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## Floral structure and development in the dioecious Australian endemic *Lomandra longifolia* (Lomandraceae)

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**Abstract.** The micromorphology and histology of the development of male and female flowers of the dioecious Australian endemic species *Lomandra longifolia* Labill. was studied by means of scanning electron microscopy and light microscopy of entire and sectioned material. Although mature flowers are functionally unisexual, in the early stages of development pistillate and staminate flowers are identical and apparently bisexual. In a sequential fashion, six perianth parts are initiated within two alternating whorls, the sepals first and the petals second; six stamens are initiated in two alternating whorls of three stamens each, the first opposite the sepals and the second opposite the petals; and last, a central gynoecium is initiated. Following initiation, the two flower types diverge developmentally when the stamens become bilobed. In male flowers, cytological analysis of the slowly growing abortive pistil shows that megasporogenesis does not occur. Pistil abortion happens before meiosis whereas the stamens continue to develop until maturity and dehiscence. In female flowers, stamen arrest occurs before the onset of meiosis in microspore mother cells, after which the pistil continues its development through megasporogenesis and megagametogenesis. In all, 14 stages of floral development of both male and female flowers have been designated. Stages 1–6 of the two flower types were common to both sexes.

### Introduction

*Lomandra* is a genus of 50 species native to Australia, of which two species extend to New Guinea and one of these to New Caledonia (Lee and Macfarlane 1986). The systematic position of this genus has long been problematic, having been placed in the families Juncaceae (Bentham 1878; Bentham and Hooker 1880), Liliaceae (Krause 1930), Xanthorrhoeaceae (Cronquist 1981), Dasygogonaceae (Dahlgren *et al.* 1985), Xanthorrhoeaceae (Bedford *et al.* 1986) and more recently Lomandraceae (Brummitt 1992; Rudall and Chase 1996) and Laxmanniaceae (APG 1998; APGII 2003). The Australian Plant Name Index (2008) currently places *Lomandra* in the family Lomandraceae.

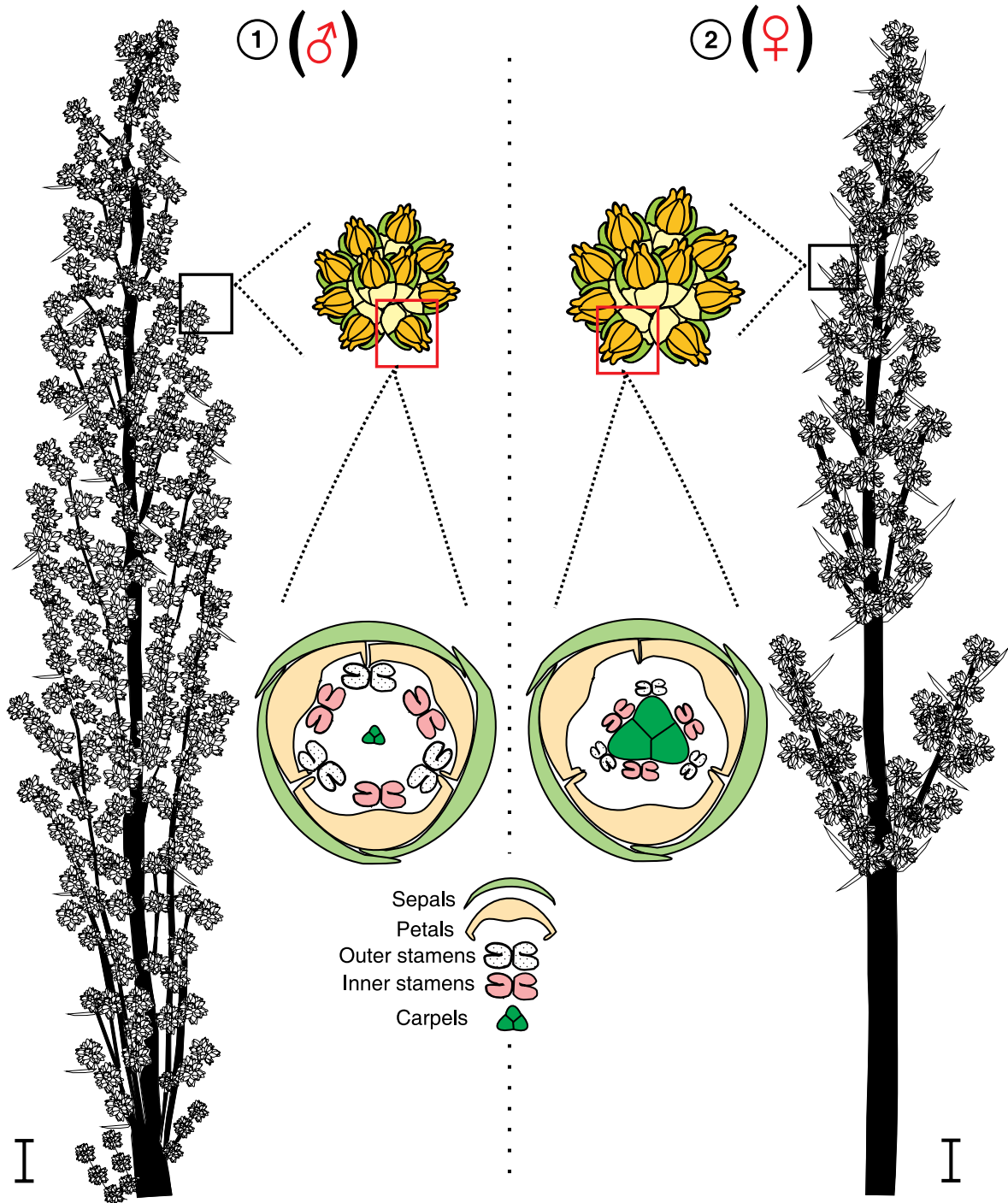
*Lomandra longifolia* is the predominant species of the genus in the eastern states of Australia, being widespread along the coast and Great Dividing Range, from north Queensland through New South Wales and Victoria to the south-east of South Australia and into Tasmania (Lee and Macfarlane 1986). *L. longifolia* is found in widely diverse landscapes, including beach sands, rich soils on creek banks and rock crevices on dry sandstone hillsides.

The versatility of this species allows it to adapt easily to almost any garden situation. It is not prone to attack by pests, and once established, demands virtually no attention (Napier-Thomson 1982). *L. longifolia* is an ideal border plant in native gardens and is highly suited for large-scale planting along roadsides and in various public works. The attractive strap-like foliage grows up to 1.2 m in height, surrounding yellow flower panicles that are mildly perfumed with a pineapple-like scent. The panicles can be used to create unique floral arrangements. *L. longifolia* is hardy by nature and tolerates dry and frosty conditions, making it increasingly popular in modern landscapes.

Developmental studies of flower morphogenesis have long been important resources for systematists and plant breeders. Most angiosperms bear morphologically and functionally hermaphroditic flowers. Unisexuality, however, is widespread in the plant kingdom (Renner and Ricklefs 1995), with dioecious species estimated to make up ~4–5% of the world's angiosperms (Richards 1986; Charlesworth 2002).

Numerous developmental studies have been conducted on species with unisexual flowers. Unisexual flowers arise in two ways (Heslop-Harrison 1964): either the flowers initiate organs of both sexes and later selectively abort those of the inappropriate sex, or organs of only one sex initiate in individual flowers. De Mason *et al.* (1982) cited more than 10 reports illustrating the first mode of unisexual-flower development, covering genera such as *Zea*, *Silene* and *Asparagus*. Examples of the second mode of development include *Cannabis sativa* (Mohan Ram and Nath 1964), *Mercurialis* spp. (Durand and Durand 1991), *Phoenix dactylifera* (De Mason *et al.* 1982) and *Spinacia oleracea* (Sherry *et al.* 1993).

In flowers that selectively abort the organs of the unwanted sex there is considerable variation in the stage at which this occurs, taking place in some species before meiosis of the microspore or macrospore mother cells and in others after meiosis but before functional pollen or embryo sacs have developed. For example, in dioecious *Silene latifolia* the developmental arrest occurs when the reproductive organs are morphologically recognisable but before full differentiation and before meiosis in the reproductive mother cells of the inappropriate sex (Ye *et al.* 1991; Grant *et al.* 1994b; Zlucovna *et al.* 2006). In the dioecious *Asparagus officinalis* (garden

