The Morphology and Anatomy of Utricularia Transrugosa Stapf.

By

J. Slinger.

INTRODUCTION.

Kamienski, in 1897, recognised five genera of the family LENTIBULARIACEAE. These are, Pinguicula, Genlisea, Polypompholyx, Utricularia and Biovularia. It is now known that all these plants are carnivorous and that prey is captured and digested by means of specialised vegetative organs which constitute the " traps ". Among the LENTIBULARIACEAE occur examples of the simplest traps (Pinguicula), the most complex of the pitfall type (Genlisea), and the trap or bladder of Utricularia which has attained a degree of structural complexity and perfection of mechanism for which there is no analogue among other plants.

The Utricularias show a wide range of variation in form and habit. The plants may be freely-floating or anchored aquatics, or epiphytic, or they may be terrestrial in wet to moist sandy soils. Among the aquatic forms are found the larger (e.g. *U. stellaris*) and the smaller species of Utricularia (e.g. *U. cymbantha*), while the terrestrial species, with few exceptions are small. The epiphytic forms are often remarkable for the size and showiness of their flowers.

The genus is of world-wide distribution, the most widely distributed species being the submerged or semi-submerged aquatics. They are found throughout North America including Greenland, in Europe and in Asia. Related species extend throughout the tropics into South America, South Africa, Australia and New Zealand. Terrestrial species are widely distributed in the tropics of the Old and New worlds.

Fig. 1 shows the distribution of Utricularia in Africa. It will be seen that aquatic and terrestrial species are well represented, while only one epiphytic species has been recorded (*U. bryophila*). Most common are the terrestrial species, of which *U. transrugosa* Stapf is an example. It will also be seen from Fig. 1 that six species, all terrestrial, are limited to Africa south of the central lake area. These species are *U. transrugosa*, *U. kirkii*, *U. capensis*, *U. livida*, *U. ecklonii* and *U. sandersonii*. Twenty-three species are restricted to west, central and northern regions, while the nine remaining species are distributed throughout Africa. Several of the African species are also found in tropical America (*U. obtusa*, *U. foliosa*, *U. subulata*), while others occur in Algiers and Portugal (*U. exoleta*) and through India to China, Malaya, and tropical Australia (*U. stellaris*, *U. striatula*).

As previously stated, *U. transrugosa* is one of the small terrestrial species of Utricularia and is found growing in vleis or boggy ground along stream banks. It will be seen from Fig. 1 A that the species has been recorded from the Transvaal and also from Southern Rhodesia near Salisbury, but is not recorded from any locality north of the Zambesi.

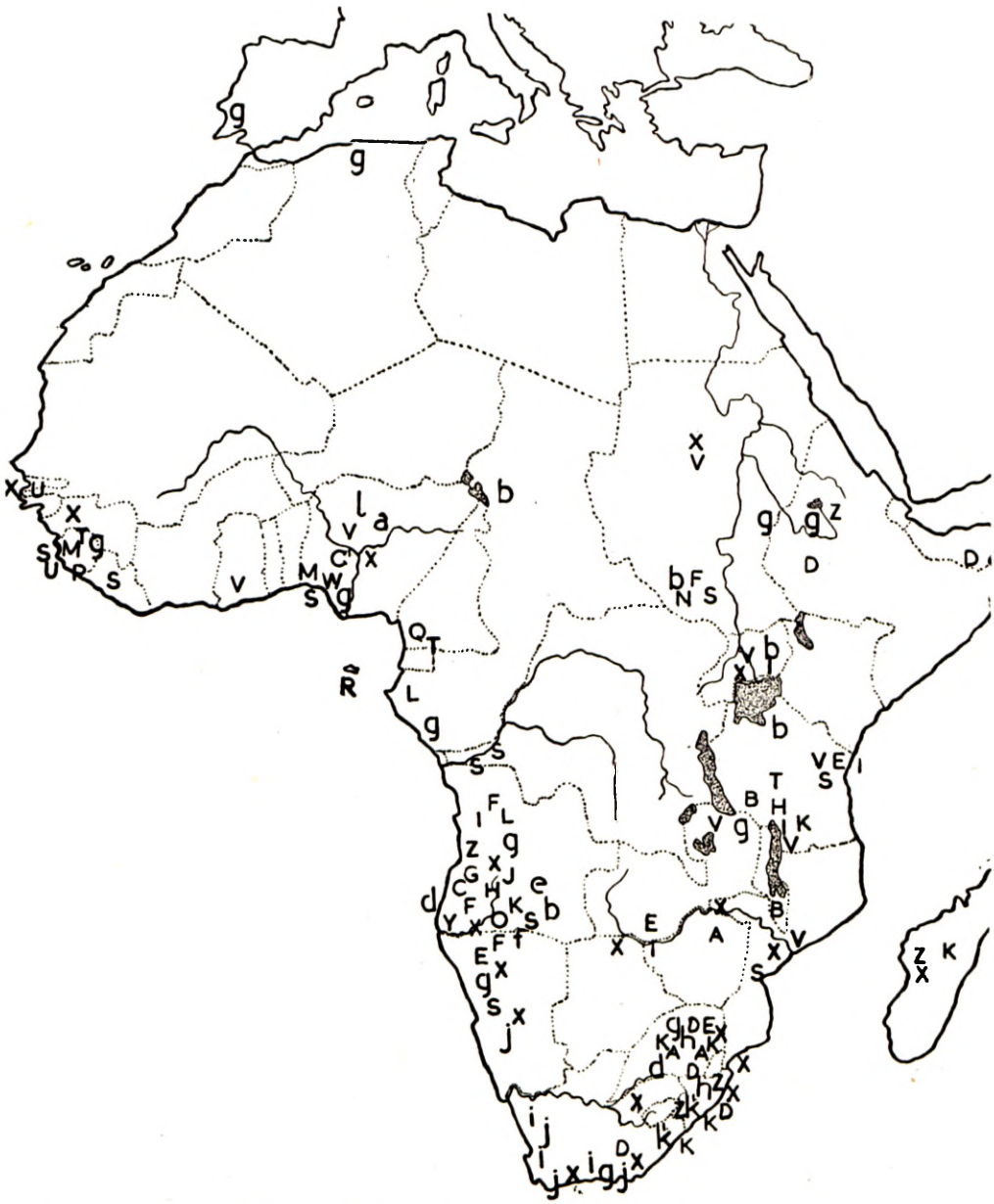

FIG. 1.—*The Distribution of the Genus Utricularia in Africa.*

(Legend for Fig. 1 at foot of page 387).

U. transrugosa was first described by O. Stapf in Flora Capensis *IV 2*, 428 (1904). His description was based on specimens from Barberton, Johannesburg and Rustenburg. Rand 727, which was collected in boggy ground near Johannesburg, was one of these and had previously been misidentified by Moore in Journal of Botany (1903), 405, as *U. sanguinea Welw.* It is the first published record of *U. transrugosa* from the Witwatersrand. Later, in 1951 and 1952, the plant was found at Bryanston, near Johannesburg, in a semi-dry vlei associatd with sedges, *Drosera cf. burkeana* and *Lobelia cf. decipiens*. It was also found in very wet mud along the bank of a nearby stream.

Legend.

A. U. transrugosa Stapf. (Ter.).
B. U. odontosperma Stapf. (Ter.).
C. U. sanguinea Oliv. (Ter.).
D. U. tribracteata Hochst. (Ter.).
E. U. kirkii Stapf. (Ter.).
F. U. exilis Oliv. (Ter.).
G. U. linarioides Welw. (Ter.).
H. U. welwitschii Oliv. (Ter.).
I. U. firmula Welw. (Ter.).
J. U. baumii Kam. (Ter.).
K. U. prehensilis E. Mey. (Ter.).
L. U. andongensis Welw. (Ter.).
M. U. spiralis Smith. (Ter.).
N. U. schweinfurthii Bak. (Ter.).
O. U. tortilis Welw. (Ter.).
P. U. micropetala Smith. (Ter.).
Q. U. manii Oliv. (Ter.).
R. U. bryophila Ridley. (Epi.).
S. U. subulata Linn. (Sub-ter.)
T. U. striatula Smith. (Ter.).
U. U. rigida Benj. (Aq.).
V. U. thoningii Schumach. (Aq.).
W. U. trichoschiza Stapf. (Aq.).
X. U. stellaris Linn. (Aq.).
Y. U. villosula Stapf. (Aq.).
Z. U. foliosa Linn. (Aq.).
a U. platyptera Stapf. (Aq.).
b U. reflexa Oliv. (Aq.).
c U. charoides Stapf. (Aq.).
d U. diploglossa Welw. (Aq.).
e U. cymbantha Oliv. (Aq.).
f. U. obtusa Swartz. (Aq.).
g. U. exoleta R. Br. (Aq.).
h. U. livida E. Mey. (Ter.).
i. U. capensis Spreng. (Ter.).
j. U. ecklonii Spreng. (Ter.).
k. U. sandersonii Oliv. (Ter.).
l. U. papillosa Stapf. (Ter.).

Aq. Aquatic including submerged, anchored or surface-floating forms.

Ter. Terrestrial.

Sub-ter. Sub-terrestrial.

Epi. Epiphytic.

In Hooker's Icones Plantarum, Tab. 2796 (1903) this plant was described as *U. livida var. transrugosa Stapf.* The two species *U. transrugosa* and *U. livida*, (Fig. 1, h) are distinguished by the markings on the palate of the lower corolla lip; in the former the palate is distinctly rugose, while in the latter it is narrower and tubercled.

