

**A palynological study of *Heliophila* (Brassicaceae) in southern  
Africa**

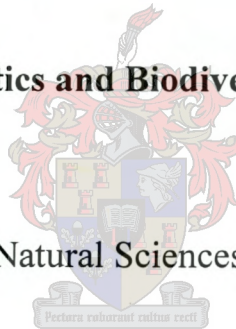
By

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## ABSTRACT

Pollen grains of the southern African genus *Heliophila* L. (Brassicaceae), were examined using light microscopy (77 taxa), scanning electron microscopy (82 taxa) and transmission electron microscopy (10 taxa). The present study emanates from a pilot study by Kose (2001) in which palynology was identified as one of the taxonomically informative data sources that can be used in the classification and subdivision of the genus *Heliophila*. The aim of this study was to assess the taxonomic significance of palynological data in the genus *Heliophila*.

All pollen grains of *Heliophila* taxa are prolate in shape and tricolpate. The exine surface sculpture varies and two main groups are identified. In the majority of the taxa the sculpture is psilate or psilate micro-perforate with suprategal spinules (Pollen Type A), whereas in the remaining taxa the sculpture is micro-reticulate to reticulate with or without suprategal spinules (Pollen Type B). Pollen Type B may be divided further into three subgroups based on the shape of the lumina and the position of the suprategal spinules. TEM results also confirm the two main groups as proposed by the SEM. The structural and sculptural features of the exine proved important in the demarcation of the pollen grains into two distinctively different pollen types and the other pollen morphological data such as pollen grain sizes, pollen grain shape are discussed in relation to the groups/subgroups of pollen types. The sculpturing types are discussed with regard to their evolution and pollination discussed with regard to plant habit and pollen size.

Cluster analysis, based on vegetative and reproductive characters, excluding palynological characters, was performed only after the pollen types were identified. These groups were well supported by plant habit, life form, leaf type, presence or absence of appendages on the filaments, indumentum of filaments and presence of the stipe on the ovary and divided the genus *Heliophila* into two main clades. It was observed that this division followed a similar pattern as the species arrangement in the classification by Marais (1970). Although the two main pollen types occur in both clusters proposed by the morphological analysis, the specific pollen types are restricted to distinct subclusters.

In conclusion, pollen data reveals that *Heliophila* is a heterogeneous genus. The present study also reveals that it is difficult to subdivide the *Heliophila* taxa into infrageneric units on the basis of palynological evidence alone. In conjunction with other data sources, it does, however appear to have systematic significance. Therefore, the results of the present palynological study can be used as an additional source of evidence to support the subdivision of the *Heliophila* taxa into infrageneric units in the near future if a better understanding and classification of the genus is to be achieved.

**Key words:** *Heliophila*, Brassicaceae, palynology and phenetic approach.

## OPSOMMING

Stuifmeelkorrels van die suider Afrikaanse genus *Heliophila* L. (Brassicaceae) is met behulp van ligmikroskopie (77 taksons), skandeerelektronmikroskopie (82 taksons) en transmissie-elektronmikroskopie (10 taksons) bestudeer. Die huidige studie is 'n uitvloeisel van 'n lootsprojek deur Kose (2001) waarin palinologie geïdentifiseer is as een van die taksonomies-belangrike data-bronne wat in die klassifikasie en subdivisie van die genus *Heliophila* gebruik kan word. Die doel van die studie was om die taksonomiese waarde van palinologiese data van die genus *Heliophila* te bepaal.

Alle stuifmeelkorrels van *Heliophila* taksons is prolaat in vorm en trikolpaat. Die oppervlak-skulptuur van die eksien varieer en twee hoofgroepe word onderskei. In die meerderheid van die taksons is die skulptuur psilaat of psilaat-mikroperforaat met supratektale uitsteeksels (stuifmeeltipe A), terwyl die skulptuur in die oorblywende taksons mikro-retikulaat tot retikulaat met of sonder supratektale uitsteeksels (stuifmeeltipe B) is. Stuifmeeltipe B kan verder in drie subgroepe, gebaseer op die vorm van die lumens en die posisie van die supratektale uitsteeksels, verdeel word. Resultate verkry vanaf die TEM bevestig die twee hoofgroepe soos voorgestel deur die SEM-studies. Die strukturele en skulpturale kenmerke van die eksien blyk belangrik te wees in die verdeling van die stuifmeelstruktuur in twee duidelike stuifmeeltipes en ander morfologiese kenmerke, byvoorbeeld die grootte en vorm van die stuifmeelkorrels word bespreek in hul verhouding tot die groepe / subgroepe van die stuifmeeltipes. Die tipes stuifmeel-skulptuur word bespreek ten opsigte van hul evolusie en bestuiwing word bespreek ten opsigte van groeiwyse en stuifmeelgroottes.

Groepanalise, gebaseer op vegetatiewe en reprodusiewe kenmerke, uitsluitend palinologiese kenmerke, is saamgestel nadat die stuifmeeltipes geïdentifiseer is. Die groepe word gevorm op grond van groeiwyse, groeivorm, blaartipe, aan- of afwesigheid van aanhangsels aan die helmdrade, die indumentum van die helmdrade en die aanwesigheid van 'n stipe op die vrugbeginsel en verdeel die genus *Heliophila* in twee hoofgroepe. Daar is waargeneem dat hierdie analise 'n soortgelyke patroon volg as die spesie-rangskikking in die klassifikasie van Marais (1970). Alhoewel die twee stuifmeeltipes in beide die morfologiese hoofgroepe aanwesig is, stem die stuifmeeltipes duidelike ooreen met die sub-groepe wat deur die morfologiese analise gevorm is.

Ten slotte, stuifmeeldata dui daarop dat *Heliophila* 'n heterogene genus is. Die huidige studie dui ook daarop dat 'n onderverdeling van *Heliophila* in infrageneriese eenhede op grond van palinologiese kenmerke alleen ook baie moeilik is, maar saam met ander kenmerke kan stuifmeeldata wel van taksonomiese waarde wees. Dus, die resultate van hierdie studie kan as 'n aanvullende bron van data gebruik word om 'n onderverdeling van die genus te steun en 'n klassifikasie van die genus daar te stel.

## CONTENTS

<b>Chapter one</b>	<b>Introduction</b>	1
1.1	Brassicaceae	1
1.2	Classification	1
1.3	Tribal Classification	3
1.4	The genus <i>Heliophila</i>	4
1.5	Pollen Character Analysis	5
1.6	Objectives	6
<b>Chapter two</b>	<b>Taxonomic review of <i>Heliophila</i></b>	7
<b>Chapter three</b>	<b>Material and Methods</b>	11
3.1	Material	11
3.2	Palynological Methods	11
3.2.1	Light microscopy	11
3.2.2	Scanning electron microscopy	12
3.2.3	Transmission electron microscopy	12
3.2.4	Analysis of Pollen data set	12
3.3	Numerical Taxonomy	17
<b>Chapter four</b>	<b>Results</b>	22
4.1	Palynological results	22
4.1.1	Size and shape	22
4.1.2	Description of pollen types	22
4.1.3	Analysis of variance (ANOVA) Test	39
4.2	Phenetic Analyses Results	41

<b>Chapter five</b>	<b>Discussion</b>	46
5.1	Introduction	46
5.2	Pollen data	47
5.1.1	Exine sculpture and structure	47
5.1.2	Pollen grain size	48
5.1.3	Pollen grain shape	48
5.1.4	Apertures	49
5.3	Cluster analysis	49
5.4	Proposed groups based on palynological and morphological data	53
5.5	Pollination	55
5.6	Evolution	56
<b>Chapter six</b>	<b>Conclusions</b>	58
<b>Chapter seven</b>	<b>References</b>	59
<b>Chapter eight</b>	<b>Acknowledgements</b>	66
<b>List of appendices</b>		
Appendix I:	<i>Heliophila</i> sections based on morphology by De Candolle	67
Appendix II:	<i>Heliophila</i> sections based on morphology by Sonder	68
Appendix III:	Raw data for cluster analysis	69
Appendix IV:	Fruit variation in <i>Heliophila</i> species	75

## CHAPTER ONE: INTRODUCTION

### 1.1 Brassicaceae, (mustard family)

Brassicaceae, sometimes referred to as the mustard family, and alternatively known as Cruciferae, is one of the four families represented in most of the different floras of the world. It is also the largest family within the order Brassicales, consisting of about 380 genera and 3300 species (Heywood, 1993). The family has a cosmopolitan distribution with the majority of species occurring in the temperate and cold regions of the Northern Hemisphere, especially in the Mediterranean region, south western and central Asia and western north America. About 34 genera (20 exotic) and 152 species (37 exotic) occur in the Southern Hemisphere and 6 genera are indigenous to South Africa (Heywood, 1978)

Many of the species of the Brassicaceae are of economic importance and are extremely valuable to the human race because of their medicinal, nutritional and ornamental properties. The family provides important food crops like radish (*Raphanus sativus* L.), cabbage (*Brassica oleracea* L.) and Chinese cabbage (*B. rapa* L.) together with condiments such as Chinese mustard (*B. juncea* L.), Black mustard (*B. nigra* L.) and White mustard (*Sinapis alba* L.), all of which are used to spice up daily foods. Seeds of members of Brassicaceae are rich in fatty acids, isothiocyanates and glucosinolate sinigrins. Vegetable oil is extracted from the seeds of several species of *Brassica*, e.g. *B. napus* L. The family is also known for several ornamentals such as *Erysimum* L. (wallflower) and *Lunaria* L. (honesty). *Heliophila longifolia* E. Mey., the blue flax, is ornamental in South Africa. *Arabis thaliana* (L.) Heynh (thale or mouse-ear cress), a Eurasian weed, is the most widely used vascular plant in molecular and experimental biology (Judd *et al.*, 1999). Ecologically, Brassicaceae is a source of nectar to pollinators like bees, flies, butterflies, moths and beetles (Strimbeck 2001) (<http://strimbeck.faculty.jsc.vsc.edu/FamilyPages/Brassicaceae/Brassicaceae.htm> ).