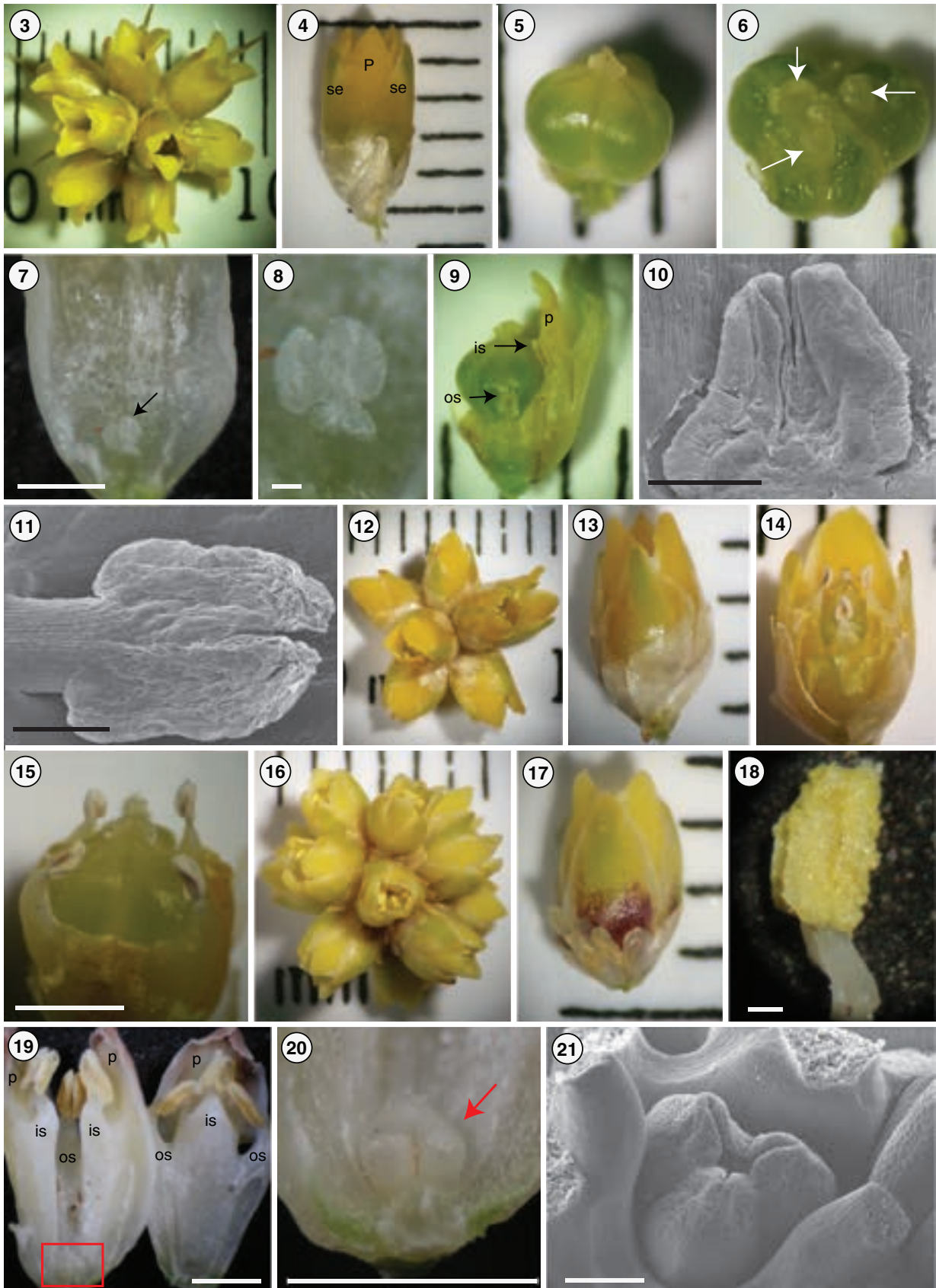


**Figs 1, 2.** Schematic representation of the inflorescences of *Lomandra longifolia* and the floral diagram for each inflorescence type. **Fig. 1.** Male inflorescence. **Fig. 2.** Female inflorescence. Scale bar for inflorescences = 10.0 mm.

asparagus) flower buds from females and males are identical in morphology until the onset of meiosis (Lazarte and Palser 1979; Bracale *et al.* 1991); thereafter, selective abortion leads to the production of unisexual flowers. Interestingly, in the male plants there is a considerable variation among different genotypes in the extent of growth of pistils, which in some remain as rudiments, whereas others develop non-functional

embryo sacs in the ovules and recognisable styles and stigmas (Galli *et al.* 1993).

In the last 20 years there has been an increasing interest in the application of molecular methods to the developmental genetics of dioecy. In a recent review, Meagher (2007) presented an interesting synthesis of evolutionary genetics and developmental genetics in relation to gender determination in



flowers. In particular, he summarised work on the role of a cascade of MADS-box genes relating to the development of successive whorls of the flower in *Arabidopsis thaliana*, *Antirrhinum majus*, *Silene latifolia*, *Rumex acetosa*, *Thalictrum dioicum*, *Asparagus officinalis*, *Populus* spp. and *Spinacea oleracea*.

Morphological and genetic studies on *Asparagus* have a special relevance to work on *Lomandra* because of the grouping of both genera in the order Asparagales (APGII 2003). MADS-box genes having a B function (Park *et al.* 2003) and an E function (Caporali *et al.* 2000) have been reported in *Asparagus officinalis*. Genes having a B function are associated with the development of stamens, C-function genes relate to the development of carpels and E-function genes appear to operate at a different level and are not specifically associated with the sexual organs (Meagher 2007).

Detailed anatomical and morphological observations of structural events leading to sexual differentiation are essential to provide the underpinning for future molecular and physiological studies of the genetic control of sexual determination in a given species. In the present paper, the development of male and female flowers of *L. longifolia* is described, with particular emphasis on the developmental events surrounding the transition from hermaphroditic to unisexual development.

## Materials and methods

### Source of material

Plants of *L. longifolia* grown by Leppington Speedy® Seedlings Pty Ltd, Leppington, New South Wales, from seed collected in the Southern Highlands region of New South Wales, were supplied in nursery trays when ~5 cm high. These were grown to maturity and maintained in the field at the Plant Breeding Institute at Cobbitty, New South Wales. Floral and fruit materials (buds, flowers and fruits) of various developmental stages of *L. longifolia* were collected periodically from this plantation (every 2 days from inflorescence emergence to seeds ripening). Individual plants often had floral and fruit material at various stages of development, ranging from

**Table 1. Some inflorescence characteristics in male and female *Lomandra longifolia***

Mean ± s.e. values were recorded for 40 inflorescences				
Sex	Inflorescence length (cm)	Peduncle length below the first branch (cm)	Peduncle width (cm)	Node no.
Female	68.39 ± 1.50	41.89 ± 1.19	1.11 ± 0.0319	10.95 ± 0.152
Male	86.87 ± 1.81	46.33 ± 1.55	1.28 ± 0.028	12.77 ± 0.29

unopened buds through to immature fruits on different inflorescences.

Buds at different developmental stages were removed from the plants and dissected under a stereomicroscope, before being studied in greater detail by scanning electron microscopy (SEM) and light microscopy of thin tissue sections. Detailed examinations were made on at least 15 specimens of floral buds for each stage. Vouchers representative of the male and female plants examined have been deposited in the Australian National Herbarium CANB (CANB 763117-CANB 763124).

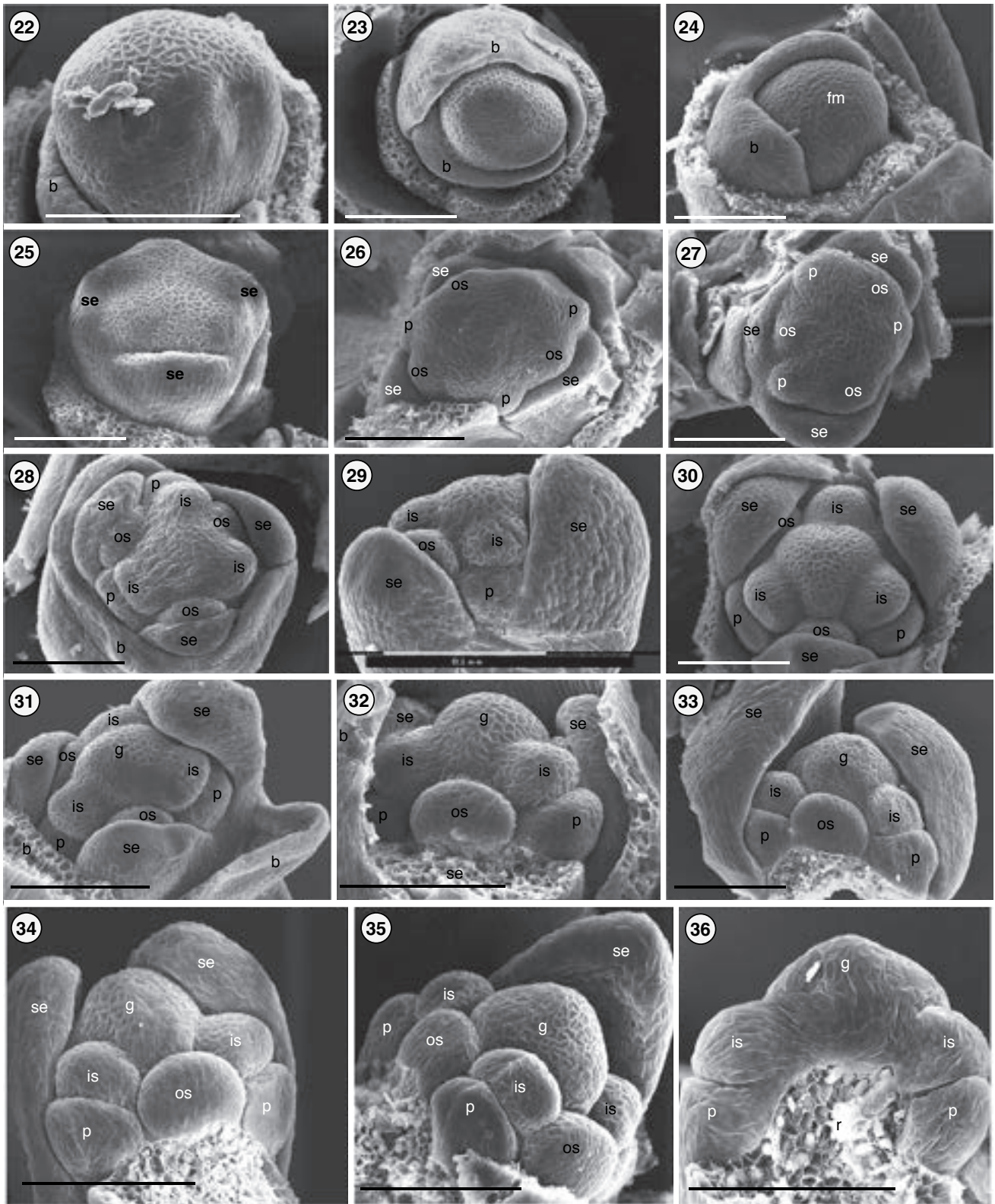
### Scanning electron microscopy

Dissected floral parts at different developmental stages were fixed in 2.5% glutaraldehyde (in 0.1 M potassium phosphate buffer, pH 7.1) at 20°C for 2 h. After rinsing in the same buffer for three intervals of 5 min, samples were dehydrated in a graded ethanol series (50, 70, 95 and 100%). Samples were then critical point dried in CO<sub>2</sub> with a BAL-TEC 030 critical point dryer (Bal-Tec, Balzers, Leichtenstein), and mounted on sticky tape affixed to aluminium stubs after they were dissected under a dissecting microscope with very fine forceps (number 5) and microneedles (250 µm) to expose the floral parts. Mounted specimens were coated with 20 nm of gold palladium in a sputter coater (Edwards E306 A: Edwards Vacuum Systems, Crawley, UK) and examined with a Philips 505 scanning electron microscope (Philips, Eindhoven, Netherlands) operating at an accelerating voltage of 15 KV. Approximately 250 samples were examined by SEM at various stages of floral development, and photographed with an installed digital camera.