Bladders, stolons, leaves, capsules and seeds were not available to Stapf for his description of *U. transrugosa*, and F. E. Lloyd (1942) in " The Carnivorous Plants " does not even refer to *U. transrugosa*. The object of this report is to give an adequate description of the anatomy and morphology of *U. transrugosa*, together with facts concerning the biology of this species which have become known during these investigations.

MORPHOLOGY.

(*a*) *VEGETATIVE CHARACTERS.*

This is a small perennial plant reaching a height of 6–10 cms. when in flower. Unless in flower, the plant is likely to be overlooked, as apart from the inflorescence, only the tips of the leaves appear above ground.

The vegetative morphology of Utricularia cannot be likened to that of any other flowering plant. In order to avoid confusion, the terms leaf, stolon and rhizoid will be used in this report, though it must be remembered that in Utricularia the distinctions between these three are ill-defined. The vegetative portions of the plant are almost entirely below soil-level, with just the tips of the recurved leaves appearing above ground. These leaves are simple, entire and spathulate in shape tapering towards the base into a cylindircal portion which is continuous with a stolon. In its expanded portion, the leaf is dark green above, paler below, often becoming reddish-brown with age. Because of the soft, succulent texture of the leaves, the dichotomously-branched midrib is often indistinct and cannot be seen at all with the naked eye in the lower cylindrical portion.

The stolons are very delicate, cylindrical, filiform strands of tissue which have a characteristic white, glistening transluscence. They are much branched, and as will be seen later, this branching follows a pattern.

U. transrugosa is rootless. The rhizoids described by Stapf are problematical. When present, they occur in tufts at the base of an inflorescence, but cannot really be distinguished morphologically from the stolons. Indeed, the vegetative structures of *U. transrugosa* are so plastic, that a rhizoid can continue growth, branch and bear leaves and thus become a stolon. The leaves too, as will be seen from Fig. 2 are no more than unbranched, expanded stolons.

Minute stalked bladders (traps) are borne in great numbers on leaves, stolons and rhizoids. There appears to be no definite arrangement of bladders; they may be opposite or alternate and are not more frequent in one region than in another. As it is difficult and inpracticable to separate the anatomy from the morphology of these bladders, this will be discussed collectively in a later section.

Numerous small glands are present over the outer surface of the vegetative parts of the plant and the epidermis is thickly cuticularised. (See anatomical section for structure of glands.)

U. transrugosa was frequently found associated with filamentous green algae (Zygnema, Oedogonium) which had become densely twisted and matted around stolons and leaves. These, at first glance, give the plant a hairy appearance, and can lead to confusion.

(b) BRANCHING, AND ORIGIN OF THE INFLORESCENCE.

In spite of the plasticity of stolons, leaves and rhizoids these organs are arranged in positions bearing a clear relationship to one another.

The main stolon (St. 1, Figs. 2 and 3) is of indefinite growth. At various points along its length it gives rise to aerial shoots which will bear flowers. These aerial shoots or aerial stems are not to be confused with the " air-shoots " of Goebel (Lloyd, p. 223). Secondary stolons (St. 2) arise at the base of an aerial shoot at right angles to the main stolon and a short distance above the junction of the aerial shoot with the main stolon. A single primary leaf (Lloyd, Plate 23, Fig. 7) is always associated with an aerial shoot and originates in an angle between St. 1 and St. 2. These leaves are circinate away from the aerial shoot.

Rhizoids are formed in consecutive pairs alternately on opposite sides of the aerial stem, the first pair being at right angles to the secondary stolons. As the rhizoids may later become stolons, in Fig. 3, at a later stage of development, R1 may be St. 3, R2 would then be R1, and so on.

Secondary stolons and secondary leaves may also arise alternately along the main stolon between aerial shoots, while tertiary stolons and leaves may arise alternately along a secondary stolon. It will be seen that in this way a much branched plant bearing inflorescences may be formed, and that the branching conforms to a pattern with unexpected regularity.

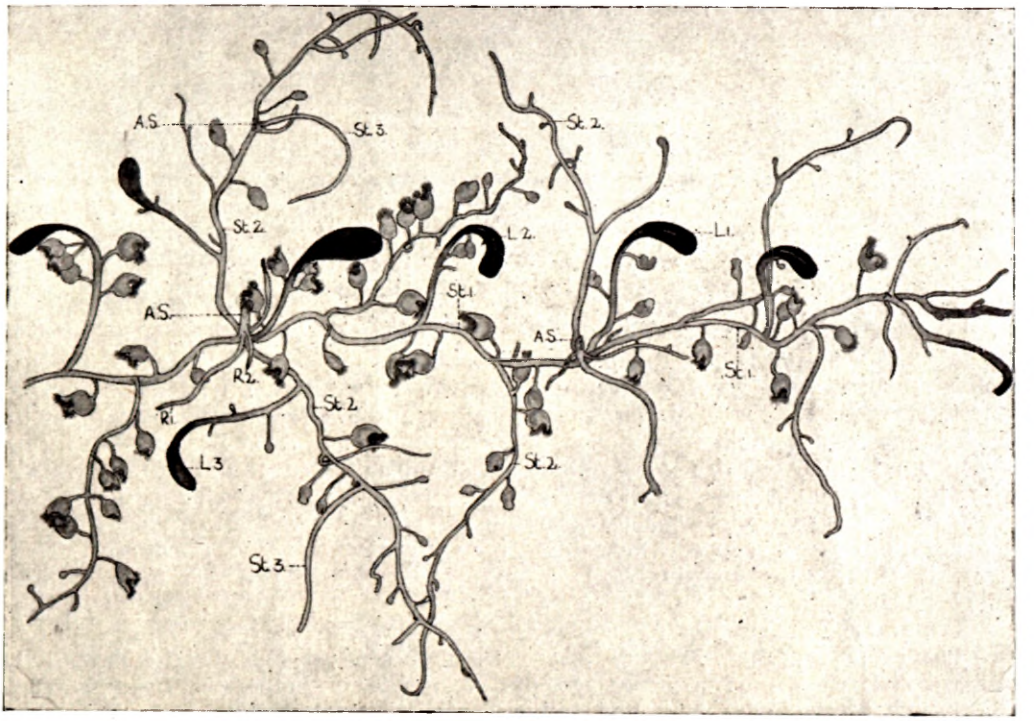

FIG. 2.—*Vegetative structures of U. transrugosa Stapf.*

The plant was freed of organic debris and soil particles with the aid of a fine jet of water.

A.S.: aerial shoot. St. 1: main stolon. St. 2: secondary stolon. St. 3: tertiary stolon. L1: primary leaf. L2: secondary leaf. R1, R2: rhizoids.

(*c*) *FLORAL CHARACTERS.*

The erect aerial stem of *U. transrugosa* bears from 1–3 personate flowers which have a delicate honey-like scent. Pedicels are about 2 mm. long and are subtended by a bract which is often recurved in the fruit. 1–2 barren bracts are found near the base of the aerial stem. Two bracteoles arise at the base of the pedicel above the fertile bract. They are narrower than this bract but of equal length. The calyx consists of two reddish-purple sepals, 3 mm. long, which are persistant and become papery in the mature fruit.

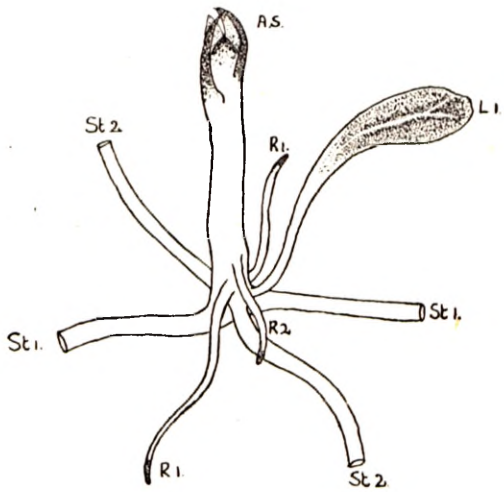

FIG. 3.—*Young inflorescence showing origin, and branching of stolons.*

This is an enlargement of a portion of the plant represented in Fig. 2. Lettering as for Fig. 2.

The corolla varies greatly in colour from mauve or purple with yellow markings on the palate, to pale yellow or white. Its upper lip is 6 mm. long and constricted in the centre, the lower half being broader than the upper half. The apical margin of this lip shows slight variation in that it is in some cases smoothly rounded and in others, emarginate. The lower lip rises from a reflexed spur, 7 mm. long, to arch forward and meet the upper lip; thence it is reflexed backwards and expands to form a platform 11 mm. broad, the margins of which are upturned. In the mouth formed with the upper lip it bears two lateral rugose swellings; each swelling usually has 20–30 transverse rugosities which are dark purple and velvety in appearance. These rugosities often converge or bifurcate forming in places a zig-zag pattern. The depression formed between the two lateral rugose swellings is variously marked with yellow patches.

Two stamens are attached to the lower lip at the mouth of the spur. When mature, they face away from the ovary and towards the mouth of the spur. Fig. 4 shows the stamen-ovary relationship in three stages of development of the flower. In (*a*), a very young bud, filaments are short and anthers very large and closely adpressed to the ovary. In (*b*) the filaments have elongated and anthers have separated and rotated so that they face each other, while in (*c*) the mature anthers have completed a rotation through 180° so that their previously outer margins become the inner margins, and the anthers face away from the ovary instead of towards it as in the bud. During this roration the filaments become twisted. When fully mature the anthers adhere at the centre of their inner margins. Dehiscence is by means of longitudinal slits.

