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# THE STRUCTURE AND DEVELOPMENT OF STOMATA IN SOME ZINGIBERALES

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ABSTRACT. The structure and development of stomata on the leaves of seventy species belonging to the order Zingiberales are described. Four types of stomata occur: paracytic, tricytic, tetracytic and polycytic. Paracytic stomata are predominant in most members of Musaceae, Cannaceae, Lowiaceae and Marantaceae; tetracytic stomata are the commonest in most members of Zingiberaceae; while in Costaceae, Strelitziaceae, and Heliconiaceae polycytic stomata are much more frequent than any other type. The development of stomata in all Zingiberales studied is perigenous. Four closely similar modes of development were observed: biperigenous, triperigenous, tetraperigenous and polyperigenous, leading to the formation of paracytic, tricytic, tetracytic and polycytic stomata respectively. The interrelationships amongst the eight families in the order are discussed in the light of the organisation of their mature epidermides and stomata.

## INTRODUCTION

The structure of the mature leaf epidermis and stomata of members of the Zingiberales have been described by many workers such as Solereder & Meyer (1930), Stebbins & Khush (1961), and Tomlinson (1956, 1969). Tomlinson (1969) reported that paracytic stomata are predominant in the Cannaceae, Lowiaceae, Marantaceae and Zingiberaceae, while tetracytic stomata are prevalent in the Costaceae, Heliconiaceae, Musaceae and Strelitiziaceae.

In a study of the stomatal structure and development in some members of the Zingiberales, Olatunji (1970) observed that tetracytic stomata in the Zingiberaceae and polycytic stomata in the Costaceae are commoner than was earlier reported.

Examination of mature stomata alone, without developmental studies, may lead to difficulty in interpreting the true structure of the stomatal complex. The importance of developmental studies in the elucidation of stomatal structure was emphasized by Tomlinson (1969, 1974) and Paliwal (1969). Few developmental studies have so far been reported for members of the Zingiberales. Stebbins and Khush (1961) studied 11 species in the order; Olatunji (1970) investigated about 305 species in the family Zingiberaceae, and Tomlinson (1974) studied the development of stomata in 11 species belonging to the order. The present investigation was therefore undertaken to extend our knowledge of the structure and development of stomata in the Zingiberales.

## MATERIALS AND METHODS

The structure and development of stomata were investigated in 70 species representing the eight families of the Zingiberales.

Leaf material of about 70 species was collected from plants that were either growing wild or were cultivated in different parts of Nigeria. Additional material was also obtained from herbarium specimens and

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plants cultivated in the glasshouses of the Royal Botanic Garden, Edinburgh. The source of material is given in Table 1 and Appendix 1.

For the study of mature stomata, portions of the mature leaves were taken from the standard median level. This is mid-way between the base and the apex of the leaf, and half-way between midrib and margin—the area where cell size is normally intermediate between the differing measurements of apical and basal portions.

Fresh materials were fixed in formalin/glacial acetic/alcohol (1:1:3 FAA) for at least six hours. Dried herbarium material was boiled in 2% sodium hydroxide solution for about 5–10 minutes: a treatment which allows the epidermis to be separated very easily in most material.

Epidermal peels of both abaxial and adaxial surfaces were made. The leaf material was placed on a glass tile, irrigated with water and the tissues above the epidermis were scraped off carefully with a scalpel or razor-blade until the epidermis underneath was reached. In some cases the hypodermis was tightly attached to the epidermis and very difficult to remove, as occurred in most members of the Marantaceae and Strelitziaceae. Epidermal preparations from herbarium or other drier specimens which are usually coloured were bleached in 2% sodium hyphochlorite or domestic 'parazone'. Before staining, epidermides were washed in several changes of water—this was particularly important with bleached preparations where all traces of bleaching agents had to be removed.

Peels were stained in 10% Delafield's haemotoxylin for 5–10 minutes, washed in water to remove excess stain, and then differentiated in 50% ethanol to which a few drops of concentrated hydrochloric acid were added. The peels were again washed several times in water, taken through the grades of alcohol up to 95% and mounted in Euparal. Many temporary preparations were also made.

For the ontogenetic studies, young developing plants or parts of plants were collected. The young leaves, which were rolled inside the concentric layers of leaf sheaths, were dissected out after slashing the stem into longitudinal halves.

Small portions of the young developing leaves were cut at different levels and fixed in alcohol-glacial acetic acid mixture (3:1) for at least six hours. In most cases it proved difficult to peel or scrape the epidermal tissue of the youngest leaves because they were brittle and delicate. Instead, portions were crushed on a slide. The epidermal preparations were mounted in 1% acetocarmine solution on a slide and warmed on a hot plate. When cooled, the slides were sealed with rubber solution or nail varnish and kept in a cool place. The slides were ready for observation after about 4–6 hours when the nuclei had become deeply stained.

Drawings and photographs were made using a Wild M20 microscope fitted with drawing and photographic attachments.

The stomatal frequency per sq. mm was calculated from an average of ten counts made on the lower surface of the leaves. The distribution of the different types of stomata was expressed as a percentage frequency of the total stomata per sq. mm. Ranges in guard cell area were calculated from a sample of ten measurements of the guard cells taken on the lower surface. Franco's (1939) formula for calculating guard cell area was used, viz: guard cell area = length × width × 0·7854 (constant).

## TERMINOLOGY

The terms used in the present article are the same as those employed previously by Metcalfe & Chalk (1950), Pant & Mehra (1965), Paliwal (1969), Fryns-Claessen & Van Cotthem (1973), and Van Cotthem (1970). Some of the terms are explained below:

*Meristemoid*: a protodermal cell that becomes distinguishable as a meristematic cell. It becomes the guard cell mother cell (gcmc).

*Neighbouring cell*: any cell lying close to the meristemoid.

*Perigene*: a neighbouring cell which by division may give rise to subsidiary cells or other cells lying close to the stomata.

*Contact cell*: the product of the division of a neighbouring cell that lies in contact with the meristemoid or guard cell.

*Stoma*: the pair of guard cells and the pore enclosed between them.

*Stomatal complex*: the stoma and the surrounding subsidiary or contact cells.

*Subsidiary cells*: the epidermal cells surrounding the mature stoma which differ in shape or size from other epidermal cells.

*Paracytic stoma*: a stoma having two visibly modified lateral subsidiary (contact) cells placed parallel to the guard cells, but not completely enclosing them.

*Tricytic stoma*: a stoma having three subsidiary (contact) cells (two lateral and one terminal).

*Tetracytic stoma*: a stoma having four subsidiary (contact) cells (two terminal and two lateral) completely surrounding the guard cells.

*Polycytic stoma*: a stoma having more than four (i.e. 5–10) subsidiary cells all of which are visibly different in shape and/or size from other epidermal cells.

## OBSERVATIONS

### MATURE STOMATA

The leaves of the Zingiberales examined are amphistomatic but stomata are much more frequent on the abaxial than the adaxial epidermis. The orientation of the stomata is unidirectional in most of the families in that the guard cells lie more or less parallel to the long axes of the veins. But in the Costaceae most of the stomata are irregulary orientated.

