The epidermal structure and ontogeny of stomata in all vegetative and floral organs of *Stenoglottis fimbriata* were investigated. Epidermal cells are polygonal or isobilateral. The leaf sheath, inner whorl of the perianth, column and tuber are astomatic. Other organs investigated are heterostomatic and of anomocytic origin. The ontogeny of stomata is perigenous and attention has been paid to various aspects of development. Abnormalities such as degeneration of guard cells, superimposed and juxtaposed contiguous stomata, persistent stomatal cell contiguous with normal stoma, and stoma with persistent intervening wall, were observed. Stomatal and epidermal cell frequency, and stomatal index are also tabulated. These characters are of taxonomic importance for the family Orchidaceae.

Die bou van die epidermis en die ontogenie van huidmondjies is by alle vegetatiewe en blomorgane van *Stenoglottis fimbriata* ondersoek. Epidermiscell is poligonaal of isobilateraal. Die blaaroskede, binneste perianthkrans, suil en knoel is sonder huidmondjies. Ander organe wat ondersoek is, is heterostomaties van anomositiese oorsprong. Die ontogienie van huidmondjies is pengeen en daar is genoeg aandag aan die verskillende aspekte van ontwikkeling gegee. Abnormaliteite soos degenerasie van sluitselle, oortoeenlikgende en teenaanliggende aangrensende huidmondjies, bywende tussenliggende wand, is waargeneem. Die grootte huidmondjies onder blomplante is ook by die spesie aangeteken. Huidmondjie-indeks word getabuleer. Hierdie kenmerke is van groot taksonomiese belang by die familie Orchidaceae.

**Keywords:** Epidermal structure, ontogeny, organographic distribution, *Stenoglottis fimbriata*, stomata.

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**Introduction**

*Stenoglottis fimbriata* Lindl. is one of the many orchid species reported from the coastal zones of Transkei (Stewart et al. 1982). Considerable work has been done on vessel structure of Orchidaceae by Cheadle & Kosakai (1982), but very little attention has been paid to the epidermal structure of orchids. Rutter & Willmer (1979) studied the epidermis of *Paphiopedilum* spp. and Hew et al. (1980) have reported on the distribution and frequency of stomata in flowers and leaves of a few tropical orchids. Williams (1979), Rasmussen (1981) and Singh (1981) studied the diversity of stomatal developments in Orchidaceae. Recently Das & Paria (1992) investigated the stomatal structure of some Indian orchids with reference to taxonomy. This investigation was undertaken to investigate the epidermal structure, organographic distribution, structure and ontogeny of stomata in *Stenoglottis fimbriata* with reference to its taxonomic significance.
Material and Methods

Plants of Stenoglottis fimbriata in blossom were collected from Port St. Johns, Langeni forest, Umtata and the Unita campus, Transkei, South Africa. Epidermal peels were taken from vegetative and reproductive parts, stained in Delafeld's haematoxylin and mounted in glycerine. Epidermal layers were also taken by gently heating the specimens in dilute nitric acid. The peels were thoroughly washed in distilled water and neutralized in dilute sodium hydroxide solution for better staining. The technique of Bhat & Etejere (1985) was also used to take imprints of the epidermis. Observations were made by using a binocular microscope Leitz Lab 11. Mean values of thirty observations of each organ were taken to calculate the stomatal frequency, stomatal index, size of the stomata in μm, frequency and size of epidermal cells. The results are given in Table 1. All the observations are supported by the diagrams, drawn by using camera lucida (Figures 1 – 20). The terminology used to explain the stomatal types is from Metcalfe & Chalk (1950) and Van Cotthem (1970).