### 1.2 Classification

In the past many different schools of thought, suggested many different affiliations of the Brassicaceae. Table 1.1 shows the taxonomic treatments of the Brassicaceae.

Table 1.1 The taxonomic treatment of the Brassicaceae (Cruciferae)

<b>Author and date</b>	<b>Order</b>	<b>Families</b>
Bentham & Hooker (1865)	Parietales	Papaveraceae, Sarraceniaceae, Capparaceae and Resedaceae
Thonner (1915)	Rhoeadales	Papaveraceae, Capparaceae, Resedaceae and Moringaceae.
Rendle (1925)	Rhoeadales	Papaveraceae, Capparaceae and Resedaceae
Lawrence (1951)	Rhoeadales	Papaveraceae, Capparaceae and Resedaceae
Melchior (1964)	Papaverales	Papaveraceae, Capparaceae, Resedaceae, Tovariaceae and Moringaceae
Hutchison (1969)	Cruciales	Cleomaceae and Oxystylidaceae
Hutchison (1973)	Brassicales	Cleomaceae and Oxystylidaceae
Dahlgren (1980)	Capparales	Capparaceae (+ Pentadiplandraceae), Resedaceae, Tovariaceae, Moringaceae, Bataceae and Koeberliniaceae
Cronquist (1981)	Capparales	Capparaceae (+ Pentadiplandraceae, Koeberliniaceae, Oxystylidaceae), Resedaceae, Tovariaceae, Moringaceae and Koeberliniaceae
Takhtajan (1987)	Capparales	Capparaceae (+ Oxystylidaceae), Resedaceae, Tovariaceae, Koeberliniaceae and Pentadiplandraceae

The delimitation of families within the different orders into which Brassicaceae has been placed seems not to be problematic. The only controversy has always been the origin of Brassicaceae, deciding whether it was derived from the Papaveraceae or Capparaceae. Lawrence (1951) postulated that Brassicaceae was derived from Capparaceae because of the similarities in the

morphology and anatomy of the androecium and gynoecium. Chemical features also support this view, as isothiocyanates are present in both Capparaceae and Brassicaceae (Harborne and Turner 1984). It differs from Papaveraceae in terms of chemistry and the presence of endospermous seeds, although there are a few resemblances in androecial and gynoecial features and the tetramerous perianth. From a palynological point of view, the pollen grains in Brassicaceae are almost similar to the grains in Capparaceae, whereas they differ from those in Papaveraceae-Fumarioideae (Erdtman, 1971). Bhattacharyya and Jonri (1998) proposed that Brassicaceae originated from Capparaceae ancestors and was better placed in the order Capparales.

Classification systems based on both morphological and molecular data place Brassicaceae in the Brassicales (Angiosperm Phylogeny Group [APG], 1998). Other families included in this order are Akaniaceae (+ Bretschneideraceae), Bataceae, Caricaceae, Emblingiaceae, Gyrostemonaceae, Koeberliniaceae, Limnanthaceae, Moringaceae, Pentadiplandraceae, Resedaceae, Salvadoraceae, Stetchellanthaceae, Toyariaceae and Tropaelaceae (APG, 1998). Results of Salvoainen *et al.* (2000a), based on molecular data, also support the inclusion of all these families, except Emblingiaceae.

The Brassicales is characterized by the presence of glucosinolates, a compound (or secondary metabolite) containing sulphur. The presence of glucosinolates is synapomorphic for members of Brassicales and thus systematically informative in the phylogeny of the order (Rodman *et al.*, 1993). The only other taxon that contains glucosinolates is *Drypetes*, a member of the Euphorbiaceae. This has led botanists to believe that mustard oils have evolved twice in two different unrelated taxa. According to the APG (1998), Brassicales is more closely related to the Malvales and Sapindales clade. However, Salvoainen *et al.* (2000b) indicated that the Brassicales is most closely related to the Malvales, and forms a sister clade to the Sapindales.

### **1.3 Tribal classification**

Diagnostic characters for the Brassicaceae are the distinctive cruciform arrangement of the petals and the tetradynamous stamens. The family is well defined and readily recognised, because of its distinctive floral characters and is regarded as a natural taxon. The tetradynamous stamens is a fixed character, and apparently a very efficient contrivance for successful pollination (Hutchison, 1969). Various attempts have been made to produce a natural subdivision of the family into tribes. Such studies have used fruit characters, embryo features, nectar glands and myrosine cells



in embryos. Schulz (1936) divided the family into 19 tribes, but Heywood (1993) regarded some of these tribes as “far from being satisfactory.” Janchen (1942) divided the family into the following 15 tribes: Stenleyeae, Pringleae, Romanschulzieae, Streptantheae, Schizopetaleae, Stenopetaleae, Sisymbriaceae, Hesperideae, Arabideae, Alysiseae, Lepidieae, Brassicaeae, Chamireae, Heliophileae and Cremolabeae. Only four of these tribes are today regarded as natural taxa, namely Brassicaeae, Lepidieae, Pringleae and Chamireae (Heywood, 1978). Two tribes, Chamireae and the Heliophileae are confined to the Cape Flora (Heywood, 1978). The endemic southern African tribe Heliophileae, as defined by Schulz (1936), includes *Cycloptychis* E. Mey. ex Sond., *Thlaspeocarpa* C. A. Smith and *Carponema* DC. (now *Heliophila* L.).

#### **1.4 The genus *Heliophila***

*Heliophila* L. is the largest genus of the Brassicaceae represented in South Africa, and occurs mainly in the Cape Flora. The genus includes ca. 75 species (Dreyer and Jordaan, 2000). The majority of the species occur in the Fynbos, but some extend also to the Karoo biomes (Goldblatt and Manning, 2000). Most of the species are endemic to southern Africa, and most occur in the winter-rainfall areas of the Northern, Western and Eastern Cape Provinces (Marais, 1970). However, some have a wider distribution.

Sonder (1860) and Marais (1970) published the most comprehensive studies of the Brassicaceae of southern Africa (including the genus *Heliophila*) to date. Their work was mainly based on morphology and little is known about palynology and karyology of the South African members of the *Heliophila*. Similarly no molecular data are available for *Heliophila*. *Heliophila* is a large genus without any infrageneric classification, which really is in need of a thorough multidisciplinary revision. However, due to limited time, the present study only dealt with pollen morphology. It is hoped that this study will form a basis for future studies dealing with different aspects of the genus.

So far little palynological work has been done on the genus *Heliophila*. Erdtman (1971) studied two species of *Heliophila*, *H. amplexicaulis* L.f., of which the pollen grains are prolate in shape and having a size of ca. 36 x 24 µm, and *H. monticola* Sond., of which the pollen grains are subprolate and about 41 x 31 µm in size. The pollen grains of both species are tricolpate. Kose (2001), in a pilot study of the pollen morphology of *Heliophila*, studied the pollen grains of 18 species with the SEM. She described two different tectum types, namely micro-rugulate with

spinules and rugulate reticulate and also showed that all pollen grains are tricolpate. These pollen morphological results (Kose, 2001) formed the basis of the present study.

## **1.5 Pollen Character Analysis**

Palynology provides a multitude of characters of potential taxonomic significance. Traditionally grain size and shape, apertures and exine structure are considered important. However, the degree of detail in which each of these features is investigated, is mostly determined by characteristics of the group under study (Dreyer, 1996).

### **1.5.1 Grain size**

Although palynological classifications are seldomly base on grain size, this character is often used as an additional feature when describing a specific pollen type. Erdtman (1971) proposed a number of pollen grain size classes, which can be used as a reference when describing pollen characters.

### **1.5.2 Grain shape**

Most palynological discussions mention pollen grain shape as possible character. Descriptions of the shapes are based on the polar view outline and/or the equatorial view outline. Erdtman (1971) and Walker and Doyle (1975) suggested a widely used range of shape classes based on the P/E ratio of grains, where the P/E ratio is the ratio of the polar axis (P) to the equatorial diameter (E). However, shape is used with caution, since it can vary considerably within one-grain type or even within a particular species (Moore & Webb, 1987 as cited by Dreyer 1996).

### **1.5.3 Apertures**

Traditionally aperture shape, number and arrangement are considered important taxonomic characters. Erdtman (1971) developed the NPC system (N = number, P = position, C = character), which is commonly used to classify pollen grains by means of their apertures.

### **1.5.4 Exine sculpture and exine structure**

The exine is defined as the outer layer of the pollen grain wall and comprises primarily of sporopollin and which is highly resistant to strong acids. Two conventions exist to describe the different exine layers. They are both summarized in Figure 1.1 to avoid confusion. The external elements located upon the tectum constitute the exine sculpture and is studied using the scanning

electron microscope (SEM) whereas the internal elements such as the rod-like columellae constitute exine structure and is studied by using a transmission electron microscope (TEM). Modern SEM and TEM techniques have facilitated detailed exine studies, which have resulted in the identification of numerous taxonomically significant characters (Luegmayer 1993). Amongst others, these include features such as the exine thickness, ratio between sexine and nexine diameters, (fig. 1.1) occurrence and type of tectum, occurrence and type of suprategal structures, presence or absence of foot layer and foot layer diameter.