The surface areas of the guard cells vary in the different species and sometimes at different positions on the same leaf. The range of surface areas of guard cells for the species investigated is given in Table 1. Guard cells with the largest surface areas were observed in the Costaceae, while those with the smallest surface areas occur in the Marantaceae.

The average stomatal frequency on the abaxial epidermis of the species investigated is also listed in Table 1. Within genera the species vary considerably in stomatal frequency. The highest stomatal frequencies were recorded in members of the Marantaceae.

The stomata observed in all families fall into four closely related types: paracytic (Fig. 1 C, E), tricytic (Fig. 1 A), tetracytic (Fig. 2 A, B) and polycytic (Fig. 2 C, D & H)—see above for definitions.

In the Musaceae, Cannaceae, Marantaceae and Lowiaceae paracytic stomata were the commonest stomatal type in the vast majority of the species investigated (Table 1). The paracytic structure of the stomata is

TABLE 1

N, Nigerian material from wild or cultivated source; EC, R.B.G. Edinb. from cultivated living material; EH, R.B.G. Edinb. herbarium material; GH, Ghana; *, see Appendix for collector's number; †, including the modified paracytic type with extra lateral subsidiary cells.

| | Source | Guard cell area $\mu m^2$ | Types of stomata (%) | | | | Av. frequ. of stomata per $mm^2$ |
|---|---|---|---|---|---|---|---|
| | | | Paracytic† | Tricytic | Tetracytic | Polycytic | |
| **Cannaceae** | | | | | | | |
| Canna chinensis Willd. | EC | 260–451 | **60** | 31 | 5 | 4 | 156 |
| C. coccinea Mill. | EH | 312–364 | **75** | 6 | 17 | 2 | 94 |
| C. flaccida Salisb. | EH* | 555–703 | **99** | 1 | | | 70 |
| C. generalis Bailey | EH* | 521–811 | **92** | 6 | 2 | | 104 |
| C. glauca L. | EH* | 260–286 | **53** | 14 | 17 | 16 | 114 |
| C. indica L. | N | 282–364 | **92** | 4 | 4 | | 98 |
| C. iridiflora Ruiz & Pav. | EC | 607–693 | **83** | 2 | 15 | | 108 |
| C. orchiodes Bailey | EC | 286–338 | **79** | 15 | 6 | | 68 |
| C. sylvestris Rosc. | EH* | 282–338 | 25 | 32 | 2 | **41** | 118 |
| **Costaceae** | | | | | | | |
| Costus afer Ker Gawl. | N | 716–885 | 7 | | 7 | **86** | 58 |
| C. arabicus L. | EC | 451–607 | | | 5 | **95** | 88 |
| C. dubius (Afzel.) K. Sch. | N | 486–651 | | | **81** | 19 | 72 |
| C. englerianus K. Sch. | N | 1457–1692 | | | | **100** | 14 |
| C. lucanusianus Braun & K. Sch. | N | 486–651 | | | 6 | **94** | 66 |
| C. macranthus K. Sch. | N | 1297–1457 | | | | **100** | 20 |
| C. sp. (cultivated Ife) | N | 451–547 | | | 6 | **94** | 64 |
| C. sp. (epiphytic) | N* | 451–547 | | | 13 | **87** | 30 |
| Tapeinochilus ananassae Haussk. | EC | 486–694 | 15 | | 18 | **67** | 67 |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Heliconiaceae** | | | | | | | |
| Heliconia bihai L. | N, EC | 282–338 | 12 | 9 | 4 | **75** | 114 |
| H. brasiliensis Hook. | EH | 217–370 | | | 17 | **83** | 152 |
| H. choconiana S. Wats | EH | 286–364 | | | 7 | **93** | 86 |
| H. psittacorum L.f. | EC | 416–547 | 5 | 3 | 15 | **77** | 78 |
| H. puberula Lindl. | EH* | 260–334 | | | 2 | **98** | 124 |
| **Lowiaceae** | | | | | | | |
| Lowia longiflora Scortech. | EC | 455–555 | **84** | 5 | 11 | | 38 |
| **Marantaceae** | | | | | | | |
| Calathea leitzii E. Morr | EC | 260–364 | **52** | 25 | 21 | 2 | 96 |
| C. lutea (Aubl.) G. F. W. Meyer | EH* | 239–312 | **71** | 16 | 8 | 5 | 210 |
| C. nigricans Gagnep. | N, EC | 364–451 | **80** | 14 | 4 | 2 | 100 |
| C. rufibarba Fenzl | EC | 260–395 | **40** | 32 | 18 | 10 | 80 |
| Donax canniformis (Forst.) K. Sch. | EH* | 217–364 | **81** | 11 | 6 | 2 | 360 |
| Hypselodelphys poggeana (K. Sch.) M. Redh. | N | 122–208 | 32 | 11 | 24 | 33 | 185 |
| Ischnosiphon sp. | EH* | 260–347 | **95** | 4 | 1 | | 146 |
| Maranta arundinacea L. | N | 260–334 | **98** | 2 | 1 | | 304 |
| Marantochloa ramosissima (Benth.) Hutch. | N* | 217–286 | **88** | 8 | 3 | 1 | 202 |
| Phrynium capitatum Willd. | EH* | 239–318 | **90** | 10 | | | 114 |
| P. parvum (Ridl.) Holtt. | EH* | 130–239 | **99** | 1 | | | 160 |
| Pleiostachya pruinosa (Reg.) K. Sch. | EH | 260–334 | **97** | 1 | 1 | 1 | 234 |
| Schumannianthus dichotomus (Roxb.) Gagnep. | EH* | 282–304 | 24 | 9 | 52 | 15 | 495 |
| Stachyphrynium tetranthum K. Larsen | EH* | 217–286 | **95** | 5 | | | 120 |
| Stromanthe sanguinea (Hook.) Sond. | EC | 425–521 | **84** | 6 | 6 | 4 | 72 |
| **Musaceae** | | | | | | | |
| Ensete gilletii (De Willd) Cheesman | N | 365–486 | 35 | 29 | 26 | 10 | 102 |
| Musa acuminata Colla | N, EC | 364–508 | **90** | 10 | | | 104 |
| M. basjoo Sieb. | EH | 281–364 | **92** | 7 | 1 | | 300 |
| M. coccinea Andr. | EH | 182–243 | **87** | 4 | 9 | | 142 |
| M. ornata Roxb. | EH* | 195–286 | **97** | 3 | | | 192 |
| M. sapientum L. × paradisiacum L. | N, EH* | 364–521 | 8 | 2 | 59 | 31 | 77 |
| M. sp. (*Yü* 20538) | EH | 286–334 | 16 | 1 | | **83** | 216 |

Table 1 (cont.)