**Table 2. Early stages of *Lomandra longifolia* male- and female-flower development**

Stage	Description
1	Vegetative/floral transition.
2	Floral meristem – sepal primordia initiated.
3	Petal and outer stamen primordia emerge. Floral meristem surface increases radially.
4	Primordia of inner stamens arise. Sepals start to arch inwards.
5	Stamen primordia well established. Petal primordia clearly separated from inner stamen primordia. Floral apex is a tall rounded dome.
6	Gynoecium initiation – flattened triangular zone, the centre persists as a small rounded dome. Sepals close to cover the reproductive organs. Inner stamens are clearly separated from the floral apex.

**Figs 3–21.** Female and male floral morphology in *Lomandra longifolia*. **Fig. 3.** A cluster of female flowers. **Fig. 4.** A single female flower. **Fig. 5.** Mature ovary. **Fig. 6.** Three ovules included within an ovary (arrows). **Figs 7, 8.** Rudimentary stamens as seen under the stereomicroscope. **Fig. 9.** A single ovary, showing the attached inner and outer stamens. **Figs 10, 11.** Rudimentary inner and outer stamens, respectively, as seen under the scanning electron microscope (SEM). **Figs 12–15.** Aberrant female flowers in *L. longifolia*, showing mature functional ovary and partly grown but non-functional stamens. **Fig. 16.** A cluster of male flowers. **Fig. 17.** A single male flower. **Fig. 18.** Stamen showing dehiscing anthers. **Figs 19–21.** Rudimentary pistil at the base of the filaments (Fig. 19, location indicated by rectangle; Fig. 20, rudimentary pistil indicated by arrow; Fig. 21, a SEM image showing rudimentary pistil). Scale bar graduation = 1.0 mm (Figs 3–9, 12–17, 19, 20) and 0.1 mm (Figs 8, 10, 11, 18, 21). is, inner stamen; os, outer stamen; p, petal; se, sepal.



*Light microscopy*

For developmental studies, individual buds, flowers and developing seeds were excised and fixed in formalin acetic alcohol (FAA; 5 parts formalin : 5 parts glacial acetic acid : 90 parts 50% ethanol (v/v/v)) and stored in 70% ethanol. They were dehydrated through the ethanol series and then embedded in paraffin with melting point 58–60°C for microtoming. Serial sections (longitudinal and transverse) cut with a rotary microtome (Spencer 820: American Optical Co, Buffalo, NY, USA) at 6–8 µm in thickness were stained with Safranin-O and Fast Green FCF (Sass 1958), dehydrated through an alcohol series to 100% ethanol and mounted with DPX (BDH, Poole, UK).

The samples were observed with normal brightfield optics with a Nikon Eclipse E800 light microscope (Nikon Optical Co, Tokyo, Japan) and photographed with a Nikon Photo Head V-TP Sencicam camera (PCO CCD imaging: PCO Imaging, Kelheim, Germany) mounted on the same microscope.

*Developmental stages*

Fourteen developmental stages were defined, six for the stages before sexual divergence (Table 2) and eight for each sex after that (Tables 3, 4).

**Results***Inflorescence and flower morphology*

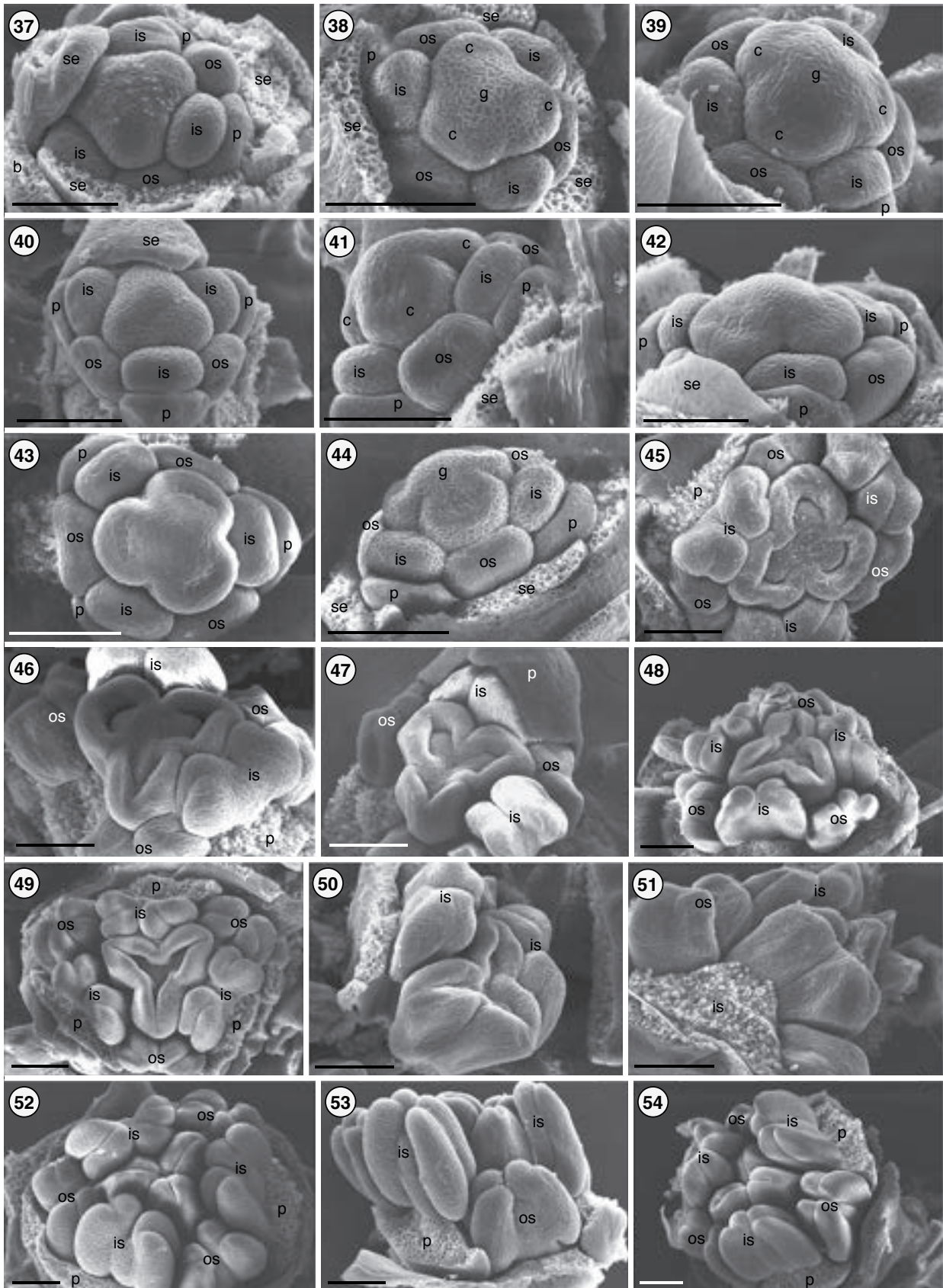
The inflorescence of *L. longifolia* is a panicle with a principal axis, from which secondary axes originate. The main axis of the panicle is terminated by a cluster of flowers, as for all the lateral axes. The degree of branching increases more or less regularly downwards from the top, so that the complete inflorescence has a conical outline, or at least primarily so (Figs 1, 2). Male inflorescences are, on average, larger than female inflorescences (Table 1).

Male flowers and female flowers are sessile and arranged in clusters borne on the highly branched panicles, each flower being

**Table 3. Later stages of development of the staminate flower in *Lomandra longifolia***

Stage	Description
7	Carpel primordia become very clear and small cavities appear near the abaxial side of each carpel. The centre of the gynoecium apex becomes more flattened than that of the female floral bud at the comparable stage. Inner and outer stamen primordia are well established but without obvious bilobing. Petals elongate but still shorter than the inner stamens.
8	The cavities on carpels become deeper and the centre of the gynoecium apex is flat. Four lobes can be seen on each stamen. Petals start to arch on the inner stamens.
9	The cavities on carpels become deeper and the side edges of the carpels start to grow and elongate inwards. The centre of the gynoecium apex becomes depressed. The four lobes of each anther are well established but all stamens are shorter than the gynoecium. Petals do not cover the outer stamens yet.
10	The upper side edges of each carpel continue to elongate and arch inwards. The centre of the gynoecium apex becomes more depressed and an ovule primordium becomes visible on the top of each carpel. The inner stamens are slightly taller than the gynoecium, whereas the outer stamens are shorter. Petals almost cover the inner stamens.
11	The upper side edges of each carpel elongate and gradually fuse, arching towards the centre of the gynoecium. Stamens continue to elongate. The inner stamens are taller than the gynoecium, whereas the outer stamens are about the same height.
12	The three carpels fuse in the centre of the gynoecium, starting from the abaxial edge and towards the centre of the gynoecium, without any sign of papillae formation on the edges of fusion. Stamens keep elongating and growing in size around the gynoecium.
13	The gynoecium stops growing. Stamens keep elongating and growing in size. The inner stamens show short filaments and are still connected to the bases of petals. Meiosis in stamens.
14	The non-functional gynoecium is situated in a canal-like structure made up of the bases of the surrounding stamens. Anthers are fully mature, showing the longitudinal dehiscent lines.

**Figs 22–36.** Scanning electron micrographs of early developmental stages of staminate and pistillate flowers in *Lomandra longifolia*. **Figs 22–24.** Stage 1, showing vegetative meristem elongation as the first sign of conversion from vegetative to floral in staminate (Figs 22, 23) and pistillate buds (Fig. 24). **Fig. 25.** Stage 2, showing initiation of three sepals. **Figs 26, 27.** Stage 3 in pistillate and staminate buds, respectively, showing, from different angles, an increase in the surface area of the floral meristem in a radial shape and the emergence of petal and outer stamen primordia. **Fig. 28.** Stage 4, showing a staminate flower, with initiation of the inner stamens and sepals starting to arch inwards. **Fig. 29.** Early Stage 5. Side view of the floral apex of staminate flower, showing a tall rounded dome, with sepals enclosing the outer stamens. **Figs 30–32.** Stage 5, showing the established inner and outer stamen primordia in pistillate (Fig. 30) and staminate flowers (Figs 31, 32). **Fig. 33.** Stage 5. Side view of the staminate bud, showing two of the three sepals arching over the developing sexual organs. **Figs 34, 35.** Stage 5. Semi-vertical view of pistillate and staminate buds, respectively, showing arrangement of the floral parts including the inner and outer whorls of stamens (cf. floral diagram in Figs 1 and 2). **Fig. 36.** Stage 5. Near-median longitudinal section, showing attachment of petals, inner stamens and gynoecium to the receptacle in a pistillate bud. Scale bar = 0.1 mm. b, bracteole; fm, floral meristem; g, gynoecium; is, inner stamen; os, outer stamen; p, petal; r, receptacle; se, sepal.