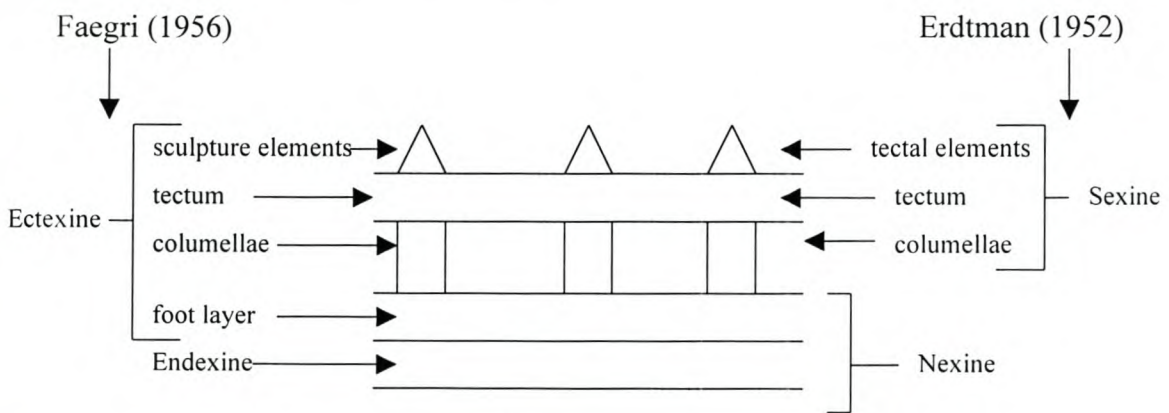


Figure 1.1 Diagrammatic presentation of the different exine layers recognised in a pollen grain wall (after Punt *et al.* 1994).

## 1.6 Objectives

The main objective of the present study was to assess the taxonomic significance of palynological characters in the genus *Heliophila*. In addition, a number of sub objectives were also set. These include:

- 1.6.1 to identify different pollen types within the genus
- 1.6.2 to provide a descriptive analysis of *Heliophila* pollen
- 1.6.3 to make suggestions towards a possible new subgeneric classification based on palynological data.

## CHAPTER TWO: TAXONOMIC REVIEW OF *HELIOPHILA*

The name *Heliophila* was derived from the Greek words “helios”, (sun) and “philos”, (loving) and was first used by Linnaeus in 1763 when he described the two species, *H. integrifolia* L. and *H. coronopifolia* L. However, the first *Heliophila* species was also described by Linnaeus, but under the name of *Cheiranthus africanus* L. (Linnaeus 1760). According to Marais (1970) *H. integrifolia* is similar to *Cheiranthus africanus*. The latter was changed to *H. africana* (L.) Marais with *H. integrifolia* as a synonym thereof (Marais 1970). Linnaeus’ son (Linnaeus f., 1781), added seven new species, of which four, namely, *H. digitata* L.f, *H. amplexicaulis* L.f., *H. pusilla* L.f. and *H. pinnata* L.f. have been retained as validly published names, whereas *H. filiformis* L.f. has been synonymized under *H. coronopifolia*. The remaining two species were transferred to other genera.

Willdenow described *H. pendula* Willd. as a new species in 1800 and added another new species, *H. crithmifolia* Willd. in 1809. De Candolle (1821) recognised 46 species including 24 newly described ones. He was the first person to attempt a subdivision of the genus. Thirty-eight of the species were placed into eight sections, while the remaining eight species were not allocated to any of the sections on the basis of plant habit and fruit characters (Appendix I). Many of the names recognised by De Candolle (1821) have since been reduced to synonymy by several authors. *H. linearifolia* DC. has been reduced to variety level as *H. linearis* (Thunb.) DC. var. *linearifolia* (Burch. ex DC.) Marais. Link (1822) recognized six species including *H. lepidioides* Link as a new species, but which has since been synonymized under *H. diffusa* (Thunb.) DC. (Marais, 1970).

Ecklon and Zeyher (1834—35) recognised 29 species, four of which were new species. Subsequently, *H. reticulata* Eckl. & Zeyh. has been reduced to a variety status as *H. linearis* var. *reticulata* (Eckl. & Zeyh.) Marais, while *H. maritima* Eckl. & Zeyh. and *H. falcata* Eckl. & Zeyh. have been reduced to the synonymy of *H. subulata* Burch. ex DC. and *H. linearis* var. *linearis* respectively. *H. brassicaefolia* Eckl. & Zeyh. has been retained as a validly published name. Steudel (1840) transferred *Cheiranthus carnosa* Thunb. to *Heliophila* as *H. carnosa* (Thunb.) Steudel. Meisner (1842) recognised 12 species, of which two were newly described, but only one, *H. brachycarpa* Meisn. has been retained as a species (Marais 1970).

Sonder (1846) recognised 54 species (of which twenty-one were newly described). Eleven of these new species have since been retained as species and *H. flacca* Sond. has been reduced to variety level as *H. diffusa* var. *flacca* (Sond.) Marais (Marais, 1970). The remaining 10 names have since been synonymized. The most comprehensive studies of *Heliophila* was done by Sonder (1860). He used fruit characters to compile a key to the South African genera and species of the Brassicaceae and subdivided 61 *Heliophila* species into six sections on the basis of plant habit and fruit characters (Appendix II).

Several new species were described after Sonder's (1860) study. Harvey (1863) described *H. scandens* Harv., which was followed by Oliver's (1894) description of *H. patens* Oliv. and Bolus' (1896) description of *H. namaquana* Bol.

Schlechter (1899) recognised 17 new species, of which only seven, namely *H. arenosa*, *H. lactea*, *H. leptophylla*, *H. linoides*, *H. macowaniana*, *H. macra* and *H. nubigena* have been retained as validly published names (Marais 1970). *H. squamata* has been reduced to a variety as *H. cornuta* Sond. var. *squamata* (Schltr.) Marais. The remaining nine species have been synonymized.

Wolley Dod (1901) added a new species, *H. tabularis*, that has been retained and Schinz (1910) described four new species. *H. tulbaghensis* Schinz has been retained as a validly published name, but the other three, namely *H. schlechteri* Schinz, *H. chamomillifolia* Schinz, and *H. rudolfii* Schinz have been synonymized to *H. coronopifolia*, *H. digitata* and *H. subulata* respectively (Marais 1970).

Schlechter (1913) described an additional 15 new species, out of which only three species, *H. gariopina*, *H. descurva* and *H. deserticola*, have been retained. *H. nigellifolia*, *H. setacea* and *H. aspera* have been reduced to variety levels as *H. seselifolia* Burch. ex DC. var. *nigellifolia* (Schltr.) Marais, *H. pusilla* var. *setacea* (Schltr.) Marais and *H. scoparia* Burch. ex DC. var. *aspera* (Schltr.) Marais respectively. The remaining species have been synonymized (Marais 1970).

Barnes (1931) described another new species *H. bulbostyla* and during the same year, 1931, Schulz described 22 species (including eleven new species and four varieties). Of the 11 new species, only four species, *H. adpressa*, *H. collina*, *H. remotiflora* and *H. ramosissima*, have

been retained. *H. marlothii* has been reduced to a variety as *H. seselifolia* var. *marlothii* (O. E. Schulz) Marais. *H. maximiliani* var. *agtertuinensis* and *H. sabulata* var. *glabrescens* have been transferred to *H. arenaria* Sond. var. *agtertuinensis* (O. E. Schulz) Marais and *H. arenaria* var. *glabrescens* (O. E. Schulz) Marais respectively (Marais 1970). Many of the species were synonymized (Marais 1970). Adamson (1941) described *H. lanceolata*, which has since been reduced to variety level as *H. pusilla* var. *lanceolata* (Adamson) Marais (Marais 1970). In 1950, Adamson recognised 25 species growing on the Cape Peninsula and apart from other characters, he used seed characters in the keys to the genera and species of *Heliophila*. He also commented that the genus is in need of a detailed revision.

Marais (1964) made several nomenclatural changes (outlined above) and described the new species *H. laciniata*. He combined *Cleome minima* Stephens, *H. pearsonii* O. E. Schulz, *H. pearsonii* var. *prageri* O. E. Schulz, *H. pearsonii* var. *edentata* Hainz and *H. smithii* O. E. Schulz together in the species *H. minima* (Stephens) Marais. He also reduced *H. aspera* Schltr., *H. nigellifolia* Schltr., *H. marlothii* and *H. squamata* Schltr. to varieties. In 1966, he described 11 new species and four new varieties and made eleven new combinations. These new varieties were *H. arenaria* var. *acocksii*, *H. elata* var. *pillansii*, *H. meyeri* var. *minor* and *H. pusilla* var. *macrosperma* and the new species were *H. alpina*, *H. cedarbergensis*, *H. cuneata*, *H. esterhuyseniae*, *H. exima*, *H. filicaulis*, *H. katbergensis*, *H. obibensis*, *H. promontorii*, *H. rimicola* and *H. schulzii*. New combinations made by him were: *H. arenaria* var. *glabrescens*, *H. arenaria* var. *agtertuinensis*, *H. linearis* var. *linearifolia*, *H. linearis* var. *reticulata*, *H. pusilla* var. *setacea* and *H. pusilla* var. *lanceolata*.

Schreiber, in her study on the Brassicaceae of Namibia in 1966, recognised eight species growing in Namibia. Marais (1970) constitutes the latest and most comprehensive study of the Cruciferae of southern Africa to date. He used fruit types in the keys to the genera and species of Brassicaceae and recognized 71 species in the genus *Heliophila*. However, he did not recognise the subdivisions of *Heliophila* as proposed by Sonder (1860), and merged the species into a massive genus without any internal structure.

After this last revision of *Heliophila*, Nordenstam (1980) made some nomenclatural changes. He changed *H. latisiliqua* E. Mey. Ex Sond. to *H. thunbergii* Steud. var. *thunbergii*, and reduced *H. macrostylis* E. Mey. ex Sond. and *H. latisiliqua* E. Mey. ex Sond. var. *macrostylis* (E. Mey. ex Sond.) Marais to variety level, as *H. thunbergii* Steud. var. *macrostylis* (E. Mey. ex Sond.) B.

Nord. In 1982, Hilliard and Burtt described the new species *H. formosa* from KwaZulu-Natal and Pienaar and Nicholas (1988) described the new species *H. cornellsbergia* from the Richtersveld. The latest addition of a new species of *Heliophila* was by Bean (1990), who described the woody species *H. ephemera*.