| | Source | Guard cell area $\mu m^2$ | Types of stomata (%) | | | | Av. frequ. of stomata per $mm^2$ |
|---|---|---|---|---|---|---|---|
| | | | Paracytic† | Tricytic | Tetracytic | Polycytic | |
| **Strelitziaceae** | | | | | | | |
| Ravenala madagascariensis Sonn. | N, EC | 260–286 | | | | **100** | 127 |
| Strelitzia augusta Thunb. | EH* | 547–651 | | | | **100** | 70 |
| S. nicolai Regel & Koch | N, EC | 382–468 | | | | **100** | 146 |
| S. reginae Banks | EC | 547–651 | | | | **100** | 80 |
| **Zingiberaceae** | | | | | | | |
| Aframomum melegueta (Rosc.) K. Sch. | N | 222–302 | 1 | 3 | **96** | | 166 |
| A. sceptrum (Oliv. & Hanb.) K. Sch. | N | 334–451 | 5 | | **95** | | 86 |
| A. sp. nov. | EC* | 216–286 | 3 | 3 | **89** | 5 | 180 |
| Alpinia purpurata (Vieill.) K. Sch. | N | 281–468 | | 3 | **97** | | 126 |
| A. vittata Bull | N | 260–416 | 31 | 23 | **46** | | 130 |
| Boesenbergia grandifolia (Val.) K. Sch. | EC* | 703–868 | | | | **100** | 36 |
| Boesenbergia sp. aff. parva (Ridl.) Merrill | EC* | 976–1171 | | | 33 | **67** | 30 |
| B. sp. aff. parva (Ridl.) Merrill | EC | 811–938 | | | | **100** | 18 |
| B. parvula Bak. | EH* | 1093–1345 | | | | **100** | 24 |
| Cienkowskiella nigerica (Hepper) Kam | N | 753–1015 | | | **98** | 2 | 52 |
| Elettaria surculosa (K. Sch.) Burtt & Sm. | EH* | 286–395 | | | **91** | 9 | 112 |
| Globba atrosanguinea Teysm. & Binn. | EC | 620–729 | | 2 | **96** | 2 | 130 |
| ? Haplochorema sp. aff. H. extensum K. Sch. | EC* | 729–911 | | | **77** | 23 | 26 |
| Kaempferia (Cienkowskiella) rosea Schweinf. | EC | 651–885 | 5 | **95** | | | 38 |
| Nicolaia elatior (Jack) Horan. | N | 508–607 | 16 | 23 | **61** | | 240 |
| Renealmia alpina (Rottb.) Maas | EH* | 395–586 | 17 | 12 | **71** | | 130 |
| R. battenbergiana Bak. | GH* | 564–698 | | | **100** | | 36 |
| R. bracteosa Griseb. | EH | 547–694 | 1 | 5 | **94** | | 126 |
| Rhynchanthus beesianus W.W. Sm. | EH* | 455–590 | | | **74** | 26 | 133 |
| Scaphochlamys perakensis Holtt. | EC | 338–425 | **36** | | 29 | 35 | 34 |
| Zingiber officinale Rosc. | N, EC | 625–737 | 19 | 23 | **58** | | 104 |
| Z. zerumbet (L.) Smith | EC | 286–338 | 5 | 8 | **87** | | 80 |

usually very distinct. The two lateral subsidiary cells associated with each pair of guard cells are smaller than, and distinctly differentiated from the ordinary epidermal cells (Fig. 1). Whereas, the terminal neighbouring cells (tnc) are often large and not easily distinguishable from the ordinary epidermal cells (Fig. 1 C, G). Other stomatal types also occur in these families but are much less frequent than the paracytic type except in a few taxa, e.g. *Musa sapientum* x *paradisiacum* and *Canna sylvestris*, where tetracytic and polycytic stomata respectively are predominant.

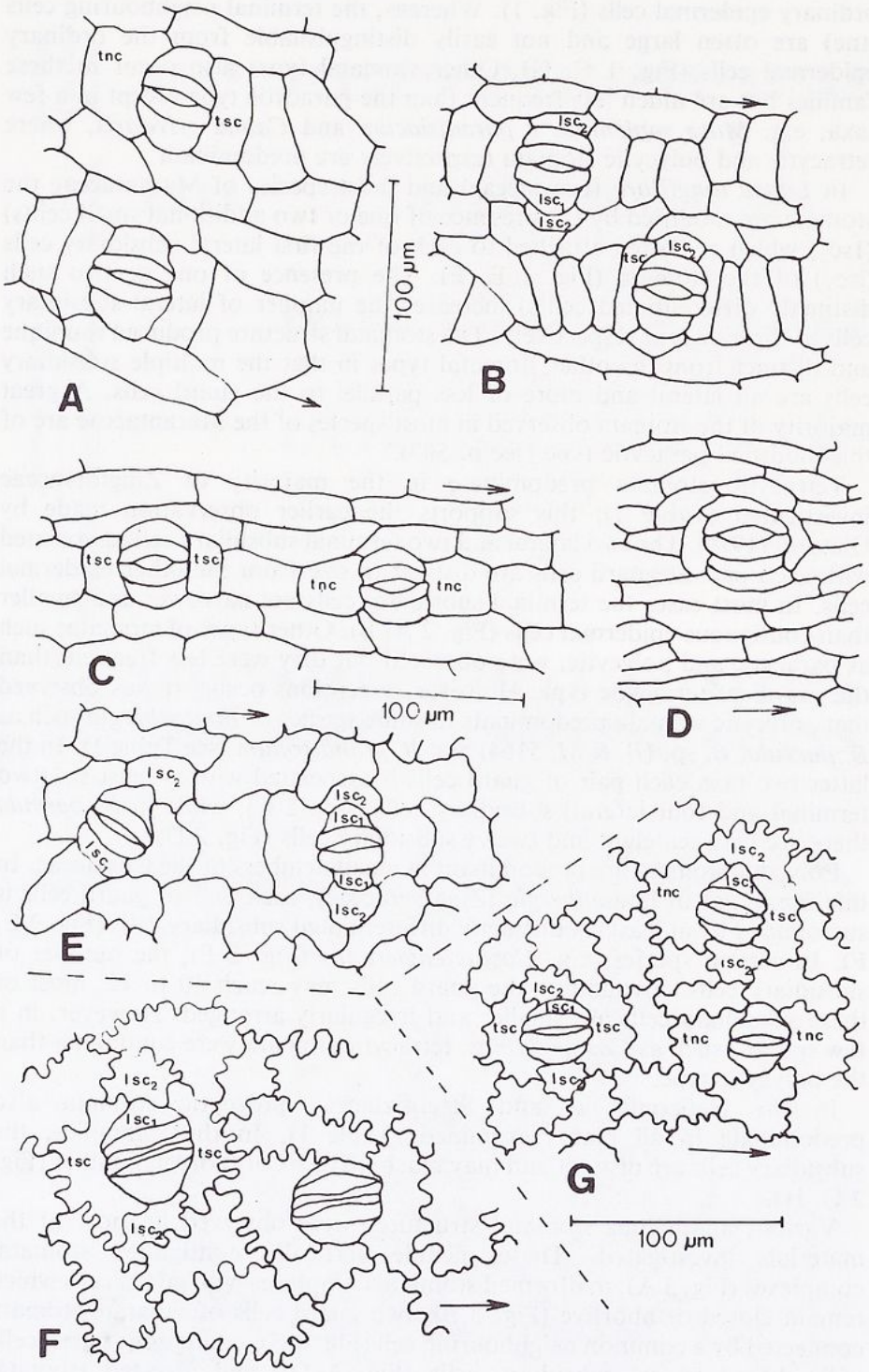
In *Lowia longiflora* (Lowiaceae) and most species of Marantaceae the stomata are modified by the presence of one or two additional small cell(s) ($1sc_2$) which are often attached to each of the first lateral subsidiary cells ($lsc_1$) of the stomata (Fig. 1 E, F). The presence of one or two such distinctly differentiated cell(s) increases the number of lateral subsidiary cells to three or four respectively. The stomatal structure produced is unique and distinct from the other stomatal types in that the multiple subsidiary cells are all lateral and more or less parallel to the guard cells. A great majority of the stomata observed in most species of the Marantaceae are of this modified paracytic type (see p. 507).

