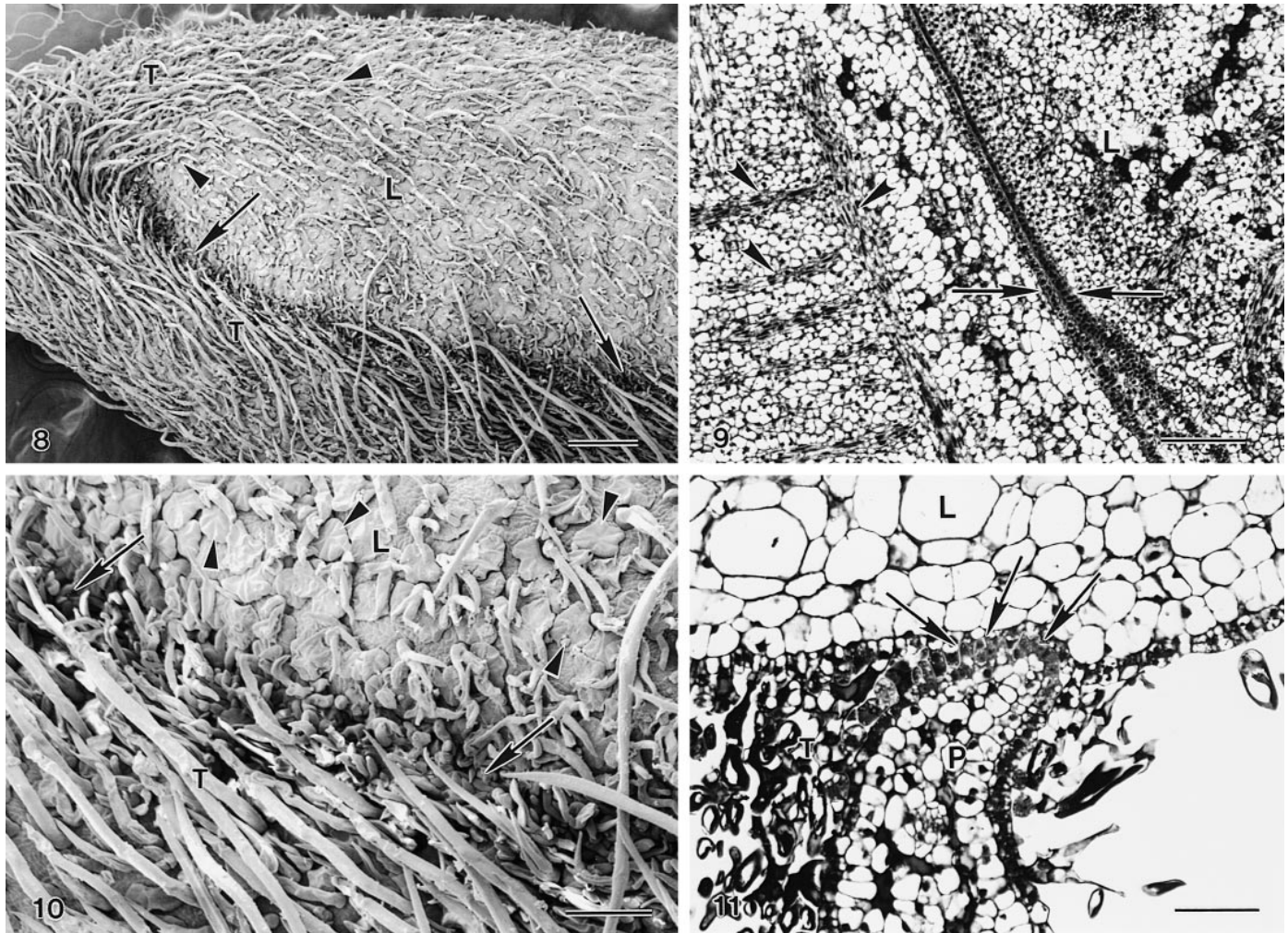

Fig. 7.   Plot of increase of pitcher length over time. Data with third-degree polynomial curve fit ($y = 7.1 \times 10^{-9}x^3 - 2.3 \times 10^{-5}x^2 + 2.6 \times 10^{-2}x + 4.6$) represent averages and standard deviations of eight pitchers. The immature pitchers opened when elongation slowed (arrow).