**Table 4. Later stages of development of the pistillate flower in *Lomandra longifolia***

Stage	Description
7	Carpel primordia become very clear and small cavities appear near the abaxial side of each carpel. The centre of the gynoecium apex persists as a small rounded dome. Inner and outer stamen primordia are well established. Each outer stamen initiates two lobes, whereas the inner stamens are still plump. Petals elongate but remain still shorter than the inner stamens.
8	The cavities on carpels become bigger and deeper. Anther bilobing can be seen on all stamens; however, the central line is clearer on the outer stamens. Petals start to arch on the inner stamens.
9	The cavities on carpels become deeper and the side edges of the carpels start to grow and elongate inward. The centre of the gynoecium becomes flattened. Anther bilobing becomes clearer on all stamens. Petals arch over the inner stamens.
10	Three ovule primordia become visible as protrusions on the top of the flattened apex of the gynoecium. Outer stamens become four-lobed but remain still shorter than the gynoecium. Petals continue to elongate.
11	Ovule primordia become very visible on top of each carpel and there is a small dome-like protrusion in the centre of the gynoecium between the three carpels. The carpel edges start to elongate and arch inwards. All anthers are four-lobed. Inner stamens elongate and become slightly longer than the gynoecium, whereas the outer stamens are shorter than the gynoecium. Petals cover almost all the sexual organs.
12	The side edges of each carpel continue elongating and gradually fuse towards the centre of the gynoecium. The four-lobed anthers are well established and keep on elongating. The inner stamens are taller than the gynoecium, whereas the outer stamens are about the same height. The filaments of the inner stamens make very little contribution to stamen elongation.
13	The three carpels fuse in the centre of the gynoecium. Stigma formation in the area of fusion between the upper edges of each carpel and papillae start to rise owing to epidermal cell elongation. Stamens shrivel around the enlarged gynoecium; the inner stamens are at the same height as the gynoecium in between the carpels, whereas the outer stamens stay shorter at the lower edge of each carpel. Meiosis in pistil.
14	The gynoecium enlarges and carpels become well established. Stamens are shrivelled or vestige-like structures. Short style formation and well established stigma.

subtended by a bract. At maturity, rudimentary sexual organs of the opposite sex can be observed in both male and female flowers (Figs 3–21).

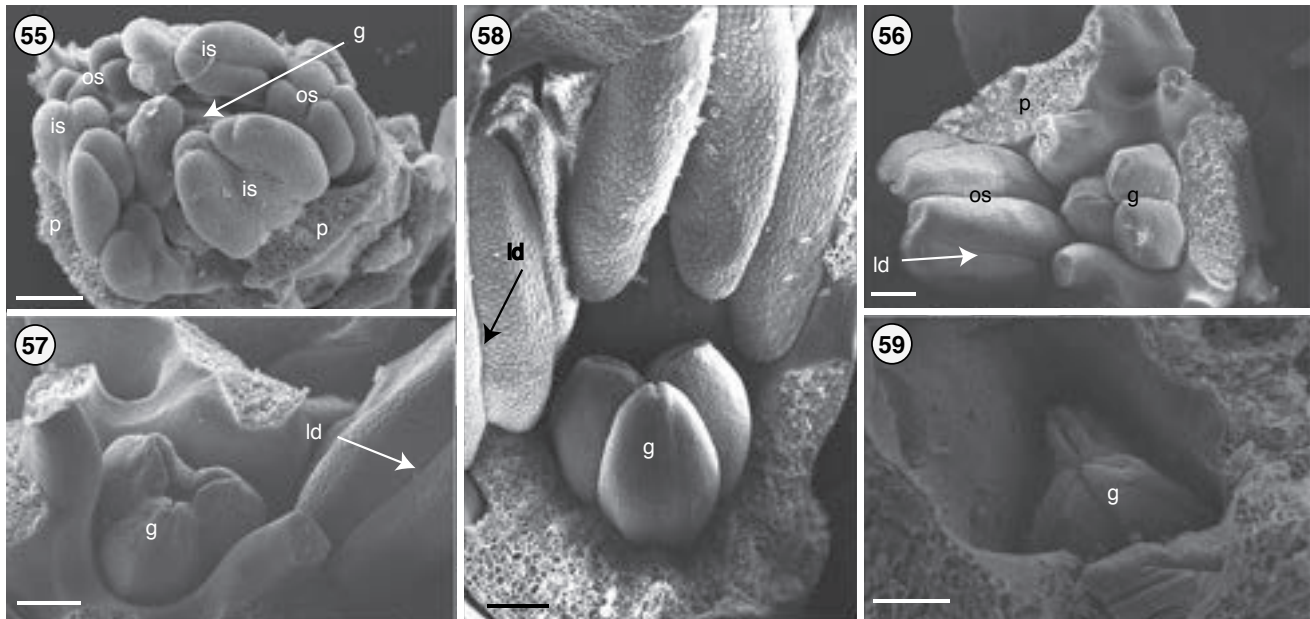
The floral diagrams of both sexes of *L. longifolia*, included in Figs 1 and 2 show two successive perianth whorls, each with three segments, within which stand six stamens belonging to two whorls of three. The stamens of the inner whorl are inserted higher on the perianth than those of the outer whorl. The three carpels of the gynoecium are united in their basal parts

which enclose the ovules, thus forming a single ovary with three locules.

#### *Early development of the staminate and pistillate flowers*

Early development of both the staminate and pistillate flowers is identical during carpel initiation and will be treated together (Table 2). In the transition from vegetative to floral bud, the vegetative meristem undergoes elongation, which is the first sign

**Figs 37–54.** Scanning electron micrographs showing Stage 6 (Figs 37–42) in the development of male and female floral buds and Stages 7–12 of male-flower development (Figs 43–54) in *Lomandra longifolia*. Stage 6 shows the gynoecium in pistillate (Figs 37, 40, 42) and staminate (Figs 38, 39, 41) buds initiated as a flattened triangular zone with a small rounded dome and initials of three carpel primordia; the inner stamens are clearly separated from the gynoecium. **Fig. 43.** Stage 7. Top view, showing the initiated carpels with a small dimple near the abaxial side of each carpel; the inner and outer stamens are well established but without obvious bilobing. **Fig. 44.** Stage 7. Side view, showing the different floral parts including a gynoecium with three carpels and a central rounded dome. **Fig. 45.** Stage 8. The cavities on carpels become deeper and the centre of the gynoecium apex is becoming flat; each stamen (inner and outer) has four lobes. **Fig. 46.** Stage 9, showing a more developed gynoecium, with cavities on carpels becoming deeper and the side edges of the carpels starting to grow and elongate inwards; the centre of the gynoecium becomes depressed and all stamens are shorter than the gynoecium. **Fig. 47.** Stage 10. Petals almost covering the outer stamens; the centre of the gynoecium apex becoming more depressed and an ovule primordium becoming visible on top of each carpel. **Fig. 48.** Stage 10. An overall view, showing the elongation of the upper side edges of each carpel arching inwards; the inner stamens are slightly taller than the gynoecium, whereas the outer stamens are shorter. **Fig. 49.** Stage 11. Top views, showing more elongation of the upper side edges of each carpel arching towards the centre of the gynoecium; the inner stamens are taller than the gynoecium, whereas the outer stamens are about the same height. **Figs 50, 51.** Late Stage 11, showing the carpels before fusion and more elongated inner stamens. **Fig. 52.** Stage 12. Complete fusion of carpels in the centre of the gynoecium. **Figs 53, 54.** Stage 12. Side views, showing enlargement and elongation of the inner and outer stamens around the gynoecium. Scale bar = 0.1 mm. b, bracteole; c, carpel; g, gynoecium; is, inner stamen; os, outer stamen; p, petal; se, sepal.



**Figs 55–59.** Scanning electron micrographs showing late developmental Stages 13 (Fig. 55) and 14 (Figs 56–59) of staminate flowers. **Fig. 55.** Top view, showing the enlarged inner and outer stamens almost covering developmentally retarded gynoecium. **Figs 56, 57.** The rudimentary pistil surrounded by the outer stamens with their longitudinal lines of dehiscence; and filaments of the inner stamens. **Fig. 58.** Fully mature anthers with their longitudinal lines of dehiscence and a completely arrested gynoecium at the stamen bases. **Fig. 59.** The non-functional rudimentary pistil is situated in a canal-like structure made up of the bases of the surrounding stamens. Scale bar = 0.1 mm. g, gynoecium; is, inner stamen; ld, line of dehiscence; os, outer stamen; p, petal.

of conversion (Figs 22–24). Three sepals are initiated first (Fig. 25). All the sepals appear to originate at the same level on the flower primordium and, thus, should be considered as forming a single whorl. The surface of the floral meristem increases radially and a whorl of three separate petals is initiated in this area, the petals alternating in position with the sepals (Figs 26, 27), although at a higher level (Fig. 29). The sepals remain small during petal initiation and start to arch inwards after all petals are formed. All three petal primordia appear to form closely in time, and before any begin to enlarge. The stamens are initiated in two whorls of three, the first (outer) in an antisepalous position, the second (inner) in an antipetalous position (Fig. 28). The two whorls of stamens are initiated separately but in quick succession. In contrast, there is a slight overlap in the time of initiation between the petal and outer stamen whorls. The outer stamens (antisepalous) remain small while the inner ones are initiated. After all six stamens have been initiated