Tetracytic stomata predominate in the majority of Zingiberaceae investigated (Table 1); this supports the earlier observation made by Olatunji (1970). The two lateral and two terminal subsidiary cells associated with each pair of guard cells are distinct in size from the other epidermal cells. In most cases the terminal subsidiary cells are narrower and smaller than contiguous epidermal cells (Fig. 2 A, B). Other types of stomata, such as paracytic and polycytic, were observed but they were less frequent than the prevalent tetracytic type. However, exceptions occur: it was observed that polycytic stomata predominate in some species of *Boesenbergia* such as *B. parvula*, *B.* sp. (*B. & M.* 5164) and *B. grandifolium* (see Table 1). In the latter two taxa each pair of guard cells is associated with at least six (two terminal and four lateral) subsidiary cells (Fig. 2 C), while in *B. parvula* there are between eight and twelve subsidiary cells (Fig. 2 D).

Polycytic stomata are predominant in most members of the Costaceae. In this family, as in *Boesenbergia* (Zingiberaceae), each pair of guard cells is surrounded by at least six distinctly differentiated subsidiary cells (Fig. 2 E, F). In certain species, e.g. *Costus engleranus* (Fig. 2 F), the number of subsidiary cells surrounding the guard cells may reach 10 to 12; most of these subsidiary cells are smaller and irregularly arranged. However, in a few species, such as *Costus dubius*, tetracytic stomata were commoner than the polycytic type.

In the Heliconiaceae and Strelitziaceae polycytic stomata also predominate in all plants examined (Table 1). In these families, the subsidiary cells are distinct and may reach up to six or more in number (Fig. 2 G, H).

Various anomalous stomatal structures were observed in most of the materials investigated. These include vertically contiguous stomatal complexes (Fig. 3 A), malformed stomatal complexes with guard cells which remain closed or abortive (Fig. 3 B), two guard cells of separate stomata connected by a common neighbouring cell (Fig. 3 C), contiguous guard cells with shared lateral subsidiary cells (Fig. 3 D), and arrested stomatal development (Fig. 3 E).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STOMATAL DEVELOPMENT

Maturation of leaf tissues in the Zingiberales follows the normal acropetal monocotyledonous pattern. The initial cells of the stomatal complexes have a rather random distribution which is, however, characteristic of each species. Their meristematic activity continues after the majority of the surrounding epidermis has matured and differentiated. The initial cell divides transversely and asymmetrically to produce two cells: the smaller of which becomes the meristemoid and the larger a neighbouring cell at the terminal (polar) side of the meristemoid.

A meristemoid is usually distinguishable from the other differentiating protodermal cells by its rounded corners as seen from surface view, dense protoplasm, relatively large nucleus and deep staining contents. These meristemoids often appear birefringent under phase contrast microscopy.

Surrounding each meristemoid are four neighbouring cells, two lateral neighbouring cells (lnc) and two terminal (often called polar) neighbouring cells (tnc)—some or all of which may divide further (Fig. 4). The developmental pathway and mature stomatal complex produced depends on the number of neighbouring cells that divide to produce subsidiary cells.

In the Zingiberales four closely similar modes of stomatal development were observed: (i) biperigenous, (ii) triperigenous, (iii) tetraperigenous, and (iv) polyperigenous development.

*Biperigenous development* (Fig. 4 A; 5 D). In biperigenous development each of the two lateral neighbouring cells divides to cut off a small cell adjacent to the gcmc. As soon as the divisions in the neighbouring cells are completed or at least well underway, the gcmc divides to produce two cells which differentiate into the guard cells of a stoma. The stomatal pore is then produced between these two guard cells. In this mode of development the two terminal neighbouring cells do not divide but differentiate into ordinary epidermal cells. The product of biperigenous development is a paracytic stoma.

The biperigenous mode of development is basic to the other three modes of development described below. The stomata in the Zingiberales are all produced by or develop through this pathway. Simple biperigenous development was observed predominantly in the Cannaceae, Musaceae, Lowiaceae and Marantaceae.

In the Marantaceae the lateral subsidiary cell or its larger sister cell produced from the division of the lateral neighbouring cell may divide to cut off another smaller cell near to the first formed subsidiary cell (Fig. 5 E, F). In some cases, the sister cell of the first division of the lsmc may differentiate straight away into a small cell which becomes noticeable as