Results

Epidermis

The leaves are hypostomatic while the bract and the outer whorl of the perianth are amphistomatic. The peduncle and ovary wall are stomatic, while the tuber, sheathing leaf base, inner whorl of the perianth and the column are astomatic. The plant organs which are stomatic show anomocytic (Figure 4), staurocytic (Figure 7), tetracytic (Figures 6 & 12) and actinocytic (Figure 19) stomata. The anomocytic stomata of Metcalfe & Chalk (1950) are the predominant type observed. Anomocytic stomata with three neighbouring cells are frequently present in all the organs investigated (Figures 8 & 15). In all cases the stomata are oriented along the long axis of the organ. Chloroplasts are clearly observed in the guard cells of the vegetative parts, but in the guard cells of the floral parts some colourless bodies are noticed. The walls of the ventral side of the guard cells are strongly thickened around the stomatal pores (Figures 2, 8 & 12). In many cases a persistent intervening wall (middle lamella) is found in the stomatal pore between the guard cells (Figures 4, 6, 7 & 8). Stomatal distribution is uniform on

guard cells and are found oriented along the long axis of the stomata (Figures 2, 5 & 6). The anticlinal cell walls are straight or slightly arched in leaves, peduncle, sheathing leaf base and at the basal part of the bract, but they are arched or sinuous in the perianth, ovary wall, column, tuber and the upper part of the bract (Figures 3 & 5). The maximum length of the epidermal cell observed is 399 μm on the adaxial side of the sheathing leaf base, while the minimum length of the epidermal cell is 78 μm found in the column. The maximum width is 162 μm on the adaxial surface of the leaf while a minimum of 44 μm is found on the adaxial side of the perianth. The highest and the lowest epidermal cell frequency per mm² is 219 and 56 on the adaxial surface of the bract and on the abaxial surface of the leaf, respectively.

Trichomes and papillae

Unicellular, uniseriate trichomes with blunt tips are observed on almost every alternate epidermal cell on the peduncle (Figure 14). Water vesicles are commonly observed on the adaxial side of the lamina, bract and perianth (Figures 4, 5, 12 & 13). Vesicular hairs are also observed on the margins of the perianth lobes (Figure 20).

Stomata

The stomata are exceptionally large in size. The plant organs which are stomatic show anomocytic (Figure 4), staurocytic (Figure 7), tetracytic (Figures 6 & 12) and actinocytic (Figure 19) stomata. The anomocytic stomata of Metcalfe & Chalk (1950) are the predominant type observed. Anomocytic stomata with three neighbouring cells are frequently present in all the organs investigated (Figures 8 & 15). In all cases the stomata are oriented along the long axis of the organ. Chloroplasts are clearly observed in the guard cells of the vegetative parts, but in the guard cells of the floral parts some colourless bodies are noticed. The walls of the ventral side of the guard cells are strongly thickened around the stomatal pores (Figures 2, 8 & 12). In many cases a persistent intervening wall (middle lamella) is found in the stomatal pore between the guard cells (Figures 4, 6, 7 & 8). Stomatal distribution is uniform on

Table 1 The organographic distribution, frequency and index of stomata mm⁻², size of guard and epidermal cells (μm), and epidermal frequency and nature of anticlinal walls in Stenoglottis fimbriata Lindl.

| Name of species | Organ       | Frequency mm⁻² | Index | Size of guard cell L w | Size of epidermal cell L w | Anticlinal walls |
|-----------------|-------------|----------------|-------|------------------------|----------------------------|------------------|
| Stenoglottis fimbriata | Lower leaf | 9              | 14    | 79 27                  | 135 98                     | Straight/arched  |
|                  | Upper leaf  | –              | –     | –                      | 217 162                    | Straight         |
|                  | Leaf sheath L. | –         | –     | –                      | 219 69                     | Straight         |
|                  | Leaf sheath U. | –           | –     | –                      | 399 81                     | Straight         |
|                  | Peduncle    | 5              | 6     | 79 22                  | 176 55                     | Straight         |
|                  | Bract L.    | 11             | 5     | 57 20                  | 90 50                      | Sinuous          |
|                  | Bract U.    | 6              | 3     | 50 23                  | 95 59                      | Arched           |
|                  | Perianth    |                |       |                        |                            |                  |
|                  | Outer L.    | 4              | 3     | 50 20                  | 102 67                     | Arched           |
|                  | Perianth    |                |       |                        |                            |                  |
|                  | Outer U.    | 2              | 1     | 43 20                  | 87 54                      | Arched           |
|                  | Perianth    |                |       |                        |                            |                  |
|                  | Inner L.    | –              | –     | –                      | 137 62                     | Sinuous          |
|                  | Perianth    |                |       |                        |                            |                  |
|                  | Inner U.    | –              | –     | –                      | 102 44                     | Sinuous          |
|                  | Ovary wall  | 4              | 4     | 59 22                  | 115 83                     | Arched           |
|                  | Column      | –              | –     | –                      | 78 54                      | Arched           |
|                  | Tuber       | –              | –     | –                      | 91 63                      | Arched           |