Figs. 8–11. Closed, immature pitchers (2 cm length). **8.** SEM micrograph of the lid over the pitcher opening. The elongated trichomes on the pitcher continue in a limited area to the lid surface (arrowheads). The interface between the peristome lip and lid is indicated (arrows). Bar = 300 µm. **9.** Longitudinal section of the peristome-lid interface lined by distinct epidermal cells (arrows). There are several parallel rows of developing vascular tissue (arrowheads) in the pitcher. Bar = 100 µm. **10.** Distinct morphologies of the lid (arrowheads) and pitcher trichomes next to the lid seal (arrows). Bar = 100 µm. **11.** Cross section of a closed pitcher with the lid tightly pressed against the pitcher wall adjacent to the developing peristome. The separation zone (arrows) and numerous trichomes are present. Pitcher interior at bottom right. Bar = 50 µm.

pitchers were also noticeably smaller than the others measured and were located toward the top of the plant while the faster developing pitchers were of a basal origin.

Pitcher lid—Light microscopy and SEM micrographs revealed the specializations of the pitcher tissue of *N. alata.* To determine the mechanism for pitcher opening, the lid–peristome junctions of immature, closed pitchers were examined. Each surface had a distinctive trichome density and morphology. The epidermis of the developing pitchers was densely covered with straight, unbranched trichomes (Fig. 8). The developing pitcher walls were heavily covered with unbranched elongated trichomes together with underlying, smaller, flat, and round trichomes (Figs. 8, 10). This trichome distribution was also present at the apex of the lid where the elongate trichomes diminished in number and were apparently replaced with stellately branched trichomes along with the flat trichomes seen on the pitcher wall (Fig. 10). In longitudinal

and cross sections the epidermal layers of the lid and peristome were tightly appressed, with a visible separation zone outlining the cutinized cell walls of the tissues (Fig. 9). The edge of the lid formed a sharp point that fit inside a recessed area on the developing peristome, with each surface having irregular, angular protrusions that neatly interdigitated with those of the opposing surface (Fig. 11). This interlocking of epidermal cells, together with trichomes that appeared interwoven, securely held the lid structure over the opening of the pitcher.