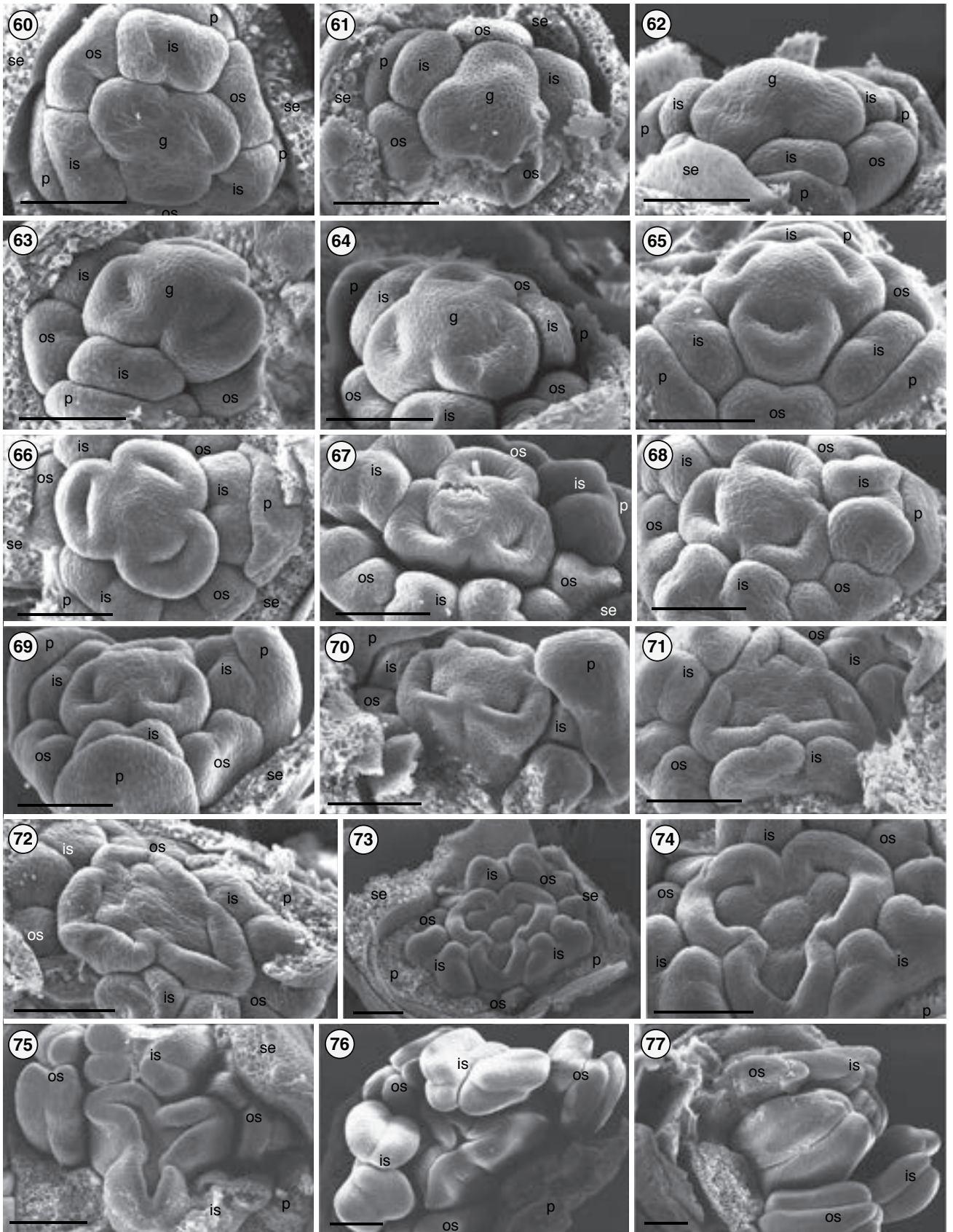
the floral apex is a tall rounded dome (Figs 29–36). The gynoecium is initiated as a flattened triangular zone at the top of the floral apex (Figs 37–42). The centre of the apex persists as a small rounded dome, which later separates the three individual carpels. Up to this stage, development of the female and male inflorescences, with the regular sequence of initiation of three sepals, three petals, six stamens and a central gynoecium, is identical, except for the larger size of the female floral organs and flower buds.

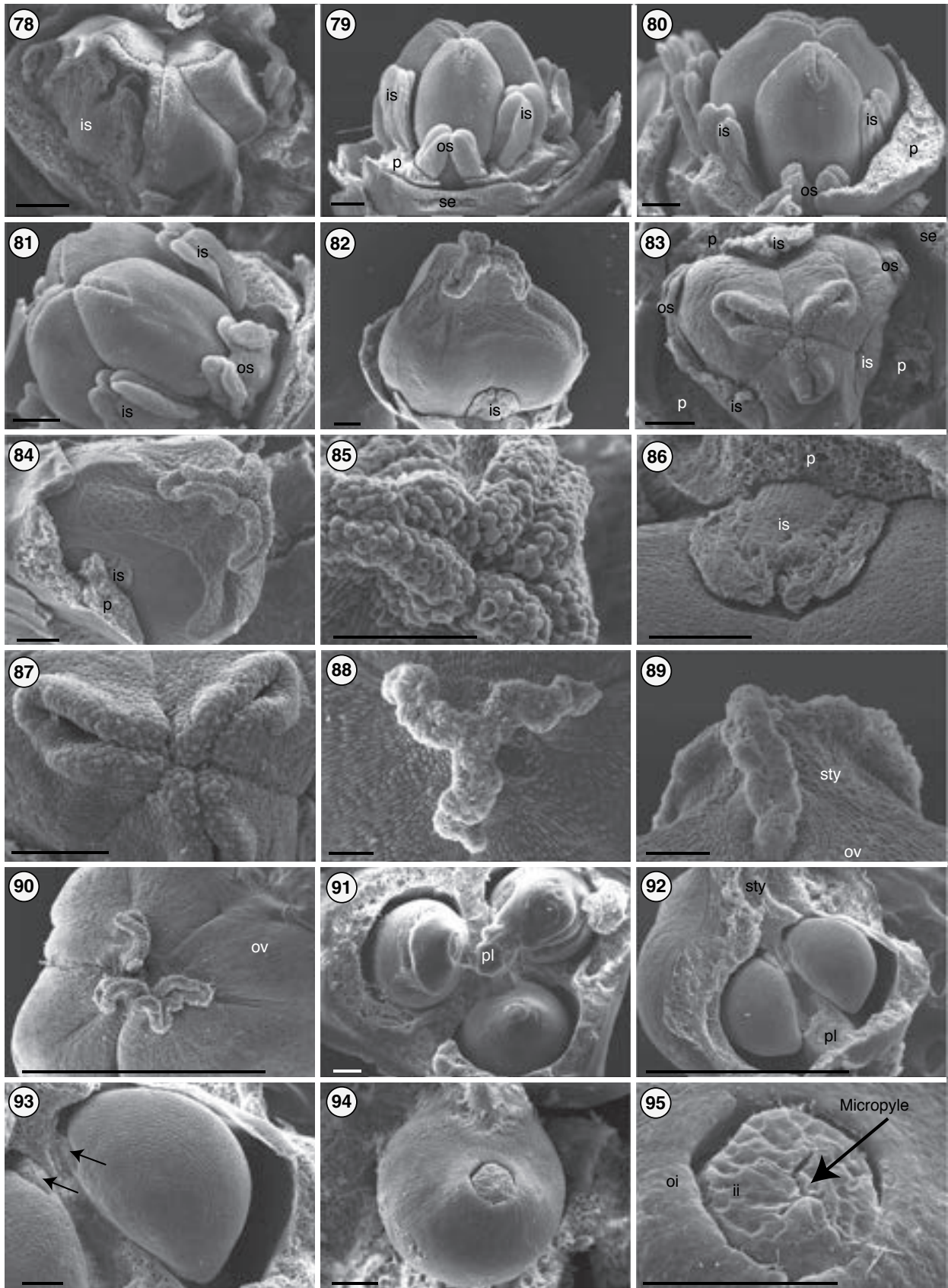
Sex determination in *L. longifolia* takes place subsequent to the six ‘bisexual’ stages, as differences become more noticeable in the morphology of the male and female floral buds.

#### Later development of the staminate flower

Tables 3 and 4 list the key differences between male and female flower buds in *L. longifolia* as revealed by SEM. Male flower buds

**Figs 60–77.** Scanning electron micrographs showing Stages 7–12 of carpel initiation and floral development in pistillate flowers of *Lomandra longifolia*. **Fig. 60.** Stage 7. Top view, showing clear bilobing in the outer stamens. **Figs 61–63.** Stage 7, showing small cavities near the abaxial side of each carpel, while the gynoecium apex persists as a small rounded dome; petals remain shorter than the outer stamens. **Fig. 64.** Stage 8. The cavities on carpels become bigger and deeper. **Fig. 65.** Stage 8, showing anther bilobing for all stamens, and deeper cavities in carpels. **Fig. 66.** Stage 9. Top view, with petals arching over the inner stamens; the cavities on carpels become bigger and deeper. **Fig. 67.** Stage 10. The inner and outer stamens become four-lobed and remain shorter than the gynoecium. **Figs 68, 69.** Stage 10. Side views, showing the enlarged inner and outer stamens. **Fig. 70.** Stage 10. Enlargement and elongation of petals to cover the inner stamens and part of the gynoecium. **Fig. 71.** Stage 10, showing a depressed and flattened surface at the gynoecium centre and the outer stamens still shorter than the gynoecium. **Fig. 72.** Stage 10. Overview of the pistillate floral bud, showing three ovule primordia as protrusions on the top of the flattened apex of the gynoecium. **Fig. 73.** Stage 11. Ovule primordia become very visible and there is a small dome-like protrusion in the centre of the gynoecium between the three carpels. Inner and outer stamens are four-lobed and the inner stamens elongate to become slightly longer than the gynoecium. **Fig. 74.** Stage 11. A magnified image of the gynoecium in Fig. 73, showing the carpel edges starting to elongate and arch inwards. **Fig. 75.** Stage 12. The side edges of each carpel keep on elongating and arching inwards. **Figs 76, 77.** Stage 12. Inner and outer stamens keep on elongating, whereas the gynoecium enlarges and the carpel edges elongate and fuse, leaving only a small opening at the centre of the gynoecium. Scale bar = 0.1 mm. g, gynoecium; is, inner stamen; os, outer stamen; p, petal; se, sepal.





at Stage 6 show the development of the three fused carpels surrounded by six stamen primordia. The three long stamens (inner stamens) initiate opposite the petals and the three short stamens (outer stamens) initiate earlier in antisepalous positions at a level on the meristem near the petal primordia.