L₄ = length; L = lower; U = upper; w = width; – = not observed.
Figures 1 – 20. 1. Lower leaf showing degenerated guard cell. 2. Lower leaf showing juxtaposed contiguous stomata and cuticular striations in the neighbouring cells. 3. Upper perianth showing sinuous anticlinal walls. 4. Upper bract showing water vesicles and persistent intervening wall in the stomatal pore. 5. Upper bract showing persistent stomatal cell contiguous with normal stoma. 6. Lower bract showing tetracytic stomata and cuticular striations in the neighbouring cells. 7. Perianth showing staurocytic stomata and a bundle of calcium oxalate crystals. 8. Lower leaf showing anomocytic stoma with three neighbouring cells and persistent intervening wall in the stomatal pore. 9. Leaf sheath showing elongated epidermal cells. 10. Lower leaf showing superimposed contiguous stomata with disintegrated common wall. 11. Epidermal cells of column with prominent nucleus. 12. Upper bract showing water vesicle. 13. Upper leaf showing epidermal cells with compressed water vesicles and extra-floral nectaries. 14. Peduncle showing unicellular trichome with blunt tip. 15. Lower leaf showing degenerating guard cells. 16 – 19. Stages of development of stomata. 20. Perianth showing vesicular hair on the margin. dgc = degenerated guard cells; psc = persistent stomatal cell; wv = water vesicle; cr = crystals; m = meristemoid; gc = guard cells; efn = extrafloral nectaries; pd = periclinal division. Magnification of Figures 1 – 20 = × 180.
leaves and peduncle, while on the bract the stomata are concentrated towards the apex and margins. It was also observed that on the ovary the stomata are concentrated towards the placental region. Bract and perianth stomatal frequency is higher on the abaxial side of the organ. The highest stomatal frequency is observed on the abaxial side of the bract with an average of 11 mm⁻² and the minimum, 2 mm⁻², is found on the adaxial surface of the outer whorl of the perianth. The highest stomatal index is 14 on the leaf and the lowest is 1 on the adaxial surface of the perianth.

Abnormalities include superimposed and juxtaposed contiguous stomata (Figures 2 & 10), stomata with a degenerating guard cell (Figures 1 & 15), a persistent stomatal cell contiguous with a normal stoma (Figure 5), and stomata with persistent intervening walls (Figures 4–8 & 12).

### Ontogeny of stomata

The ontogeny of stomata follows the perigenous type. Any young epidermal cell (protoderm cell) is able to divide unidirectionally by a periclinal division and the smaller daughter cell with a high concentration of cytoplasm is differentiated into a meristemoid (Figures 17 & 18). The meristemoid develops directly into two guard cells by an anticlinal division (Figure 16). It was also observed that a protoderm cell developed directly into a meristemoid, and such a meristemoid is hexagonal in shape (Figure 19). It has been noticed that the shape and the position of the periclinal wall formed to separate the meristemoid from the protoderm cell, determine the number of epidermal cells which surround the stoma. In the early stages of development a meristemoid may be triangular (Figure 17), rectangular (Figure 18), pentagonal (Figure 19) or hexagonal (Figure 19) in shape. The surrounding epidermal cells are capable of periclinal division (Figure 19). It is clearly seen that a meristemoid invariably undergoes an anticlinal division to produce two guard cells.