Peristome and nectaries—In immature pitchers the peristome region was infolded and completely contained within the sealed pitcher opening (Fig. 4). Nectaries developing within the peristome were characterized by small, darkly staining, thin-walled cells (Fig. 12). Although somewhat indistinct at this stage, each nectary became much more defined as the pitcher matured. During development, the nectaries appeared slightly sunken
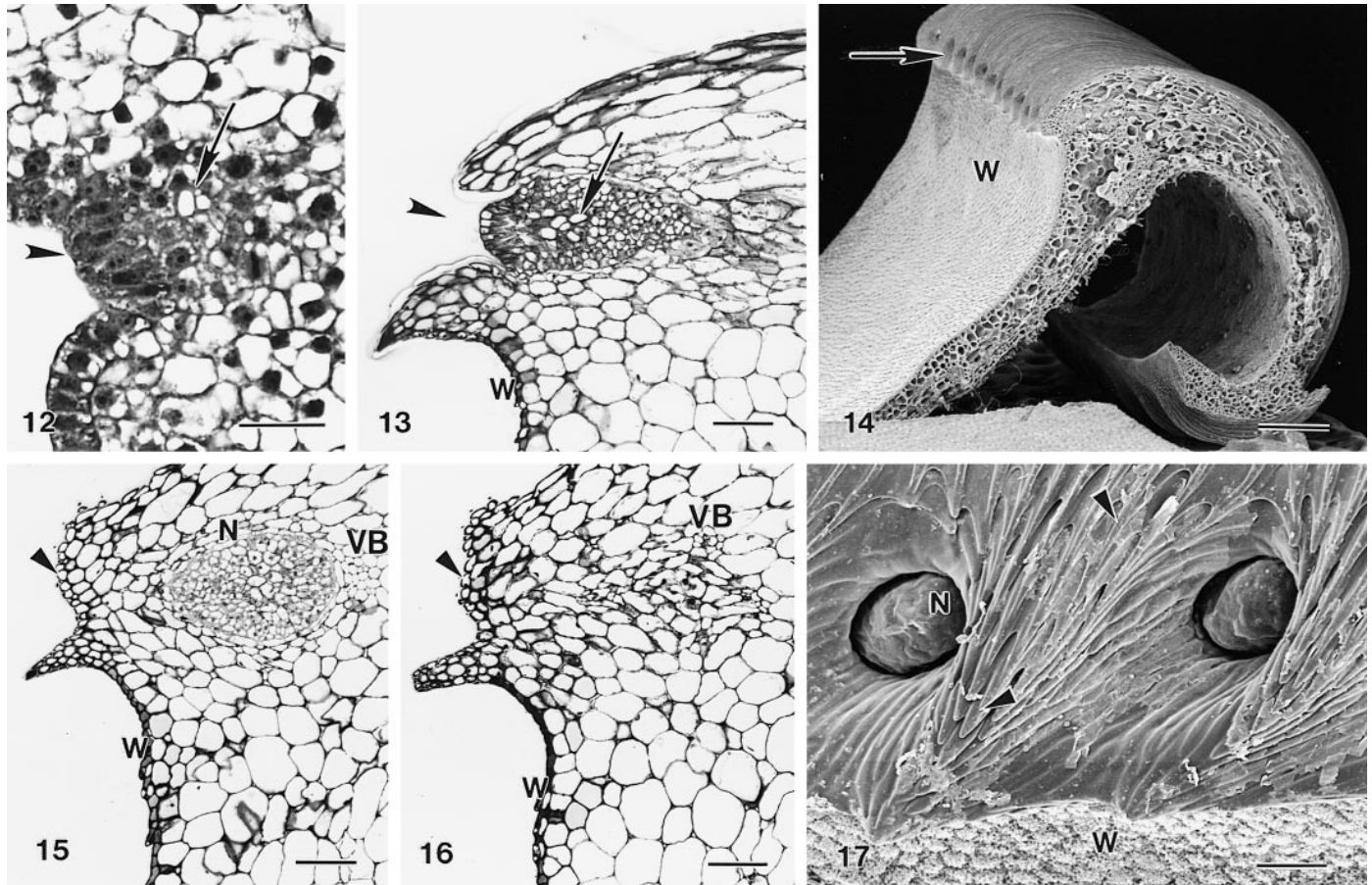
Figs. 12–17.   Peristome nectary development. **12.** Elongating peristome region from a closed pitcher (2 cm length). The tip of the nectary contains elongated, densely cytoplasmic cells (arrowhead) with some differentiation of the underlying nectariferous tissue including a small cluster of vacuolated cells (arrow). Bar = 25 μm. **13.** Mature peristome nectary. The columnar-shaped head cells open into a cavity in the peristome (arrowhead). The underlying small, densely staining cells form a teardrop shape. A small cluster of vacuolated cells are under the tip cells (arrow). Bar = 100 μm. **14.** SEM image of a mature pitcher showing a piece of peristome with attached inner pitcher wall. The ridged edge has numerous nectary cavities that line the pitcher opening (arrow). Bar = 500 μm. **15.** Near-median section of peristome nectary. The nectary apex and peristome opening are no longer visible, but the specialized underlying nectary cells are evident. The overlapping peristome epidermal cells are indicated (arrowhead). Bar = 100 μm. **16.** Cross section of the peristome next to the region in Fig. 15. The specialized nectary cells are no longer visible, indicating they not interconnected. Bar = 100 μm. **17.** Face view of peristome nectaries within the epidermal crypts. The peristome surface has long overlapping cells (arrowheads) in contrast to the wax-covered epidermal cells under the peristome lip. Bar = 50 μm.

into the tissue with a more apparent multicellular structure (Fig. 13). At maturity, the peristome had a highly ridged appearance (Fig. 14) with the nectaries located within deep depressions (Figs. 13, 17). The nectaries expanded and appeared as large clusters of small, isodiametric cells capped by an outer row of oblong cells (Fig. 13). The middle of both the immature and mature glands had a cluster of slightly larger cells with prominent vacuoles (Figs. 12, 13). Nectary tissue underlying the exposed region was quite large (Fig. 15), but each gland was separate (Fig. 16). Peripheral to the nectaries were small vascular bundles that comprised predominantly phloem tissue (Figs. 15, 16). Based on the position of the nectaries and the peristome tip within the closed pitcher, the mature peristome appeared to develop as a result of cell expansion on the upper surface to push the tip out and curl it around on the outside of the trap (Fig. 14).

Upper pitcher—Immediately below the peristome and continuing down approximately one-third of the trap is a zone characterized by irregularly spaced, ridged cells interspersed among flattened ones (Figs. 18–19). In the SEM these cells resembled single guard cells surrounding apparent stomates. However, serial cross sections of the region showed the epidermis was continuous with no pores (Fig. 21). These ridged cells included a cluster of three distinct cells: a laterally elongated epidermal cell with a small, triangular (in cross section) cell at its tip, overlapping an adjacent small epidermal cell (Fig. 22). The exterior, or abaxial, surface had fully formed stomata flanked by guard cells (Fig. 21). The ridged cells were particularly numerous immediately below the inner edge of the peristome. Also, the cells in this zone were coated by a thick layer of epicuticular wax forming loose scales (Figs. 19–20).