The names of taxa studied for the present project are based on the study by Marais (1970).

## DECLARATION

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and that I have not previously in its entirety or in part submitted it at any other university for a degree.

Signature M. J. van der Merwe

Date 6<sup>th</sup> February 2003



## CHAPTER THREE: MATERIAL AND METHODS

### 3.1 Material

Herbarium specimens representing 87 of the recognised taxa of *Heliophila* were selected (Table 3.1) and requested on loan from the Bolus (BOL) and Compton (NBG) Herbaria in Cape Town and supplemented by material from National Herbarium Pretoria (PRE). The work by Marais (1970) was used as a basis for selecting the specimens and an effort was made to select specimens annotated or seen by Marais to minimize the likelihood of misidentification. Mature flower buds were removed from herbarium specimen and anthers were carefully dissected from the buds.

### 3.2 Palynological Methods

The taxa included in the present study were awarded taxon numbers, which were used throughout the investigation. Due to inclusion of varieties, the system deviates from the one used by Marais (1970). The reader is therefore cautioned to refer to table 3.1 of the present study to avoid confusion. No suitable specimens or pollen material could be found for the following taxa: [2], [34], [44], [67] and [73].

Both acetolysed and unacetolysed material was used in the present study. Most of the pollen grains used in the SEM study were acetolysed using the following procedure modified from Radford *et al.*, (1974). Anthers were suspended in glacial acetic acid for a period of 1 hr at room temperature, centrifuged and decanted. The anthers were then lightly crushed against the wall of the tube with a glass rod. About 5ml of 9:1 solution of acetic acid anhydrite and sulphuric acid was added to each tube and the tubes were heated in a water bath (100<sup>0</sup> C) for 10 minutes, stirring each a few times with its own glass rod. Specimens were allowed to cool in cold water, rinsed three times with distilled water and then finally rinsed with 95% ethanol.

#### 3.2.1 LM

The specimens listed in Table 3.1 were studied using a light microscope except *H. obibensis*, *H. patens*, *H. concatenata*, *H. tabularis*, *H. leptophylla*, *H. descurva*, *H. katbergensis*, *H. filicaulis*, and *H. esterhuyseniae*, which lacked specimens or suitable pollen grains to be measured. Unacetolysed pollen grains were mounted on slides in glycerine jelly under cover slips and

studied within 48 hours after mounting. Measurements of the polar and equatorial axes were made with the aid of Nikon ECLIPSE E400 light microscope, based on 30 grains per taxon. Only 15 grains of *H. cuneata* were measured, due to limited material. Pictures were taken at fixed magnifications with the aid of a Nikon Coolpix 990 digital camera.

### 3.2.2 SEM

The SEM study formed the core of the investigation and included all specimens that were available and listed in Table 3.1. Acetolysed grains were transferred and mounted on a standard steel stub and air-dried, whereas unacetolysed pollen grains were mounted onto the stubs using double-sided tape. Both acetolysed and unacetolysed material was sputter-coated with a gold-palladium layer and viewed using LEO 1430VP Scanning Electron Microscope. The wall structure of pollen grains was studied and electron micrographs were taken at fixed magnification in order to enable comparisons between the different taxa.

### 3.2.3 TEM

A total of 10 specimens (marked with \* in Table 3.1) were selected for the TEM study. Unacetolysed material was used for TEM analysis. The material was fixed in a 2.5% glutaraldehyde solution in a 0.075M phosphate buffer at pH 7.4 for two days. It was then fixed in a watery 0.1% OsO<sub>4</sub> solution for one hour, dehydrated in a graded acetone series and embedded in epoxy resin. Ultra-thin sections of pollen grains were cut using a glass knife on a Reichert Ultracut microtome. The sections were stained with uranyl acetate and lead citrate, and photographed using a Zeiss EM 109 transmission electron microscope.

### 3.2.4 Analysis of Pollen data set

Pollen morphologists routinely measure polar (P) and equatorial (E) axes and place pollen grains into the size classes defined by Erdtman (1971), Walker and Doyle (1975) and Nilsson and Praglowski (1992). This grouping of the pollen into arbitrary classes may or may not correspond to statistically significant differences between and among the taxa within a data set (Morton & Kincaid, 1995). As a result of this, in the present study pollen variability was determined by using one-way analysis of variance (ANOVA). The ANOVA test was performed for pollen size in terms of polar measurement and equatorial diameter measurements. The measurements for each taxon were entered into the Microsoft Excel package with P and E in separate columns and corresponding with the pollen type in a separate column. The data were imported into the

STATISTICA 5.0 Software package (STATISTICA, Statsoft 1984 – 1995), in which separate analysis were conducted for between main type variations and among main and subtypes variations.

Table 3.1 Specimens used for the palynological studies of the *Heliophila* species (names and arrangement of taxa according to Marais 1970). \* Specimens used for TEM studies. <sup>1</sup> Acetolysed and unacetolysed specimen used for SEM. <sup>2</sup> only unacetolysed specimen used. <sup>3</sup> only acetolysed specimen used for SEM.

TAXON	TAXON No.	COLLECTOR	COLL. No.	HERB.
<i>H. amplexicaulis</i> L.f. <sup>1</sup>	1.1	Compton	11048	NBG
<i>H. obibensis</i> Marais	2.1	-	-	-
<i>H. gariiepina</i> Schltr. <sup>1</sup>	3.1	Thompson & Le Roux	319	NBG
<i>H. namaquana</i> Bolus <sup>1</sup>	4.1	Thompson	2382	NBG
<i>H. bulbostyla</i> Barnes <sup>1</sup>	5.1	Compton	3801	BOL
<i>H. adpressa</i> O. E. Schulz <sup>1</sup>	6.1	Van Niekerk	273	BOL
<i>H. pectinata</i> Burch. ex DC. <sup>1</sup>	7.1	Marais	1416	NBG
<i>H. pubescens</i> Burch. ex Sond. <sup>1</sup>	8.1	Levyns	1643	BOL
<i>H. collina</i> O. E. schulz <sup>1</sup>	9.1	Lavis	19824	BOL
<i>H. laciniata</i> Marais <sup>1</sup>	10.1	Le-Roux	2959	NBG
<i>H. deserticola</i> Schltr. <sup>1</sup>	11.1	Bolus	BH 27352	BOL
<i>H. seselifolia</i> Burch. ex DC. var. <i>seselifolia</i> <sup>1</sup>	12.1	Thomas	s.n.	NBG
<i>H. seselifolia</i> var. <i>nigellifolia</i> (Schltr.) Marais <sup>1</sup>	13.1	Mathews	BH 27360	BOL
<i>H. seselifolia</i> var. <i>marlothii</i> (O. E. Schulz) Marais <sup>1</sup>	14.1	Acocks	24428	PRE
<i>H. variabilis</i> Burch. ex DC. <sup>1</sup>	15.1	Leighton	1198	BOL
<i>H. minima</i> (Stephen) Marais <sup>1</sup>	16.1	Pearson	7868	BOL
<i>H. crithmifolia</i> Willd. <sup>1</sup>	17.1	Hutchinson	373	BOL
<i>H. trifurca</i> Burch. ex DC. <sup>1</sup>	18.1	Schlieben	8904	BOL
* <i>H. latisiliqua</i> E. Mey. ex Sond. var. <i>latisiliqua</i> <sup>1</sup>	19.1	Esterhuysen	5989	BOL

TAXON	TAXON No.	COLLECTOR	COLL. No.	HERB.
<i>H. latisiliqua</i> var. <i>macrostylis</i> Marais <sup>1</sup>	20.1	Page	14361	BOL
<i>H. pinnata</i> L.f., <sup>1</sup>	21.1	Leipoldt	3972	BOL
<i>H. pusilla</i> L.f. var. <i>pusilla</i> <sup>1</sup>	22.1	Esterhuysen	20334	BOL
<i>H. pusilla</i> var. <i>macrosperma</i> Marais <sup>1</sup>	23.1	Thompson	2324	NBG
<i>H. pusilla</i> var. <i>setacea</i> (Schltr.) Marais <sup>1</sup>	24.1	Stevens & Gloves	8696	BOL
<i>H. pusilla</i> var. <i>lanceolata</i> (Adamson) Marais <sup>1</sup>	25.1	Pillans	3907	BOL
<i>H. patens</i> Oliv.	26.1	Schlechter	3482	PRE
<i>H. diffusa</i> (Thunb.) DC. var. <i>diffusa</i> <sup>1</sup>	27.1	Walgate	s.n.	BOL
<i>H. diffusa</i> (Thunb.) DC. var. <i>diffusa</i> <sup>3</sup>	27.2	Pillans	s.n.	BOL
<i>H. diffusa</i> var. <i>flacca</i> (Sond.) Marais <sup>1</sup>	28.1	Parker	4247	BOL
<i>H. pendula</i> Willd. <sup>1</sup>	29.1	Lewis	BH 27365	BOL
* <i>H. meyeri</i> Sond. var. <i>meyeri</i> <sup>1</sup>	30.1	Pillans	4033	BOL
<i>H. meyeri</i> var. <i>minor</i> Marais <sup>1</sup>	31.1	Jooste	171	NBG
<i>H. coronopifolia</i> L. <sup>1</sup>	32.1	Esterhuysen	23371	BOL
<i>H. concatenata</i> Sond. <sup>1</sup>	33.1	Compton	18567	NBG
<i>H. tabularis</i> W. Dod.	34.1	-	-	-
<i>H. acuminata</i> (Eckl. & Zeyh.) Steud. <sup>1</sup>	35.1	Leipoldt	3935	BOL
<i>H. macowaniana</i> Schltr. <sup>1</sup>	36.1	Leighton	1098	BOL
<i>H. promontorii</i> Marais <sup>1</sup>	37.1	Compton	20177	BOL
* <i>H. digitata</i> L.f. <sup>1</sup>	38.1	Schlechter	4944	BOL
<i>H. digitata</i> L.f. <sup>3</sup>	38.2	Compton	15075	NBG
<i>H. refracta</i> Sond. <sup>1</sup>	39.1	Bolus	12598	BOL
<i>H. schulzii</i> Marais <sup>1</sup>	40.1	Schlechter	11147	BOL
<i>H. lactea</i> Schltr. <sup>1</sup>	41.1	Mathews	BH 27345	BOL

