DIPLOMARBEIT

Titel der Diplomarbeit

„Development of unisexual flowers and dioecy in *Schlegelia* (Schlegeliaceae, Lamiales)“

verfasst von

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angestrebter akademischer Grad

Magistra der Naturwissenschaften (Mag. rer. nat.)

Wien, 2013

Studienkennzahl:	A 190 445 333
Studienrichtung lt. Studienblatt:	Lehramtsstudium UF Biologie und Umweltkunde, UF Deutsch
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1. Introduction

Dioecious plants evolved independently in different lineages on repeated occasions and unisexual plant species occur all over the world (Geber, Dawson, & Delph, 1999; Ainsworth, 2000). However, the proportion of dioecious plant species of the world's flora is assumed to be rather small (Yampolsky & Yampolsky, 1922; Heslop-Harrison, 1957; Frankel & Galun, 1977; Bawa, 1980). In 1995, Renner and Ricklefs did a survey searching for the total number of dioecious plant species among the estimated total number of 240.000 flowering plant species. Their results show that out of the total of 13.479 genera, 7, 1% (959) comprise unisexual plant species (Renner & Ricklefs, 1995). Most of these genera are paleotropically (402) or neotropically (217) dispersed (Renner & Ricklefs, 1995).

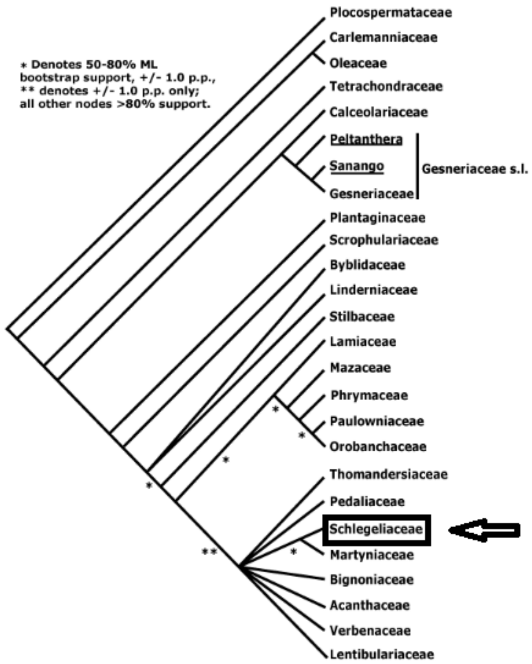


Figure 1 Phylogenetic tree of the order Lamiales; arrow indicating the current position of Schlegeliaceae (Stevens, 2001 onwards)

If one concentrates on tree species in a Central American tropical lowland rain forest, 23% of the woody species appear to be dioecious, with individual male and female plants (Bawa, 1992).

Dioecy often seems to be associated with unspecialized, white to pale yellow or green flowers (Bawa, 1980; Vamosi, Otto, & Barrett, 2003). This inconspicuousness is correlated with floral rewards that are available to various insects (Bawa & al., 1985), so the floral visitors are often assumed to be small including many generalized insect pollinators (Bawa & Opler, 1975; Bawa, 1980; Lloyd, 1982; Bawa, 1994). Other correlations with dioecy include fleshy fruits (Givnish, 1980), animal dispersal (Bawa, 1980; Givnish, 1980; Renner & Ricklefs, 1995), woody growth (Bawa & Opler, 1975; Bawa, 1980; Vamosi, Otto, & Barrett, 2003; Fromhage & Kokko, 2010), tropical distribution (Bawa, 1980; Givnish, 1980;

Renner & Ricklefs, 1995), island habitats (Bawa, 1982; Baker & Cox, 1984) and climbing growth form (Renner & Ricklefs, 1995).

As dioecy evolved independently in different lineages (Geber, Dawson, & Delph, 1999) there are many hypotheses concerning the evolutionary pathways that may have led to dioecy. Based on the fact that there often remains a rudimentary gynoecium in staminate flowers and a staminodial androecium in female flowers (Bawa & Opler, 1975) it seems clear that hermaphroditism generally is the starting point for the evolution of dioecy (Baker, 1967; Raven, 1973; Bawa & Opler, 1975; Bawa, 1980). Possible evolutionary pathways to dioecy may then have led via gynodioecy (Ross, 1970), monoecy (Lewis, 1942; Renner & Ricklefs, 1995) or heterostyly (Baker, 1958; Beach & Bawa, 1980).

As Darwin (1877) stated: "There is much difficulty in understanding why hermaphroditic plants should ever have been rendered dioecious". Since then, many questions concerning the reasons for the evolution of dioecy have been raised and discussed.

Many authors assumed selection for outcrossing as the main reason to promote dioecy (Ross, 1970; Janzen, 1977; Charlesworth & Charlesworth, 1978; Thomson & Barrett, 1981; Vamosi, Otto, & Barrett, 2003). As only female plants can benefit from outcrossing (Lloyd, 1982), there have to be other forces besides outcrossing concerning the evolution of dioecy such as sexual selection (Janzen, 1977; Bawa, 1980; Givnish, 1980), resource allocation (Maynard-Smith, 1978; Bawa, 1980; Bawa & Beach, 1981; Thomson & Brunet, 1990; Ainsworth, 2000) seed dispersal (Bawa, 1980; Wilson & Harder, 2003; Fromhage & Kokko, 2010), pollination (Bawa, 1980), predation (Bawa, 1980), a shift in ecological conditions (Sakai & Weller, 1999) and sexual specialization (Darwin, 1876; Freeman & al., 1997; Ainsworth, 2000).

The focus of this study is the Neotropical family Schlegeliaceae, which was recognized at the family level by Reveal in 1995. The family comprises four genera (with 37 accepted species names). The genus *Gibsoniothamnus* consists of 12 species, which can be found from southern Mexico over Central America to northern Colombia (Burger & Barringer, 2000). The genera *Exarata* and *Synapsis* each comprise only one species. *Synapsis ilicifolia* is endemic to the Oriente Province in Cuba (Gentry, 1980) and *Exarata chocoensis* is exclusive to the pluvial forests of the Chocó region in Ecuador and Colombia (Gentry, 1992). This latter species is most likely closely related to the genus *Schlegelia* (Gentry, 1992), which comprises 23 species (The Plant List, Version 1. , 2010) that are distributed from Mexico to the West Indies, over Central America to Brazil (Burger & Barringer, 2000).

In the descriptive-taxonomic literature (e.g. (Burger & Barringer, 2000)), Schlegeliaceae are described as having bisexual flowers and it was a surprise when during a field course in Costa Rica in February 2011, *Schlegelia parviflora* was found to have functionally unisexual flowers and the plants to be dioecious (pers. obs. Jürg Schönenberger).

As the genus *Schlegelia* has been - as Olmstead et al (1995) put it - “bounced back and forth” between Scrophulariaceae (Monachino, 1949; Thieret, 1967; Williams, 1970; Armstrong, 1985) and Bignoniaceae (Gentry, 1980) and just recently been put into Schlegeliaceae (Reveal, 1995), it is important to study the different species of this genus to gain more information about their evolutionary background. Even after morphological and molecular analyses (Olmstead & al., 1993; Olmstead & Reeves, 1995; Olmstead & al., 2001; Oxelman & al., 2005; Schäferhoff & al., 2010) several “difficult” smaller groups including Schlegeliaceae are left in uncertain positions within the Lamiales.

However, Schäferhoff et al. (2010) recently found weak support that Schlegeliaceae can be seen as a sister group to Martyniaceae, a view, which is currently supported by Stevens and the angiosperm phylogeny website. (Stevens, 2001 onwards).

The literature concerning *Schlegelia parviflora* is very scarce. To my knowledge, only Burger and Barringer (2000) and Woodson et al. (1973) described the habit, leaves, flowers and fruits under this name. I could not find any information concerning the breeding system of *Schlegelia parviflora*. Our discovery that *Schlegelia parviflora* is dioecious offers the opportunity to learn more about the evolution of dioecy in the Tropics and therefore it is important to study the plant as carefully as possible. The likely reason for why dioecy was not discovered earlier in *Schlegelia parviflora* is that both sexes are expressed in the flowers, i.e. the female flowers produce relatively large staminodes and the male flowers distinct pistillodes.

In my thesis I want to discuss the following hypotheses and questions:

1) Is *Schlegelia parviflora* truly dioecious?

2) As mature male and female flowers contain relatively large organs of the opposite sex, I hypothesize that both in male and female flowers, stamens as well as carpels are initiated in a similar way during early floral development and that a differentiation into functionally male and female flowers occurs relatively late during floral development. In what way and at what stage of floral development does this differentiation happen?

3) What are the morphological or functional differences between male and female flowers at anthesis, respectively? I hypothesize that there are no distinct differences in gross floral shape and appearance between male and female flowers as they need to be visited by the same pollinators.

Relatively little is known about the biology of tropical plants and with my thesis I want to accentuate the need for research and investigation in the tropics.

2. Material and Methods

2.1. Field observations

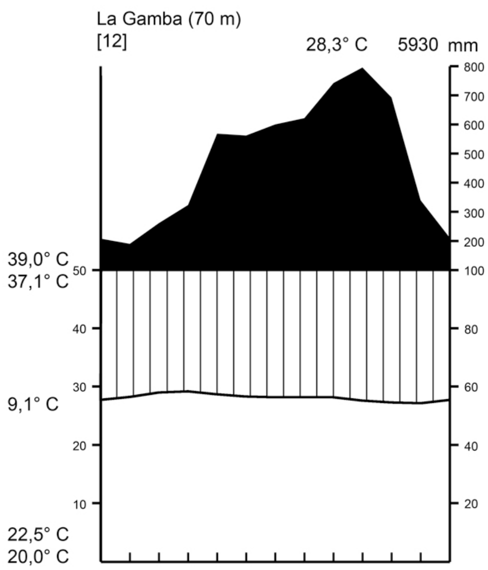


Figure 2 Climate graph of La Gamba: http://www.lagamba.at/researchdb/pagede/wirueberuns/klimadiagramm%201998_2010.jpg

Field observations were conducted in and around the Tropical Research Station La Gamba in South West Costa Rica from the beginning of February to the end of March 2012. This time of the year has been chosen because February and March are the driest months of the year (Figure 2; (Weissenhofer & Huber, 2001)). Another reason was the assumption of Burger and Barringer (2000) that tropical low-land plants flower often from December to February. The Tropical Research Station lies in the Golfo Dulce region, which is one of the most humid places in Costa Rica. The rainfall is very much influenced by the mountains adjacent to the Golfo Dulce region (Weissenhofer & Huber, 2001). As I have learned during my stay, rainfall was not much appreciated by flowers or buds of *Schlegelia parviflora* and it could happen that many of them would lie on the ground, washed down by the rain, without ever getting the chance to blossom.

Schlegelia parviflora exhibits a broad variation in habit and leaf as well as inflorescence morphology. Individuals can be epiphytic shrubs, small trees or as in La Gamba, Costa Rica, woody vines or lianas (Burger & Barringer, 2000). Burger and Barringer (2000) assume that they are flowering and fruiting throughout the year. In my study area, *Schlegelia parviflora* stopped flowering in the middle of March. In order to recognize this species in the field, it is good to know its general morphology.

2.1.1. Methods

The data for my thesis were collected during my stay in the Tropical Research Station La Gamba on living plants of *Schlegelia parviflora*. In addition, I collected buds, flowers and fruits, which were fixed in formalin-acetic acid-alcohol (FAA) and subsequently stored in 70% ethanol for further investigations in Vienna.

Field observations in La Gamba:

Figure 3: Male *Schlegelia parviflora* flower. A to E: petal lobes; Scale bars 1. and 3. indicate the petal lobe lengths of an adaxial petal; 2. indicates the length of the median abaxial petal.

Several individuals of *Schlegelia parviflora* have been located on the grounds of the tropical research station and close by along the road to the Esquinas Rainforest Lodge. These sites have been chosen, because individuals of *Schlegelia parviflora* were exposed on single trees growing at a height between two to ten metres, allowing me to get bud and flower samples by hand or with the help of a pair of lopping shears. As a first step, these samples have been studied and measured thoroughly in the laboratory of the research station in order to get an overview of the floral structure in male as well as in female flowers. I collected ten male and female flowers, respectively, from a total of ten individuals (five male, five female). I have measured calyx length, corolla tube length, petal lobe lengths, and length of stamens and pistils. The first parameter to measure was the length of the calyx. Then I cautiously separated the calyx and the corolla and measured the corolla tube length. The petals were measured as demonstrated with red lines (1., 2., 3.) in Figure 3. As the two adaxial petals (A and B) seem to be closer together than the other three (C, D and E), I decided to measure one of them from the tip to the point where it touches its adjacent petals. (1. and 3.) The third measurement was taken from petal D (the median abaxial petal). Subsequently, I cut away the corolla and measured the style length in female flowers and the stamen lengths in male flowers.