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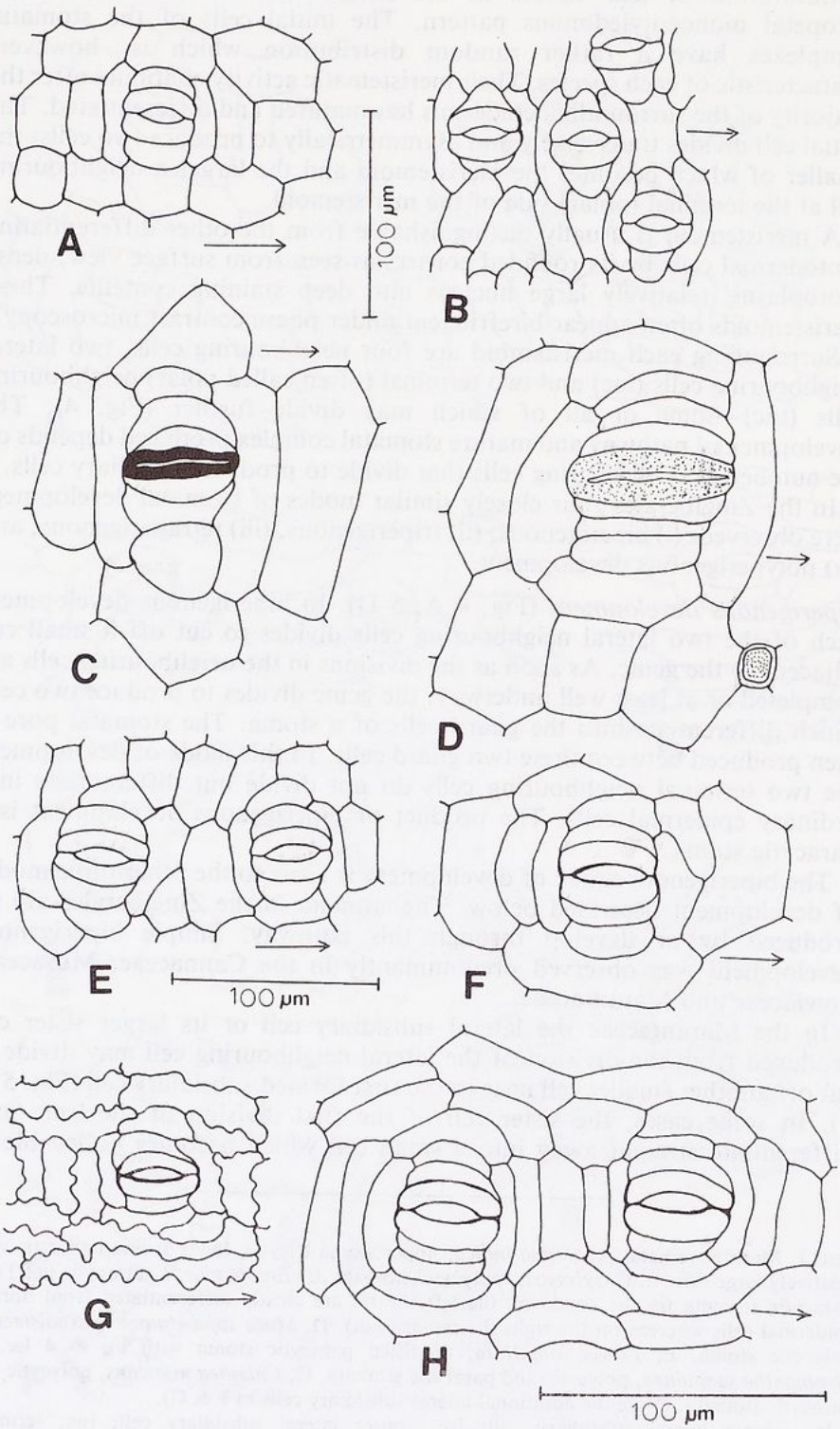
FIG. 1. Mature stomata. A, *Canna indica*, upper stoma tricytic, lower stoma tetracytic with relatively large tscs. B, *C. sylvestris*, polycytic stomata. C, *Ensete gilletii*, tetracytic (left) and paracytic stomata (in the stoma on the left the tsc are clearly differentiated from normal epidermal cells whereas on the right the tnc are not). D, *Musa sapientum* × *paradisiacum*, polycytic stoma. E, *Lowia longiflora*, modified paracytic stoma with up to 4 lsc. F, *Stromanthe sanguinea*, polycytic and paracytic stomata. G, *Calathea nigricans*, polycytic and paracytic stomata. (Note the additional lateral subsidiary cells in F & G).

$lsc_1$, inner lateral subsidiary cell; $lsc_2$, outer lateral subsidiary cell; tnc, terminal neighbouring cell; tsc, terminal subsidiary cell. (The arrows indicate the long axes of the veins).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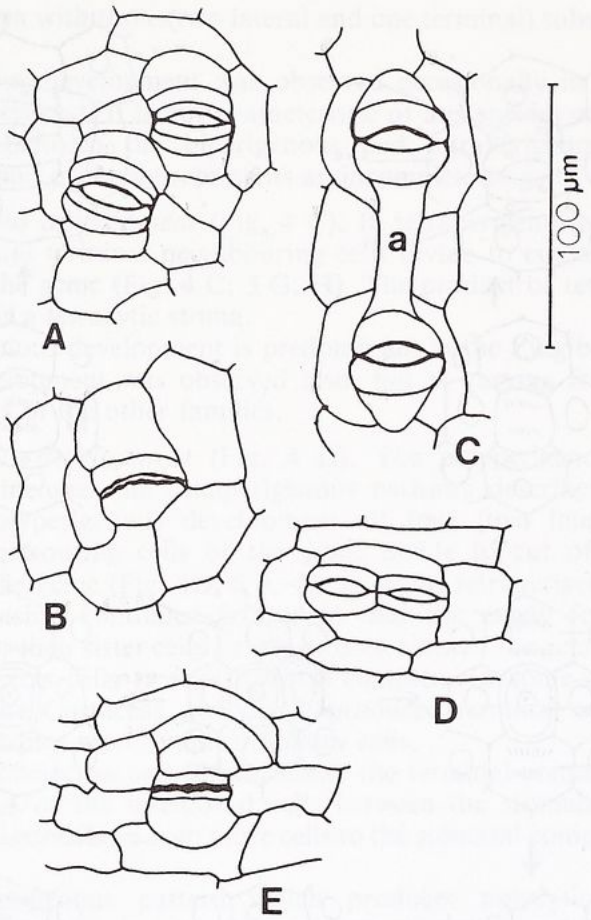



FIG. 3. Anomalous stomata. A, *Costus arabicus,* vertically juxtaposed stomata; B, *Costus* sp., guard cells with unopened stomatal pore; C, *Globba atrosanguinea,* one lateral subsidiary cell (a) shared between two stomata; D, *Musa ornata,* contiguous guard cells with lateral cells shared; E, *Ensete gilletii,* aborted guard cells.

part of the stomatal complex. The number of recognisable subsidiary cells in most species of Marantaceae varies from three to five and in the majority of cases all are produced laterally to the guard cells. Paliwal (1969) recognised this pattern as one of his two forms of tetraperigenous development but since it is essentially similar to the biperigenous pattern I prefer to keep it here.

*Triperigenous development* (Fig. 4 B). The two lateral neighbouring cells and one terminal neighbouring cell divide to produce two lateral and one terminal cell near the gcmc. The other terminal neighbouring cell of the gcmc remains undivided and differentiates into an ordinary epidermal cell.

FIG. 2. Mature stomata. A–D, Zingiberaceae: A, *Globba atrosanguinea,* tetracytic stoma; B, *Nicolaia elatior,* tetracytic stomata; C, *Boesenbergia* sp. aff. *parva* (*B. & M.* 5164), polycytic stoma; D, *B. parvula,* polycytic stoma. E & F, Costaceae: E, *Costus afer,* polycytic stomata; F, *C. engleranus,* polycytic stoma. G, *Heliconia bihai* (Heliconiaceae), polycytic stoma. H, *Strelitzia nicolai* (Strelitziaceae), polycytic stomata. (The arrows indicate the direction of the long axes of the veins).