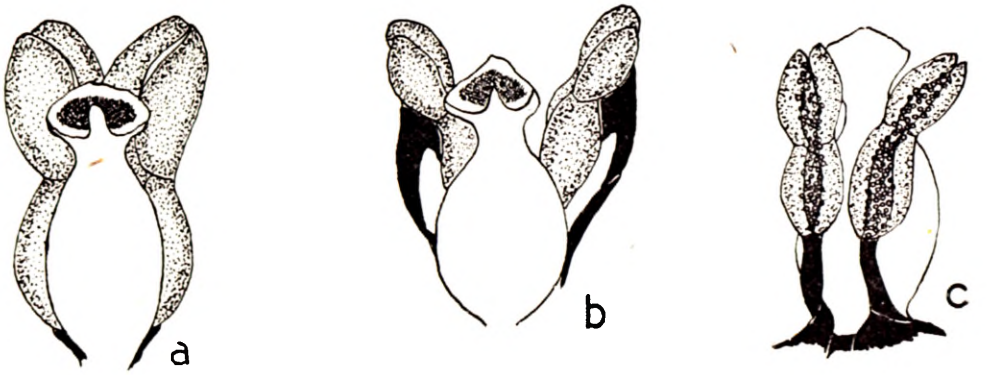

FIG. 4.—*Stamen-Ovary Relationship.*
a, b and c are drawn from flowers in progressive stages of development. Note broad point of attachment of anther to filament.

The ovary is globose and constricted at the apex into a short style which broadens out into a 2-lobed stigma, the upper lobe of which is narrow and acute and is adjacent to the upper corolla lip. The lower lobe is wide and fan-shaped and bears the stigmatic surface.

The fruit is a capsule with numerous seeds tightly packed on a free central placenta. Seeds are angular, predominantly wedge-shaped (Fig. 5) and the placenta has become convoluted and folded between them, so that when all the seeds are removed the placenta has the appearance of a fine honeycomb. The calyx and the ovary wall become papery in the fruit and dehiscence is by means of irregular longitudinal tears.

FIG. 5.—*Seeds of U. transrugosa Stapf in various positions.*
The broad, flattened surface of the seed faces externally (430 × nat.).

ANATOMY.

The microscopic and macroscopic structures of this plant proved to be different from other angiosperms. The anatomy of aerial shoot, leaf, stolon and bladder was studied. Observations were made primarily from microtomed sections, 15 μ and 20 μ thick. Material which had been preserved in 4 per cent formalin was embedded in 55 paraffin wax and the sections were later stained with safranin and fast green. As

the tissues of Utricularia are so delicate, hand sections and dissections were necessary to substantiate evidence from prepared slides. Especially in the case of bladders, microtomed sections should not be relied upon, as tearing and displacement of tissues might have lead to a wrong interpretation of bladder structure.

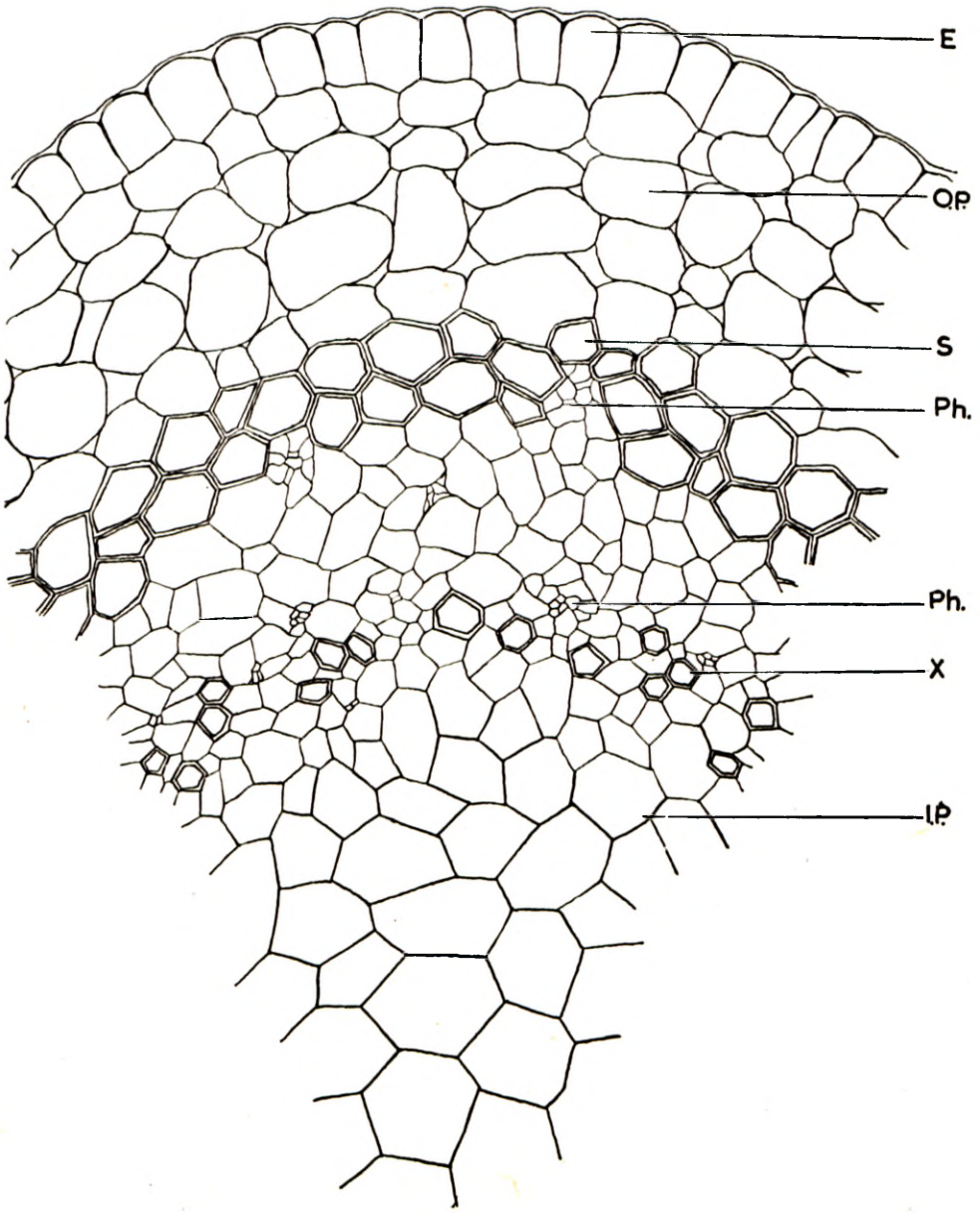

FIG. 6.—*Portion of transverse section through aerial stem of Utricularia transrugosa Stapf.*
E: epidermis. O.P: outer parenchyma (chloroplasts are not shown).
S: sclerenchyma band. Ph.: phloem. X: xylem vessel.
I.P: inner parenchyma.

(a) ANATOMY OF THE AERIAL SHOOT.

Epidermal cells of the aerial shoot are large and heavily cuticularised. Their radial walls appear to be thickened towards the outside. Many stomata are present, particularly towards the base of an aerial shoot. (See leaf anatomy for structure of stomata.)

Immediately within the epidermis is a zone of parenchymatous tissue about 4 cells wide. These cells are rounded and arranged in longitudinal rows with numerous intercellular air-spaces. Chloroplasts are abundant in this region. No distinct endodermis could be seen. The outer parenchyma zone was bounded internally by a complete sclerenchyma band, 1–3 cells wide.

The vascular tissues of *U. transrugosa* are embedded in a groundwork of angular parenchyma cells, which are smaller towards the sclerenchyma zone becoming larger towards the centre of the stem. No air-spaces are present between these cells.

Xylem vessels are arranged in a circular zone a short distance from the sclerenchyma band. Vessels are scattered, occurring singly or in pairs. Thickening of their walls is either spiral or annular and both types of thickening may occur together in a single