I also carefully checked the sex of the harvested flowers in order to confirm that *Schlegelia parviflora* is indeed a dioecious plant. Where it was possible I traced back the individual's stems and branches to its roots to confirm that the whole plant is either male or female. I measured the corolla tube length, the calyx length and the single petal lengths in both male and female flowers. Furthermore, I made detailed sketches and pictures, evaluated the floral scent and collected buds, flowers and fruits from different male and female individuals. It was important to collect buds in all different sizes, allowing me to investigate the development of unisexual flowers in *Schlegelia*.

Further fieldwork included observations of the course of floral anthesis and pollination. These observations were made on two mobile platforms (ca. 4 m above ground), specially built for my needs. These platforms were placed under a female and a male individual of *Schlegelia parviflora,* respectively. Female individuals were marked with a red woollen thread, males with a yellow thread. The same method was used to mark single buds on which I wanted to investigate the anthesis early in the morning. Altogether I marked three female buds and five male buds.

I also conducted a stigma receptivity test to check whether the reduced stigma in male flowers is functioning.

2.2. Investigated individuals

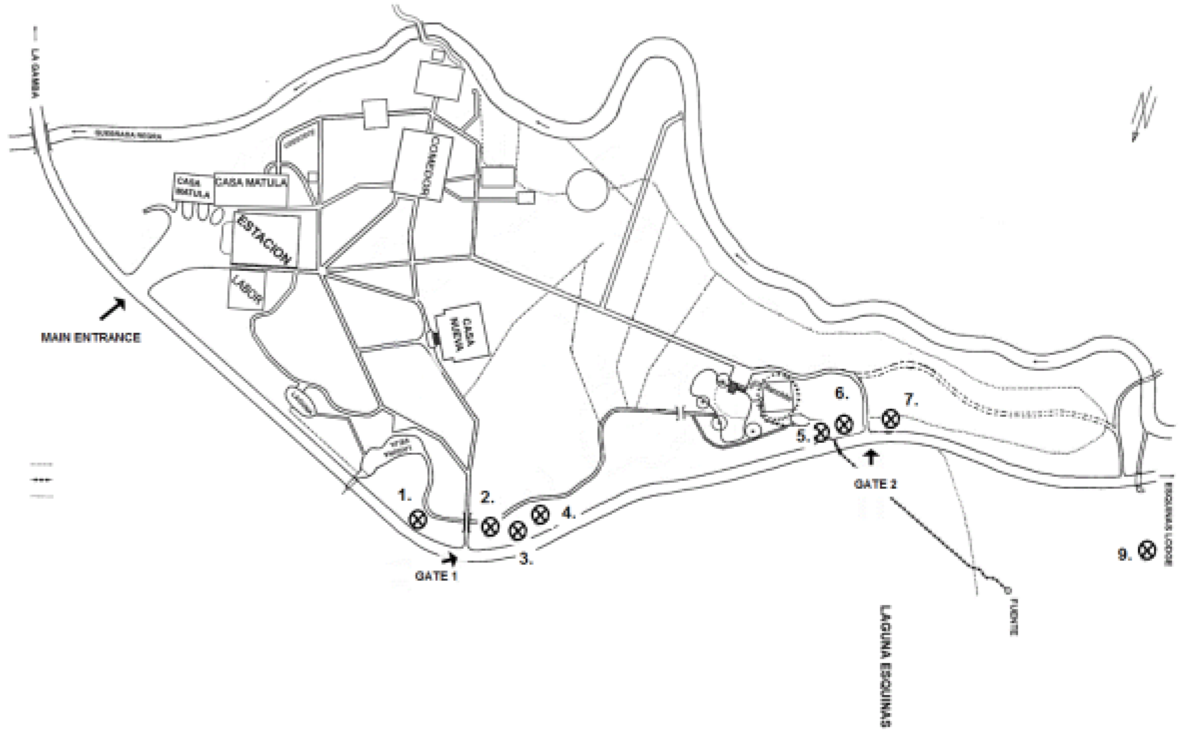


Figure 4 Map of the Tropical Research Station La Gamba and its surroundings. The crosses (x) indicate the different sites where *Schlegelia parviflora* can be found.
Modified: http://www.lagamba.at/researchdb/pagede/besucher/ausstattung/stationsplan_a4.jpg

Site 1

This individual grows to a height between five and six metres nearby the road. The plant was fruiting and flowering at the time of observation. On close observation, the flowers turned out to be all pistillate, i.e. the plant turned out to be female. Stigmas were partly exserted from the otherwise closed buds, probably one day before anthesis.

Site 2

Two *Schlegelia parviflora* individuals are growing on the same host plant to a height between eight and ten metres. From the view of the research station garden you can only see the female *Schlegelia parviflora*, so you have to have a look around the host plants stem to see that there is in fact a male individual, too. The male *Schlegelia parviflora* grows higher than the female one and can be seen easily from the road.

Site 3

Apparently several individuals growing on the same tree, some of them are even growing from tree to tree. Some branches can be reached easily to a height of three metres, many buds can be seen, fewer blossoms. At a height of nine metres more flowers are in bloom. All flowers found and collected at this site were proven to be from male *Schlegelia* individuals.

Site 4

The host plant is only reachable through the garden of the research station. There are several individuals growing at that site and some lianas can be retraced to site number three. Male and female *Schlegelia parviflora* individuals can be found at this site.

Site 5

On this spot I have found a *Schlegelia parviflora* which was not in bloom, so I could not use it for further investigations.

Site 6

This individual is at eye level, easily reachable and therefore easily identified as a female plant of *Schlegelia*. Only few flowers but plenty of fruits were present at the time.

Site 7

This female individual grows on an isolated tree, so that I could easily follow the liana to its roots and know for sure that it is just one individual. All of the flowers that have been found and observed turned out to be female. Flowering branches were easily reachable by hand, but only a few branches were in bloom.

Site 8

The host plant, a huge tree, can be found in the garden of the Esquinas Lodge. It is likely that there are more individuals of *Schlegelia parviflora* growing on it, but it was almost impossible to retrace the individual branches to their roots. Nevertheless, all of the flowers collected and investigated were found to be male. As it happens, this tree with lots and lots of *Schlegelia* growing on it has been a perfect candidate for further observations, because it grows on flat and solid ground, where it was secure to build a platform. Another advantage of this site has been that the lianas grow even in the shadow and are easily reachable at a height of about three to ten metres.

Site 9

The eight sites described above have been south of the road from the tropical research station to the Esquinas Rainforest Lodge. This site can be found on the north side of the road in the garden of the Esquinas Lodge surrounded by their guest houses. *Schlegelia parviflora* grows on a huge tree at a height of about ten metres. Through binoculars I could see that it was in full bloom, but it was necessary to bring the lopping shears to cut down some samples to categorize it as a male *Schlegelia* individual.

Site 10

Whereas the other sites can be found while walking down the road, you have to go on the Fila trail to find this female *Schlegelia parviflora*. As we found it, it was in full bloom, easily reachable and growing in the shadow of its host tree, so we decided that this would be the second perfect spot for further investigations on a platform.

2.3. Morphological analysis

Back in Vienna, I dissected male and female buds from 1mm to 13 mm under a stereo microscope in order to expose stamens and gynoecium of *Schlegelia parviflora*. The same was done for anthetic flowers. Furthermore, I cut through 3 mm, 7 mm and 13 mm male respectively female buds to determine the position of the stamens and the gynoecium during the floral development. For scanning electron microscopy, the dissected material was dehydrated in an ethanol series (70%, 86% and 96%) and acetone, criticalpoint dried, and sputter coated with gold and subsequently studied on a Jeol JSM-6390 field emission scanning electron microscope.

2.4. Terminology

As *Schlegelia parviflora* is a dioecious plant, I want to explain the terminology concerning the sexual systems used in my thesis. In dioecious populations there are individuals with functional male flowers and other individuals with functional female flowers. In my thesis I refer to them as either male or staminate plants and female or pistillate plants. Staminate plants have flowers with a functional androecium with stamens producing pollen but without a functioning gynoecium (Sakai & Weller, 1999). Pistillate plants have flowers with a functional gynoecium, including pistil(s), ovule(s), but without a functioning androecium (Sakai & Weller, 1999). For simplicity´s sake and being aware that stamens are always male and carpels always female, I use the terms "male stamens", "female stamens" and "male carpels", "female carpels", respectively, in order to refer to the two types of reproductive organs in male and female flowers, respectively.

3. Results

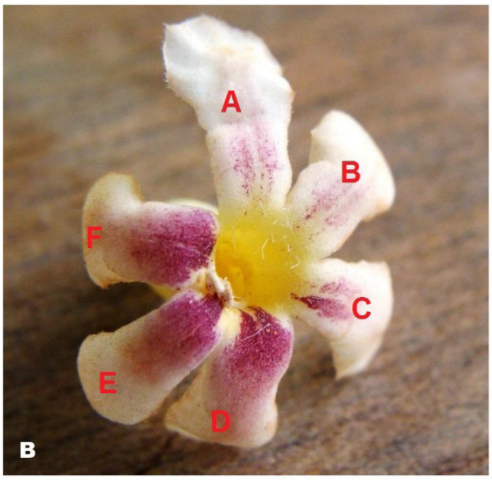
3.1. Breeding System and general description

All the individuals studied are functionally unisexual, i.e. *Schlegelia parviflora* seems indeed to be dioecious. In no case did I find male and female flowers on the same individual neither did I find any fruits on an individual with male flowers. Where it was possible to trace back the plant's stem to its roots, I can doubtless say that the investigated individuals where either male or female.

The following description is based on Burger and Barringer (2000) and complemented with my own observations in the field.

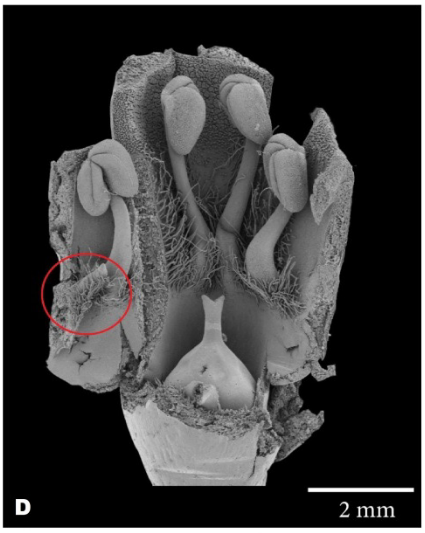
Leaves vary in their size, but the shape stays almost the same. They have got distinct petioles and their leaf blades are elliptic to ovate-elliptic. The apex is bluntly acute or slightly rounded and the leaves are glabrous on both sides.

Male *Schlegelia parviflora* individual



Male *Schlegelia parviflora* flower with 6 petals (A-F)

Fruits of *Schlegelia parviflora*



Red circle: Sterile stamen in an anthetic male flower

Figure 5: A: Flowering branches of a male *Schlegelia parviflora*; B: Male *Schlegelia parviflora* flower with 6 purple tinged petals (red A-F); C: Greenish, purple fruits of *Schlegelia parviflora*; D: Red Circle indicates the sterile stamen in an anthetic male flower.

Flowers are arranged in dense racemose or paniculate axillary clusters (Figure 5, Picture A). Leaves with inflorescences occur every five to ten centimetres on the woody branches of the liana. The quantity of flowers per inflorescence varies a lot, as the flowers drop down shortly after anthesis. Five green and glabrous sepals are united into a thick cupulate calyx, which covers the sympetalous part of the corolla in the open flower. The five petals are white with purple tinged lobes (Figure 5, Picture B), which probably act as nectar guides helping the pollinators to find their reward. The nectary chamber is hidden below numerous hairs inserted at level of stamen insertion on the corolla tube leaving only a very small and narrow entrance to the chamber. One of the five petals (not always in the same position) is always slightly wrinkled (Figure 5, Picture B, petal A), most likely being the innermost petal in the closed bud.