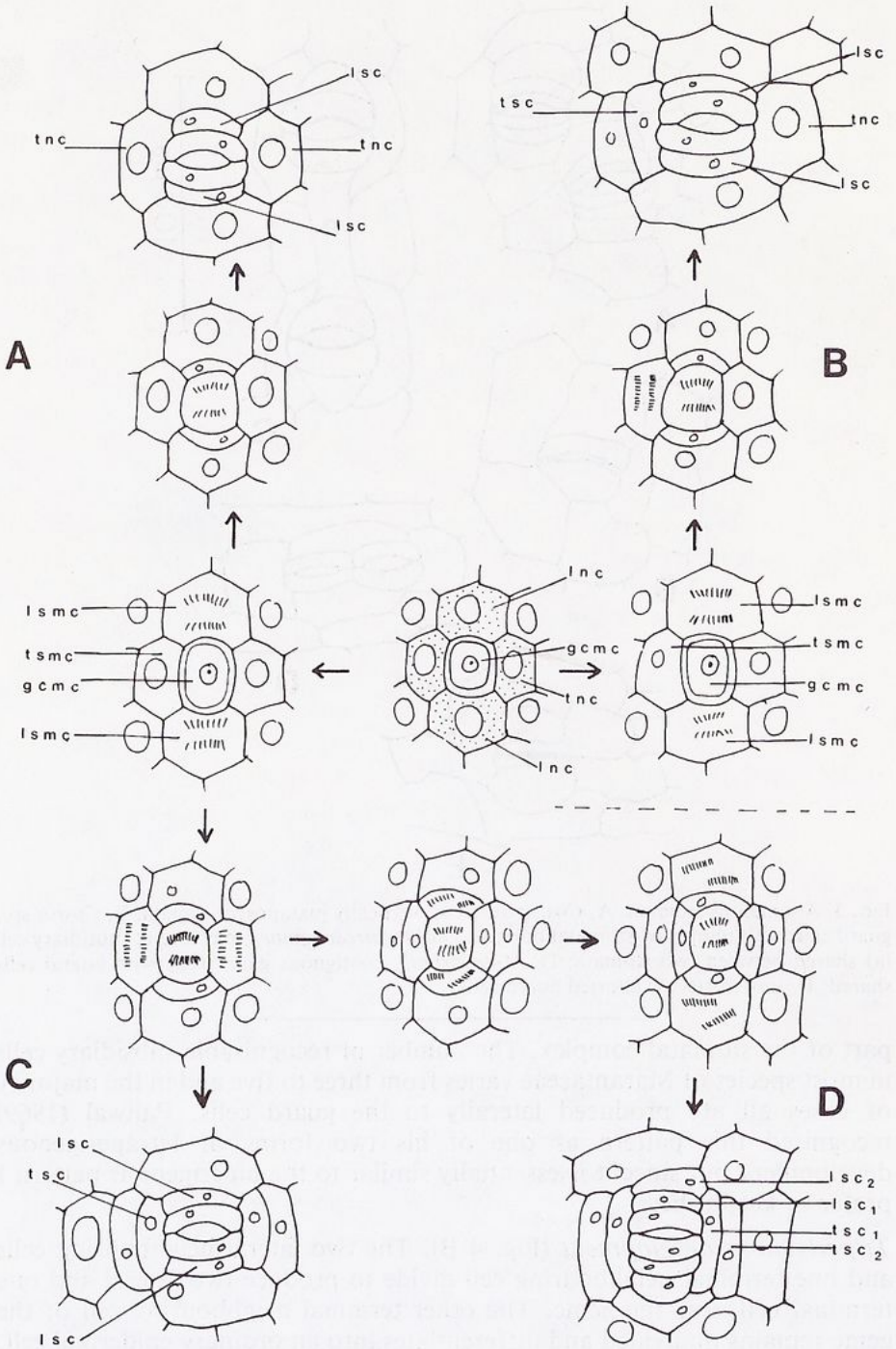



FIG. 4. Diagram showing modes of stomatal development in the Zingiberales: A, biperigenous; B, triperigenous; C, tetraperigenous; D, polyperigenous.

gcmc, guard cell mother cell; lnc, lateral neighbouring cell; lsmc, lateral subsidiary cell mother cell; lsc, lateral subsidiary cell; $lsc_1$, inner lateral subsidiary cell; $lsc_2$, outer lateral subsidiary cell; tnc, terminal neighbouring cell; tsmc, terminal subsidiary cell mother cell; tsc, terminal subsidary cell; $tsc_1$, inner terminal subsidiary cell; $tsc_2$, outer terminal subsidiary cell.

A tricytic stoma with three (two lateral and one terminal) subsidiary cells is produced.

Triperigenous development was observed occasionally in some of the material investigated. It is not characteristic of any species or family. It is intermediate between the biperigenous and tetraperigenous mode of development and probably represents an incomplete stage of the latter.

*Tetraperigenous development* (Fig. 4 C). In tetraperigenous development both lateral and terminal neighbouring cells divide to cut off small cells surrounding the gcmc (Fig. 4 C; 5 G, H). The product of tetraperigenous development is a tetracytic stoma.

Tetraperigenous development is predominant in the Zingiberaceae. This mode of development was observed also, but at varying frequencies, in some members of the other families.

*Polyperigenous development* (Fig. 4 D). The polyperigenous mode of development includes the hexaperigenous pathway described by Paliwal (1969). In polyperigenous development all four (two lateral and two terminal) neighbouring cells of the gcmc divide to cut off small cells surrounding the gcmc (Fig. 4D; 6 A–F), as in the tetraperigenous pattern. However, division continues further in that the newly formed lateral contact cells or their sister cells on one or both sides of the gcmc may divide, thus adding extra cells to the stomatal complex. In some cases, as was observed in the Costaceae, the newly produced terminal cells may also divide and produce more small subsidiary cells.

In the Heliconiaceae and Strelitziaceae the terminal contact cells, their sister cells and/or the intercostal cells between the stomata may divide transversely to contribute even more cells to the stomatal complex (Fig. 6 C, D, E & F).

The polyperigenous pattern which produces polycytic stomata is prevalent among species of *Boesenbergia* (Zingiberaceae), Costaceae, Heliconiaceae and Strelitziaceae. This mode of development was also observed in a few cases in other families.