TAXON	TAXON No.	COLLECTOR	COLL. No.	HERB.
<i>H. linoides</i> Schltr. <sup>1</sup>	42.1	Duthie	1586A	BOL
<i>H. remotiflora</i> O. E. Schulz <sup>1</sup>	43.1	Perold	1528	BOL
<i>H. leptophylla</i> Schltr.	44.1	-	-	-
<i>H. arenosa</i> Schltr. <sup>1</sup>	45.1	Mauve & Oliver	194	NBG
<i>H. arenaria</i> Sond. var. <i>arenaria</i> <sup>2</sup>	46.1	Leipoldt	20756	BOL
<i>H. arenaria</i> var. <i>acocksii</i> Marais <sup>2</sup>	47.1	Leipoldt	3960	BOL
<i>H. arenaria</i> var. <i>glabrescens</i> (O.E. Schulz) Marais <sup>2</sup>	48.1	Compton	15019	NBG
<i>H. arenaria</i> var. <i>agtertuinensis</i> (O.E. Schulz) Marais <sup>2</sup>	49.1	Johnson	185	NBG
<i>H. descurva</i> Schltr. <sup>2</sup>	50.1	Taylor	4036	NBG
<i>H. affinis</i> Sond. <sup>2</sup>	51.1	Schlechter	11171	BOL
<i>H. africana</i> (L.) Marais <sup>2</sup>	52.1	Eames	s.n.	BOL
<i>H. brassicaefolia</i> Eckl. & Zeyh. <sup>2</sup>	53.1	Galpin	1710	BOL
<i>H. cinerea</i> Marais <sup>2</sup>	54.1	Compton	6020	NBG
<i>H. cuneata</i> Marais <sup>2</sup>	55.1	Taylor	8529	BOL
<i>H. linearis</i> (Thunb.) DC. var. <i>linearis</i> <sup>2</sup>	56.1	Burt-Davy	14269A	BOL
<i>H. linearis</i> var. <i>linearifolia</i> (Burch. ex DC.) Marais <sup>2</sup>	57.1	Parker	s.n.	BOL
<i>H. linearis</i> var. <i>reticulata</i> (Eckl. & Zeyh.) Marais <sup>2</sup>	58.1	Muir	1442	BOL
<i>H. cornuta</i> Sond. var. <i>cornuta</i> <sup>2</sup>	59.1	Bond	1062	NBG
<i>H. cornuta</i> var. <i>squamata</i> (Schltr.) Marais <sup>2</sup>	60.1	Leighton	21194	BOL
<i>H. elata</i> Sond. var. <i>elata</i> <sup>2</sup>	61.1	Barker	4745	BOL
<i>H. elata</i> var. <i>pillansii</i> Marais <sup>2</sup>	62.1	Lewis	BH 27109	BOL
<i>H. sabulata</i> Burch. ex DC. <sup>2</sup>	63.1	Levyns	10140	BOL
* <i>H. suavissima</i> Burch. ex DC. <sup>1</sup>	64.1	Esterhuysen	20561	BOL

TAXON	TAXON No.	COLLECTOR	COLL. No.	HERB.
<i>H. carnosa</i> (Thunb.) Steud. <sup>2</sup>	65.1	Leipoldt	3911	BOL
* <i>H. rigidiuscula</i> Sond. <sup>2</sup>	66.1	Pegler	259	BOL
<i>H. katbergensis</i> Marais	67.1	-	-	-
<i>H. exima</i> Marais <sup>2</sup>	68.1	McDonald	724	NBG
<i>H. scandens</i> Harv. <sup>1</sup>	69.1	Thode	9242	NBG
<i>H. glauca</i> Burch. ex DC. <sup>1</sup>	70.1	Esterhuysen	6942	BOL
* <i>H. glauca</i> Burch. ex DC. <sup>1</sup>	70.2	Bond	222	NBG
<i>H. brachycarpa</i> Meisn. <sup>1</sup>	71.1	Compton	19642	NBG
<i>H. macrosperma</i> Burch. ex DC. <sup>1</sup>	72.1	Bayliss	2257	NBG
<i>H. filicaulis</i> Marais	73.1	-	-	-
* <i>H. tulbaghensis</i> Schinz <sup>1</sup>	74.1	Esterhuysen	9080	BOL
<i>H. rimicola</i> Marais <sup>1</sup>	75.1	Erasmus	131	NBG
<i>H. esterhuyseniae</i> Marais <sup>1</sup>	76.1	Esterhuysen	30359	BOL
* <i>H. dregeana</i> Sond. <sup>1</sup>	77.1	Leighton	1263	BOL
<i>H. tricuspidata</i> Schltr. <sup>1</sup>	78.1	Oliver	11181	NBG
<i>H. cedarbergensis</i> Marais <sup>1</sup>	79.1	Esterhuysen	350555	BOL
<i>H. scoparia</i> Burch. ex DC. var. <i>scoparia</i> <sup>1</sup>	80.1	Kerfoot	K5425	NBG
* <i>H. scoparia</i> var. <i>aspera</i> (Schltr.) Marais <sup>1</sup>	81.1	Compton	12875	NBG
<i>H. callosa</i> (L.f.) DC. <sup>1</sup>	82.1	Wolley Dod	474	BOL
* <i>H. nubigena</i> Schltr. <sup>1</sup>	83.1	Esterhuysen	14043	BOL
<i>H. alpina</i> Marais <sup>3</sup>	84.1	Hilliard & Burt	7088	PRE
<i>H. elongata</i> (Thunb.) DC. <sup>3</sup>	85.1	Kensit	s.n.	BOL
<i>H. elongata</i> (Thunb.) DC. <sup>3</sup>	85.2	Walters	421	NBG
<i>H. macra</i> Schltr. <sup>3</sup>	86.1	Burman	1125	BOL
<i>H. ramosissima</i> O. E Schunz <sup>3</sup>	87.1	Guthrie	3735	NBG

TAXON No. = Taxon number; COLL. No. = Collector number; HERB. = Herbarium

### 3.3 Numerical Taxonomy (Phenetic analyses)

Numerical taxonomy was introduced by Sneath and Sokal (1962) in order to facilitate arrangement of biological materials at the species level. The phenetic approach is one of the methods of numerical taxonomy in which a tree diagram (phenogram) is constructed by considering phenotypic similarities. The characters of all individuals known as Operational Taxonomic Units (OTUs) are compared to one another based on degrees of similarity and/or dissimilarity and expressed in the form of a cluster diagram (Stuessy, 1990).

Sokal and Sneath (1963) indicated that useful phenetic or taxonomic information may already exist and may merely require extraction from literature or may have to be discovered entirely or partly *de novo*. In the present study, the revision of *Heliophila* by Marais (1970) was used as a basis to identify, extract and code morphological characters from which a complete character list was compiled (Table 3.2). According to Sokal and Sneath (1963), at least 60 characters are desirable for a cluster analysis and less than 40 characters would not be acceptable. A total of 72 characters were used in the present study. Qualitative characters were coded for phenetic analysis by identifying character variation for each character among *Heliophila* taxa. The observed variation was then partitioned into discrete characters and their component states. The different taxa were coded for the presence or absence of such characters, 1 for presence and 0 for absence (Appendix III). The data were prepared for analysis by creating a data file matrix in the Microsoft office Excel package, which was then imported into the STATISTICA 5.0 Software package (STATISTICA, Statsoft 1984 – 1995) and NTSYS pc 21 computer programs.

Cluster analysis (CA) was carried out to assess and analyse the resemblances between *Heliophila* species using both the STATISTICA and NTSYS pc 21 computer programs. *H. tabularis* and *H. concatenata* were excluded as Operational Taxonomic Units (OTUs), because for *H. tabularis* some of the important characters were missing, whereas *H. concatenata* represents a group species with considerable internal variation. The cluster analysis does not assume any *a priori* grouping of objects or variables, but it groups objects or variables on the basis of overall similarity among them. It does not provide reliable evidence of evolutionary relationships either, or form a sound basis of phylogenetic classification. However, it still has several important roles in taxonomic work, particularly in identification and determining whether samples of specimens comprise one or more distinct entities. When dealing with qualitative data using character states coded 0 or 1, the association coefficient, which measure the agreement between pairs of OTU's

over an array of two-state or multi-state characters, is used. There are several coefficients that can be used such as the simple matching coefficient, which takes absences into account and the Jaccard coefficient, which does not take absences into account. According to Sneath and Sokal (1973), the simple matching coefficient is equal to the squared Euclidean distance based on unstandardised character states, which can take the value of 0 or 1. Therefore in the present study, the squared Euclidean distance was employed as a measure of similarity/dissimilarity in the STATISTICA computer program, whereas the simple matching coefficient was employed as a measure of similarity in the NTSYS pc 21 computer program. This measurement of distance or similarity acts as the distance of similarity or dissimilarity to link particular taxa together or cluster them. The amalgamation linkage rule of unweighted pair-group method using arithmetic average (UPGMA) was used in both computer programs, resulting in similar phenograms depicting similarities between the OTUs (Figure 4.6). The UPGMA model is based on joining OTUs to existing clusters on the basis of their average (mean) distance to the other members of that cluster (Quicke, 1993). The average linkage method was chosen because it is known to give a higher value for the cophenetic correlation coefficient than other methods. The higher the correlation, the less distortion occurs and presumably the better the classification. The cophenetic correlation coefficient is a measure of agreement between the similarity values implied by the phenogram and those of the original similarity matrix and in the present study the value was obtained by using the NTSYS pc 21 programme.