Even though most of the flowers are pentamerous, I have often found flowers with six petals (Figure 5, Picture B; the number of other floral organs are apparently stable) on male as well as on female *Schlegelia* individuals.

Flowers have a didynamous androecium, which means that two out of the four stamens are slightly longer than the other two (Figure 5, Picture D). The fifth median stamen is reduced and sterile (red circle, Figure 5, Picture D).

Young fruits are greenish, globose berries (Figure 5, Picture C), which become purple or deep red and contain numerous seeds. As for the conspicuous colour, I assume that the seeds are dispersed by animals, especially birds.

3.2. Comparative floral morphology of anthetic male and female flowers

Looking at flowering branches and the inflorescence clusters, it appears that flowers and buds are more abundant on male *Schlegelia* plants than on female individuals.

On first sight, male and female flowers look very much alike (Figure 6, Pictures A and B). However, on closer inspection a different picture arises.

Figure 6: A: Anthetic male flower; B: Anthetic female flower

Except for a small difference in the mean length (see Table 1), no differences can be found in the calyx between male and female flowers. The male corolla can be distinguished from the female corolla as the purple nectar guides on the petals are more distinct than the ones on the female petals.

The androecium is reduced in female flowers and non-functional, i.e. pollen is not produced. In male flowers, the androecium is fully developed, whereas the style is reduced and the stigma is underdeveloped, i.e. non-functional. The fifth median, sterile, reduced stamen can be found in male as well as in female flowers.

As the stamens in the male flowers are slightly exserted from the corolla tube, pollinators or other flower visitors must touch the anthers with their head or their proboscis to get the floral reward, which is provided in the form of nectar in a proximal nectar chamber.

When looking just at a female flower, one might mistake it for a functional bisexual flower because on first sight, the stamens look normally developed, just slightly shortened. In male flowers, however, the pistil appears quite distinctly reduced and looks non-functional (Figure 9, Pictures E and F). So comparing the reduced gynoecium in staminate flowers and the reduced androecium in pistillate flowers, the androecium in female flowers seems to be fuller developed than the gynoecium in male flowers.

3.2.1. Morphometric measurements

The average calyx length in female flowers is 0,85 cm and is slightly longer than in male flowers with 0,8 cm (see Table 1). The average female flower corolla tube length is about 0,86 cm, whereas the male flower corolla tube length is slightly shorter with 0,82 cm. The measured male petal lobe lengths were all longer than the female petal lengths, so the male corolla as a whole is bigger than the female corolla of *Schlegelia parviflora*. The style in female flowers is nearly the double in length as in male flowers. The same ratio counts for the average stamen length, where stamens in male flowers are twice as long as in female flowers.

Table 1

	Female flowers	Male flowers
Average calyx length	0,85 cm	0,8 cm
Average corolla tube length	0,86 cm	0,82 cm
1. Average petal length	0,72 cm	0,77 cm
2. Average petal length	0,67 cm	0,72 cm
3. Average petal length	0,43 cm	0,60 cm
Average style length	0,45 cm	0,21 cm
Average stamen length	0,23 cm	0,42 cm

3.2.2. Stigma receptivity test

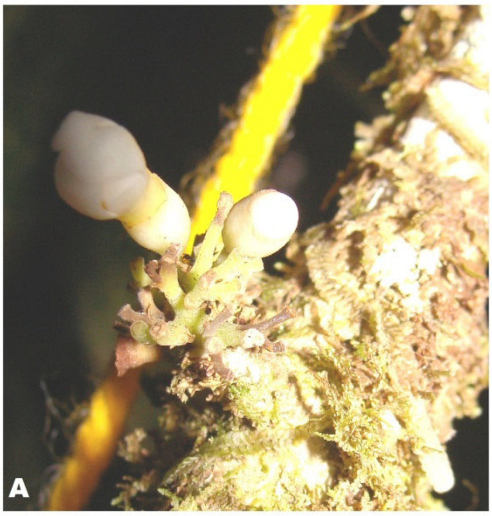
The stigma receptivity - test on the reduced stigma of male flowers has been inconclusive, because either there has been no reaction at all, or the test strip reacted to general enzymes from the plant and not to specific enzymes from the stigma. The same test was conducted with female *Schlegelia parviflora* flowers and it did not work either, and as they have to be receptive at least during a short period of the day to develop fruits, my conclusion has been, that there was something wrong with the test strips. Maybe the test strips I brought from Austria were too old to function correctly, and as I've performed this test on living material in La Gamba, Costa Rica, there has been no chance to get new ones.

3.3. Anthesis

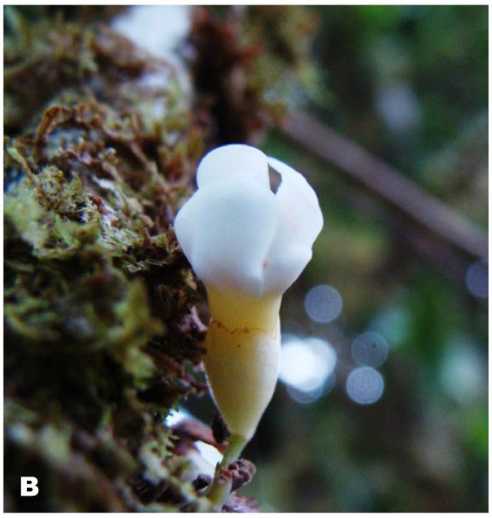
To investigate timing and duration of anthesis, it was necessary to get up early in the morning. The day before, I marked fully developed buds, which I assumed to flower the next day, with a woollen thread.

I started with a female individual because most of the female individuals around the research station started fruiting already and only few buds remained to be investigated. After three days of observing the marked buds, where nothing happened at all, the buds simply dropped on the ground without blooming at all. No female buds remained to observe anthesis.

Fortunately, there were still abundant male buds and I could observe the full anthesis there.



Time: 05:34 Beginning of anthesis. Petals still largely closed. The first petal, which covers the other four, is slightly opening up.



Time: 05:44 Right petal adjacent to the first is moving away from it.

Time: 06:09 Petals still bending towards each other, but one can see gaps between the petals.

Time: 06:41

Time: 07:04 All five petals are separated from each other, but still slightly crooked.

Time: 07:15 Flower is fully open

Figure 7: A to F: Beginning of anthesis of a male *Schlegelia parviflora* flower

3.4. Floral scent

To define the floral scent of *Schlegelia parviflora*, I collected already opened flowers and smelled them. Afterwards I transferred them to a closed test glass for about 30 minutes to get a more intense odour. Floral scent descriptions are always very subjective, so I gave the samples to some colleagues and let them describe what they've smelled.

First of all, no differences in floral scent were to be found between male and female *Schlegelia parviflora.* Flowers of both sexes smelled very sweet and are reminding of honey. Sitting on the observation platforms you could smell the scent, even without getting close to the flowers.

3.5. Flower visitors

Honeybee foraging nectar of a female *Schlegelia parviflora* flower.

Ant (red circle) crawling on a female *Schlegelia parviflora*, while a honeybee is foraging nectar in a different flower.

Hummingbird sitting on a *Schlegelia parviflora* branch.

Butterfly foraging nectar of a female *Schlegelia parviflora* flower.

Figure 8: A to D: Diverse flower visitors

Many visitors were observed on female and male *Schlegelia parviflora* plants. However, which of these really function as pollinators cannot be said without further investigations. Due to lack of time, visitors were not categorized exactly. No differences between the sexes have been noted.

Ants were found on each *Schlegelia* plant (Figure 8, Picture B), no matter whether it was in full bloom, fruiting or in vegetative state. Sometimes they were carrying away fallen flowers or fruits. They were not aggressive when I marked single flowers or collected my samples. The ants could be found on flowers, buds, berries, branches and stem of *Schlegelia parviflora*.

While sitting on the platform, I could see hawk-moths flying rapidly from flower to flower drinking nectar. Stingless bees, a hummingbird (Figure 8, Picture C), and bumble-bees were observed through field glasses foraging for nectar from *Schlegelia*. Most likely the hummingbird was stealing the nectar, because it destroyed most of the flowers by picking at them.

The large majority of flower visitors, however, consisted of honey bees. Sitting under the trees you could hear them buzzing around. The average visiting time per flower was three to five seconds, whereat the bee was clinging to lobe D (median petal of lower lip) with its hind legs, using E and C for stabilisation (Figure 3; Figure 8, Picture A). This way, as can be seen in Figure 8, Picture A, the honeybees forage nectar by sticking their head into the flower. Because they touch the stamens (in male flowers), as well as the stigma (in female flowers) with their head, it can be assumed that bees are not only visitors but also pollinators.

3.6. Comparative floral development of male and female flowers

The following pictures were taken of dissected male and female buds measuring from 1 mm to 13 mm in order to investigate floral development. Furthermore, longitudinal sections were made through male and female buds measuring 3 mm, 7 mm, and 13 mm, respectively, in order to get a better impression on the relative size and arrangement of the stamens and the gynoecium during floral development.

As shown in Figure 10, Pictures A-D, the stamens (anthers) in the female buds are bigger than the anthers in the male buds during early developmental stages. Only later do the stamens in the female buds (Figure 11, Pictures B and D) stop developing and the stamens of male flowers (Figure 11, Pictures A and C) catch up in size and finally become bigger than those of female flowers (Figure 11). Stamen filaments stay short throughout early stages and development starts only in male and female buds of approximately 7 mm in length (Figure 12, Pictures A and B). In Figure 12 you can also see the first real difference in stamen size in Pictures C and D, where the male stamens (filament as well as the anthers) are getting bigger than the female ones. Starting from 8 mm, the filament is growing in both male and female *Schlegelia parviflora* buds, but with a decelerated stamen growth in female buds. Finally, the stamens in anthetic male flowers are twice the size of stamens in anthetic female flowers as shown in Figure 14, Pictures C and D.

While the differences in stamen size can only be seen starting from a bud size of circa 4mm (Figure 11), the differences in pistil habit can already be recognized from the earliest stages of development (Figure 15, Pictures A,B). In female buds, the ovary is bigger, the style already slightly longer, and the stigma lobes are expanded. The main difference between a male and a female gynoecium (From Figure 15, Pictures A, B to Figure 16, Pictures E, F) is not the length but the width and breadth. The female style gets distinctly longer starting from 8mm bud size (Figure 17, Picture D), whereas the gynoecium in the male bud remains the same size from 4mm (Figure 16, Picture A) to an anthetic flower. (Figure 20, Picture A). In an anthetic female flower, the pistil is twice the size of the pistil in an anthetic male flower. Whereas the staminodes in a female flower just seem a little bit shorter than the functional stamens in a male flower, the pistillode in an anthetic male flower differs conspicuously from the functional pistil in an anthetic female flower. The pistil is thicker, broader and longer than the pistillode. You can see that the stigma in male flowers consists out of two lobes, but they are not as expanded or developed as the stigma lobes in the female flowers (Figure 20, Pictures A and B). In fact the male stigma does not change at all during the whole floral development.