### Discussion

This study is concerned with details of the epidermal structure, the organographic distribution and the ontogeny of stomata of *Stenoglottis fimбриata* Lindl. The large epidermal cells of the leaf are oriented along the long axis of the organ, a typical monocotyledonous character (Esau 1960). Haberlandt (1918) reported that the external wall of the epidermal cells of certain leaves and petals is raised in the form of papillae and these papillae may have a function of concentrating light rays. Esau (1960) classified trichomes into four morphological categories. One of them is termed a water vesicle, an enlarged epidermal cell. The dome-shaped water vesicle observed on the leaf, bract and perianth of this species may also have a function of concentrating light, as this plant is generally seen growing in the shade. The large number of extra-floral nectaries found on the leaves may attract pollinating insects.

The orchid flowers exhibit a very low stomatal frequency and our observations conform with those of Hew et al. (1980). This investigation shows that *Stenoglottis fimбриata* exhibits exceptionally large stomata in all the organs studied. It seems that there is no consistency in the occurrence of stomata in the floral parts of orchids. Hsiang (1951) has reported that flowers of *Cattleya labiata* are devoid of stomata. Hew et al. (1980) observed the presence of stomata on all floral parts including the column of *Arachnis* sp. and *Aranda* sp., but this study on *Stenoglottis fimбриata* reveals that the column and inner whorl of the perianth are devoid of stomata. The presence of a persistent intervening wall in some of the stomatal cavities is a unique feature in the species. It may be due to the lack of sufficient pectinate production in the guard cells which is responsible for dissolving the middle lamella. Rutter & Willmer (1979) reported the presence of lipid droplets in the guard cells of *Paphiopedilum* spp. It is possible that the colourless bodies observed in the floral guard cells of *Stenoglottis fimбриata* could be lipid droplets. The presence of a persistent stomatal cell contiguous with the normal stoma may reveal that a guard mother cell is capable of further division to form two daughter guard mother cells and then they may develop into two superimposed contiguous stomata. In the observed case one of the daughter guard mother cells would have remained persistent. The orderly arranged cuticular striation noticed in the epidermal cells around the guard cells may have certain physiological functions which deserve further investigation. The factors that bring about stomatal abnormality are variously interpreted. According to Morgan (1934), it may be due to 'cytoplasmic heterogeneity', while McClintock (1956) attributes it to 'gene action'. Bunting (1952) suggested that aberrations are due to 'extrinsic factors'. Recently, Bhat (1992) reported that the presence of pathogens increases the stomatal abnormalities considerably in *Brassica oleracea* infected by *Peronospora parasitica*. Inamdar et al. (1977) reported that gamma irradiation causes a number of stomatal aberrations. It is clearly indicated that some extrinsic factors such as radiation, pathogens and even pollutants may be able to trigger an intrinsic instability which may finally lead to aberrations.

This study of the ontogeny of stomata in the species reveals that the developmental pattern of the stomata is of the tricytic type. Pant (1965); the shape and the position of the periclinal wall which separates the meristemoid from the protoderm determine the number of epidermal cells that surround the stoma. If the meristemoid is formed as a result of a 'U'-shaped periclinal wall towards one end of the protoderm, the meristemoid gets a triangular shape. The meristemoid divides anticlinally and develops into two guard cells which will be surrounded by three epidermal cells. Patel & Inamdar (1971) classified this type of stoma in dicotyledons as anomocytic. Pant & Kidwai (1966) and Farooqui (1981) termed this type tricytic. If the meristemoid is formed as a result of an oblique periclinal wall, the meristemoid gets a rectangular shape surrounded by four epidermal cells. At maturity it develops into a tetracytic stoma. If the meristemoid is formed as a result of a straight periclinal wall connecting the lateral walls of the protoderm, a pentagonal meristemoid is formed which is surrounded by five epidermal cells which will become an anomocytic stoma with five epidermal cells. If the hexagonal protoderm develops directly into a meristemoid, the meristemoid will be hexagonal in shape which may give rise to an anomocytic stoma with six epidermal cells. This type of perigenous development where the entire epidermal cell becomes a protoderm and directly gives rise to a meristemoid without undergoing any periclinal or anticlinal division, is being reported for the first time. This type of stoma can give rise to the actinocytic type if the walls of the neighbouring cells of the stoma are radiated. This observation shows that actinocytic stomata are common in the species studied and in other orchids under investigation.