Table 3.2: A list of morphological characters which were used in the assessment of variation among selected *Heliophila* taxa for cluster analysis.

CHARACTER No.	CHARACTER DESCRIPTION	CHARACTER STATE
1	Plant herb	Present/absent
2	Plant shrub	Present/absent
3	Plant annual	Present/absent
4	Plant perennial	Present/absent
5	Plant with annual shoots	Present/absent
6	Leaf simple	Present/absent
7	Leaf compound	Present/absent



CHARACTER No.	CHARACTER DESCRIPTION	CHARACTER STATE
8	Leaf shape linear	Present/absent
9	Leaf shape lanceolate	Present/absent
10	Leaf shape filiform	Present/absent
11	Leaf shape oblanceolate	Present/absent
12	Leaf shape linear-oblanceolate	Present/absent
13	Leaf shape linear-filiform	Present/absent
14	Leaf margin entire	Present/absent
15	Leaf margin lobed	Present/absent
16	Leaf margin pinnate	Present/absent
17	Leaf margin pinnatirite	Present/absent
18	Leaf glabrous all over	Present/absent
19	Leaf hairy all over	Present/absent
20	Leaf hairy adaxially	Present/absent
21	Leaf with two minute stipules present	Present/absent
22	Leaf with two minute stipules absent	Present/absent
23	Sepal shape oblong	Present/absent
24	Sepal shape elliptic	Present/absent
25	Sepal shape oblong-ovate	Present/absent
26	Sepal shape linear-oblong	Present/absent
27	Sepal shape obovate-oblong	Present/absent
28	Two outer sepals cucullate	Present/absent
29	Two outer sepals thickened	Present/absent
30	Two outer sepals horned	Present/absent
31	Two inner sepals saccate or not	Present/absent

CHARACTER No.	CHARACTER DESCRIPTION	CHARACTER STATE
32	Sepal margins membranous	Present/absent
33	Petals papillate	Present/absent
34	All petals with appendages	Present/absent
35	Two petals with appendages	Present/absent
36	All petals without appendages	Present/absent
37	Short filaments < 3 mm long	Present/absent
38	Long filaments < 4 mm long	Present/absent
39	Filaments appendages present in all stamens	Present/absent
40	Filaments appendages present in two short stamens	Present/absent
41	Filaments appendages absent	Present/absent
42	All filaments papillated near base	Present/absent
43	Two short filaments papillated	Present/absent
44	Ovary shape linear	Present/absent
45	Ovary shape oblong	Present/absent
46	Ovary shape obovate	Present/absent
47	Ovary shape linear-oblong	Present/absent
48	Ovary with or without a stipe	Present/absent
49	Ovules number less than 20 per ovary	Present/absent
50	Stigma shape capitate	Present/absent
51	Fruit shape linear	Present/absent
52	Fruit shape subcylindrical	Present/absent
53	Fruit shape ovate	Present/absent
54	Fruit shape lanceolate	Present/absent
55	Fruit shape elliptic-lanceolate	Present/absent

CHARACTER No.	CHARACTER DESCRIPTION	CHARACTER STATE
56	Fruit shape linear-oblong	Present/absent
57	Fruit shape oblong	Present/absent
58	Fruit with a stipe	Present/absent
59	Fruit margin entire	Present/absent
60	Fruit margins moniliform/submoniliform	Present/absent
61	Fruit with 1 vein	Present/absent
62	Fruit with 1 to 3 veins	Present/absent
63	Fruit with 3 veins	Present/absent
64	Fruit venation reticulate	Present/absent
65	Seed shape oblong	Present/absent
66	Seed shape subcircular	Present/absent
67	Seed shape circular	Present/absent
68	Seed shape oval	Present/absent
69	Seed shape oval-elliptic	Present/absent
70	Seed narrowly margined	Present/absent
71	Seed with winged margins	Present/absent
72	Seed with immarginate margins	Present/absent

## CHAPTER FOUR RESULTS

### 4.1 Palynological Results

#### 4.1.1 Size and shape

The size, shape and type of pollen grains of each taxon are summarized in Table 4.1. The method used and duration of preparation may cause considerable variations in the shape of pollen grains. However, in the present study, the shape of the pollen grains was determined by studying unacetolysed pollen grains with the LM and all the samples were treated identically. It is therefore reasonable to regard the observed shapes as trustworthy. The shape of the pollen grains of *Heliophila* are prolate (Figure 4.1A, C—E) and can be subdivided into subprolate, euprolate and perprolate, with the majority of taxa having a euprolate shape. The polar axis ranges from 15.00—52.50  $\mu\text{m}$  and the equatorial diameter from 11.25—33.00  $\mu\text{m}$ . The descriptions of the pollen grain shape are based on the polar view outline (amb) and /or the equatorial view. During the present study, shape classes, as revised by Walker and Doyle (1975), were used. The pollen grains of all the *Heliophila* taxa studied are tricolpate (Figure 4.1B)

#### 4.1.2 Description of pollen types

The description terminology of Punt *et al.* (1994) was used in this study. Two different types of tectum sculpturing were observed and their descriptions were based on LM, SEM and TEM results. The tectum is either psilate or psilate micro-perforate, with suprategal spinules (Figures 4.1 C—H) or micro-reticulate to reticulate with or without suprategal sculpturing (Figures 4.2—4.4). These two types are classified as: -

Pollen Type A            Psilate or psilate micro-perforate with suprategal spinules

Pollen Type B            Micro-reticulate to reticulate, with or without suprategal spinules

Pollen type A is by far the most common type among the taxa examined (Table 4.1). Pollen type B may again be subdivided into three subtypes, with relative demarcations.

**Pollen type A      Tectum psilate or psilate micro-perforate with suprategal spinules (Figure 4.1)**

**LM**

Pollen grains are relatively large (polar axis: 26.25—52.50  $\mu\text{m}$  and equatorial diameter: 15.00—33.00  $\mu\text{m}$ ). The shape of the pollen grains ranges from subprolate to perprolate, with the majority being euprolate and perprolate, elliptic in equatorial view (Figures 4.1A, C—E) and rounded in polar view (Figure 4.1B).

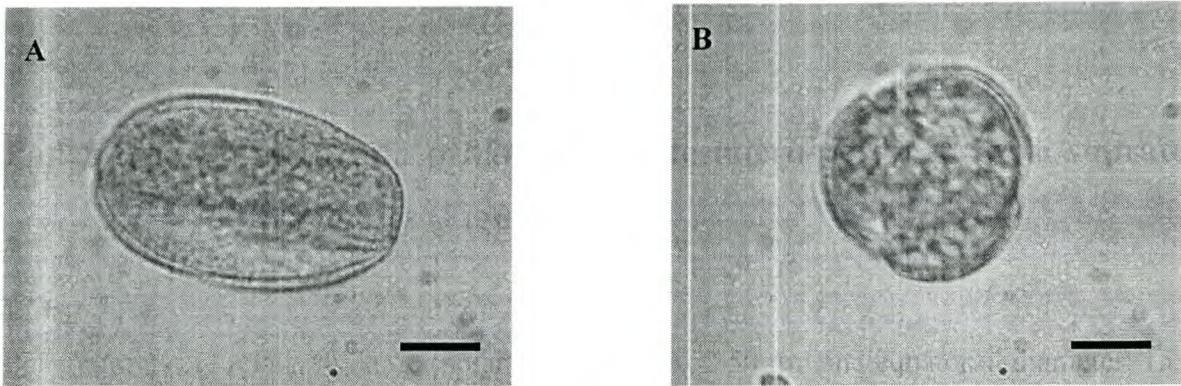


Figure 4.1(A & B) *Heliophila* pollen grains, type A. LM: - (A) equatorial view of pollen grain of *H. arenosa* (Mauve & Oliver 194), (B) polar view of pollen grain of *H. arenosa* (Mauve & Oliver 194). Scale bars A & B = 10  $\mu\text{m}$ .

**SEM**

**Pollen** tectate (Figures 4.1 I & J). **Tectum** psilate, with or without perforations and with numerous or scattered conical, suprategal spinules. The colpi appear as long furrows that are parallel to the polar axis and stretches along almost the entire length of the grain. The colpi are always fairly sunken (Figures 4.1 C—E).

**TEM (Figures 4.1 I & J)**

**Exine** 0.9—1.0  $\mu\text{m}$  thick, consisting of comparatively thick ectexine (suprategal spines, tectum, columellae, foot layer), a much thinner endexine and thicker intine (Figures 4.1 I & J). **Suprategal spinules** triangular, apices acute. **Tectum** 0.15—0.20  $\mu\text{m}$  thick, slightly thicker than foot layer, continuous, outer and inner surfaces smooth to undulating. **Columellae** well developed, half of the total thickness of the exine, simpli-columellate; columellae 0.45—0.50  $\mu\text{m}$  in height, erect, cylindrical in cross section; intercolumnellar spaces broad and filled with a dark-stained substances. **Foot layer** 0.08—0.15  $\mu\text{m}$  thick, uneven and continuous. **Endexine** 0.15  $\mu\text{m}$ , continuous, undulating, black in colour. **Intine** about twice as thick as foot layer, continuous, even to undulate.