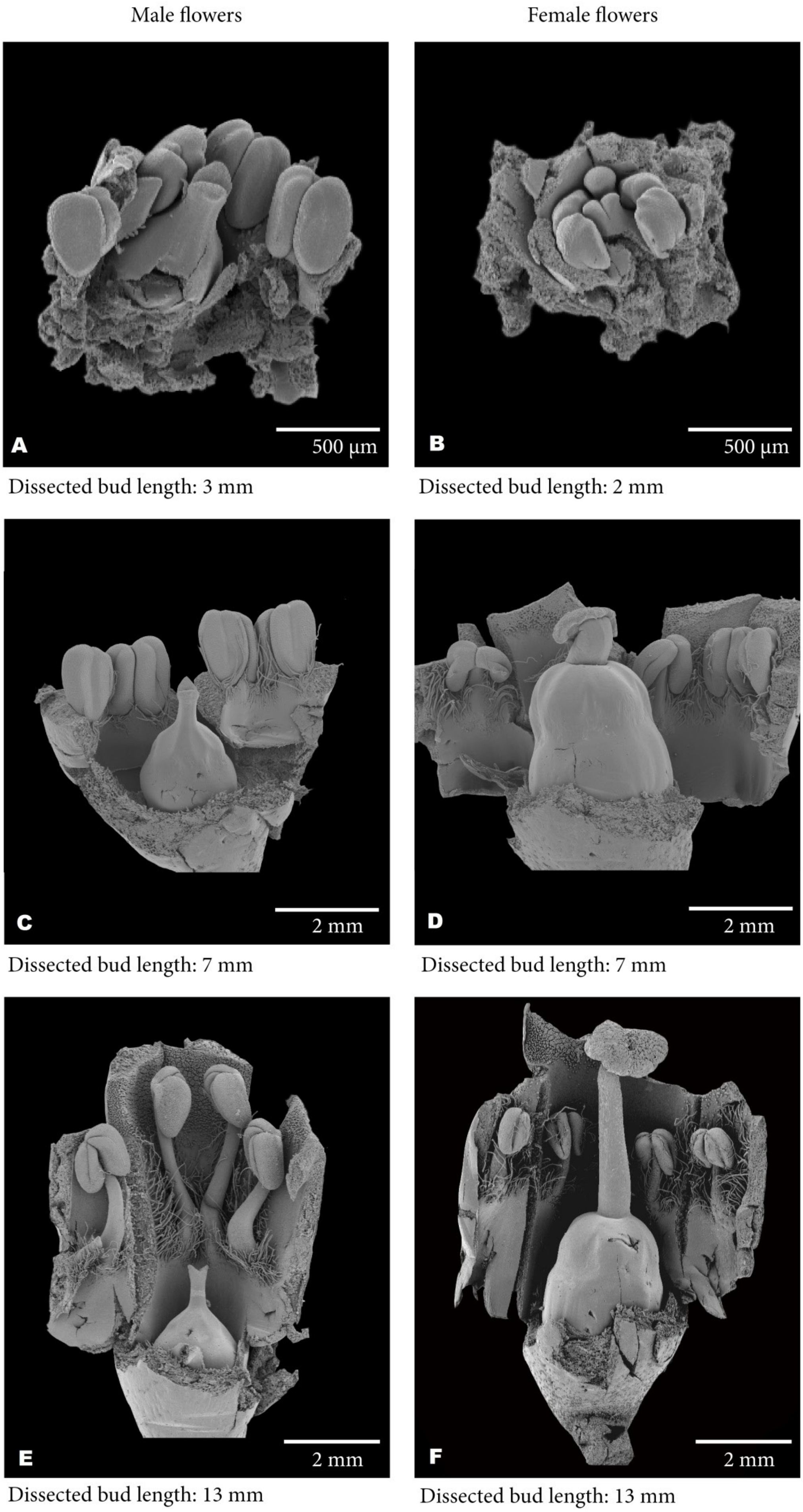


Figure 9 Longitudinal sections through male and female *Schlegelia parviflora* buds (2;3 mm to 13 mm)

Stamens of male flowers | Stamens of female flowers

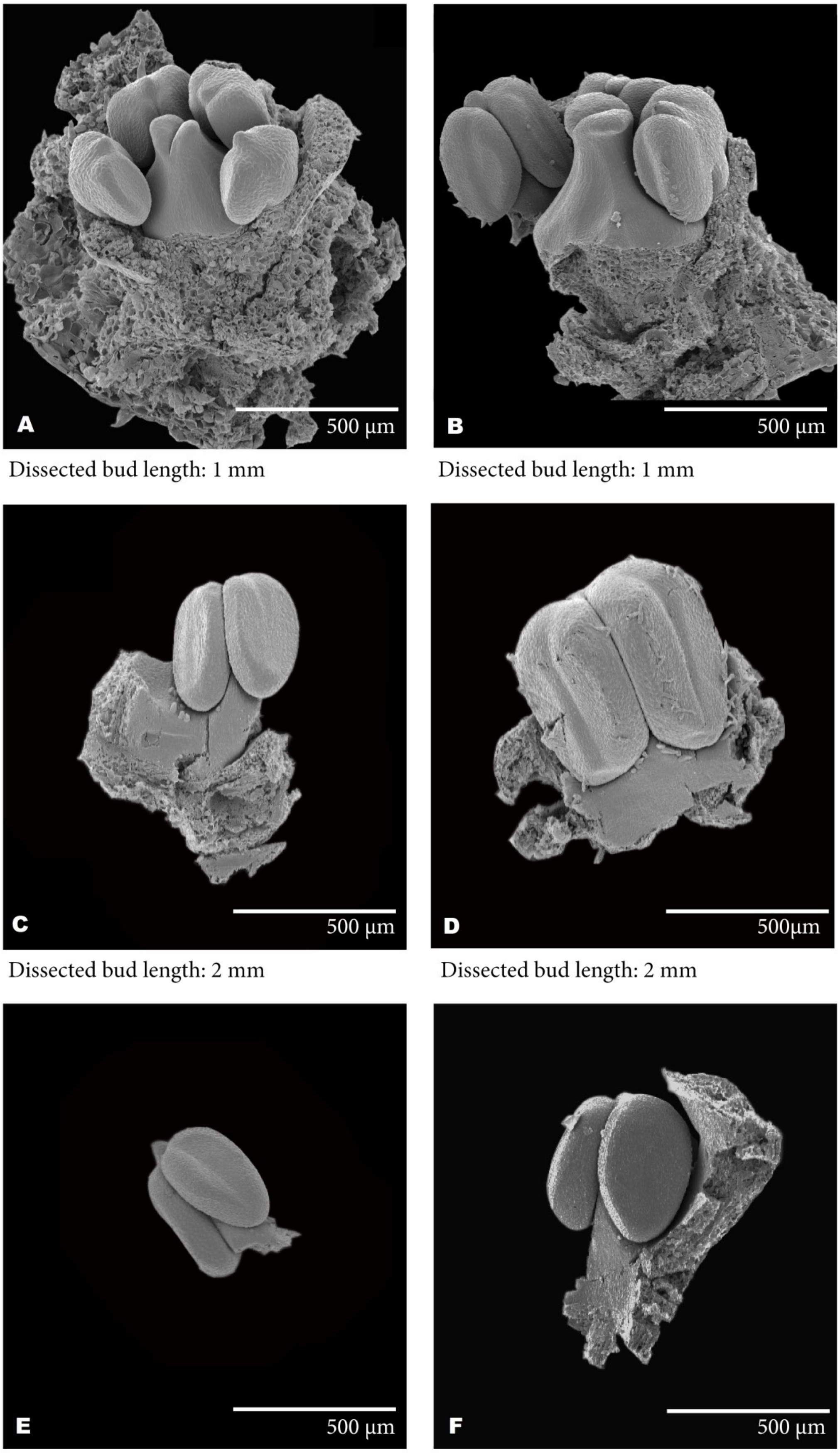


Figure 10 Stamens of male and female *Schlegelia parviflora* buds (1mm to 3mm)

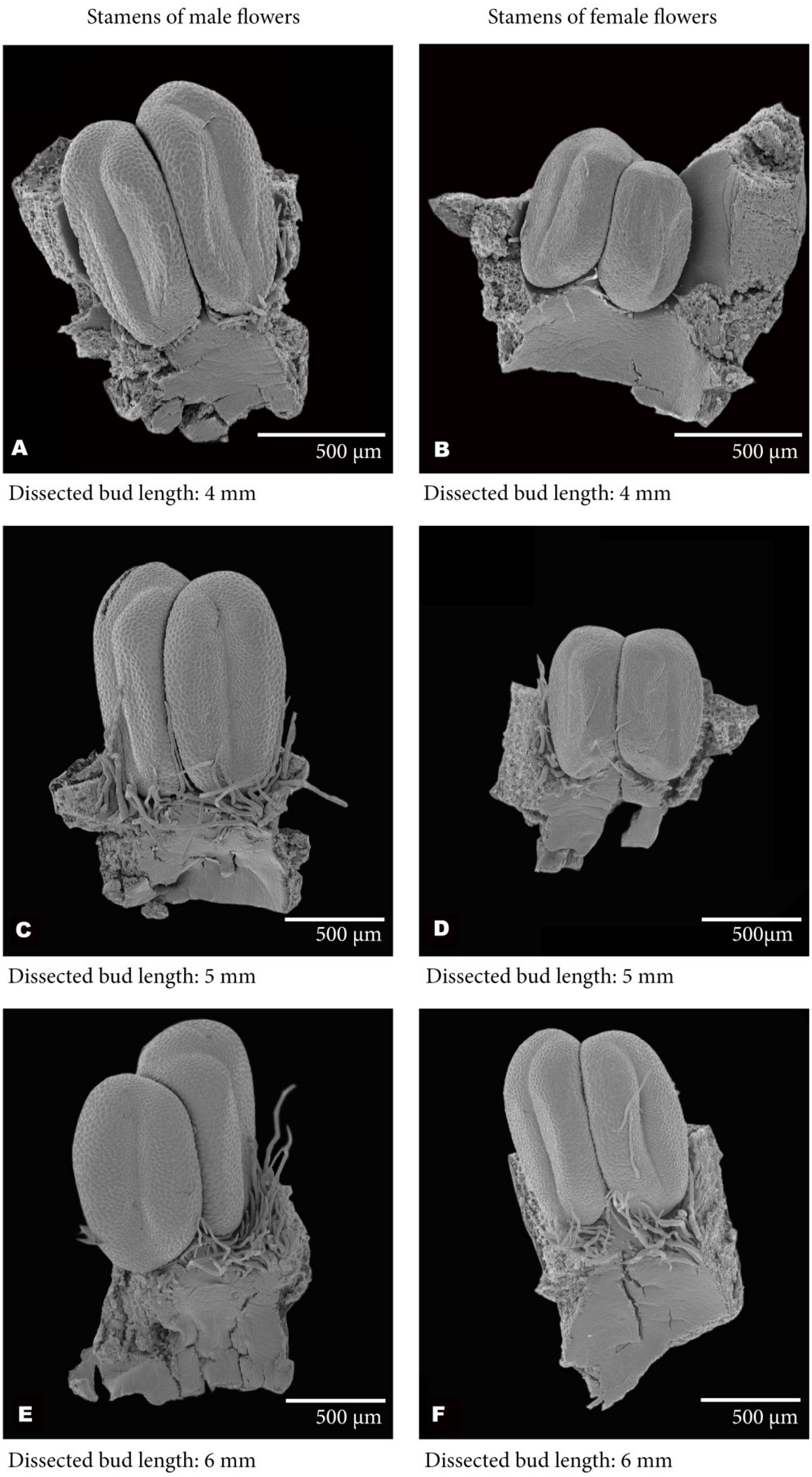


Figure 11 Stamens of male and female *Schlegelia parviflora* buds (4mm to 6mm)