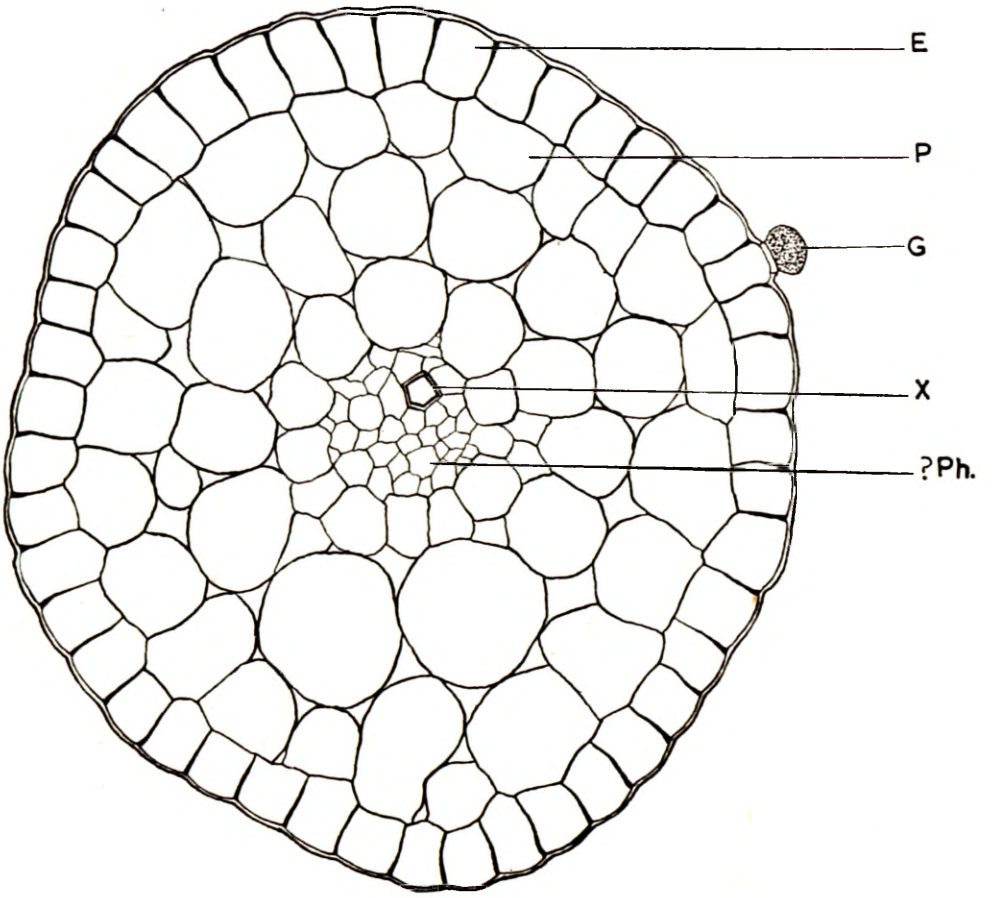

FIG. 7.—*Transverssection the rough stolon of U. transrugosa Stapf.*
E: epidermis. P: parenchyma. G: gland. X: xylem vessel. ?Ph.: phloem.

vessel element. Small, thin-walled patches of tissue are found scattered in the angular parenchymatous region near the vessels, and similar patches occur abutting on the sclerenchyma zone. These patches were interpreted as phloem.

(*b*) *ANATOMY OF THE STOLON.*

The epidermis and parenchyma of the stolons are similar to those found in the aerial shoot. In Fig. 7, one of the glands, which occur abundantly on the outer surface of the stolon, is shown. The inner basal cell is embedded in the epidermis; the middle cell is narrow and collar-like and bears the single rounded capital cell, which is not cuticularised.

Towards the centre of a stolon, parenchyma cells become smaller and closely packed. Here they surround a strand of thin-walled, tightly packed, angular cells in which a single xylem vessel is embedded. The xylem vessel is similar to those found in the aerial shoot. It is suggested that the angular cells may be phloem tissue.

(*c*) *ANATOMY OF THE LEAF.*

The leaf, as stated previously, is no more than an expanded stolon.

Fig. 8 shows that the central strand of a stolon continues into a leaf and there it divides dichotomously once, or more rarely, twice. The single xylem vessel, present in the stolon, passes into a leaf but terminates before the vascular strand branches into two, the veins consisting thereafter of angular cells only. Chloroplasts are numerous in the parenchyma cells of the leaf. Mesophyll is undifferentiated.

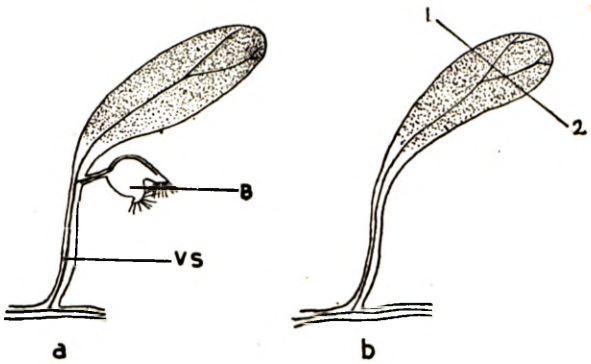

FIG. 8.—*Schematic representation of leaves of U. transrugosa Stapf.*
B: bladder. VS: vascular strand.

Epidermis and glands are similar to those described for stolons. Stomata are found towards the apex of the upper surface on the leaf, and very occasionally on the lower surface. They are not present in the lower cylindrical portion. This limited occurrence of stomata is to be expected as most parts of the leaf are usually embedded in mud or soil. The stomata are slightly sunken (Fig. 9) and, as will be seen from Fig. 10, they conform to the anomocyclic or "Ranunculaceous" type of Metcalfe and Chalk (1950) in that they are surrounded by cells which are indistinguishable in size, shape or form from those of the remainder of the epidermis.

The leaf epidermis does not strip readily, thus in order to study stomata, leaves were soaked overnight in Eau de Javelle and then the internal parenchymatous tissue

was scraped away, leaving the epidermis intact. For material which has been preserved in formalin, previous soaking in Eau de Javelle can be omitted.

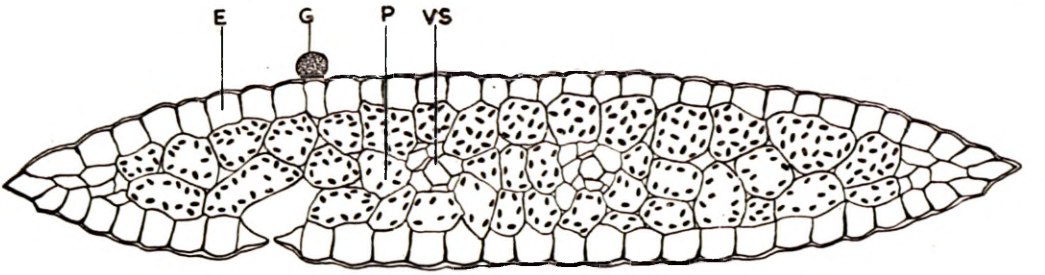

FIG. 9.—*Transverse section through upper portion of leaf.*

This section is cut through the line 1–2 of Fig. 8 b.

E: epidermis. G: gland. P: parenchyma with chloroplasts. VS: vascular strand.

Note absence of xylem vessels in this region.

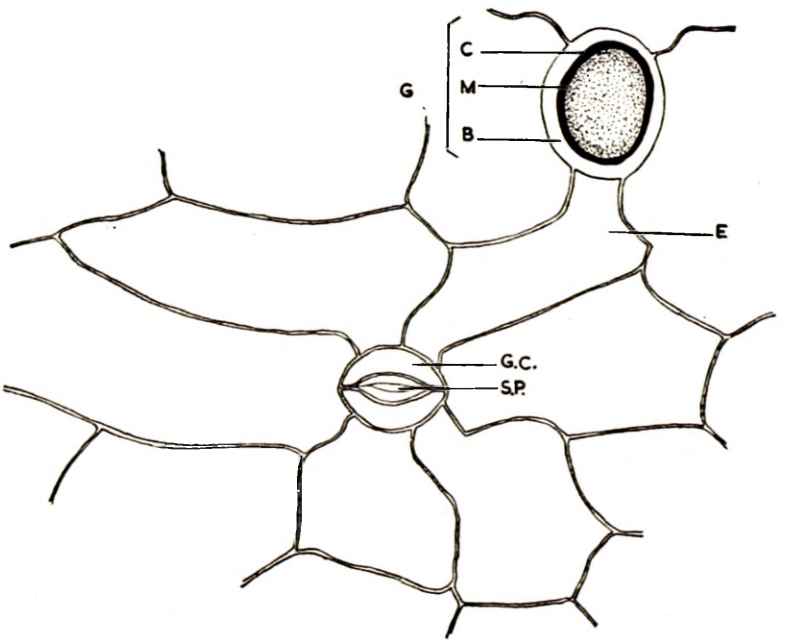

FIG. 10.—*Epidermis, showing stoma and gland in surface view.*

G: gland, which consists of C: capital cell, M: middle cell and B: basal cell. G.C: guard cell. S: stoma pore. E: epidermal cell.

The middle cell of the gland is represented by a dark line due to refraction caused by the spherical capital cell.

THE MORPHOLOGY AND ANATOMY OF THE BLADDER.