Stellate crystals, presumably of calcium oxalate, were found throughout the pitcher tissue and in greater number in the basal region (not shown). The pitcher tissues were also filled with large idioblast cells (Fig. 23), an unusual structure with dense deposits of cell wall thickenings. To-
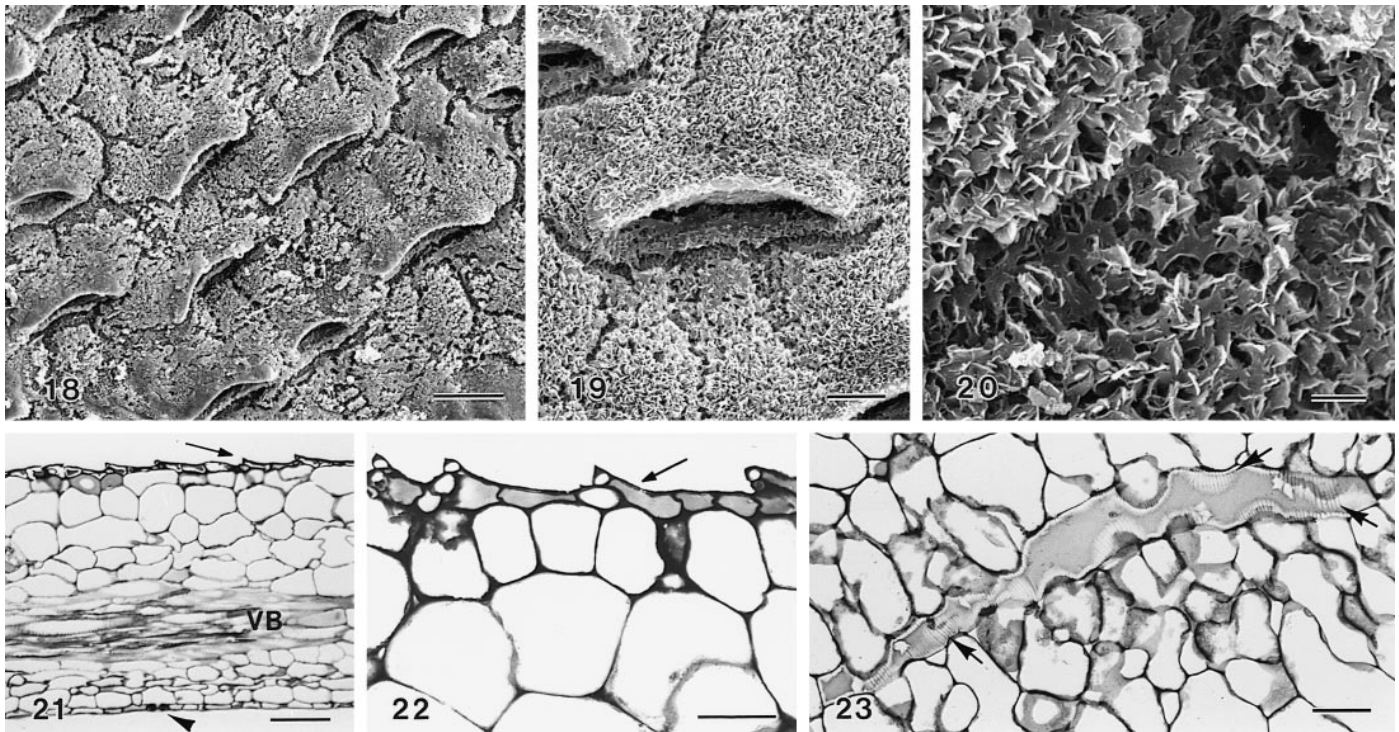
Figs. 18–23. Upper region of the pitcher. **18.** The surface of the inner pitcher epidermis below the peristome. The irregular, lunate-shaped lips are directed downward. Bar = 10 μm. **19.** Higher magnification of a lunate cell that resembles a single guard cell around a stoma. An irregular epicuticular wax is apparent. Bar = 10 μm. **20.** High magnification of the thick waxy scales that coat the upper one-third of the pitcher. Bar = 2.5 μm. **21.** Cross section of the upper pitcher with raised cells (arrow). The pitcher base is oriented to the left. The underlying epidermal cells lack an aperture. In comparison, a stomate on the abaxial surface is visible (arrowhead). Bar = 100 μm. **22.** The unusual lunate cell complex. The protruding ridges are formed by an elongated cell (arrow) with a small tip cell raised over a small epidermal cell. Bar = 50 μm. **23.** An example of the large, darkly staining idioblasts that are found throughout the pitcher. The walls have annular cell wall thickenings (arrows) that may give support to the pitcher walls. Bar = 50 μm.

luidine blue stained the thickenings a light blue, indicating they contained lignin. Their function may be to support the pitcher tissues.