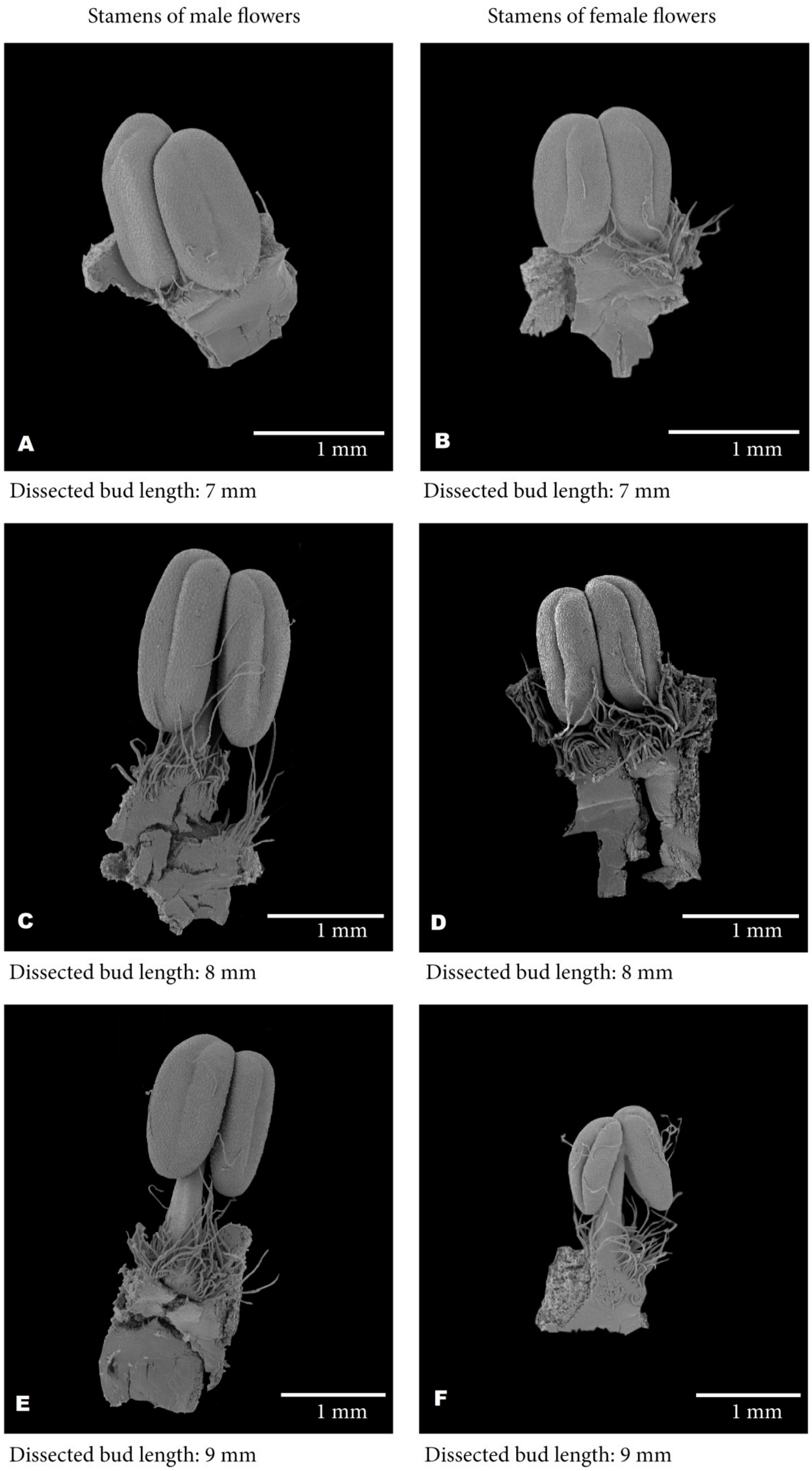


Figure 12 Stamens of male and female *Schlegelia parviflora* buds (7mm to 9mm)

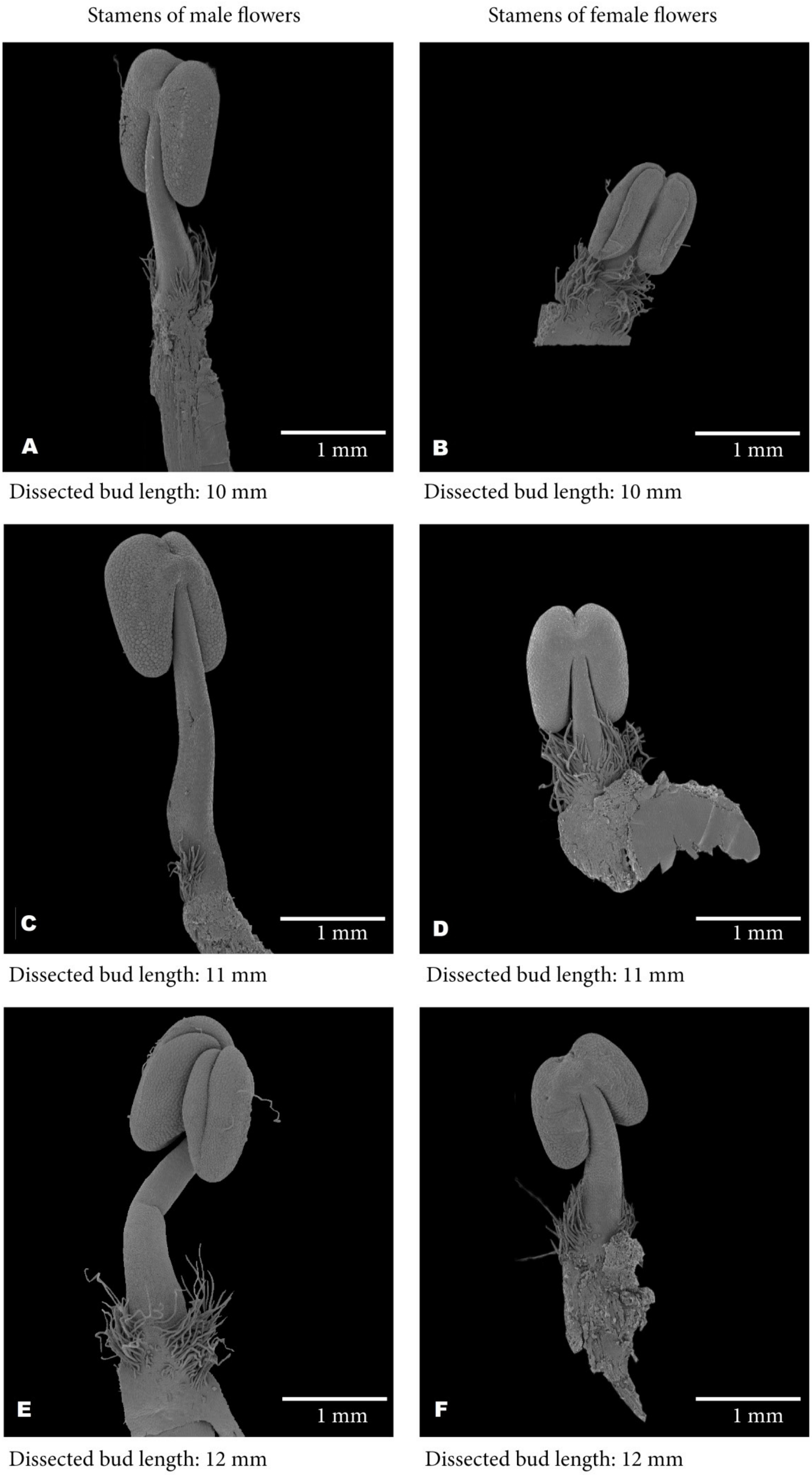
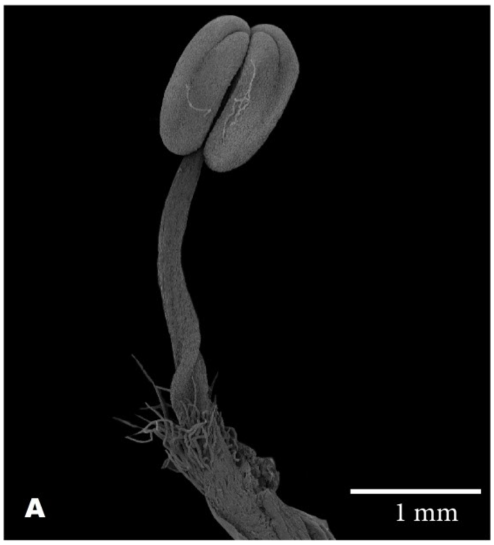
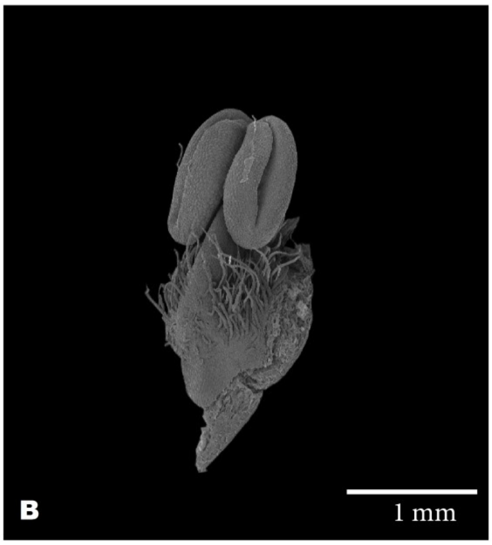


Figure 13 Stamens of male and female *Schlegelia parviflora* buds (10 mm to 12 mm)

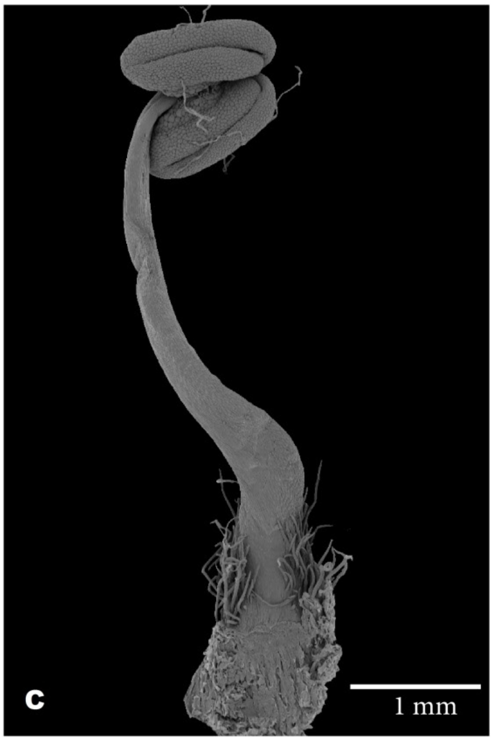
Stamens of male flowers | Stamens of female flowers



Dissected bud length: 13 mm



Dissected bud length: 13 mm



Dissected fully developed male flower

Dissected fully developed female flower

Figure 14 Stamens of male and female *Schlegelia parviflora* buds (13mm to fully developed flower)