It has already been noted that the bladders or traps are present in large numbers on stolons and leaves of *U. transrugosa*. They are small ovoid-shaped structures, 2 mm. long and 1 mm. broad, and are slightly flattened on the sides. They are attached to a stolon or leaf by a narrow stalk which varies considerably in length. The cells at this point of attachment are slightly thickened and possibly prevent the bladder from being torn away. The stalk is attached near the base of one end of the bladder, while at the opposite end the walls of the bladder are produced into an upper and a lower lip. The two lips are joined laterally by folds of tissue which form the cheeks. These structures together form a protective funnel-like entrance to the mouth of the bladder. Lloyd has stated that the stalk end of the bladder is ventral, the mouth end dorsal (p. 233). It is more convenient however to describe dorsal and ventral surfaces of the bladder to correspond with upper and lower lips. From Fig. 11 it will be seen that the lips carry numerous glandular hairs which interlock with one another across the mouth entrance. Each hair consists of 4 cells. The lowermost or " wall cell " is a prolongation of an epidermal cell. This is followed by a basal cell, a narrow cylindrical middle cell, and a capital glandular cell (see Fig. 12 b). The glandular hairs are arranged in six longitudinal rows on both upper and lower lips. On the cheeks, glandular hairs are shorter and uniseriate. The large number, and

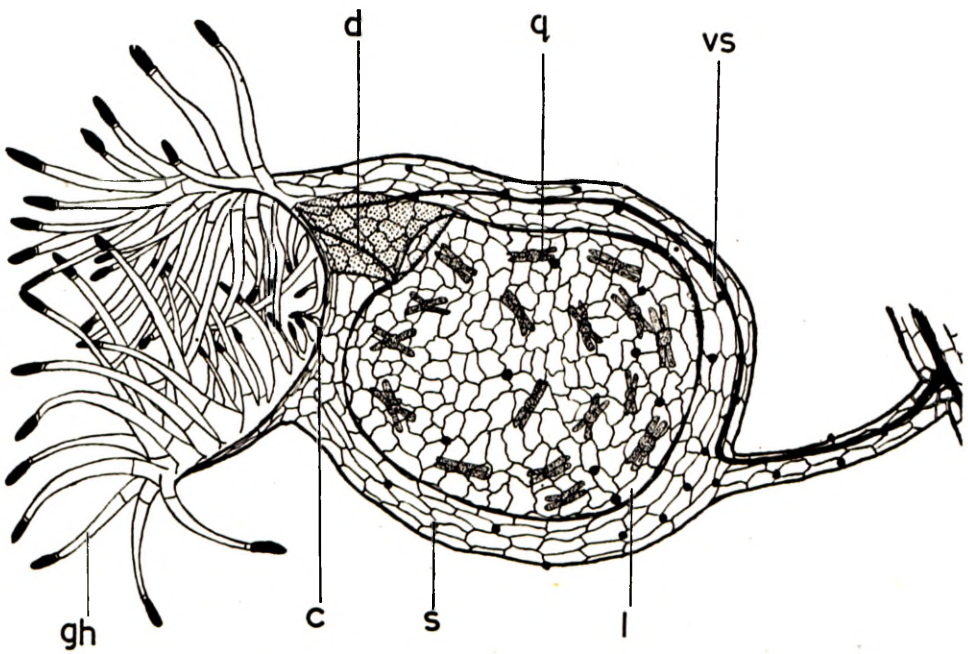

FIG. 11.—*Cellular representation of bladder of U. transrugosa Stapf.*

Only the outer wall is shown with the quadrifids being visible because of transparency of the bladder wall. The stippled area in the upper left of the bladder is the mouth region.

d: door. q: quadrifid. c: cheek. s: outer spherical gland. l: the shaded circular line indicates extent of lumen. vs: vascular strand. gh: glandular hair.

(NOTE.—Soaking bladders overnight in 20 per cent citric acid solution assists considerably in cleaning them from soil, detritus, algae, etc.)

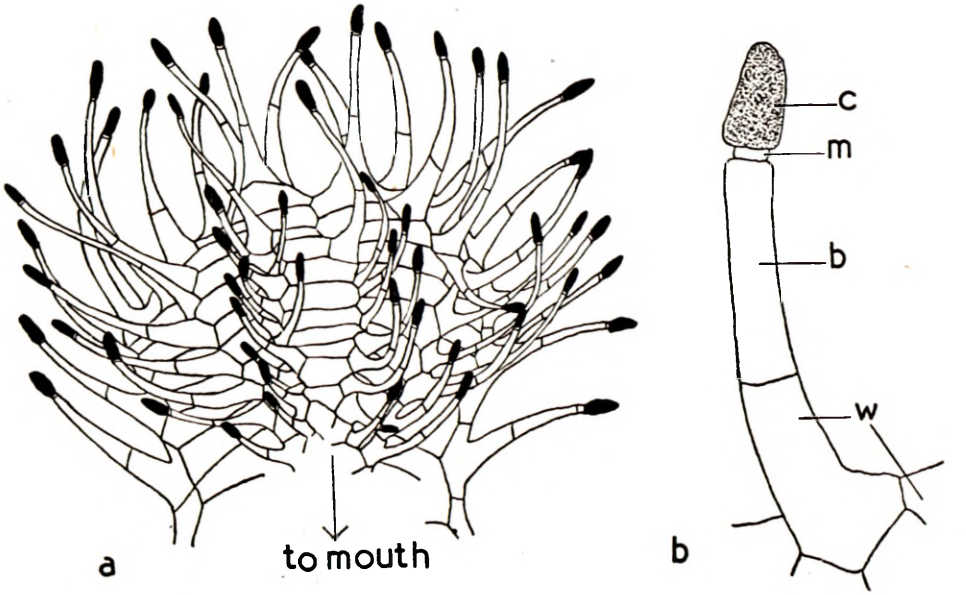

FIG. 12 (a).—*Upper lip of bladder seen from below.*
(b).—*A single glandular hair from lip of bladder.*
w: " wall cell ". b: basal cell. m: middle cell. c: capital cell.

interlocking positions of these glandular hairs probably serve two functions: (*a*) they form a protective covering to the mouth and prevent soil particles and detritus from clogging the entrance; (*b*) they assist in maintaining a continuous film of water around the trap mechanism.
the mouth. Water is essential to the proper functioning of the trap mechanism.

There are no antennae or bristles (Lloyd, p. 233) on the lips of the bladder of *U. transrugosa.*

The bladder wall is composed of two layers of cells except in the lip region where it becomes thicker. The outer wall layer is continuous with the epidermis of stolon or leaf. Scattered over this outer layer are the small, 3-celled, spherical glands which are present over the rest of the underground plant (see Fig. 7). The cells of this layer are elongated along the profile of the bladder (Fig. 11) and are smaller and compressed in the cheek region, but become more equidimensional and wavy-walled on the

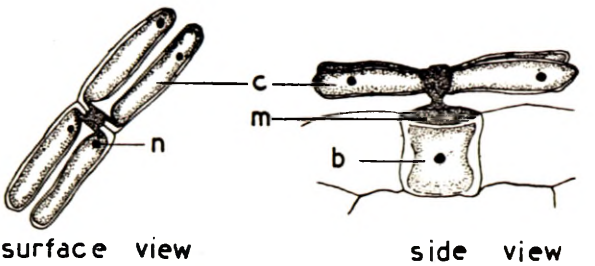

FIG. 13.—*Quadrifids in surface and side view.*
c: projection of capital cell. m: middle cell. b: basal cell. n: nucleus.

flattened sides of the bladder. These irregular walls may assist in retaining bladder shape during action and resetting of the trap when the sides are alternately convex and concave.

The cells of the inner wall layer are in general larger in surface area than the outer layer cells. In the lip and cheek region the two layers are separated by one to many, large, thin-walled parenchymatous cells. The two walls enclose a central cavity or lumen which is reached from the outside through the mouth.

A single vascular strand passes into the bladder through the stalk, travels dorsally in the wall layers without branching, and finally terminates in the tissue of the upper lip.

Projecting into the lumen are glandular hairs bearing two or four armed capital cells. Darwin called them bifids and quadrifids (1888). The bifids are limited to the mouth region. Each gland arises from a cylindrical basal cell and has a disc-shaped middle cell, and a 2- or 4-armed capital cell which is devoid of cuticle. The arms of the quadrifids are equal in length and are not spreading (Fig. 13).

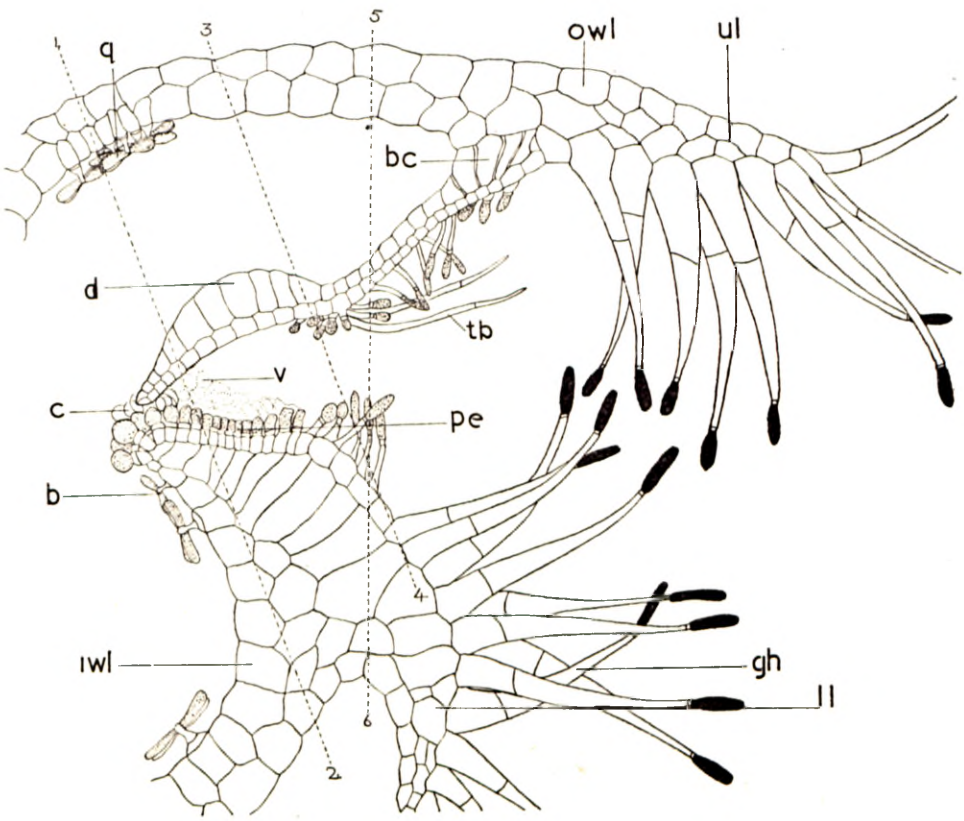

FIG. 14.—*Longitudinal section through mouth region of bladder.*

owl: outer wall layer. iwl: inner wall layer. q: quadrifid. b: bifid. d: door. v: theoretical position of velum. pe: pavement epithelium. c: balloon-like cuticles. ul: upper lip. ll: lower lip. gh: glandular hair. tb: tripping bristle. bc: buttress cell.