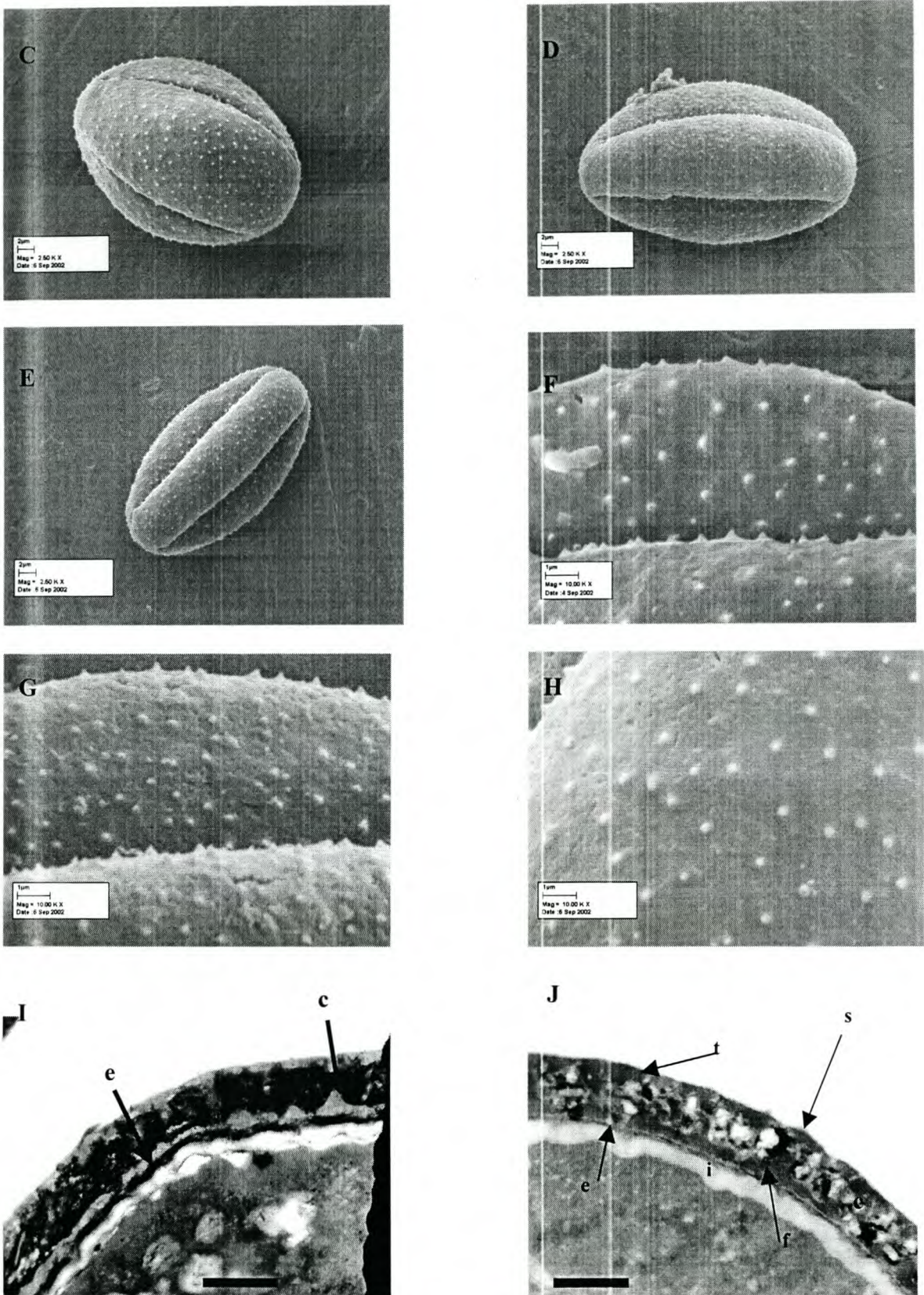


Figure 4.1(C—J) *Heliophila* pollen grains, type A. SEM: - (C) pollen grain of *H. seselifolia* var. *nigellifolia* (Mathews s.n.), (D) pollen grain of *H. patens* (Schlechter 3482), (E) pollen grain of *H. pusilla* var. *macrosperma* (Thompson 2324), (F) mesocolpium of the pollen grain of *H. collina* (Lavis 19824), (G) mesocolpium of the pollen grain of *H. digitata* (Schlechter 4944), (H) mesocolpium of the pollen grain of *H. seselifolia* var. *nigellifolia* (Mathews s.n.). TEM: - (I) Wall section of the pollen grain of *H. rigidiuscula* (Pegler 259). (J) Wall section of the pollen grain of *H. suavissima* (Esterhuysen 20561) showing tectum (t), columellae (c), foot layer (f), endexine (e) intine (i) and spinules partially (s). Scale bars I & J = 1  $\mu$ m.

**Pollen type B** Tectum micro-reticulate to reticulate, with or without supratectal spinules (Figures 4.2 to 4.4).

**Pollen type B<sub>1</sub>** Tectum micro-reticulate, homobrochate isodiametrically perforated without supratectal structures (Figure 4.2)

## LM

Pollen grains smallest of all the different types (polar axis: 15.00—27.50 (-35)  $\mu\text{m}$  and equatorial diameter: 11.25—21.25  $\mu\text{m}$ ). Pollen grain shape ranges from subprolate to euprolate, elliptic to oval in equatorial view (Figures 4.2 A & C) and rounded in polar view (Figure 4.2B).

## SEM

**Pollen** semitectate (Figures 4.2 C—D). **Tectum** micro-reticulate to reticulate, homobrochate, isodiametrically perforated, sparsely granular. **Lumina** are round and less than 1  $\mu\text{m}$  in diameter (Fig 4.2 D). **Muri** smooth, 0.3  $\mu\text{m}$  thick. Colpi appears as long thin furrows that are parallel to the polar axis and stretches along almost the entire length of the grain. The colpi are fairly sunken (Figure 4.2 c).

## TEM (Figures 4.2 E & F)

**Exine:** 0.5—0.6  $\mu\text{m}$  thick, consisting of comparatively thick ectexine (tectum, columellae, foot layer), a much thinner endexine and thin intine. **Tectum:** 0.15  $\mu\text{m}$  thick, almost of the same thickness as the foot layer, discontinuous, outer and inner surfaces smooth to undulating. **Columellae** well-developed, occupying almost half of the total thickness of the exine, simpli-columellate; columellae 0.3  $\mu\text{m}$  in height, erect, cylindrical in cross-section; intercolumnellar spaces broad and empty in most areas. **Foot layer:** 0.15  $\mu\text{m}$  thick, uneven and continuous. **Endexine** continuous, but not as prominent as in other pollen types. **Intine:** 0.075—0.08  $\mu\text{m}$  thick, continuous, even to undulate.

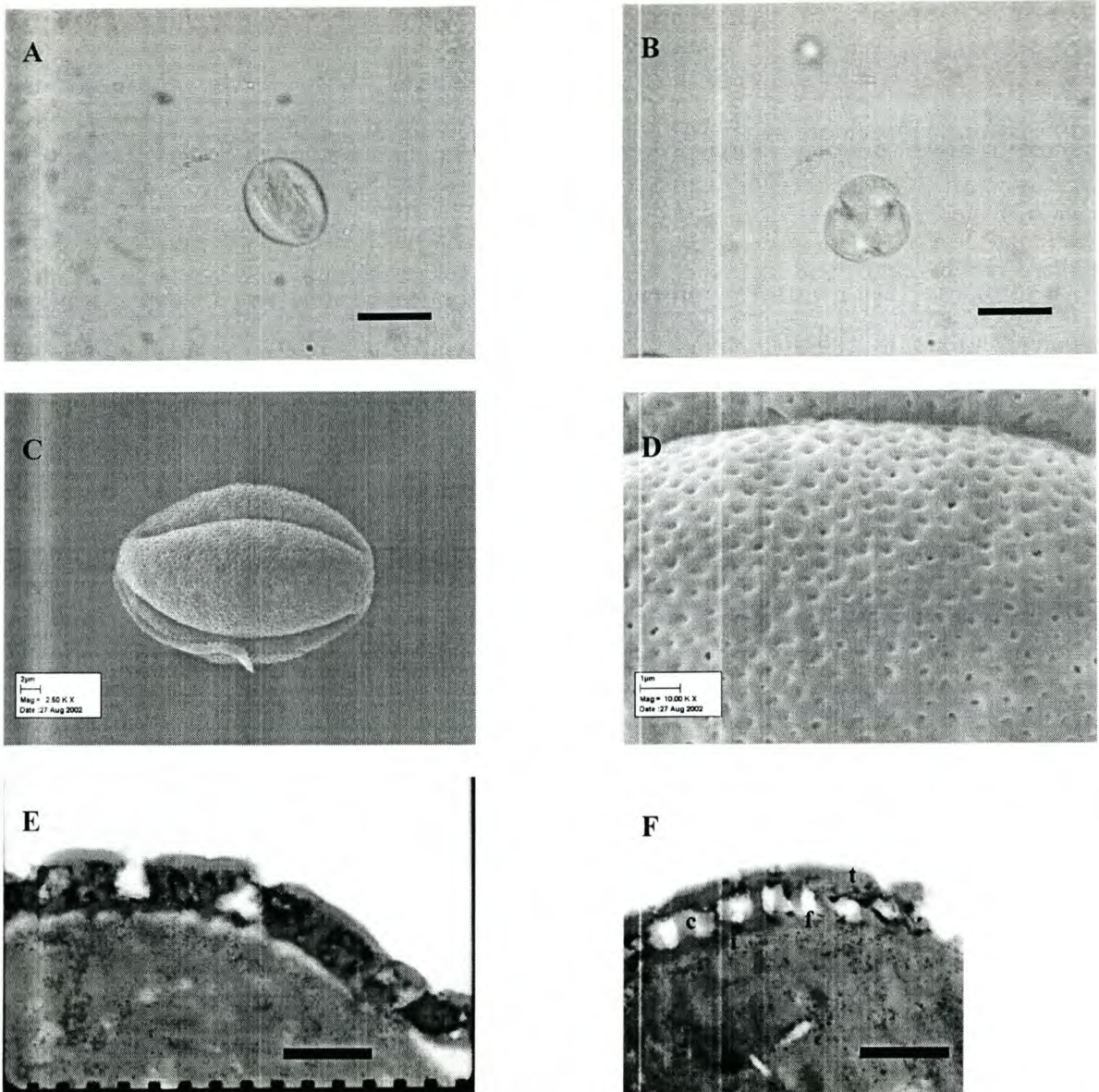


Figure 4.2 (A—F) *Heliophila* pollen grains, subtype B<sub>1</sub>. LM: - (A) equatorial view of pollen grain of *H. brachycarpa* (Compton 19649), (B) polar view of pollen grain of *H. brachycarpa* (Compton 19649). SEM: - (C) pollen grain of *H. scandens* (Thode 9242), (D) mesocolpium of the pollen grain of *H. scandens* (Thode 9242). TEM: - (E & F) Wall sections of pollen grains of *H. glauca* (Bond 222) showing tectum (t), columellae (c), foot layer (f) and intine (i). Scale bars A & B = 10 µm, E & F = 1 µm.