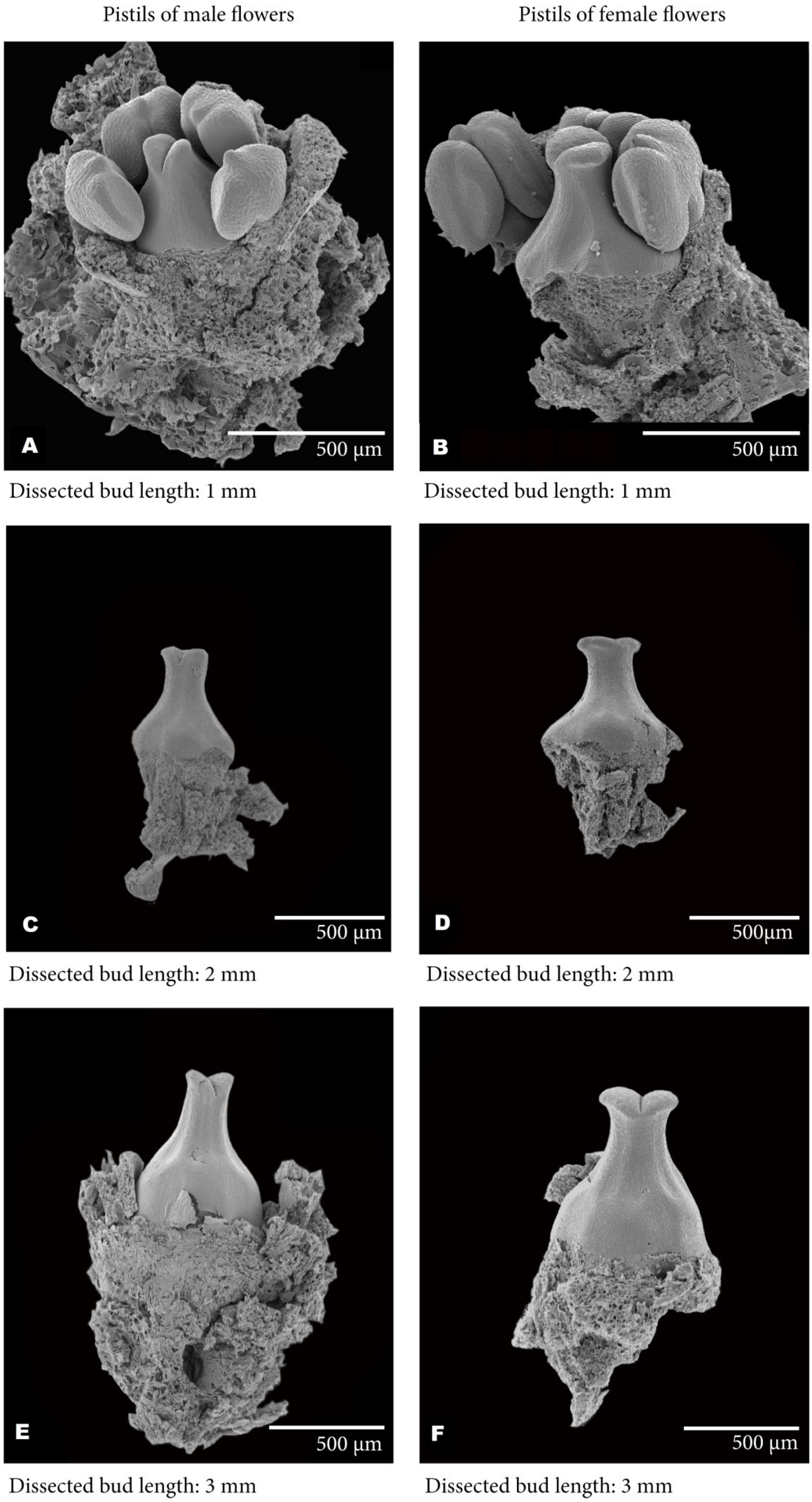
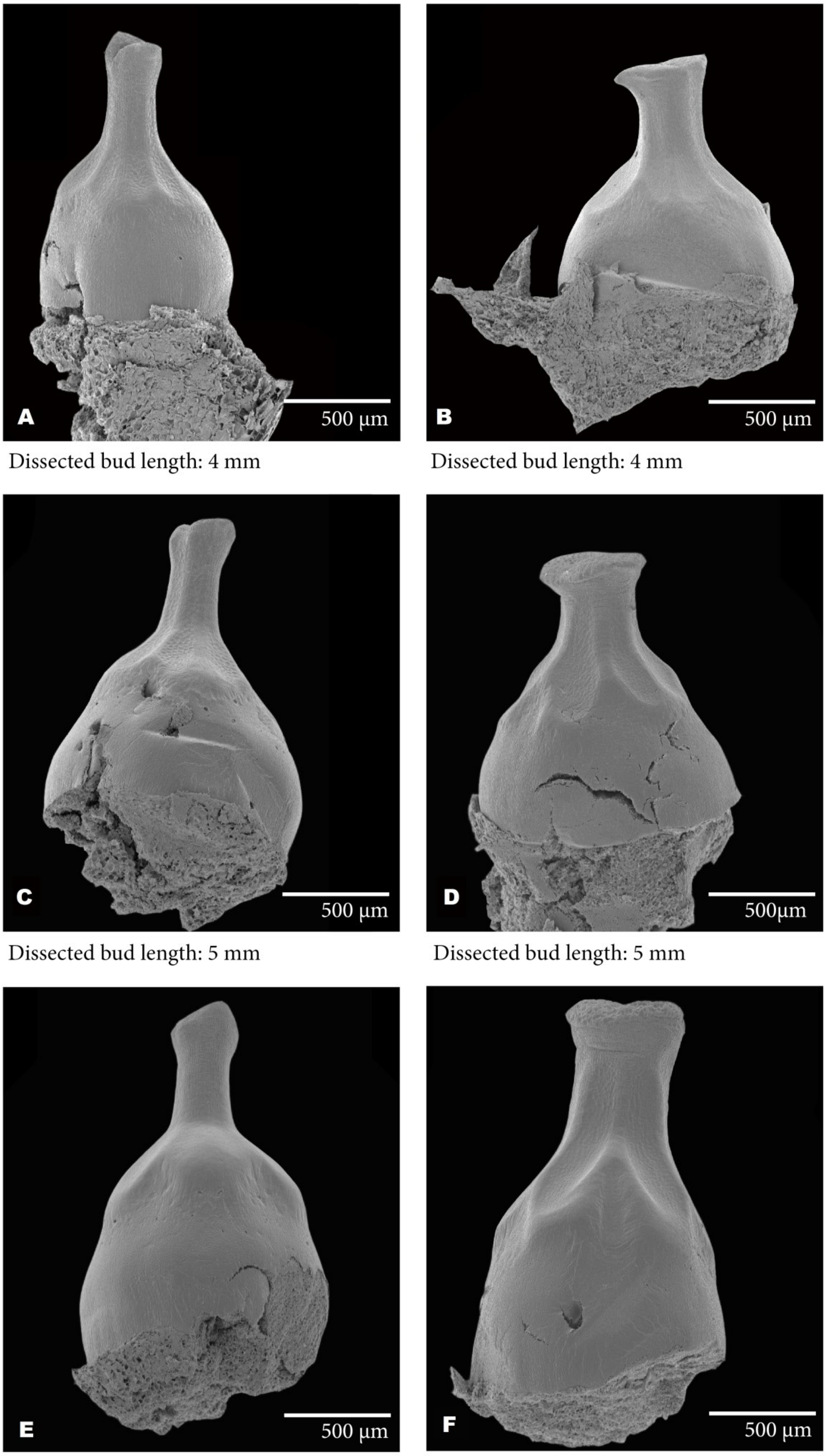


Figure 15 Pistils of male and female *Schlegelia parviflora* buds (1 mm to 3 mm)

Pistils of male flowers | Pistils of female flowers



Dissected bud length: 6 mm | Dissected bud length: 6 mm

Figure 16 Pistils of male and female *Schlegelia parviflora* buds (4 mm to 6 mm)

Pistils of male flowers

Pistils of female flowers

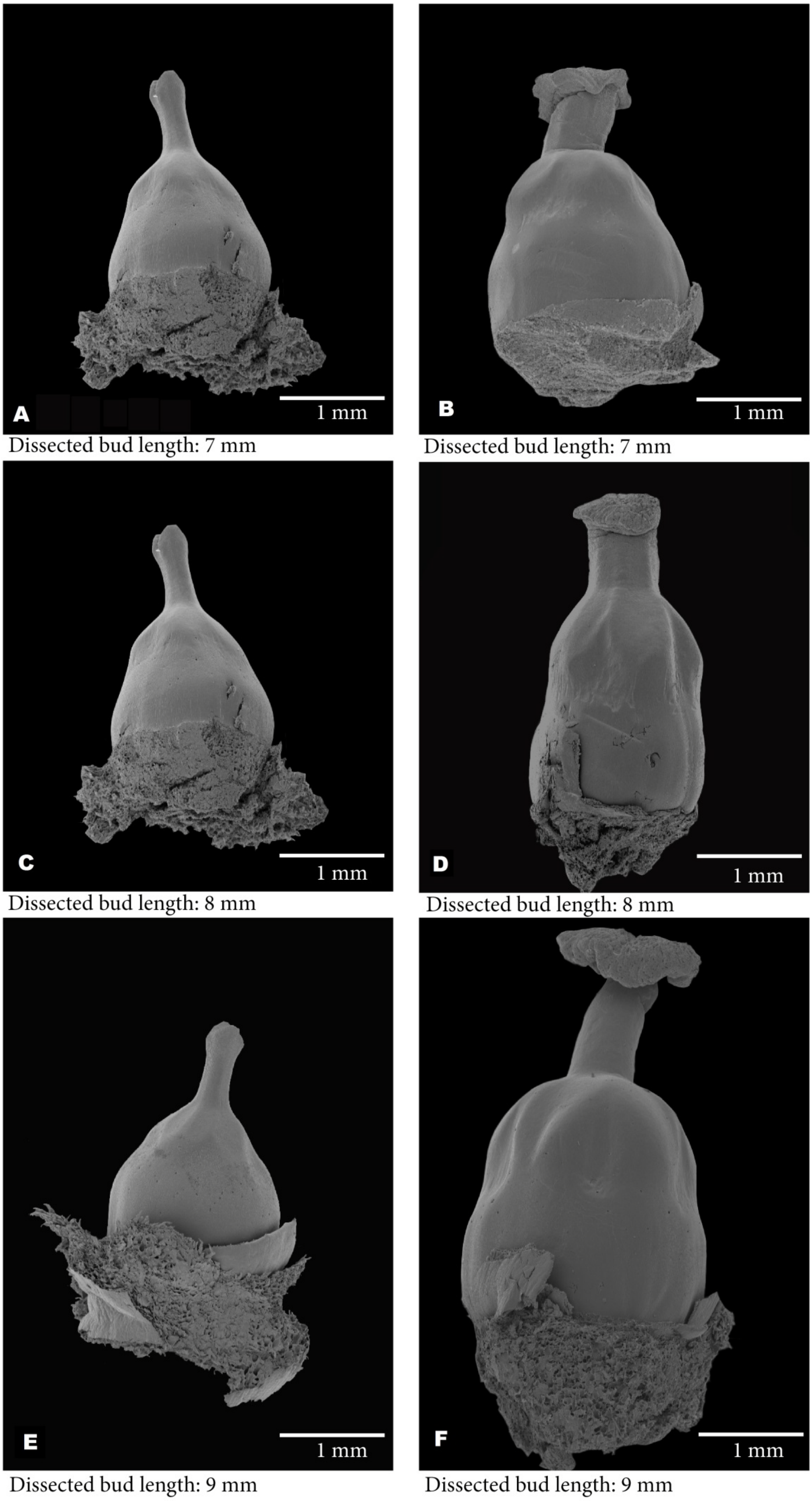


Figure 17 Pistils of male and female *Schlegelia parviflora* buds (7 mm to 9 mm)

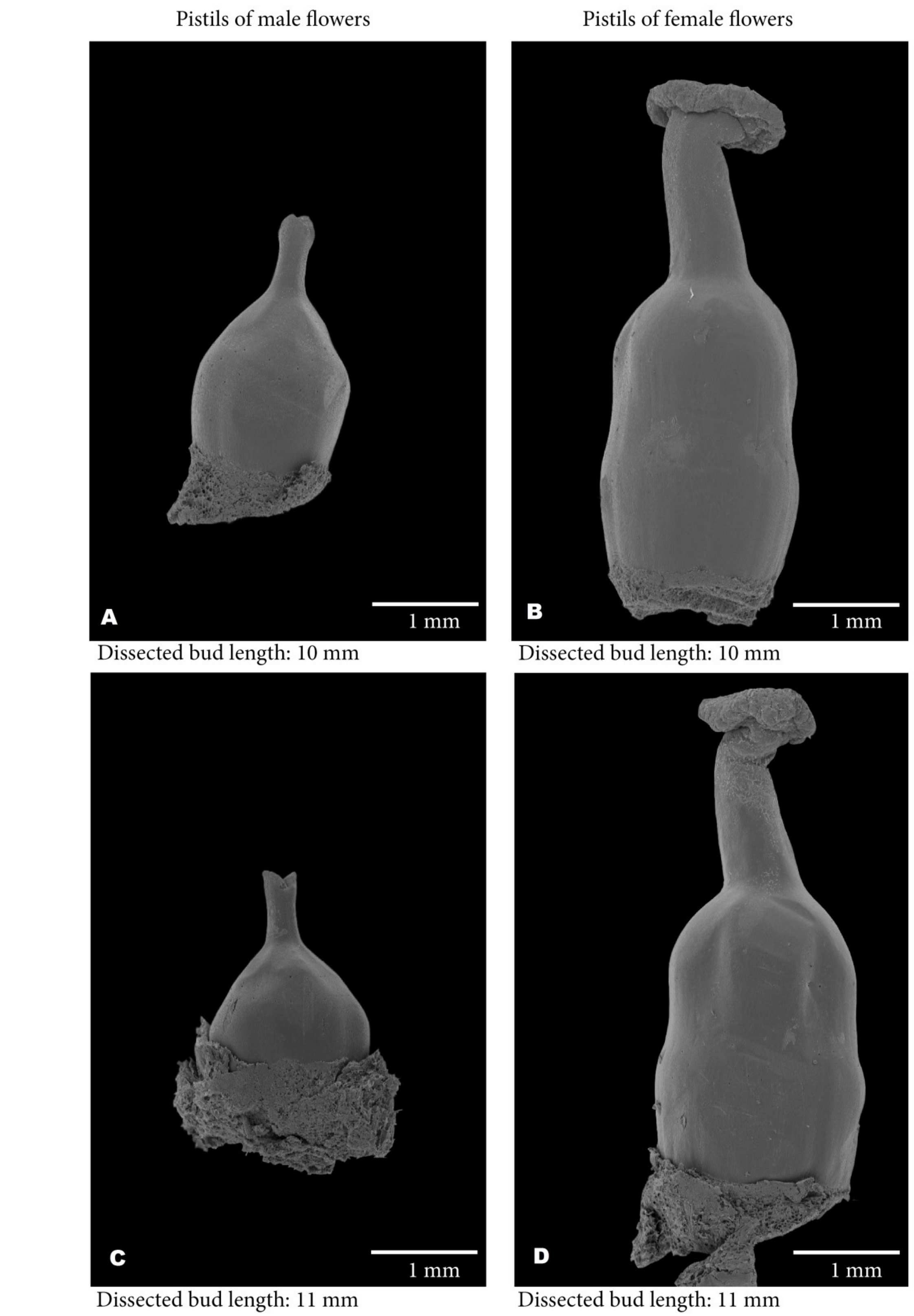


Figure 18 Pistils of male and female *Schlegelia parviflora* buds (10 mm to 11 mm)