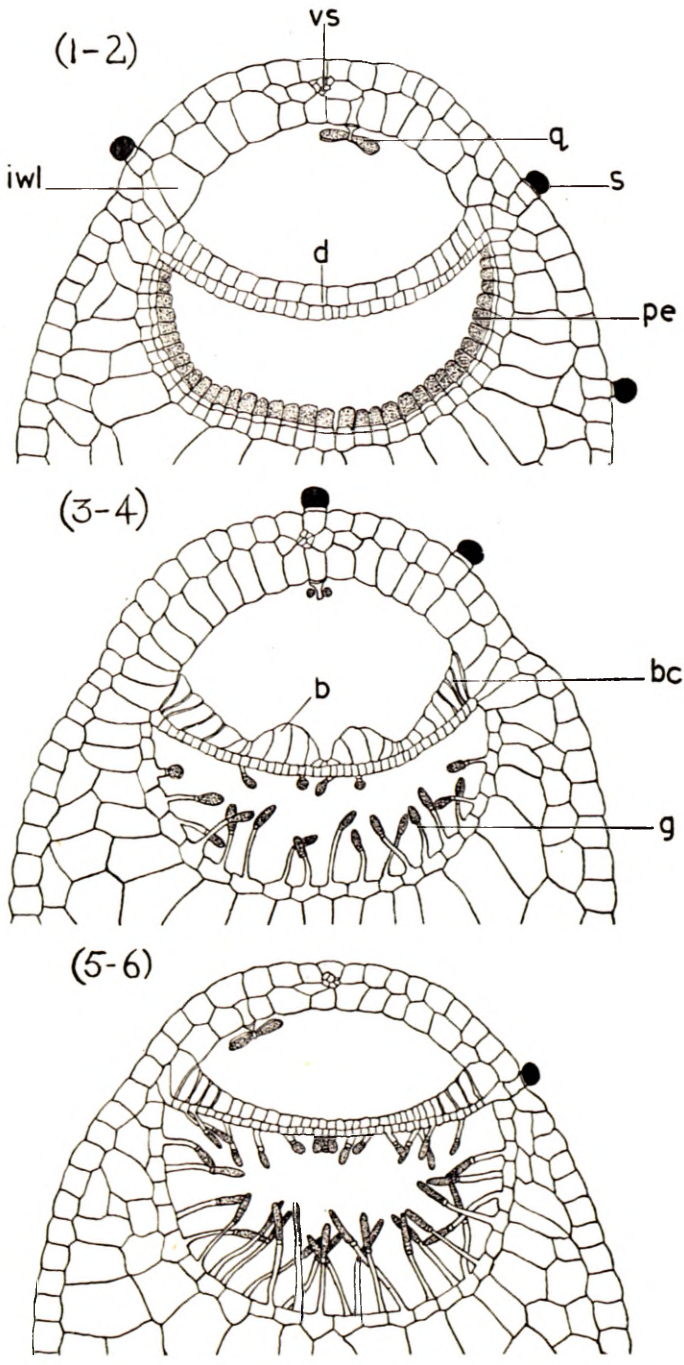

FIG. 15.—*Sections through bladder along the lines* 1–2, 3–4, *and* 5–6 *of Fig.* 14.

vs: vascular strand. q: quadrifid. s: outer spherical gland. pe: pavement epithelium. iwl: inner wall layer. d: door. g: gland on threshold.

The basic structure of the mouth region can be studied from Figs. 14, 15 and 16. In longitudinal section it will be seen that the upper and lower lips project beyond the rest of the bladder and that they are several cells in thickness. The lower lip also extends back in towards the lumen of the bladder as a wedge-shaped shelf—the collar or threshold. The bladder entrance is guarded by two valves; a larger one, the door, hanging obliquely inwards at an angle of 45°, and a smaller one, the velum. The door is an oval flap of tissue which hangs downwards from the inner margin of the upper lip. Its base rests against the threshold (Fig. 14). In its upper half, the sides of the door are attached to the cheeks, which are the flaps of tissue connecting upper and lower lips. In its lower half, the sides of the door are attached to the " pavement epithelium " (of Goebel), which is a glandular layer arising from the threshold. In this way the cheeks and pavement epithelium form a tunnel-like entrance with the door hanging obliquely across the tunnel and being attached on all sides except at the base.

The angle at which the door hangs downwards is about 45°, and this together with the general structure of the mouth region, classifies the bladder of *U. transrugosa* as one of the " long tubular entrance " type of Lloyd (p. 258).

From Fig. 15 it will be seen that the door in transverse section is not flat but it hangs like a hammock, the concave side being towards the upper lip, the convex side towards the lower lip. Fig. 14 shows the door in longitudinal section. It is evidently curved in this plane also.

In lower surface view, Fig. 16, it will be seen that the door is roughly oval in shape with the lower free edge truncated and appearing slightly concave due to flattening of the door in making the preparation for Fig. 16. Two distinct regions are visible; an upper and a lower. The lower half of the door is composed of small, thin-walled cells and is non-glandular. Cells in the upper half of the door are somewhat large and bear numerous glands. These glands radiate outwards from a central point where they are very short, and are also found on the invaginated cheeks and on the inside of upper and lower lips where they are much longer. The glands are slightly different in structure from other glands of *U. transrugosa* in that they are " sessile " i.e. the normal basal cell is lacking, and they arise directly from prolongations of the cells which produce them. The capital cells of these glands in the central region of the door are small and spherical, but in the upper and outer regions of the door they are greatly elongated. From the central point at the junction of upper and lower halves of the door and from which the glands radiate outwards, arise two long, stiff, tapering bristles. Very rarely, three of these bristles were seen. They form the tripping mechanism. Each bristle is of two cells, the upper being much smaller than the lower, and the lower cell arising directly from the door without any bulbous swelling at its base. These bristles in all cases, projected outwards and upwards towards the upper lip.

In longitudinal section (Fig. 14) the door is seen to consist of two layers of cells; an outer one from which the glands and tripping bristles arise, and an inner layer of cells. Two distinct regions of the door are again visible; an upper glandular, and a lower non-glandular region. In the upper portion, cells of the inner layer are slightly broader than outer layer cells (in a ratio of about 2: 1) and are similarly deeper, except at the point of attachment of the door to the upper lip where cells of the inner layer are about three times as deep as the outer layer cells. In the central region of the door the cells of both layers are approximately equidimensional.

The lower non-glandular portion of the door has a characteristic and peculiar structure, similar to that found in *U. kirkii* Stapf. (Lloyd, p. 232, 260, Plate 33.) Cells of the inner door layer are greatly enlarged and form two lateral bulges separated by a narrow groove (Fig. 15). In Fig. 14 one of these bulges is shown in longitudinal section.

The door is attached to the rest of the bladder along a semicircular line, and considerable stress is placed on cells along these lines of attachment during action of the trap.

This is compensated for by the presence of thickened " buttress " cells in the inner layer of the door. These buttress cells are shown in surface view in Fig. 16. They are visible because of transparency of the outer cell layer of the door. It was not possible to confirm Lloyd's interpretation of these " buttress " cells. He states that " the cells are constricted at regular intervals " and that the spaces between the