Digestive glands—Of particular interest was the lower, glandular region of the pitcher, which is the zone of secretion and absorption (Juniper, Robins, and Joel, 1989). The epidermal surface of immature pitchers had small, indistinct oval depressions in which the glands were forming (Figs. 24–25). Mature glands, in contrast, were present within defined depressions of the epidermis and were partially covered by many small epidermal lips (Fig. 26) that, in orientation, resembled the stomatal-like ridges in the region beneath the peristome. The gland surface was uninterrupted by visible breaks or gaps. The glandular surface of fresh, hydrated samples examined under reduced vacuum in the SEM was similar to fixed and dried tissues (Figs. 24–25).

In the smallest immature pitchers examined (1 cm), the epidermis had small, dark-staining cells periodically interspersed with lighter staining cells that resembled meristemoids (Fig. 27). Larger traps (4 cm) had clusters of 4 to 5 cells wide that had divided once periclinally (Figs. 28–29). This gave the appearance of placing the epidermis below a single row of protoglandular cells. The mature glands consisted of three cell layers (Figs. 30–31) above a depression of cells continuous with the epidermis (Fig. 31). The outermost layer had 40–60 small colum-

nar-shaped cells (Fig. 32), while immediately underneath were 16 densely cytoplasmic rectangular cells (Fig. 33). The third cell layer of the gland had 8–12 cuboidal cells (Fig. 34) that rested on a row of small vacuolate cells (Fig. 35). The base of the glands was at the same level of the vasculature and appeared to be in direct contact or interconnected with the tracheary elements (Figs. 31, 34–35). At a level just below the glands there were numerous xylary endings (Fig. 31).

The gland cells generally had thicker cell walls compared to the surrounding cells. Toluidine blue staining suggested the basal gland cell walls were impregnated with a suberin-like material to form an endodermal layer. Fresh hand sections and unstained plastic sections were examined for autofluorescence of suberin or cutin. As expected, the epidermal cuticle was highly autofluorescent and it continued over the top of the gland (Fig. 36). In addition, the lateral walls of the basal cells and the second row of gland cells also fluoresced compared to the underlying mesophyll (Fig. 36), indicating suberized regions.

DISCUSSION

The pitchers of *Nepenthes alata* are highly modified leaves adapted to effectively attract, capture, and kill arthropods, then break down and absorb the nutrients therefrom. The anatomy of this species resembles that which
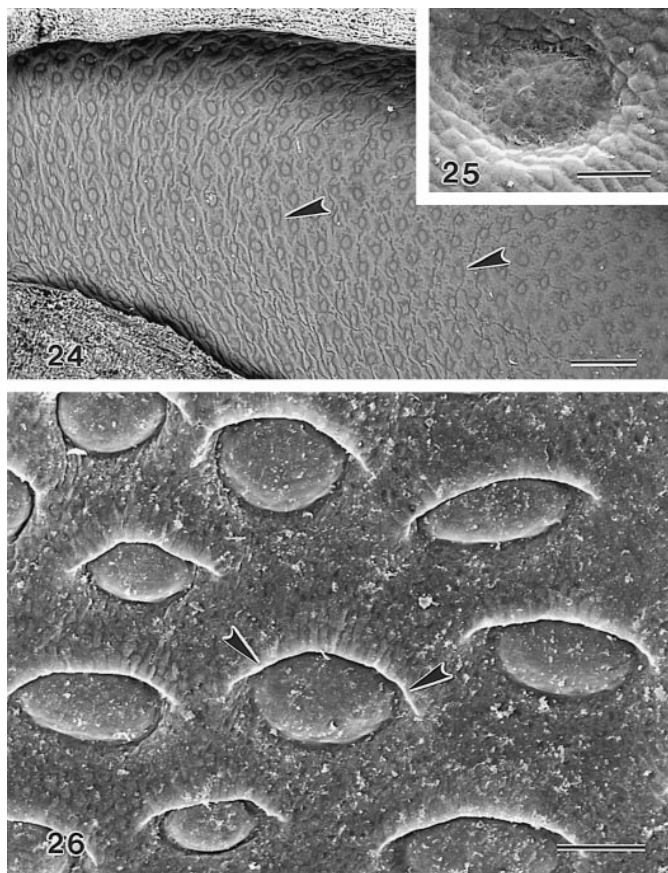
Figs. 24–26.   SEM of the digestive glands. **24.** Basal region of the inner surface of an immature closed pitcher (2 cm). The numerous small depressions (arrowheads) are the developing digestive glands. Unfixed, hydrated sample. Bar = 200 μm. **25.** Developing digestive gland. The gland rests within a shallow symmetrical depression of the epidermis. Fixed sample. Bar = 25 μm. **26.** Digestive glands from a mature pitcher. Epidermal ridges (arrowheads) overarch the upper gland surfaces. Bar = 100 μm.

is common to the genus. The structural features involved in carnivory, which were the focus of this study, included extrafloral nectaries 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. This paper is the first to examine the development of these pitchers in general and their characteristic structural adaptations to their carnivorous habit.