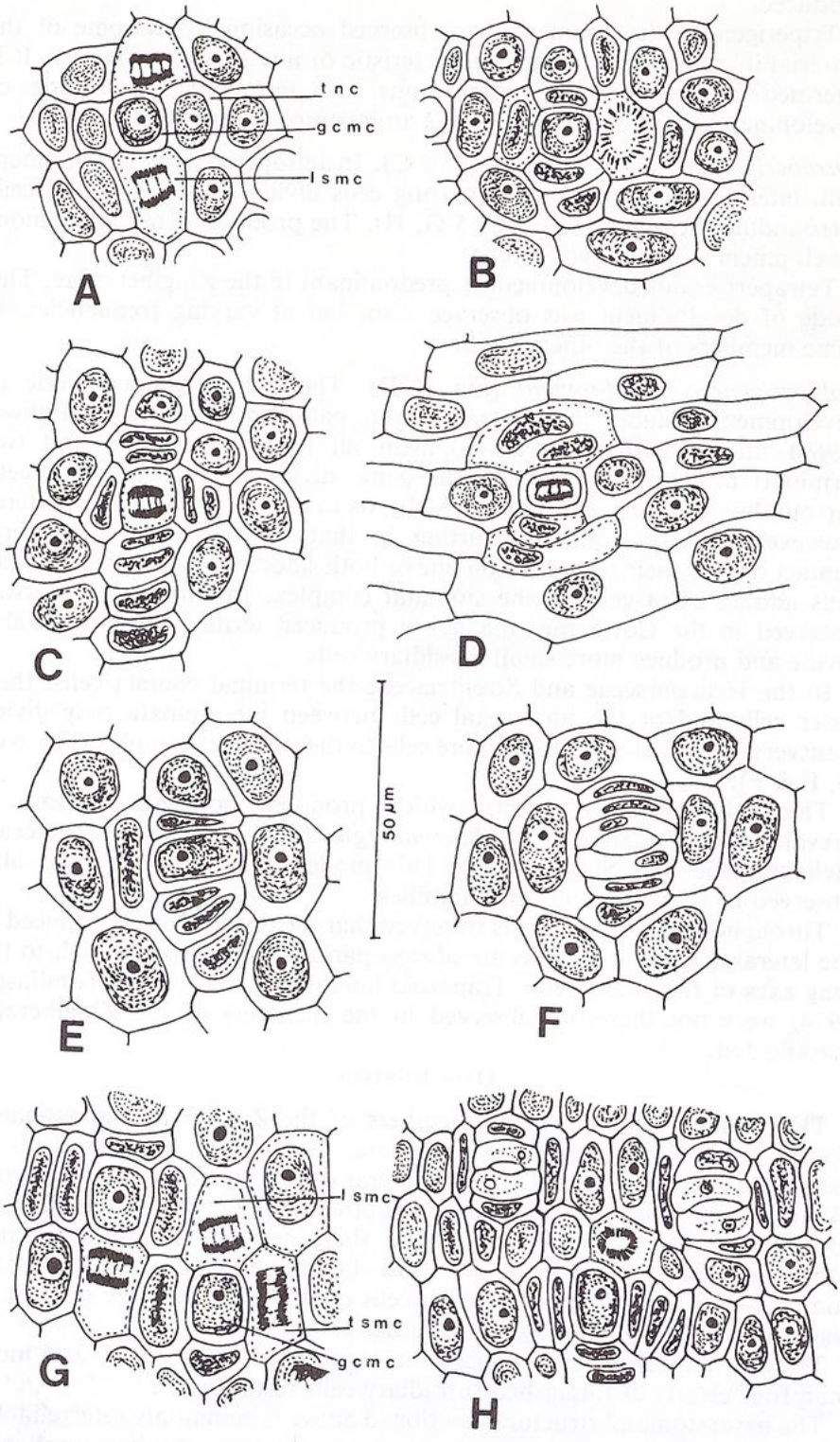
Throughout this study it was observed that the division lines produced in the lateral neighbouring cells are always parallel, or nearly parallel, to the long axes of the guard cells. Trapezoid lateral subsidiary cells (Tomlinson 1974) were not therefore observed in the members of the Zingiberales investigated.

## DISCUSSION

The stomata observed in all members of the Zingiberales investigated possess a similar fundamental structure. Each pair of guard cells is associated with two, clearly distinct, lateral subsidiary cells. The four types of stomata observed differ from one another in the number of additional subsidiary cells. In paracytic stomata there are no other recognisable subsidiary cells apart from the two laterals basic to the stomatal complex—the terminal neighbouring cells of the guard cells are not in any way different from other epidermal cells.

Tricytic, tetracytic, and polycytic stomata have three, four, and more than four clearly distinguished subsidiary cells respectively.

The basic stomatal structure mentioned above is commonly referred to as the paracytic pattern. It is prevalent among members of other monocoty-


tnc
gcmc
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A
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50 µm
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tsmc
gcmc
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ledonous groups such as the Commelinales (Tomlinson 1969), Poaceae, Cyperaceae, Alismataceae and Butomaceae.

The present study shows that paracytic stomata predominate in Musaceae, Cannaceae, Lowiaceae and Marantaceae. This observation agrees, apart from the Musaceae, with the earlier findings of Tomlinson (1969) who did not examine the species of Musaceae used in this study. However, tetracytic stomata were common in *Musa sapientum* x *paradisiacum*, agreeing with Tomlinson's findings.

The predominance of paracytic stomata in Cannaceae, Lowiaceae, Marantaceae and some members of the Musaceae seems to indicate these families are more closely related to each other than they are to the remaining four families in the order. The presence of three or four lateral subsidiary cells in each of the paracytic stomata, leading to the formation of modified paracytic stomata, is a common feature in Marantaceae. This family is also characterized by the presence of sinuous epidermal cell walls, a feature it shares with some members of Heliconiaceae and Strelitziaceae (Tomlinson 1969).

The epidermis of the families Musaceae, Strelitziaceae, and Heliconiaceae are dominated by longitudinally extended cells, while transversely extended cells are restricted, more or less, to areas near the stomata (see Tomlinson 1969). Polycytic stomata of essentially similar type are predominant in most members of Strelitziaceae and Heliconiaceae but in the Musaceae paracytic stomata are the dominant type.

The Zingiberaceae is distinct from all the other families of Zingiberales in many anatomical features such as the presence of oil cells in the leaf and the differentiation of the epidermis into distinct costal and intercostal areas (Tomlinson 1969). The present investigation shows that tetracytic stomata are also much more common in this family than in other Zingiberales: this agrees with Olatunji (1970) who indicated that tetracytic stomata were more prevalent in the Zingiberaceae than was earlier reported by Tomlinson (1969).

The results of this study support Tomlinson's (1969) recognition of the Costaceae as a family distinct from the Zingiberaceae. Stomata are predominantly polycytic in Costaceae whereas tetracytic stomata predominate in the Zingiberaceae.