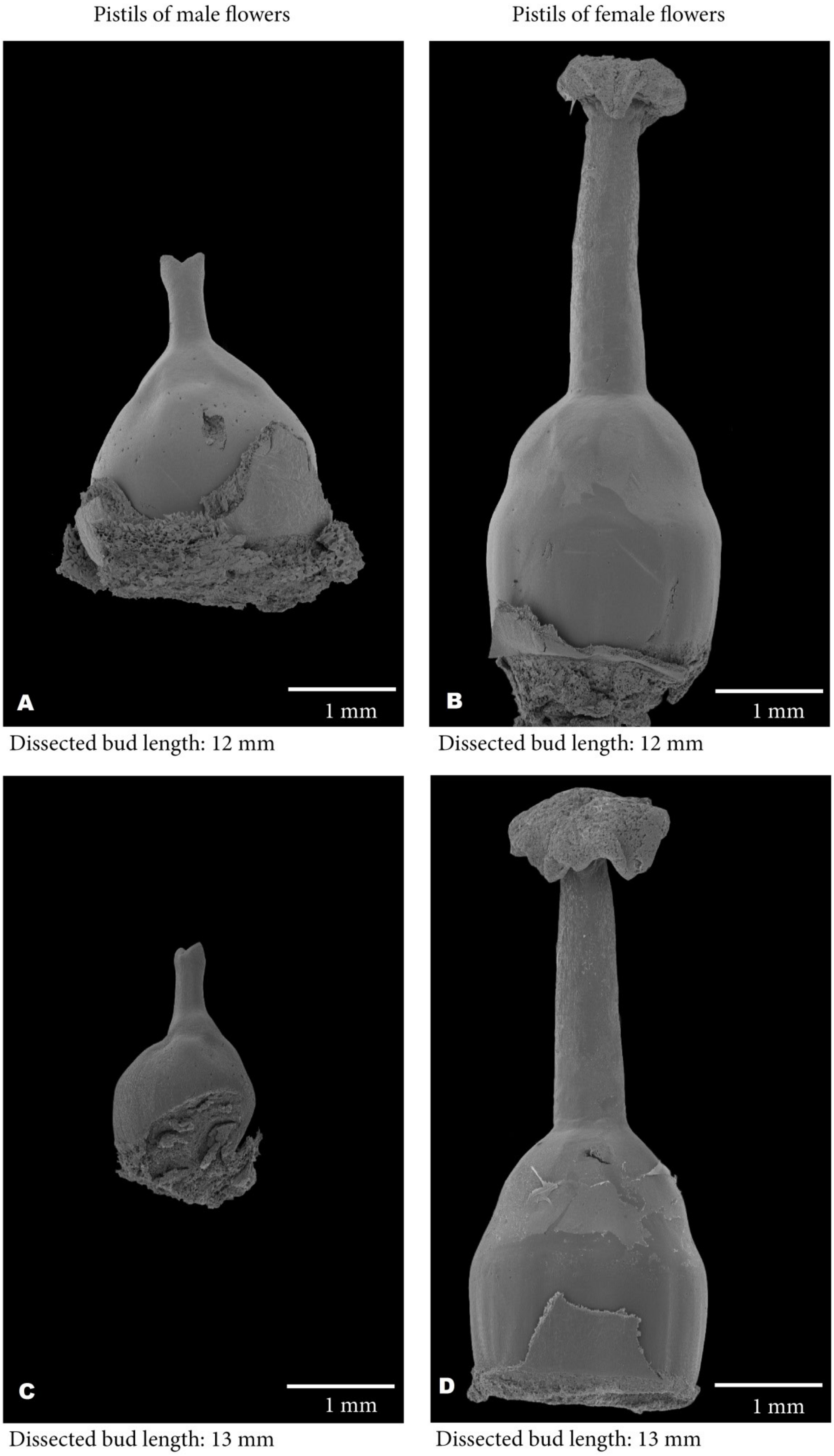


Figure 19 Pistils of male and female *Schlegelia parviflora* buds (12 mm to 13 mm)

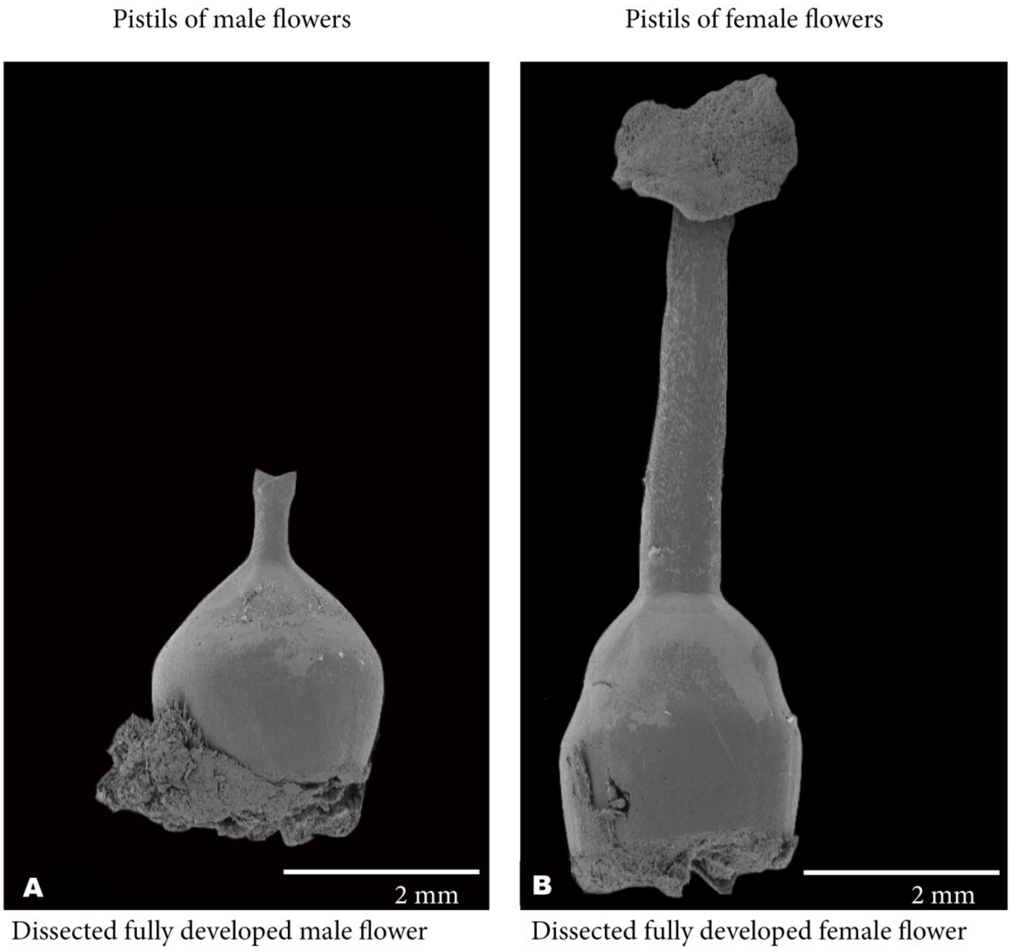


Figure 20 Pistils of male and female *Schlegelia parviflora* flowers

4. Discussion

Evolution of dioecy in Schlegelia parviflora

Dioecy seems to be rare in Lamiales. In fact, it has apparently only evolved in some species of the Lamiaceae (Harley & al., 2004) (e.g. in the genus *Lepechinia*), Oleaceae (Green, 2004) and Plocospermataceae (Struwe & Jensen, 2004).

However, neither of these families currently belongs to the same clade as Schlegeliaceae, and no dioecious species are known for the families, which do belong in the same clade (Thomandersiaceae, Pedaliaceae, Martyniaceae, Bignoniaceae, Acanthaceae, Verbenaceae, Lentibulariaceae (Stevens, 2001 onwards)).

According to Charlesworth (1991) there had to be two evolutionary steps from a hermaphroditic to a unisexual flower. The first adjustment is either a mutation producing male sterility or female sterility. As gynodioecious populations are more common in nature (Lloyd, 1975), it is assumed that the first mutation causes male sterility. The second change concerns the female sterility, which can occur instantly or step by step over the gynodioecious, subdioecious to the fully dioecious flower pathway (Dellaporta & Calderon-Urrea, 1993).

According to the system by Mitchell & Diggle (2005), *Schlegelia parviflora* has type I unisexual flowers (Mitchell & Diggle, 2005), which means that the development of stamens and pistils is initiated in both, female and male flowers (Figure 10, Pictures A and B), but is instantly "followed by the termination of development in one or the other organ set" (Mitchell & Diggle, 2005). In *Schlegelia parviflora* the development of the staminodes does not really terminate instantly in female flowers but appears to stop only in 9 mm buds, because from this size on, the length of the staminodes stays more or less the same (Figure 12, Picture F). As for the pistillode in a male flower, the development seems to stop much sooner at a bud size of 5 mm (Figure 16, Picture C). As can be seen in Figure 9, Pictures E and F, fully developed male flowers of *Schlegelia parviflora* consist of a functional androecium with a reduced non-functional gynoecium and fully developed female flowers have a functional pistil and four reduced non-functional stamens. Because of these rudimentary androecial and gynoecial organs it is assumed that the evolutionary background of these type I flowers is hermaphroditic (Bawa & Opler, 1975; Bawa, 1980). It can be seen in anthetic flowers (Figure 9, Pictures E and F) that the pistillate flowers look more like hermaphroditic flowers than the staminate flowers of *Schlegelia parviflora*. Floral rewards for pollinators are nectar (in female as well as in male flowers) and pollen (only in male flowers). Considering sexual selection as a driving force behind dioecy, female plants had to come up with some "tricks" to convince pollinators that they offer a reward comparable to that of male plants. In fact, if male and female flowers look and smell the same, pollinators do not "know" that they will only receive nectar but no pollen from pistillate flowers. So, I assume that the main function of the big staminodes is deceiving pollinators.

Another reason for the later termination of the staminodal development could be that nectar robbers should not be able to get to the floral reward easily. Even though that the entrance to the nectary chamber in *Schlegelia parviflora* is quite narrow and hidden below numerous hairs inserted at the level of stamen insertion, the big staminodes could be another hindrance to potential nectar robbers as they narrow down the entrance to the flower and may therefore preclude some visitors from reaching the nectar without actually pollinating the flowers.

Another reason to believe in sexual selection when it comes to dioecy, is that in *Schlegelia parviflora*, the corollas of male plants - or at least the corolla lobes, which are mainly responsible for the floral display - are slightly bigger (see Table 1) than those of female flowers. In addition, the pinkish nectar guides are more distinct (Figure 6, Picture A) and the inflorescence clusters comprise more flowers than in female *Schlegelia* individuals. Bawa (1980) and Beach (1981) stated that bigger inflorescences would attract more pollinators, which in fact respond strongly to alterations in floral presentation. More and showier flowers signal that it is more rewarding for pollinators if they would fly to these plants. So pollinators would be attracted to these plants earlier in the day and more often, the conclusion following that these plants "transmit more genes via pollen than via ovules" (Bawa & Beach, 1981), bringing a clear advantage for the establishment of males and the dioecious breeding system. This seems to be a reasonable explanation for the fact that flowering male *Schlegelia parviflora* plants are showier than female plants even if on first sight individual male and female flowers look very much alike.

Features associated with dioecy in Schlegelia parviflora

Since dioecy is more common in tropical than in temperate climates (Bawa, 1980; Givnish, 1980; Renner & Ricklefs, 1995) and since there is a general lack of research in the tropical flora, it is no surprise that we found a believed "bisexual" (Burger & Barringer, 2000) plant to be actually dioecious. As mentioned in the introduction, dioecy is often associated with various other morphological and ecological traits. Some of those can be found in *Schlegelia parviflora* as well.