The pitchers initiated as small flattened structures at the tips of tendrils. The organization of the vascular bundles, in a ring with the xylem oriented toward the pitcher lumen (not shown), indicated that the interior of the pitcher is the adaxial surface, typical of an epiascidiate leaf. This supports Arber's (1941) assertion that the pitchers of the genus evolved through the infolding of a leaf with the adaxial surface to the inside of the pitcher. The pitchers grew uniformly to maturity with the timing of lid opening coinciding with the completion of the growth phase (Fig. 7). Logically, it is advantageous for the pitchers to be fully formed before the lid opens to allow prey to access the pitcher in order to limit herbivory or loss of nectar. Macfarlane's (1908) observation that *Nepenthes* pitchers may be mono-, di-, or trimorphic on a single

plant explains the spread found in the growth data. Specifically, larger pitchers, presumably for trapping crawling insects, develop first towards the base of the plant, followed by the appearance of smaller upper pitchers for the capture of flying insects (Juniper, Robins, and Joel, 1989).

An examination of the lid and peristome junction in an immature pitcher found the two tissues to be closely appressed, but separated by a distinct boundary (Figs. 8–11). Tapered edges of the lid fit neatly into shallow depressions of the peristome and, together with numerous epidermal trichomes, tightly sealed the pitcher opening. While there have been reports of yeast and bacteria in the pitcher fluid of *Nepenthes* (Hepburn, 1918; Shivas and Brown, 1989), the fluid in closed pitchers lacks micro-organisms (Hepburn, 1918), thus demonstrating the tightness of the lid seal. Since the lid remained closed until the end of the pitcher growth, the development of the lid and peristome must have been synchronous (Lloyd, 1942). As the pitcher neared maturity, the peristome, which up to this point protruded only to the inside of the pitcher (Lennon, 1995), expanded outward causing a disconnection of the lid. The outer epidermis of the pitcher and the apical one-third of the lid both had long, unbranched trichomes. Thus, superficially the lid represents a folded flap originating from the side of the pitcher rather than being a unique organ.

The extrafloral nectaries in the peristome were large teardrop-shaped glands that extended far back into the rim. Similar peristome nectaries have also been reported in *N. rafflesiana* (Pant and Bhatnagar, 1977) and *N. maxima* (Juniper, Robins, and Joel, 1989). There has been speculation that the nectaries may interconnect to form a continuous glandular ring around the pitcher rim (Juniper, Robins, and Joel, 1989). However, serial sections of the peristome in *N. alata* showed the nectaries were separated by parenchyma cells interspersed with vascular bundles (Figs. 15–16). The peristome nectaries had small bundles of phloem tissue around their periphery. The lack of xylem is somewhat unusual for nectaries, which typically are supplied by both vascular tissues (Elias, 1983). The nectary structure appeared more specialized than other nectaries supplied exclusively by phloem (Davis, Peterson, and Shuel, 1986). Further, the cluster of vacuolated cells that form early under the columnar-shaped tip cells may function as a nectar collection site. While the nectar secreted by *Nepenthes* has not been studied (Juniper, Robins, and Joel, 1989), one would expect these structurally elaborate glands to produce a sugar-rich, modified fluid favored by foraging insects. Their position, on the downward-facing edge of the protruding peristome lip, lures and holds insects in a precarious position from which they ultimately fall into the trap (Ratsirarson and Silander, 1996).