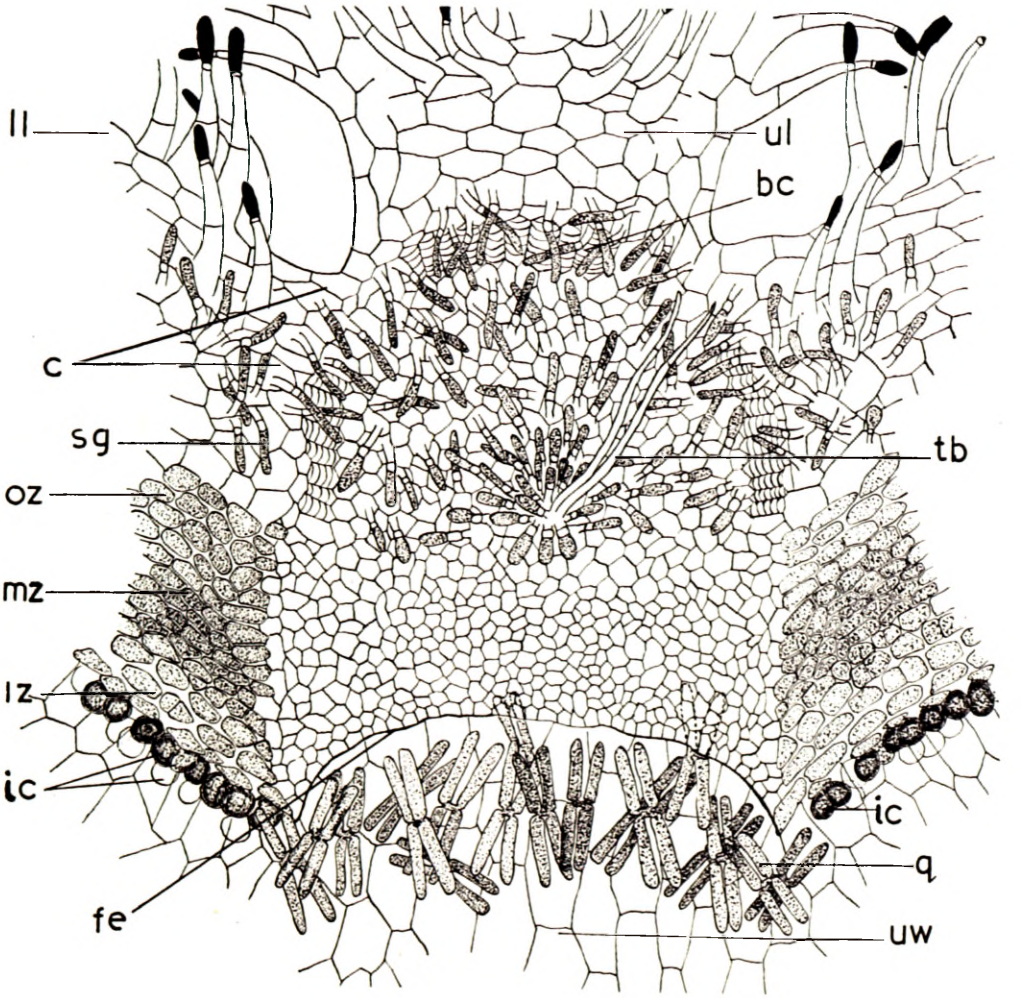

FIG. 16.—*Door of bladder in lower surface view.*

This preparation was obtained by slitting the threshold along a median line and spreading it out on either side of the door. This was then viewed flattened back against the upper wall and lip of the bladder.

ll: lower lip. c: cheek. sg: sessile gland. oz, mz, iz: outer, middle and inner zones of pavement epithelium. ic: balloon-like cuticles from inner zone. fe: free edge of door. uw: upper wall of bladder. q: quadrifid. tb: tripping bristle. bc: buttress cell. ul: upper lip.

constrictions are mistaken for single cells. (See Lloyd, p. 250 and Plate 33, No. 2.) Microtomed sections failed to confirm this and the view is expressed that these are indeed single cells. They are shown in transverse and longitudinal sections in Figs. 15 and 14. Buttress cells are not found where the door is attached to the pavement epithelium.

The pavement epithelium is a glandular layer arising from the threshold. The glands of the pavement epithelium are also sessile in that their lowermost cells are the outer layer of the threshold, and a true basal cell is lacking. In Fig. 14 it can be seen that these glands are tightly packed together in the central region, becoming wider spaced and separated from each other at either end. Three zones are thus distinguishable; an outer, middle and inner zone.

The velum described by Lloyd (p. 247) was not seen intact during these investigations. Its theoretical position has been indicated by dotted lines in Fig. 14. The velum is formed by the cuticles which are shed from the capital cells of the pavement epithelium glands. These cuticles remain attached to one another and to the capitals of the outer zone glands, and form a thin membranous flap which covers the free edge of the door. It is only the cuticles of the outer and middle region glands which form this velum, while those from the inner zone of glands behave individually. These enlarge and burst forming a cushion of balloon-like tissue which blocks the small chink between the edge of the door and the pavement epithelium glands. These were observed and have been represented in Figs. 14 and 16.

Thus it may be seen that the bladders of *Utricularia transrugosa* are structurally and anatomically complex. This allows for the intricate mechanism of trap action, which has been fully described by Lloyd.

POINTS OF BIOLOGICAL INTEREST.

(*a*) *PERENNATION.*

It has been established during these investigations that *U. transrugosa* is perennial under field and laboratory conditions.

A block of earth, about 10 inches × 10 inches × 10 inches was dug from the vlei where *U. transrugosa* has previously been collected. At the time of removing the earth (May, 1952) there were no visible signs of the presence of the plant on the surface of the vlei. The earth was kept in the laboratory at room temperature (24–28° C.) and was watered daily with tap water. No artificial nutrients were supplied to the soil. After two months (July, 1952) the plant had not reappeared in the vlei, but small green leaves appeared on the surface of the soil which had been kept in the laboratory. The plant from which these leaves were produced was then dissected free of soil. A considerably thickened stoloniferous structure was noticed, and from this arose " new " stolons bearing young green leaves. Clinging to the thickened portion were the remains of old, decayed stolons and leaves. The regular branching previously described for *U. transrugosa* Stapf was not evident in this perennating portion of the plant. As thickened structures were not found to be present in dissections of mature flowering plants, one may assume that as the young plants establish themselves they either break away from the thickened portion or this shrivels due to depletion of food reserves. Plants grown in this way in the laboratory did not develop rhizoids and all the bladders present were empty. Thus all nutrients for the developing plant must have come from the thickened stoloniferous portion or have been absorbed from the soil through the non-cuticularised capitals of the spherical glands.

Under field conditions, too, similar thickened perennating parts of the plant were found. A very young plant of which only the tips of the leaves were visible, was taken from mud along the banks of a stream and dissected free of soil and detritus. Fig. 17 shows two of the thickened areas found on this plant. It was noted that young stolons arising from these thickened parts were green, especially at the rounded growing

apices. The preparation from which Fig. 17 was drawn was left soaking in tap water in the laboratory for two weeks, after which time the small green outgrowth (Fig. 17, z) had developed from one end of the thickened portion.

The ability to develop perennating structures is not an uncommon phenomenon among the Utricularias. *U. globulariaefolia*, a fairly large terrestrial species, becomes perennial by its stout, tough stolons, similarly to *U. transrugosa*. In *U. volubilis* the plant body is an upright corm which grows at the top and dies behind. From the corm are produced numerous leaves. Wager (1928) described the ability of *U. stellaris* to form resting buds towards the end of a season. These buds were then able to develop the following spring. Many species of Utricularia have now been found to produce resting buds and the structural differences of these buds have been used as a basis for classification of the species (Rossbach, 1939).

(*b*) *GERMINATION.*

All efforts to germinate seeds of *U. transrugosa* were unsuccessful. Seeds were selected, and sterilised by soaking for 2 mins. in mercuric chloride (0·1 per cent in 50 per cent alcohol). They were then washed in sterile distilled water and left to soak overnight under sterile conditions.

Seeds were then placed on moist sterile filter paper, or on agar in petri plates, 10 seeds per plate, and were kept in the laboratory at room temperature which fluctuated between 24–28 C. Two kinds of agar were used; Difco bean pod agar which had

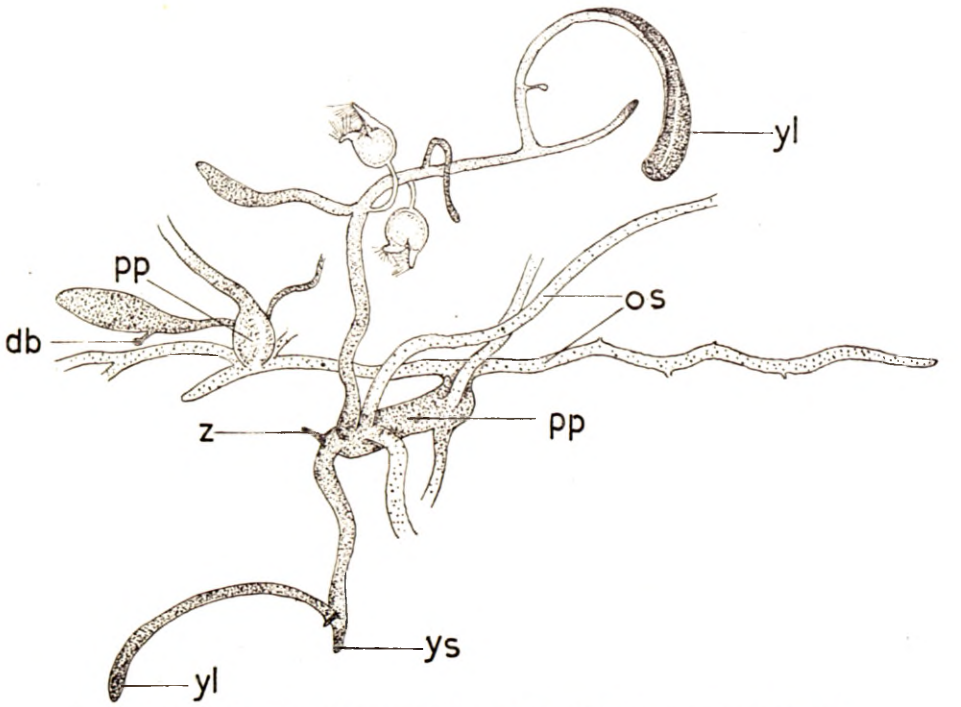

FIG. 17.—*Portion of perennating plant of U. transrugosa Stapf.*
(Taken from natural habitat.)

The density of stippling is proportional to the greenness of the plant areas.

pp: perennial portion. yl: young leaf. yz: young stolon. os: old stolon. db: developing bladder. z: young green shoot (see text).

a final pH of 5·3, and a second medium prepared as follows: 150 g soil, taken from the vlei in which the plants had been found growing, was thoroughly shaken up with 150 ml distilled water, and left to soak for 3 hrs. The suspension was then filtered and 2 per cent agar was added to the liquid obtained. The final pH of the medium was between 4·6–4·8.