At Stage 7, the carpels grow up in a cup-shaped manner, apparently initiated with a small dimple near the abaxial side of each carpel (Figs 43, 44). The centre of the apex of the gynoecium has a little convexity at this stage. The stamen primordia are cylindrical and plump without any sign of lobe initiation. At Stage 8, the carpels grow so that the apex of the gynoecium becomes completely flat and the cavities on the abaxial side of the carpels become deeper. Meanwhile, four lobes are initiated on each stamen, including both the inner and the outer stamens (Fig. 45).

After Stage 8, the preformed gynoecial initials cease development, while the stamens continue to develop to sexual maturity. At a comparable stage in the female floral bud, the stamen initials cease development, whereas the gynoecium continues to develop to sexual maturity.

At Stage 9, the formation of locular grooves on the stamens and the establishment of four-lobed stamens precede the complete enclosure of the stamens by the petals. The cavities on carpels become deeper and the side edges of the carpel start to grow and elongate inwards (Fig. 46). The gynoecium at this stage shows a depression at the centre; this depression constitutes a major difference between the male and female floral buds at the same developmental stage. This depression becomes even deeper at Stage 10 (Figs 47, 48), and an ovule primordium becomes barely visible on the top of each carpel (Fig. 47). The upper edges of the carpel continue to elongate and the petals and stamens continue to develop and extend, such that each stamen primordium is enclosed completely within one petal.

By Stage 11 (Figs 49–51), the stamens have enlarged considerably whereas the gynoecium shows the occurrence of slower carpel development. At Stage 12, the three carpels fuse in the centre of the gynoecium while the stamens keep elongating (Figs 52, 53). The gynoecium stops growing (absence of further carpel development) at Stage 13, at a time when the stamens show considerable elongation and growth owing to enlargement of the anthers in all stamens as well as filament elongation for the first time in the inner stamens (Figs 54, 55). Finally, Figs 56–59 show a male flower at Stage 14, at which point male-flower development is almost complete. As the male flowers mature, the carpel primordia undergo a process of degeneration that is evident from as early as Stage 13. Anthers dehisce along the sutures formed at the anther margins (Figs 56–58).

#### Later development of the pistillate flower

Female flower buds at Stage 7 show morphology similar to male flower buds, except that the centre of the apex of the gynoecium persists as a small rounded dome, and the outer stamens start bilobing earlier than those in the male flower buds, which was unexpected (Figs 60–63). However, at Stage 8 all stamens become bilobed (Figs 64, 65) at the time when all stamens are four-lobed in the male flower bud (Fig. 45). At Stage 9, anther bilobing becomes clearer on all stamens, the cavities on carpels become deeper and the centre of the gynoecium becomes flattened (Fig. 66).

At Stage 10, three ovule primordia become visible as protrusions on the top of the flattened apex of the gynoecium. The outer stamens become four-lobed but are still shorter than the gynoecium (Figs 67–72). All anthers become four-lobed at Stage 11 (Figs 73, 74). The inner stamens elongate and become slightly longer than the gynoecium. The ovule primordia on top of each carpel, as well as a small dome-like protrusion on the centre of the gynoecium between the three carpels, become very visible (Figs 73, 74).

At Stage 12, through intercalary growth, the carpels fuse along the margin of the adaxial groove. Later development, in this case, leads to a basal ovarian portion in each of the three carpels (Figs 75–77). At the end of this stage, the stamen initials cease development, whereas the gynoecium continues to develop to sexual maturity. Stage 13 shows three fused carpels and shrivelled stamens around the enlarged gynoecium (Figs 78–81). The carpels develop stigmas on their surfaces and papillae start to appear as epidermal cell elongations.

By Stage 14, the carpels have enlarged considerably, whereas the stamens remain shrivelled at the bases of the well established carpels. The extension of the distal portion at Stage 14 forms a short style and stigmatic region (Figs 82–89). The stigmas at this stage are highly papillated and receptive to pollen. The mature ovule shows a micropyle that is formed by the inner integument which protrudes through the outer integument (Figs 94, 95); this is in accord with the situation described by Rudall (1994) for *L. integra*, *L. hastilis* and *L. purpurea*, and corresponds closely to the typical Asparagalean type. After fertilisation, the trilocular gynoecium matures with considerable increase in size into a capsular fruit that encloses three seeds (one seed per locule) (Figs 90–92). Each seed is connected to the placenta by a very short funicle (Fig. 93). At maturity, the *L. longifolia* fruit (capsule) dries and dehisces along the sutures formed at the carpel margins (Fig. 96).

**Figs 78–95.** Scanning electron micrographs showing Stages 13 and 14 of pistillate-flower development and the morphological features of the pistil at pollination and post-fertilisation stages (Figs 89–95) in *Lomandra longifolia*. **Fig. 78.** Stage 13. Complete fusion of the three carpels and the appearance of papillae in the area of fusion. **Figs 79–81.** Stage 13. Shrivelled stamens around the enlarged gynoecium. **Figs 82, 83.** Stage 14. Side (Fig. 82) and top (Fig. 83) views, showing a well developed carpel with prominent stigmatic surfaces resting on a very short style (see also Figs 124, 125). The stamens are shrivelled at the base of the gynoecium. **Fig. 84.** Stage 14. Highly papillate stigma. **Fig. 85.** Stage 14. A magnified portion of the stigmatic surface in Fig. 84, showing papillose cells (see also Figs 126, 127). **Fig. 86.** Stage 14. Shrivelled inner stamens. **Figs 87, 88.** Stage 14. Immature (Fig. 87) and mature receptive (Fig. 88) stigma. **Fig. 89.** Mature pistil, showing the ovary (ov), short style (sty) and stigma with papillose cells. **Figs 90–93.** Post-fertilisation mature fruits, showing the locules, the single seed within each locule and the axile placenta (pl). **Fig. 91.** Transverse section. **Fig. 92.** Longitudinal section. **Fig. 93.** Magnified view of the right-hand side of Fig. 92, showing short funicle (arrow). **Fig. 94.** Mature ovule showing the micropylar region. **Fig. 95.** Details of the micropylar region, showing the inner (ii) and the outer (oi) integuments. Scale bar = 0.1 mm (Figs 78–89, 91, 93–95); 1 mm (Figs 90, 92). ii, inner integument; is, inner stamen; oi, outer integument; os, outer stamen; o, ovary; p, petal; pl, axile placenta; se, sepal; sty, style.

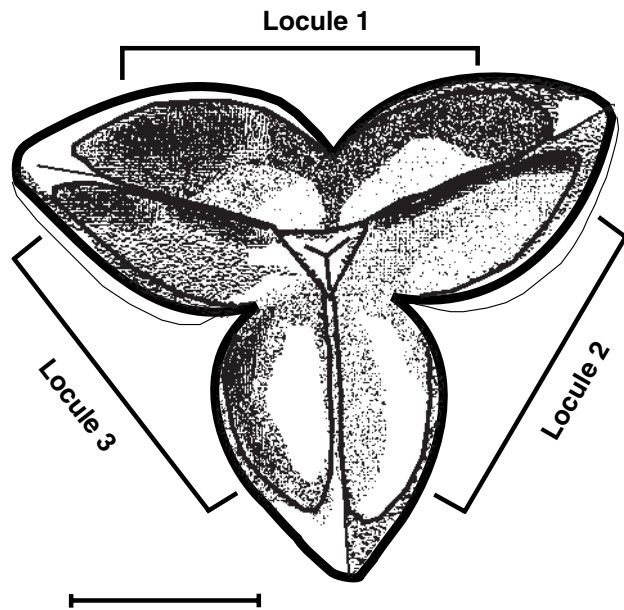


Fig. 96. Schematic representation of a dehiscent capsular fruit of *Lomandra longifolia*. Scale bar = 5 mm.

#### Histology of the staminate and pistillate flowers

A comprehensive analysis of male- and female-flower formation requires correlation of the results of the SEM investigation reported above, with a histological examination of selected developmental events as shown in Figs 98, 127.

Stage 1 is characterised by transformation of vegetative meristems into floral meristems in the developing inflorescence (Figs 98, 99). This is followed by the initiation of five whorls representing sepals, petals, outer stamens, inner stamens and the gynoecium, in that order (Figs 100–103).

In *L. longifolia*, the paired bracteoles around each flower become massive, flat-topped and fuse early, with an overlap between each pair over the floral summit (Fig. 104). They continue to enlarge as a long, narrow-tapered tube.

Cytological analysis of the slowly growing abortive pistil of male flowers shows that megasporogenesis does not occur (Figs 105–112). Pistil abortion happens before meiosis, whereas the stamens continue to develop until maturity and dehiscence. In female flowers, stamen arrest occurs at an early stage before the onset of meiosis in microspore mother cells (Figs 113–117), following which the pistil continues its development through megasporogenesis and megagametogenesis (Figs 118–123).

Details of the stigma and style, highlighting the glandular and papillose stigmatic surface, are shown in Figs 124–127.

#### Discussion

Our light stereomicroscopic observations of the mature flowers (Figs 7–9, 19, 20) showed that arrested stamens were present in female flowers and that the male flowers contained a rudimentary gynoecium.