The area immediately below the peristome to approximately one-third the length of the pitcher was characterized by a thick wax and scattered, protruding epidermal cells with the concave ridge oriented downward. Referred to as lunate cells (Lloyd, 1942; Pant and Bhatnagar, 1977) or a transformed stomatal complex (Fig. 2.18e in Zeigler, 1987), the function of these cells is a mystery (Juniper, Robins, and Joel, 1989). Lloyd (1942) reviewed several suggestions including secretion of wax or water
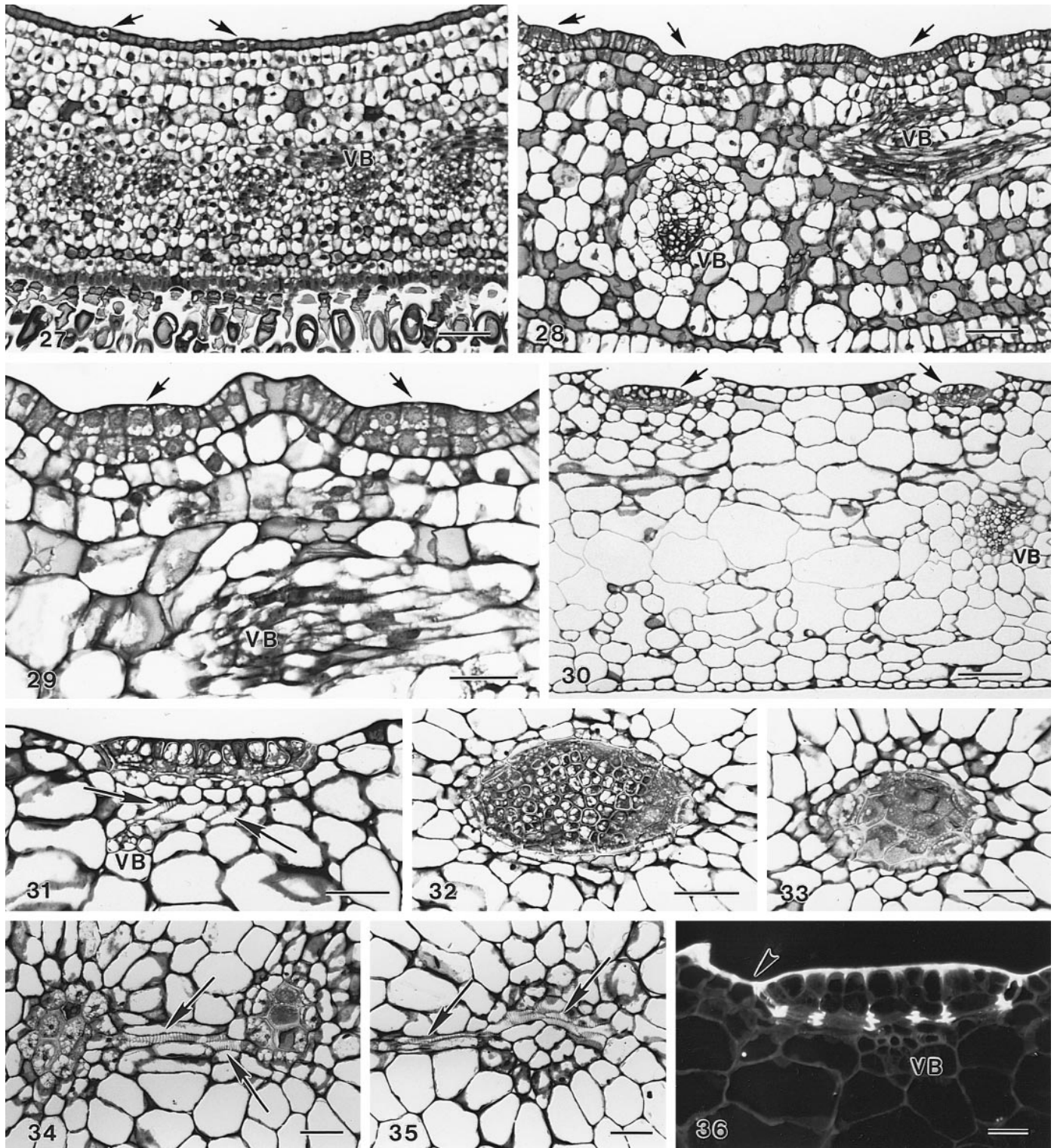
Figs. 27–36. Development of the digestive glands. **27.** Cross section of an immature closed pitcher (1 cm). The inner epidermis has small, darkly staining isodiametric cells interrupted by slightly larger cells that are lighter staining (arrows). The outer epidermis is densely covered with trichomes. Bar = 50 μm. **28.** Cross section of a slightly larger pitcher (2 cm). Three developing glands are visible within the epidermis (arrows). Note the underlying vascular tissue. Bar = 50 μm. **29.** Higher magnification of developing glands (arrows). Four epidermal cells divided periclinally, while the surrounding epidermal cells remain a single cell layer. Bar = 25 μm. **30.** Off-median longitudinal section through the basal region of a mature pitcher. The raised lip cells are visible over two glands (arrows). Bar = 100 μm. Figs. 31–35. Bar = 50 μm. **31.** Median section through a gland. There are four distinct cell layers. The top row has slightly elongated cells with thick walls and rests upon two rows of smaller densely staining cells. The base of the gland resembles a layer of epidermal cells and is closely associated with xylary traces (arrows). **32.** Tangential section through the top layer of a gland. The ~60 cells in the region are small compared to the surrounding epidermal cells. **33.** Tangential section

and gas exchange. In agreement with observations made by Lloyd (1942), we found no pore associated with the structure. Thus, a role in secretion is not likely.