Such features that can be found in *Schlegelia parviflora* include a climbing growth form (Renner & Ricklefs, 1995) and woody growth (Bawa & Opler, 1975; Bawa, 1980; Vamosi, Otto, & Barrett, 2003; Fromhage & Kokko, 2010). Sometimes the female and male lianas were growing on the same host tree, a circumstance which makes these unisexual individuals look like one monoecious individual. The climbing habit allows these lianas to separate their sexes, but at the same time to grow in an intimate spatial relationship. This allows the plants to profit from the benefits of shared maternal and paternal costs (Lloyd 1982) for reproductive success provided by a dioecious breeding system. At the same time, it assures avoiding selfing (Ross, 1970; Janzen, 1977; Charlesworth & Charlesworth, 1978; Thomson & Barrett, 1981; Vamosi, Otto, & Barrett, 2003) and yet the highest chances of being pollinated.

Staminate and pistillate flowers of *Schlegelia parviflora* perfectly fit the "Bawa hypothesis" (Bawa & Opler, 1975; Bawa, 1980; Beach, 1981), being rather small (at anthesis ca. 14 mm; other Lamiales have often large and showy flowers), white with purple tinged lobes and pollinated by "small generalist insects" (Bawa, 1980), i. e. bees. The floral morphology and size fit well with the anatomical shape of the bees (Figure 8, Pictures A and B), which are attracted by the honey-flavoured smell and the floral display. In addition, *Schlegelia parviflora* also produces fleshy and most likely animal-dispersed fruits, which is another feature often found in dioecious plants.

Floral development

As for the fact that female anthers do not get bigger after a bud size of 4 mm during floral development (only the filament and the hairs grow), I assume they have to compensate this fact with a rapid growth during early bud development (1-3mm), so to look like real stamens and not just staminodes at anthesis. If you just compare the female and male anthers with each other, the female thecae are almost as big as the male thecae at an anthetic stage (Figure 14, Pictures C and D).

Another interesting feature concerns the development of the male stigma, as it does not change its appearance during bud development. Inside a 1mm bud you can see the two stigma lobes, but they are not expanding or developing; only the style and the ovary grow until a bud size of 5 mm. This may be due to the fact that except for the production of nectar in the base of the ovary, the gynoecium in a male flower has no other function. Comparative floral development of male and female flowers shows that the sexual differentiation in male flowers starts earlier during floral development and is more strongly expressed also at anthesis than in female flowers.

Conclusions

To sum up my findings, I can state that *Schlegelia parviflora* is indeed dioecious. I found individuals with solely male flowers or female flowers, respectively. Some branches could not be traced back to their roots, so it might be possible that there are individuals with flowers of the other gender. However, that seems unlikely as no such case could be found.

My hypothesis that male and female flowers are similar during early floral development but differentiate during later stages is confirmed. Interestingly, stamens in female flowers tend to be bigger in very young buds (1 to 3 mm) than stamens in young male flowers. At a bud size of about 4 mm, the male stamens grow dramatically and outmatch the female ones. However, at anthesis the staminodes in female flowers are not much smaller than the stamens in male flower and may function in deceiving pollinators and/or stop nectar robbers.

Concerning my third research question, there are no distinct differences in gross floral shape and appearance between male and female flowers, except the fact that male corollas are slightly bigger and the nectar guides tend to be more distinct.

Future work

Since we know now that *Schlegelia parviflora* is dioecious, we should lay our eyes on other species in the genera *Schlegelia*, *Gibsoniothamnus*, *Exarata* and *Synapsis* in order to search for other dioecious species, which have not been recognised before.

Although I did my best to study *Schlegelia parviflora* as thoroughly as possible, there still remain many unanswered questions. First of all, there is a need of identifying the actual phylogenetic position of Schlegeliaceae within Lamiales, because only then we can make qualified statements concerning the ecological and morphological traits believed to be correlated with dioecy within this species.

Further investigations should also be made concerning the anthesis in female flowers of *Schlegelia parviflora*, which I could not make, due to the fact that I began too late in the flowering season with this project and all the female individuals in the surroundings of the research station were fruiting and no buds were left to observe anthesis.

Other studies can be made about the quantity and quality of nectar in male and female flowers, respectively, as pistillate flowers offer solely nectar.
With the right tools it would be possible to investigate individuals which grow high up in the trees, to verify and confirm my investigations conducted at eye- or platform level.
Last but not least, it would be interesting to investigate the genetics of this species and the background of sex determination. Maybe this could give further insights as to why *Schlegelia parviflora* became dioecious.

5. Acknowledgements

First of all, I owe sincere and earnest thankfulness to my supervisor Jürg Schönenberger, who was there whenever needed.
I am grateful to all the people working at the Tropical Research Station La Gamba, especially Eduardo Gerardo Aráuz Suárez and José Luis Sánchez Jiménez who built my observation platforms in less than a day, which allowed me to start immediately with my observations.
Furthermore, I would like to thank Susanne Sontag for introducing me to the laboratory and all the techniques necessary for my investigations in Vienna.
My thanks also go to Andrea Frosch-Radivo, Susanne Pamperl and Ursula Schachner, who provided me with necessary material and answers to all of my questions.
My gratitude also goes to the University of Vienna for granting me two scholarships for my research (KWA and Förderungsstipendium nach dem StudFG).
Finally, I would also like to thank MINAE (Ministerio de Ambiente y Energía) in San José, Costa Rica for the research and export permits.

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7. Appendix

7.1. Abstract

Schlegelia parviflora is a tropical liana belonging to Schlegeliaceae, a family which, despite molecular analyses, still remains in an uncertain phylogenetic position within the Lamiales. During a field course, conducted in Costa Rica in 2011, it was observed that this species might be dioecious, an unknown fact in the literature. Dioecy is a relatively rare phenomenon and therefore it is important to study species with this breeding system in order to better understand the driving forces behind its evolution.

Field observations were conducted in and around the tropical Research Station La Gamba, Costa Rica in February and March 2012. Collected plant material was brought to Vienna for further investigations.

Results show that *Schlegelia parviflora* is indeed a dioecious plant. The androecium is reduced in female flowers and non-functional, i.e. pollen is not produced. In male flowers, the pistil is reduced and the stigma is underdeveloped, i.e. non-functional.

The results of the morphological investigations are that the calyx and the corolla tube are generally slightly longer in female than in male flowers. However, the petal lobes, which are mainly responsible for the floral display, are longer in male than in female flowers. In addition, male individuals bear generally more flowers per inflorescence than female plants, which is well in accordance with earlier hypotheses on the evolution of dioecy.

In order to document floral development, scanning electron microscope pictures were taken from stamen and pistils dissected from male and female buds 1 to 13 mm in length. The same was done for anthetic flowers. The comparative analyses show that flowers of both sexes have the same basic floral organization and are similar to each other during early developmental stages. During subsequent developmental stages a differentiation into functionally male and female flowers takes place. At anthesis, however, female flowers of *Schlegelia parviflora* look almost like normal hermaphroditic flowers, in which both stamens and carpels appear fully developed, whereas in male flowers the gynoecium is clearly reduced.

Stamens in male flowers are twice as long as staminodes in female flowers. However, this size difference is mainly due to a reduced filament length; the anthers of male and female flowers, respectively, are almost equal in size. A possible function of these large staminodes might be to convince pollinators that they also offer a pollen reward (and not only nectar) comparable to that of male plants.

Flower visitors, such as honey bees, stingless bees, hummingbirds, hawk-moths or ants are probably attracted by the honey flavoured floral scent, which is produced by male and female flowers, respectively. My observations point to honey bees as pollinators. My thesis provides a detailed description of a dioecious plant and its early floral development, thus helping in interpreting as to why dioecy evolved in this species.

7.2. Deutsche Zusammenfassung

Schlegelia parviflora (Schlegeliaceae) ist eine tropische Lianenart, deren Verwandtschaftsverhältnisse trotz molekularbiologischer Untersuchungen innerhalb der Ordnung Lamiales noch nicht geklärt sind.

Während eines Praktikums in Costa Rica, entdeckte man, dass diese Art zweihäusig sein könnte, was in der Literatur bis dato unbekannt war. Da Diözie weltweit ein eher seltenes Phänomen darstellt, ist es wichtig Arten mit diesem Geschlechtssystem genauer zu erforschen. Morphologische Untersuchungen wurden im Februar und März 2012 rund um die Tropenstation La Gamba, Costa Rica durchgeführt. Gesammeltes Pflanzenmaterial wurde nach Wien transportiert, um unter dem Rasterelektronenmikroskop genauer untersucht zu werden.

Dabei stellte sich heraus, dass es sich bei *Schlegelia parviflora* tatsächlich um eine zweihäusige Art handelt, wobei in den weiblichen Blüten reduzierte, nicht-funktionelle Staubblätter (ohne Pollen) entdeckt wurden. In den männlichen Blüten gibt es zusätzlich zu den vollentwickelten Staubblättern, einen reduzierten Stempel, wobei hier die Narbe nicht rezeptiv ist und sich somit aus den männlichen Blüten keine Früchte entwickeln können.

Die morphologischen Untersuchungen ergaben, dass die durchschnittliche Kelchlänge beziehungsweise auch die Kronröhrenlänge bei weiblichen Blüten länger ist als bei männlichen Blüten, wohingegen die freien Teile der Petalen, die den Schauapparat ausmachen, bei männlichen Blüten stärker ausgeprägt sind. Hinzu kommt dass die männlichen Infloreszenzen im Allgemeinen aus mehr Blüten bestehen als die weiblichen, was mit früheren Hypothesen zur Evolution von Diözie im Einklang ist.

Um die frühen Entwicklungsstadien der Blüten zu dokumentieren, wurden rasterelektronenmikroskopische Aufnahmen wurden von Staubblättern und Stempeln gemacht, die zuvor aus weiblichen beziehungsweise männlichen Knospen mit einer Größe von 1mm bis 13 mm präpariert wurden. Die vergleichende Analyse zeigt, dass die Blüten beider Geschlechter die gleiche Blütenorganisation aufweisen und sich in frühen Entwicklungsstadien sehr ähnlich sind, sich nachfolgend aber in funktionell männliche bzw. funktionell weibliche Blüten entwickeln. Zur Blütezeit sehen weibliche Blüten allerdings aus, als wären sie normale hermaphroditische Blüten mit entwickelten Staub- und Fruchtblättern. In männlichen Blüten hingegen, ist das Gynözeum deutlich reduziert. Staubblätter in männlichen Blüten sind zweimal so lang wie die Staminodien in weiblichen Blüten. Dieser Größenunterschied kommt aber nur durch die Reduzierung der Filamentlänge zustande. Die Größe der Antheren ist in männlichen, sowohl als auch in weiblichen Blüten gleich. Eine mögliche Erklärung für die Größe der Staminodien ist, dass Bestäuber getäuscht werden sollen, die sich nicht nur Nektar als Belohnung erwarten sondern auch Pollen, der aber in weiblichen Blüten nicht produziert wird. Der Stempel in weiblichen Blüten ist zweimal so lang, breiter und robuster als der rudimentäre Stempel in männlichen Blüten.

Blütenbesucher wie Honigbienen, stachellose Bienen, Kolibris, Schwärmer oder Ameisen werden vermutlich durch den honigsüßen Duft, der von den weiblichen sowohl als auch von den männlichen Blüten ausgeht, angelockt. Ausgehend von meinen Beobachtungen werden Honigbienen als Bestäuber angenommen.

Meine Diplomarbeit bietet eine detaillierte Beschreibung einer zweihäusigen Pflanze und ihre frühe Entwicklung, welche dabei hilft zu verstehen, warum sich Diözie in dieser Art entwickelt hat.

7.3. Persönliche Danksagung

Mein größter Dank gebührt an dieser Stelle meinen Eltern, die mir durch ihre finanzielle und mentale Unterstützung ein reibungsloses und erfolgreiches Studium gewährleistet haben.
Vielen Dank auch an meine Schwester Carina, die mir immer durch Rat und Tat zur Seite gestanden ist.
Auch meinen StudienkollegInnen beziehungsweise FreundInnen gebührt ein großes Dankeschön: die gegenseitige Motivation während des Studiums und die gemeinsame Zeit in den Bibliotheken Wiens, vor allem in der Diplomarbeitsphase (speziell an Christina, Valerie und Maria), wird mir in Erinnerung bleiben.
Auch wenn man sich nicht regelmäßig sieht, heißt das nicht, dass man nicht immer füreinander da ist: Danke Klaus.
Zum Schluss noch ein herzliches Dankeschön an Kerstin, die meinen Aufenthalt in Costa Rica mit ihrer Anwesenheit bereichert hat.

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