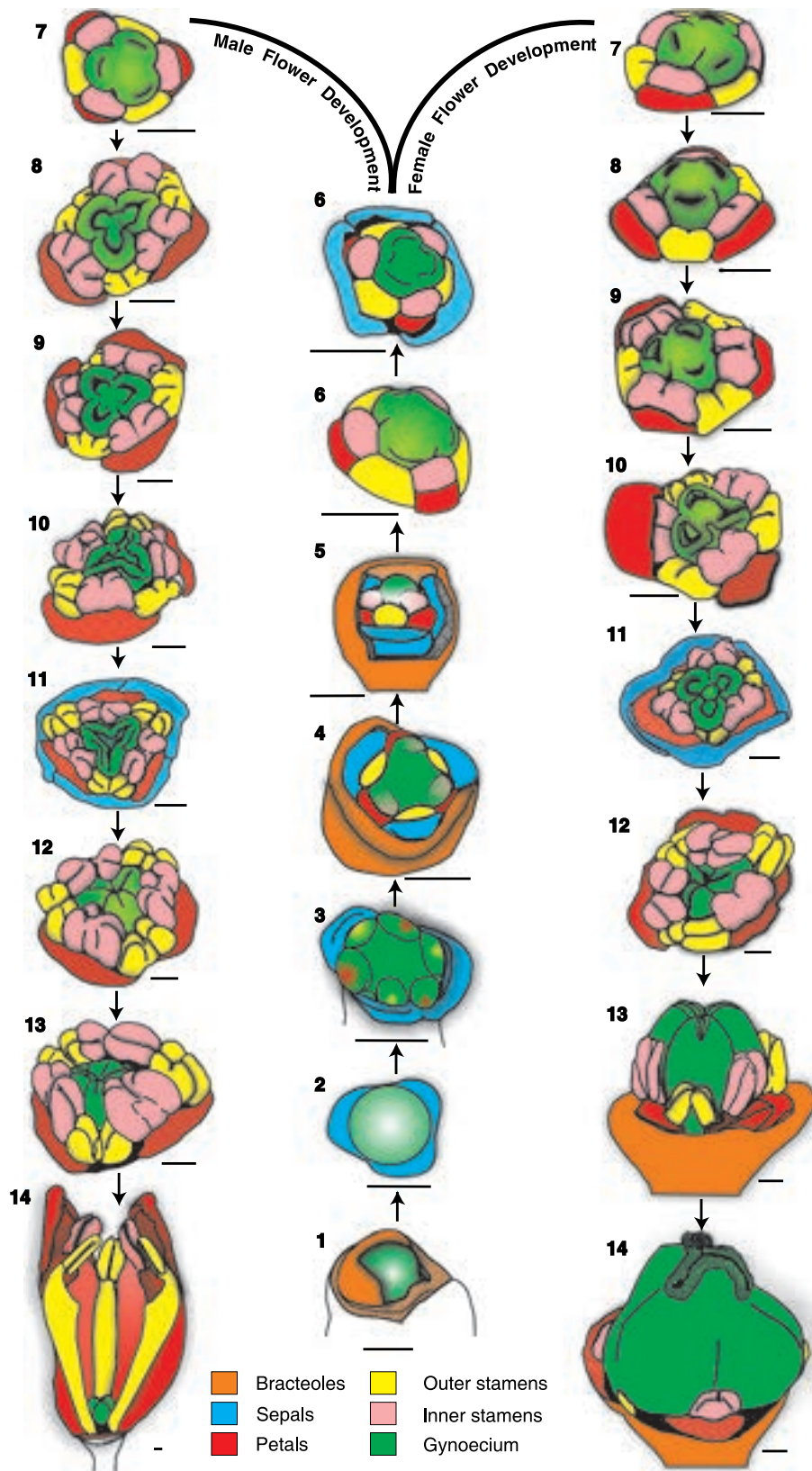
However, a detailed comparison of the development of male and female flowers by SEM revealed initial morphological differences between the sexes and between the times when the

rudimentary sex organs become arrested in development. The first morphological feature distinguishing male- and female-flower development became clear at Stage 7. The arrest of the pistil development of male flowers occurs just before meiosis. Similarly, the development of stamens of female flowers is abruptly arrested before meiosis and the stamen rudiments degenerate as the female flower matures further.

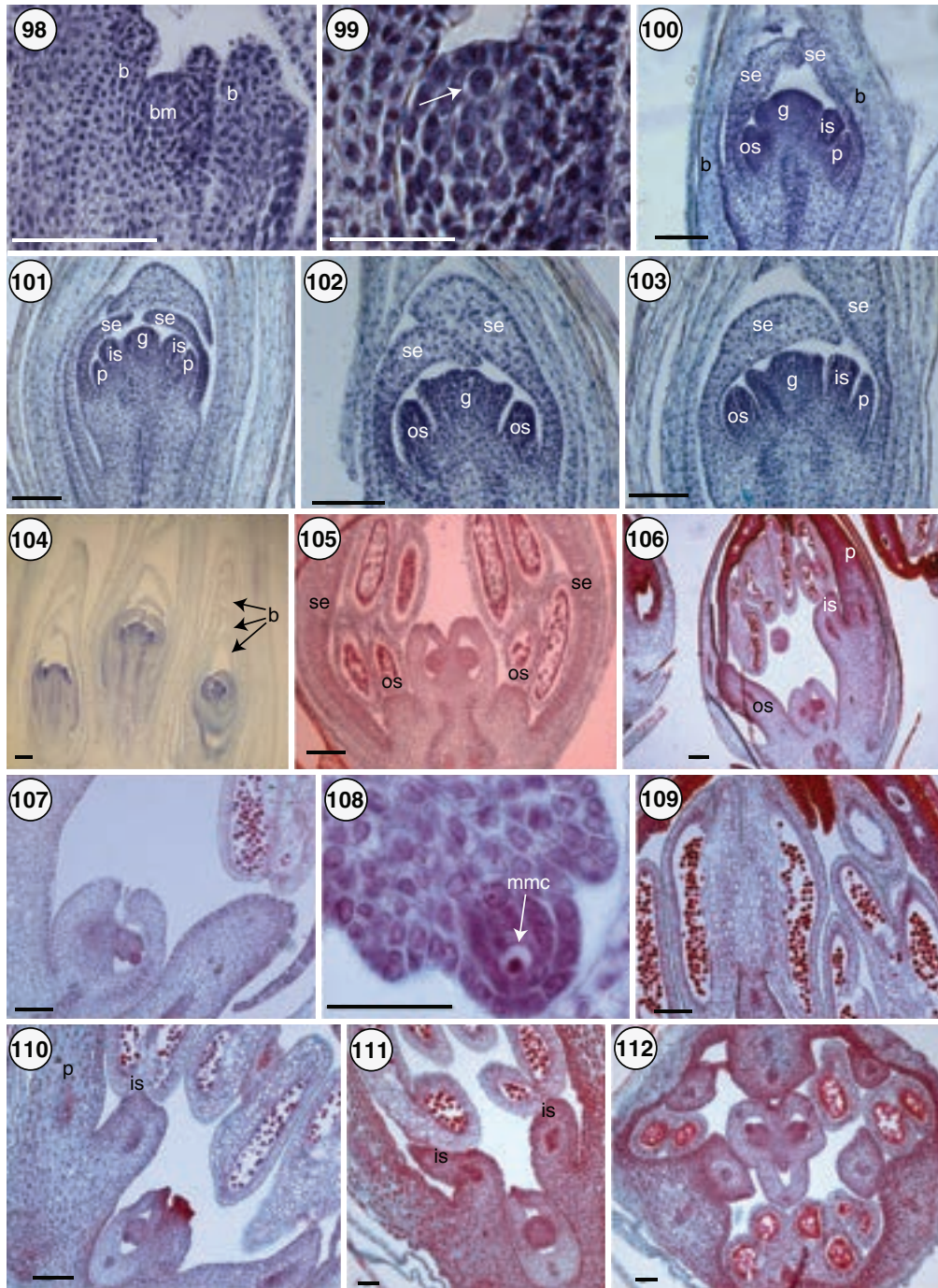
Exceptions to this pattern are apparently rare, with only 1 in >1000 female plants examined being found in which cessation of stamen growth occurred at a later stage. In this plant (Figs 12–15), the stamens are conspicuous at the time the flower opens (Fig. 15), even though they are somewhat shrivelled, smaller and paler than normal stamens (Fig. 15 v. Fig. 19). The anthers are completely devoid of pollen grains, suggesting that in this case there was a significant departure from the close link between failure of meiosis and commencement of stamen decline observed in the rest of the population. Older inflorescences on the same plant had abundant fruits and seeds, confirming its status as a fully functional female. No exceptions to the normal pattern were observed in the male plants. The occurrence in a population of >1000 female plants of one plant with stamens suggests that there is either a small degree of developmental plasticity, or, as the plants were raised from seed, a limited amount of genotypic variation in the developmental sequence. It is interesting to note that in *Asparagus officinalis*, Galli *et al.* (1993) found that variations from the normal patterns were confined to the male flowers.

Despite the gross morphological differences at maturity in the male and female flowers of maize (*Zea mays*), white campion (*Silene latifolia*), *Asparagus* (*Asparagus officinalis*) and *L. longifolia*, early events in the flower formation are remarkably similar and all flowers are initially bisexual (perfect) and morphologically indistinguishable. However, the transition from the bisexual to the unisexual state becomes visible at different developmental stages in different species. In *L. longifolia*, the first sign of sexual dimorphism between the two sexes appears in the female flower when the stamens become bilobed (Fig. 60), whereas the male flower at a comparable stage shows cylindrical and plump stamen primordia, without any sign of lobe initiation (Fig. 37). In *A. officinalis*, the first morphological feature distinguishing male- and female-flower development becomes clear when the style begins to enlarge in female flowers (Caporali *et al.* 1994, fig. 3H). In *S. latifolia*, the first gender-specific differences are seen when the stamen primordia appear in flowers of both sexes (Grant *et al.* 1994b, figs 5b, f, j).