Other pitcher plants, including species of *Sarracenia* and *Darlingtonia,* also have elongated hairs that point downward near the trap opening to capture insects (Juniper, Robins, and Joel, 1989). The lunate cells in *Nepenthes* may be an abbreviated homologue. While the number of species examined is low, the lunate cells are only missing from *N. ampullaria* (Pant and Bhatnagar, 1977). However, the effective trapping structure in *Nepenthes* in this zone is the epicuticular wax together with the ridged cells. If the wax is mechanically removed, ants are able to climb out (reviewed in Lloyd, 1942). Thus, the lunate cells by themselves are not an effective deterrent to prey escape.

The structure of the wax resembles the reticulate organization modeled by Juniper, Robins, and Joel (1989) for an unspecified *Nepenthes* species. However, unlike the wax examined by these authors, which was thermolabile and not readily detectable by SEM, the wax of *N. alata* appeared more stable under the electron beam. The increased thermostability may reflect a difference in composition. Only *Nepenthes* × *williamsii* has been examined for the composition of the epicuticular wax (data from Table 6.2 in Juniper, Robins, and Joel, 1989).

The digestive glands at the base of the pitcher developed from single protodermal cells to form a sessile gland within a slight epidermal depression. While the exact sequence of cell divisions was not determined, regular combinations of periclinal and anticlinal divisions would form the early cluster of four cells arranged in two layers. Repeated divisions progressed to form the symmetrical gland organization. SEM observations of immature pitchers showed the glands as oval depressions, which were indistinct and not yet fully delineated from the epidermal cells. The glands in mature pitchers, in contrast, were clearly separate from the epidermis. In addition, only the mature glands had lunate cells partially covering the gland head cells, a feature present in other *Nepenthes* species (Lloyd, 1942; Adams and Smith, 1977; Pant and Bhatnagar, 1977). The placement and orientation of the lunate cell may prevent insects from using the glands or epidermal cavity as footholds for escape (Lloyd, 1942).

The digestive glands were closely associated with vascular bundles (Figs. 31, 34; Stern, 1917; Anderson, 1994), and terminal ends of tracheids ended directly beneath the gland bases. Immature, closed pitchers held several millimetres of fluid that may have come directly from the transpiration stream through the glands. This does not rule out the addition of solutes by the glands; in this sterile fluid Lüttge (1964) found proteinases. In most carnivorous plants the digestive glands develop an endodermal layer that restricts apoplastic flow (Fahn, 1979; Fineran and Gilbertson, 1980; Heslop-Harrison and Heslop-Harrison, 1981). The cutin or suberin in this layer

was clearly visible by autofluorescence, and to a more limited degree by toluidine blue staining, in the mature glands of *N. alata.* The data indicated the epidermis had a thick cuticle that extended over the top of the digestive glands. Cuticular gaps over the head cells were not detected in the SEM, though it has been difficult to detect these in most carnivorous species (Joel and Juniper, 1982) except in the tentacles of *Drosera* (Williams and Pickard, 1974). *Nepenthes* reportedly have a very loose cuticle covering the digestive glands, made of individual cuticular droplets (Fig. 8.9F in Juniper, Robins, and Joel, 1989). Collectively the droplets give the appearance of an uninterrupted cuticle layer. This needs further confirmation by TEM (transmission electron microscopy) examination of the glands.

In summary, highly specialized peristome nectaries, peculiar ridged cells covered with an epicuticular wax, and large multicellular glands make the pitchers of *N. alata* remarkably adapted for the attraction, capture, and digestion of insects. Pitcher elongation was rapid until the peristome expanded to release the lid structure. The development of the digestive glands was synchronous with the vascular tissue to form an interconnected matrix for efficient trap filling and likely uptake of digested insect remains. The exact route of nutrient transport and the role of the glands in uptake and secretion will be examined in a later study.

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through the middle layer of gland cells. The 16 cells are densely cytoplasmic and heavily suberized. **34.** Basal gland region. The eight cells in each gland are interconnected by xylary traces. **35.** Tracheary elements (arrows) just underlying the gland base. **36.** Autofluorescence of suberin and cutin. The epidermal cuticle (arrowhead) is continuous with the gland exterior. The adjoining top walls of the columnar gland cells are suberized as are the walls of the basal cells. Bar = 25 μm.

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