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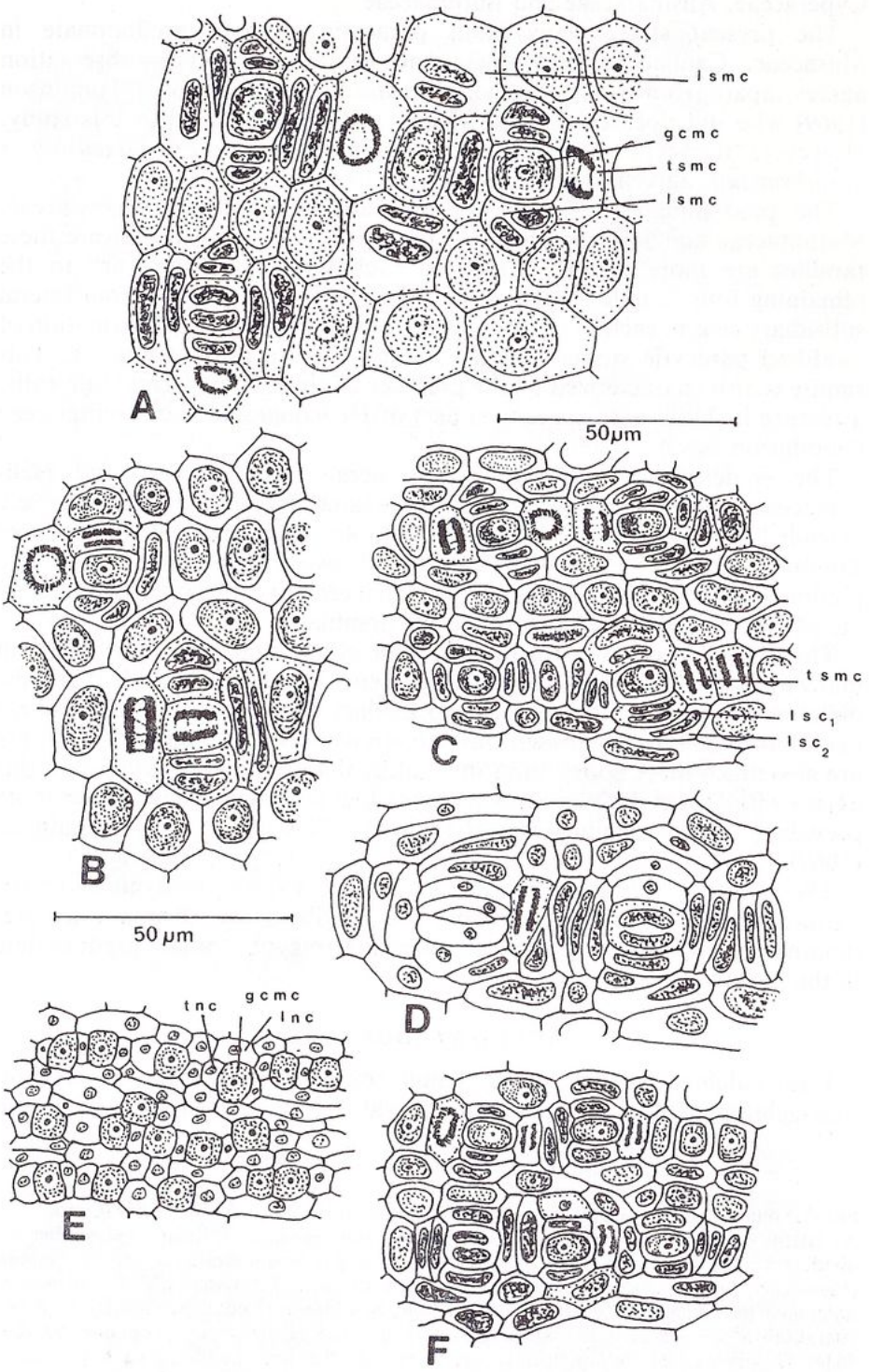
FIG. 5. Some stages in stomatal development of Zingiberales: A–C, *Canna indica* (Cannaceae): A, lateral subsidiary cell mother cell dividing; B, one terminal subsidiary cell mother cell dividing; C, guard cell mother cell dividing, formation of tetracytic stoma. D, *Musa acuminata* (Musaceae), biperigenous development, formation of paracytic stoma. E & F, *Stromanthe sanguinea* (Marantaceae), modified biperigenous development, note the formation of two lateral subsidiary cells on either side of the stoma in F. G & H, *Aframomum* sp. nov. (*H. & B.* 4634) (Zingiberaceae), tetraperigenous development, the four perigenes each produce a subsidiary cell.

gcmc, guard cell mother cell; lsmc, lateral subsidiary cell mother cell; tnc, terminal neighbouring cell; tsmc, terminal subsidiary cell mother cell.


lsmc
gcmc
tsmc
lsmc
A
50µm
B
50 µm
C
tsmc
lsc1
lsc2
D
tnc
gcmc
lnc
E
F

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## APPENDIX

Collectors' numbers of material of known wild origin

CANNACEAE

*Canna flaccida* Salisb., *Tesiang Ying* 433; *C. generalis* Bailey, *Nash* 1654; *C. glauca* L., *Britton & Rusby* 1281; *C. sylvestris* Rosc., *Sintenis* 4015.

COSTACEAE

*Costus* sp. (epiphytic), *W. S. Sanford* 5824.

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FIG. 6. Some stages of polyperigenous development in Zingiberales: A, *Boesenbergia* sp. aff. *parva* (*B. & M.* 5164) (Zingiberaceae). B, *Costus afer* (Costaceae). C & D *Strelitzia nicolai* (Strelitziaceae), note the divisions in the intercostal cells between stomata. E & F, *Heliconia bihai* (Heliconiaceae), note the divisions in the intercostal cells between stomata.

gcmc, guard cell mother cell; lnc, lateral neighbouring cell; $lsc_1$, inner lateral subsidiary cell; $lsc_2$, outer lateral subsidiary cell; lsmc, lateral subsidiary cell mother cell; tnc, terminal neighbouring cell; tsmc, terminal subsidiary cell mother cell.

HELICONIACEAE

*Heliconia puberula* Lindl., *Britton & Rusby* 411.

MARANTACEAE

*Calathea lutea* (Aubl.) G. F. W. Meyer, *Proctor* 2869; *Donnax canniformis* (Forst.) K. Sch., *Thompson* 90; *Hypselodelphys poggeana* (K. Sch.) M. Redh., *Lufadeju* 16; *Ischnosiphon* sp., *Britton & Rusby* 2201; *Marantochloa ramosissima* (Benth.) Hutch., *Guile* 2004; *Phrynium capitatum* Willd., *K. Larsen* 2339; *P. parvum* (Ridl.) Holtt., *Merrill* 7219; *Schumannianthus dichotomus* (Roxb.) Gagnep., *Kerr* 1857; *Stachyphrynium tetranthum* K. Larsen, *K. Larsen* 2653.

MUSACEAE

*Musa ornata* Roxb., *Lace* 5408; *M. sapientum* L. × *paradisiacum* L., *McClure* 7995.

STRELITZIACEAE

*Strelitzia angusta* Thunb., *Cooper* 1225.

ZINGIBERACEAE

*Aframomum* sp. nov., *Hilliard & Burtt* 4634; *Boesenbergia grandifolia* (Vah.) K. Sch., *Burtt* 2430; *B.* sp. aff. *parva* (Ridl.) Merrill, *Burtt* 5164; *B. parvula* Bak., *Keenan et al.* 1399; *Elettaria surculosa* (K. Sch.) Burtt & Sm., *Burtt* 4664; ?*Haplochorema* sp. aff. *H. extensum* K. Sch., *Burtt & Woods* 2700; *Renealmia alpina* (Rottb.) Maas, *Sintenis* 2608; *R. battenbergiana* [Cummins ex] Bak., *Hall & Enti* GC.37404, 35531; *Rhynchanthus beesianus* W.W. Sm., *Forrest* 15830.