It was expected that as the plants grew in acid soils, media having low pH values would be conducive to the germination of seeds. Unfortunately no definite reason is known for the failure of germination.

(*c*) *POLLINATION.*

The manner in which pollination is effected in *U. transrugosa* can only be conjectured. The floral characteristics of the plants are highly suggestive of adaptions to cross-pollination by insects. The colour of the flowers together with their honey-like scent would act as a lure to insect visitors, which would then be directed toward the mouth of the flower by the conspicuous transverse rugosities. In entering the mouth of the flower the insect would brush against the broad upwardly facing stigmatic lobe, and on continuing downwards into the spur of the flower it would collect pollen from the anthers. It is of interest, that if the upper corolla lobe is slightly depressed (as by the entry of an insect) the anthers are drawn away from the mouth of the spur and face obliquely upwards thus being in a position which makes it impossible for an insect to enter or lave the spur without contacting the anthers.

In retreating from a flower the insect would brush the non-receptive underside of the stigmatic lobe and self pollination would be avoided.

(*d*) *VARIATION IN NATURE.*

Several instances of variation within the species *U. transrugosa* have already been given and need only be summarised here. The most conspicuous variation occurs in flower size and colour, the purple flowers being generally the larger. The apical margin of the upper corolla lip varies in shape.

Vegetative structures are more constant however, the only variations that have been noted being the length of bladder stalk, and the presence of 2 or 3 tripping bristles on the door of the bladder.

Kamienski (1895) and Oliver (1867) have both noted a wide range of variation in floral characteristics within the species of Utricularia. This variability is of importance when one studies the characteristics which are used to distinguish species of Utricularia especially in the case of *U. livida*/*U. transrugosa* and *U. kirkii* or in the case of *U. capensis*/*U. brachyceras* and *U. ecklonii*. This will be treated more fully in the following discussion.

(*e*) *FOOD OF* U. TRANSRUGOSA *STAPF.*

If one accepts that Utricularias obtain or supplement their nitrogenous food requirements by the capture and digestion of animal prey, for which processes water is essential, the survival of *U. transrugosa* in an acid semi-dry vlei is intersting. Examination of bladders from this plant, however, showed that although soil water content was so low, the bladders were functioning efficiently. Decayed animal remains were found in most bladders and appeared to consist generally of small arthropod larvae, crustaceans and nematodes. In some cases prey was extracted alive from the bladders.

An attempt to grow Utricularias in the laboratory was only partially successful. The plants were kept in a trough containing peat and large quartzite pebbles, and were watered regularly. The water was taken from an outdoor alga-tank which contained abundant small animal and plant life. The Utricularias remained alive, but only one plant produced an aerial stem, which however failed to bear flowers even though watered with beef extract solution.

(f) PHENOLOGY.

U. transrugosa has a relatively short flowering period (about 3 weeks) and its time of flowering is largely dependent on adequate early summer rains. The plant was collected at Bryanston in September, 1951, after good rain, but in 1952 two flowering periods were noticed. In August and September 1952, a very dry period, isolated dwarf specimens were collected from the vlei and stream at Bryanston. Later in the year (November), after rains had fallen, flowers were again collected from the stream locally. Unfortunately, the vegetation covering the original vlei was burnt between September and November and the vlei has now completely dried out. *U. transrugosa* did not reappear in this site, even after heavy rains, and whether its disappearance is temporary or not cannot be told until the following summer.

It was evident that the long dry period in 1952 had a deleterious effect on the flowering of *U. transrugosa*, which had flowered far more abundantly in 1951.

DISCUSSION.

The most striking point arising out of these investigations is the anatomical and morphological similarity between *U. transrugosa* Stapf and *U. kirkii*, Stapf. In his analysis of the genus in Flora Capensis, Stapf separates the two species by flower size, *U. kirkii* being considerably smaller, and by the presence of rugosities on the palate of *U. transrugosa* contrasted with the minute tubercles in *U. kirkii*. However, as many species of Utricularia show variation in flower size, the only tangible difference between *U. transrugosa* and *U. kirkii* is the presence of rugosities or tubercles on the palate. That this is the principal difference is further substantiated by Lloyd, whose figure illustrating the bladder of *U. kirkii* shows this structure to be almost indistinguishable from the bladders of *U. transrugosa*, described in this report. Both species are terrestrial, and Fig. 1 shows that they have been recorded from similar localities i.e. in the Transvaal, and a short distance from the Zambesi in Central Africa. Lloyd, (p. 260) has further stated, with regard to the bladder, that " *U. kirkii*, occurring in Central Africa, is apparently unique ". Also (p. 232) " *U. kirkii* is an African species with apparently few associates if any, and has a distinct form of trap ". The two lateral bulges formed by the inner cells of the door (called tubercles by Lloyd), have not yet been described for any other species. Bladders of *U. transrugosa* and *U. kirkii* are also similar in that they lack a " doorstep " at the point where the lower lip turns inwards to produce the pavement epithelium. In other species of Utricularia, the lower lip forms a non-glandular, flat, steplike ridge of tissue leading to the pavement.

Two alternatives present themselves either: (*a*) *U. transrugosa* is a large flowered form of *U. kirkii*, or (*b*) the two species are a vicarious pair.

The earlier indentification of *U. transrugosa* as *U. livida var. transrugosa* leads one to consider the differences between the two species *U. livida* and *U. transrugosa*. Again it is found that the distinguishing features are corolla size, and the presence of tubercles on the palate of *U. livida* and of rugosities in *U. transrugosa*. Unfortunately no details of bladder structure are available for *U. livida*. If bladders were examined and found to conform to the *U. kirkii*/*U. transrugosa* type, an interesting position, worthy of further investigation, would arise. *U. exilis* Oliver from Flora of Tropical Africa (Fig. 1, F) which is very similar to *U. kirkii* and has a smooth palate, is of the same affinity also.

Miss L. Stevens (1938) attempted to unravel the relationship between the species *U. capensis*/*U. brachyceras*/*U. ecklonii*. Stapf (1904) maintained that *U. brachyceras* was a short spurred form of *U. ecklonii* and that *U. capensis* was a distinct separate species. Miss Stevens implies, however, that *U. ecklonii* is a growth form of *U. capensis* and should be sunk in that species' while *U. brachyceras* is quite distinct from the combined *U. capensis—U. ecklonii*. Her opinion is based on relative sizes of the stigmatic lobes.

The work of Lloyd, however, has made it obvious that in cases of close floral similarity, differentiation of species cannot be based on floral characteristics alone. The pattern of branching and bladder structure are sufficiently constant and different between species, to be used as features for taxonomic classification.

Although Lloyd does not refer to *U. transrugosa*, his descriptions of bladder types occurring among the genus Utricularia have proved useful guides.

SUMMARY.

A morphological and anatomical description of the floral and vegetative characters of *Utricularia transrugosa* Stapf is given.

Anatomical details of the bladder place it in the " long tubular entrance " group of Lloyd, and show a striking similarity between *U. transrugosa* and *U. kirkii* Stapf.

Biological findings indicate that *U. transrugosa* is perennial, and like many Utricularias shows considerable variation in flower size and colour.

ACKNOWLEDGEMENTS.

The writer is grateful to Professor N. P. Badenhuizen for permission to carry out these investigations in the Botany Department, University of the Witwatersrand, and to Dr. H. B. Gilliland for his constant guidance and assistance in collecting material.

The Chief, Division of Botany, and the Director, Transvaal Museum are thanked for the use of the facilities of the National Herbarium and of the Transvaal Museum respectively.

BIBLIOGRAPHY.

COMPTON, R. H..... 1909. The Morphology and Anatomy of *Utricularia brachiata*, Oliv. New Phytologist **8**, 117.

DARWIN, C..... 1888. Insectivorous Plants (London).

KAMIENSKI, F..... 1902. Utricularia in Bot. Jahrb. **33**, 92.
1897. Utricularia in Engler-Prantl Nutur. Pflanzen. IV Teil IIIb abt., 118

Lloyd, F. E..... 1942. The Carnivorous Plants (Waltham, Mass.).

METCALFE, C. R., AND CHALK, L..... 1950. Anatomy of Dicotyledons, **2**, 993.

OLIVER, D..... 1867. African Lentibulariae. J. Linn. Soc. **9**, 153.

ROSSBACH..... 1939. Winter buds among Utricularia. Rhodora **41**, 114.

SOLREDER, H..... 1908. Systematic Anatomy of Dicotyledons, **1**, 694.

STAPF, O..... 1904. Lentibulareaceae in Fl. Cap. (London) **IV. 2**, 429.

STAPF, O..... 1906. Lentibulareaceae in Fl. Trop. Afr. (London), **IV. 2**, 468.

STAPF, O..... 1931. Lentibulareaceae in Fl. W. Trop. Afr. (Lndon) **2**, 232.

STAPF, O..... 1916. Utricularia papillosa Stapf. Kew Bull. **41**.

STEVENS, E. L..... 1938. Notes on three South African Utricullariae. Journ. S.A. Bot.

WAGER, V. A..... 1928. Notes and descriptions of a Few Uncommon Water Plants. Trans. Roy. Soc. S.A. **16**, 191.

WEINTROUB, S..... 1933. A Preliminary Account of Aquatic and Sub-aquatic Vegetation and Flora of the Witwatersrand. J. Ecol. **21**, 44.