In all cases the meristemoid undergoes an anticlinal division to produce guard cells. This consistent anticlinal division of the meristemoid for the development of the guard cells explains the typical homogeneous orientation of the stomata on the organs. The epidermal cells surrounding the stoma are able to divide further by periclinal division. Baranova (1992) recognized fourteen morphological types of stomata and she gives equal status to tetracytic, actinocytic, encyclocytic and stephanoctytic types, although, she does suggest that the above-mentioned stomata can be treated as variant forms of the anomocytic type. This investigation shows that *Stenoglottis fimбриata* represents anomocytic, tetracytic, stauroctytic and actinocytic types, all of anomocytic origin. Baranova (1992) supported the view of Takhtajan (1966) and Cronquist (1988) that the paracytic stomata are the most primitive ones. The total absence of paracytic stomata in the species investigated is of taxonomic importance. According to Stebbins & Khush (1961), no subsidiary cells have been noticed in the four species of orchid investigated. Garay (1972) reported that
guard cells without subsidiary cells are characteristic of the Orchidales. Rasmussen (1981) reported a hemimenesogenous type of stomatal development in the species of Orchidoideae, while our present observations confirm only the perigenous type of stomatal ontogeny of Pant (1965). Therefore, clearly, the Orchidaceae predominantly exhibit anomocytic stomata although, there is a wide range of variation in the stomatal structure and mode of development. Further investigations are necessary to reach a clear conclusion as to the epidermal characters which may be of taxonomic importance.

Acknowledgement
R.B. Bhat acknowledges the University of Transkei, Umtata, South Africa, for financial assistance.

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Fruit structure of the genus Cassinopsis Sond. (Icacinaeae) in Africa

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Received 9 November 1993; revised 8 December 1993

Fruit development and structure of the two African species of Cassinopsis were examined by light microscopy. Both species are characterized by drupes: ovate-oblong, laterally compressed and yellow-orange in C. ilicifolia, oblong and black in C. tinifolia. The exocarp is uniseriate and develops solely from the outer epidermis of the ovary. A parenchymatous mesocarp with vascular bundles and scattered druse crystals of calcium oxalate is derived from the ground tissue of the ovary wall. A uniseriate parenchymatous (C. ilicifolia) (lignified in C. tinifolia) endocarp s. str. develops from the inner epidermis of the ovary wall. A lignified stone is derived mainly from the inner zone of the mesocarp. The outer surface of the stone is of taxonomic significance, being smooth in C. ilicifolia and longitudinally ribbed in C. tinifolia. Differences in fruit structure, combined with other characters, suggest that the two African species are not taxonomically closely related. Their generic status requires further comparative study, particularly with species of Cassinopsis in Madagascar.

Die vrugontwikkeling en vrugstuktuur van die twee Afrika-spesies van Cassinopsis is met behulp van ligmikroskopies ondersoek. Beide spesies word gekenmerk deur steenvrugte: ovaal-langwerpig, lateraal saamgedruk en geel-oranje by C. ilicifolia, langwerpig en swart by C. tinifolia. Die eksokarp is uniseriaal en ontwikkeld slegs uit die buitenste epidermis van die vrugbeginsel wand. 'n Parenchymatiese endokarp s. str. (C. ilicifolia) (gelignifieerd by C. tinifolia) ontstaan vanaf die binneste epidermis van die vrugbeginselwand. 'n Gelignifieerde steen is