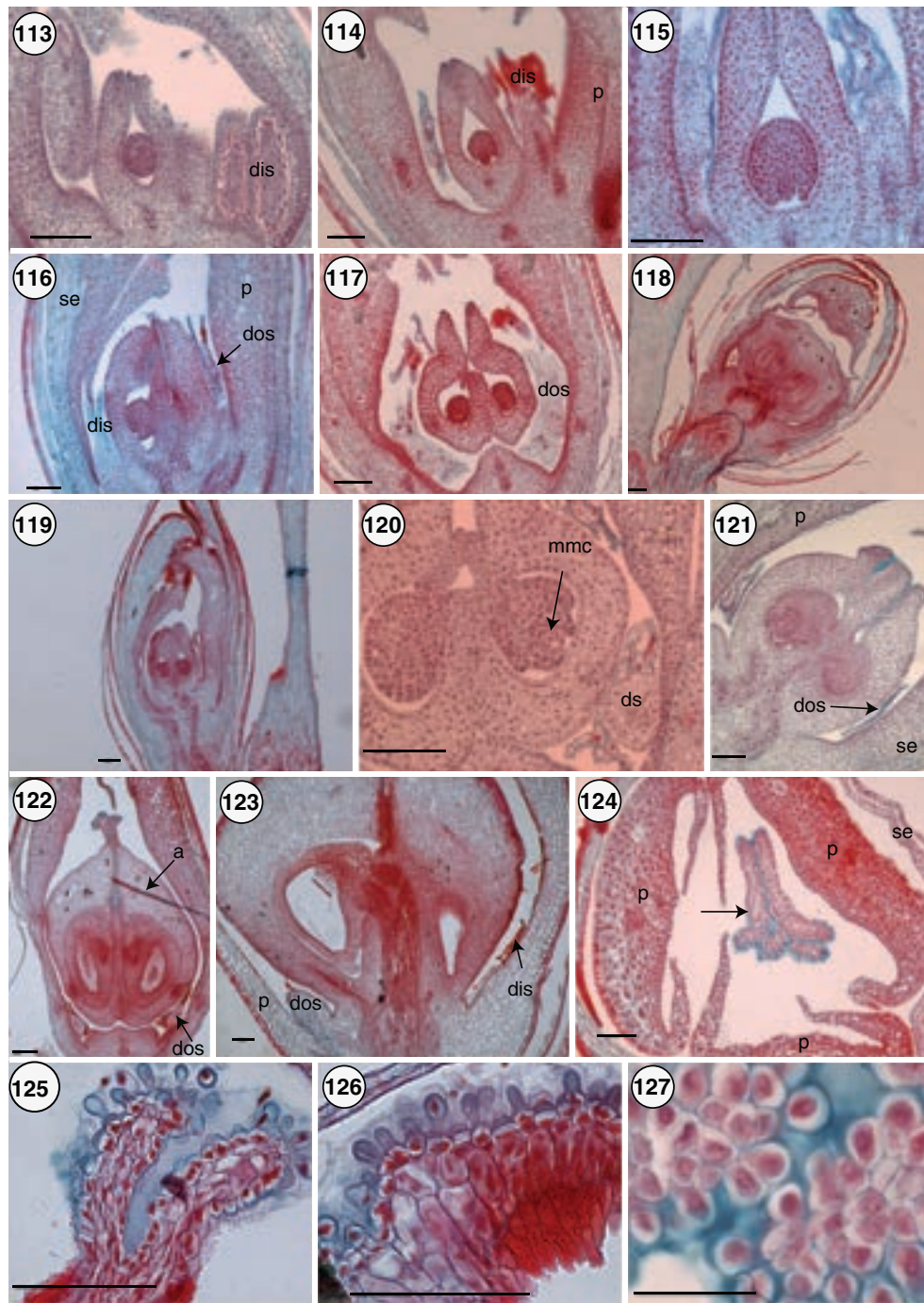
Sex determination in flowers of maize, a monoecious species, involves the selective abortion of either the female- or male-organ primordia within a bisexual floral meristem (Cheng *et al.* 1983). After initiation, pistil primordia, while still very small and internally undifferentiated, abort in tassel flowers, whereas stamen primordia in the ear commence degenerating before the development of a recognisable endothecium (Cheng *et al.* 1983). In *S. latifolia*, the gynoecium is arrested in male flowers during stamen maturation, before meiosis, whereas stamen arrest commences in female flowers during gynoecium maturation, well before meiosis of the megaspores (Grant *et al.* 1994b). In *A. officinalis*, the flowers develop as hermaphrodites until the onset of meiosis; the anthers of the female are arrested at this stage and subsequently collapse (Caporali *et al.* 1994), whereas the



**Fig. 97.** Comparison of the development of the staminate (♂) and pistillate (♀) flowers of *Lomandra longifolia*, showing the different developmental Stages 1–14. Scale bars = 0.1 mm.



**Figs 98–112.** Histological sections of early stages of floral development in both male and female floral buds (Figs 98–104) and late stages of floral development in male floral buds of *Lomandra longifolia* (Figs 105–112), showing the abortion of the pistillate organs and further development of the staminate organs. **Fig. 98.** Flower-bud primordium after conversion from vegetative meristem to floral meristem (cf. floral bud meristem in Fig. 23). **Fig. 99.** Magnified apical meristem of Fig. 98, showing an enlarged cell (arrow). **Fig. 100.** Pistillate floral bud at Stage 6. **Figs 101–103.** Pistillate bud at Stage 6 in different sectioning planes showing the gynoecium together with two petals and two inner stamens in Fig. 101, two outer stamens in Fig. 102, and with a petal and an outer and inner stamen in Fig. 103. **Fig. 104.** Groups of floral buds enclosed within pairs of bracteoles. **Fig. 105.** Longitudinal section showing ovule primordia inside two locules of the aborted ovary, while stamens continue their development to release the microspores after the dissolution of callose walls. **Fig. 106.** The complete arrest of the pistil while the stamens continue their development to produce mature pollen grains. **Fig. 107.** Inhibition of megasporogenesis (note the production of mature pollen grains in the upper right side of the image). **Fig. 108.** Close-up view of the ovule in Fig. 107, showing megaspore mother cell without any further development. **Fig. 109.** Close-up view of anthers in Fig. 106, showing mature pollen grains. **Figs 110, 111.** Different sectioning planes for similar buds. **Fig. 112.** Cross-section of male floral bud, showing mature stamens and aborted pistil (cf. floral diagram in Fig. 1). Scale bar = 0.1 mm. b, bracteole; bm, bud meristem; g, gynoecium; is, inner stamen; mmc, megaspore mother cell; os, outer stamen; p, petal; se, sepal.



**Figs 113–127.** Histological sections of the late stages of floral development in female floral buds of *Lomandra longifolia*, showing the arrest of stamens before the onset of meiosis in both the microspore mother cells and the megaspore mother cells (Figs 113–119), followed by megasporogenesis and pistillate-flower development and growth (Figs 120–127). **Fig. 113.** Female floral bud, showing the inner stamens before the onset of meiosis. **Figs 114, 115.** The degenerating inner stamens. **Fig. 116.** An oblique longitudinal section of the degenerating inner and outer stamens. **Fig. 117.** Degenerating outer stamens. **Figs 118, 119.** Longitudinal sections of entire female floral buds, showing the degenerating outer stamens. **Fig. 120.** Transverse section of the female floral bud, showing ovules (one with megaspore mother cell visible) and degenerating stamens before the onset of meiosis. **Fig. 121.** Longitudinal section of the female floral bud after megasporogenesis, showing the degenerating outer stamens. **Fig. 122.** Female floral bud at an advanced stage of development, showing the degenerated outer stamens. **Fig. 123.** Longitudinal section of a fruit (post-fertilisation) with degenerating inner and outer stamens. **Fig. 124.** Cross-section of a mature ovary, showing the stigmatic surface (arrow). **Fig. 125.** Closer view of the stigma in longitudinal section, showing glandular and papillate cells on the surface. **Fig. 126.** Glandular and papillate cells in longitudinal section of the stigmatic surface. **Fig. 127.** Close-up surface view of a portion of stigma, seen from above. Scale bar = 0.1 mm. a, artefact; ds, degenerating stamen; dis, degenerating inner stamen; dos, degenerating outer stamen; mmc, megaspore mother cell; se, sepals; p, petals.

carpels of the male flower are usually arrested before meiosis. However, Galli *et al.* (1993) showed that in some male genotypes the arrest occurs so late that formation of ovules with non-functional embryo sacs takes place. As summarised in Fig. 97, in male flowers of *L. longifolia* pistil abortion happens before meiosis in the microspore mother cells, the stamens continuing to develop until maturity and dehiscence; in female flowers, stamen arrest occurs before the onset of meiosis in microspore mother cells. Dellaporta and Calderon-Urrea (1993) stated that the timing of suppression of the inappropriate sexual organs in the species discussed above, from earliest to latest, is in the order *Zea mays*, *S. latifolia*, and *A. officinalis*. On the basis of our observations, *L. longifolia* would fit between *S. latifolia* and *A. officinalis*.

The nature of floral structure and development in *L. longifolia* represented by the arrest of the inappropriate sexual organs at an intermediate stage of floral development suggests that this species could be a convenient model species for the study of some aspects of sex determination in dioecious plants. However, genetic mechanisms of sex determination are highly variable as they appear to have evolved separately in individual families (Janousek 1996) and there are even some cases of different types of sex determination in species within the same family (e.g. in the Caryophyllaceae, *Melandrium album* v. *Silene otites* (Grant *et al.* 1994a)). These mechanisms can be based either on the action of particular loci or on the presence of sex chromosomes, with a growing body of information pointing to the probability that MADS-box genes with different developmental expressions play a primary role in sex determination of flowers in dioecious species (Meagher 2007). At this stage, nothing is known of the genetics of sex determination in *Lomandra* at either cytological or molecular levels. In addition to its theoretical interest, information obtained from sex-determination studies can be utilised to increase the efficiency of breeding new horticultural cultivars of dioecious plants such as *L. longifolia* and its relatives, as well as facilitating self-fertilisation when breeding for heterosis.

No developmental studies of this kind have been reported on members of the family Lomandraceae or related families such as Xanthorrhoeaceae. Given the unsettled nature of the classification of *Lomandra* at family level, there is an urgent need for comparative developmental studies to assist in the clarification of affinities within and between the genera.

## Conclusions

We report for the first time a detailed analysis of flower organogenesis in male and female flowers of *L. longifolia* by SEM and light microscopy of entire and sectioned material. To facilitate comparison with studies on other species, the developmental sequence was divided into defined stages from the appearance of the floral meristem to the stage of pollen shedding and stigmatic receptivity in the respective sexes.

Although mature flowers are functionally unisexual, early development is similar in staminate and pistillate flowers. Pistillate and staminate flowers are identical and apparently bisexual at early stages. The two flower types diverge developmentally when the stamens become bilobed. The arrest of the development of the inappropriate sexual organs happens before meiosis and degeneration to rudimentary structures occurs towards maturity of the functional sexual organ.

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