**Pollen Type B<sub>2</sub> Tectum micro-reticulate to reticulate, heterobrochate, with numerous supratectal spinules near colpi. (Figure 4.3)**

#### LM

Pollen grains relatively smaller than those of Pollen Type A, (polar axis: 26.25—42.50 µm and equatorial diameter: 17.50—33.00 µm). The shape of the pollen grains range from subprolate to euprolate, with the majority being euprolate, elliptic in equatorial view (Figures 4.3A, C & D) and rounded in polar view (Figure 4.3B).



## SEM

**Pollen** semitectate (Figures 4.3 C—G). **Tectum** micro-reticulate to reticulate, heterobrochate, with numerous suprategal spinules near the colpi (Figure 4.3F). **Lumina** mostly angular and less than 1  $\mu\text{m}$  in diameter (Figures 4.3 E—G). **Muri** smooth, 0.5—0.6  $\mu\text{m}$  thick. Colpi long thin furrows, parallel to the polar axis and stretches along almost the entire length of the grain. The colpi are fairly sunken (Figures 4.3 C & D).

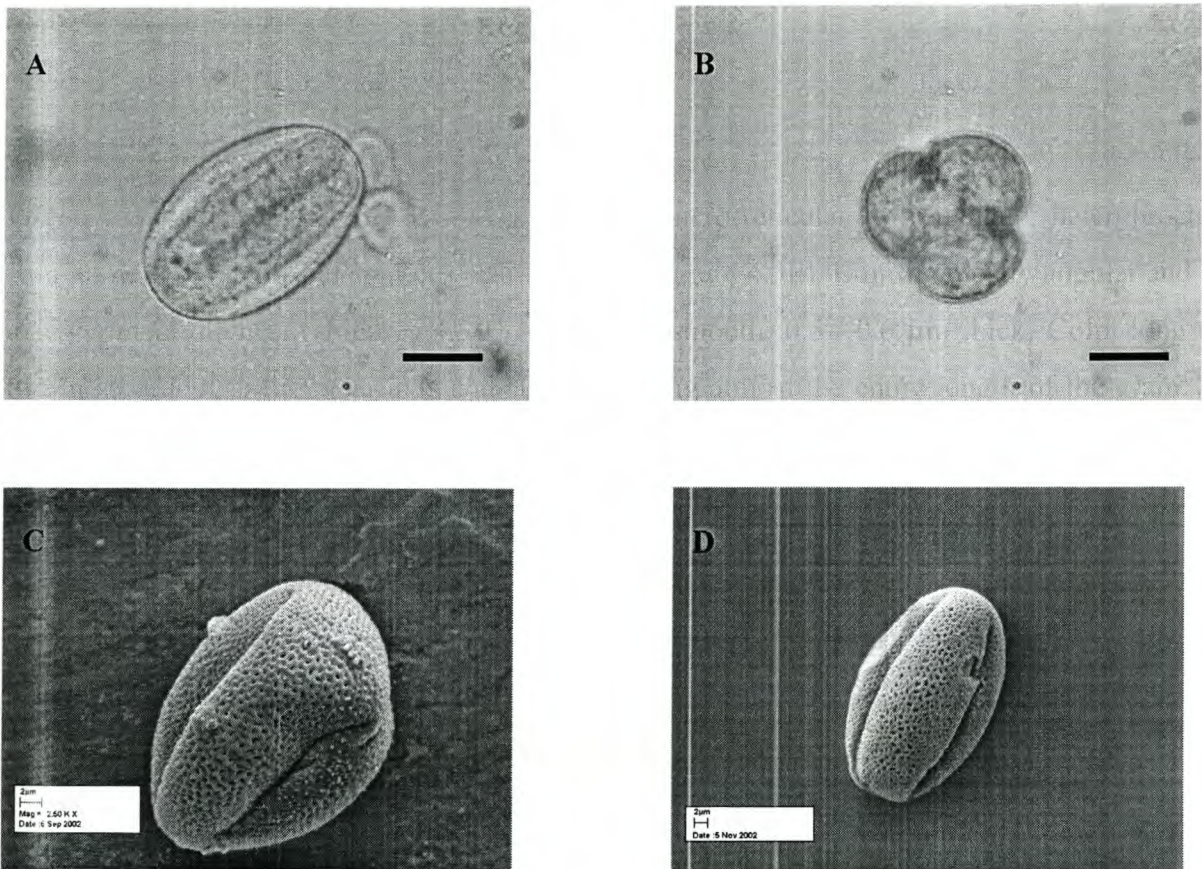


Figure 4.3 (A—D) *Heliophila* pollen grains, subtype B<sub>2</sub>. LM: - (A) equatorial view of the pollen grain of *H. pendula* (Lewis s.n.), (B) polar view of the pollen grain of *H. meyeri* var. *minor* (Jooste 171). SEM: - (C) pollen grain of *H. diffusa* var. *diffusa* (Pillans s.n.), (D) pollen grain of *H. pendula* (Lewis s.n.), Scale bars A & B = 10  $\mu\text{m}$ .

## TEM (Figure 4.3 H)

**Exine:** 0.7—0.8  $\mu\text{m}$  thick, consisting of comparatively thick ectexine (suprategal spines, tectum, columellae, foot layer), a much thinner endexine and thin intine. Suprategal spinules triangular, apices acute. **Tectum:** 0.15  $\mu\text{m}$  thick, slightly thicker than foot layer, discontinuous, outer and inner surfaces smooth to undulating. **Columellae** well-developed, more than half of total thickness of the exine, simpli-columellate; columellae 0.42  $\mu\text{m}$  in height, erect, cylindrical in

cross section; intercolumnellar spaces broad and partially filled with a dark-stained substances. **Foot layer:** 0.11  $\mu\text{m}$  thick, uneven and continuous. **Endexine:** 0.07  $\mu\text{m}$  thick, continuous, black in colour. **Intine:** 0.07—0.14  $\mu\text{m}$ , continuous, even to undulate.

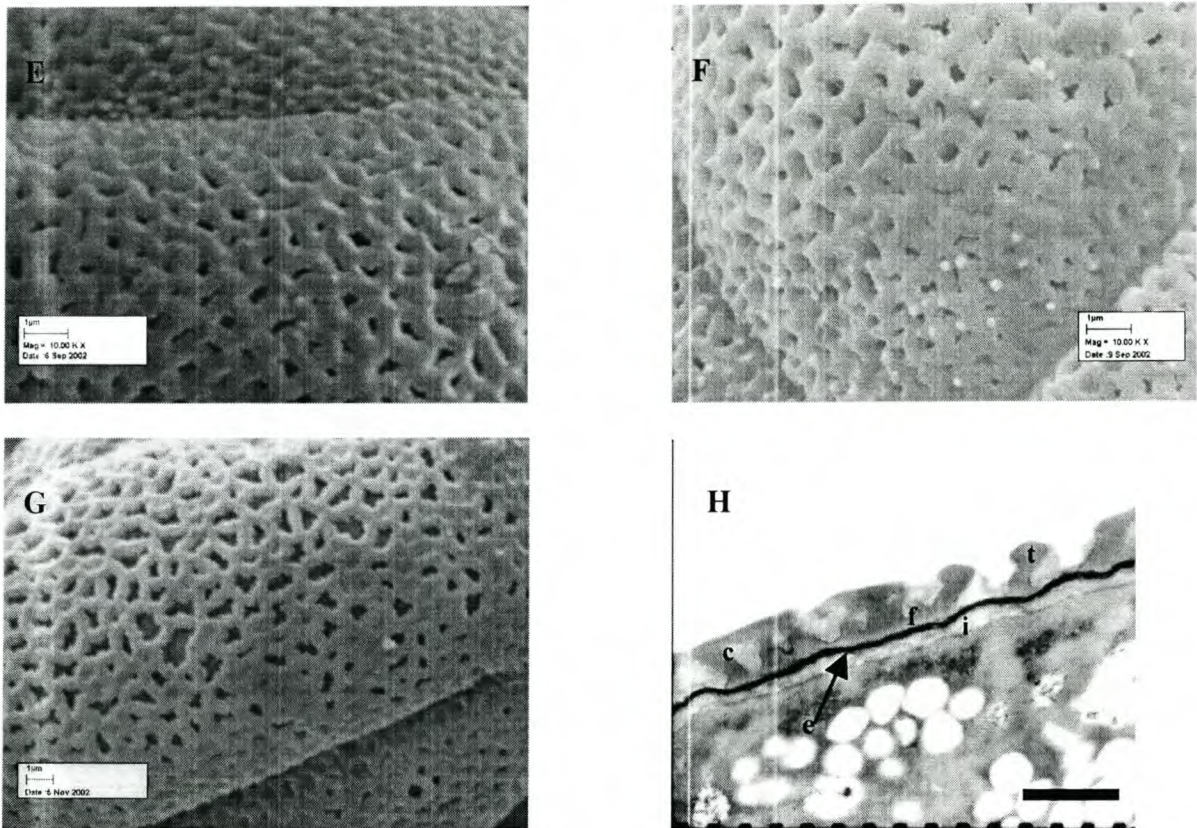
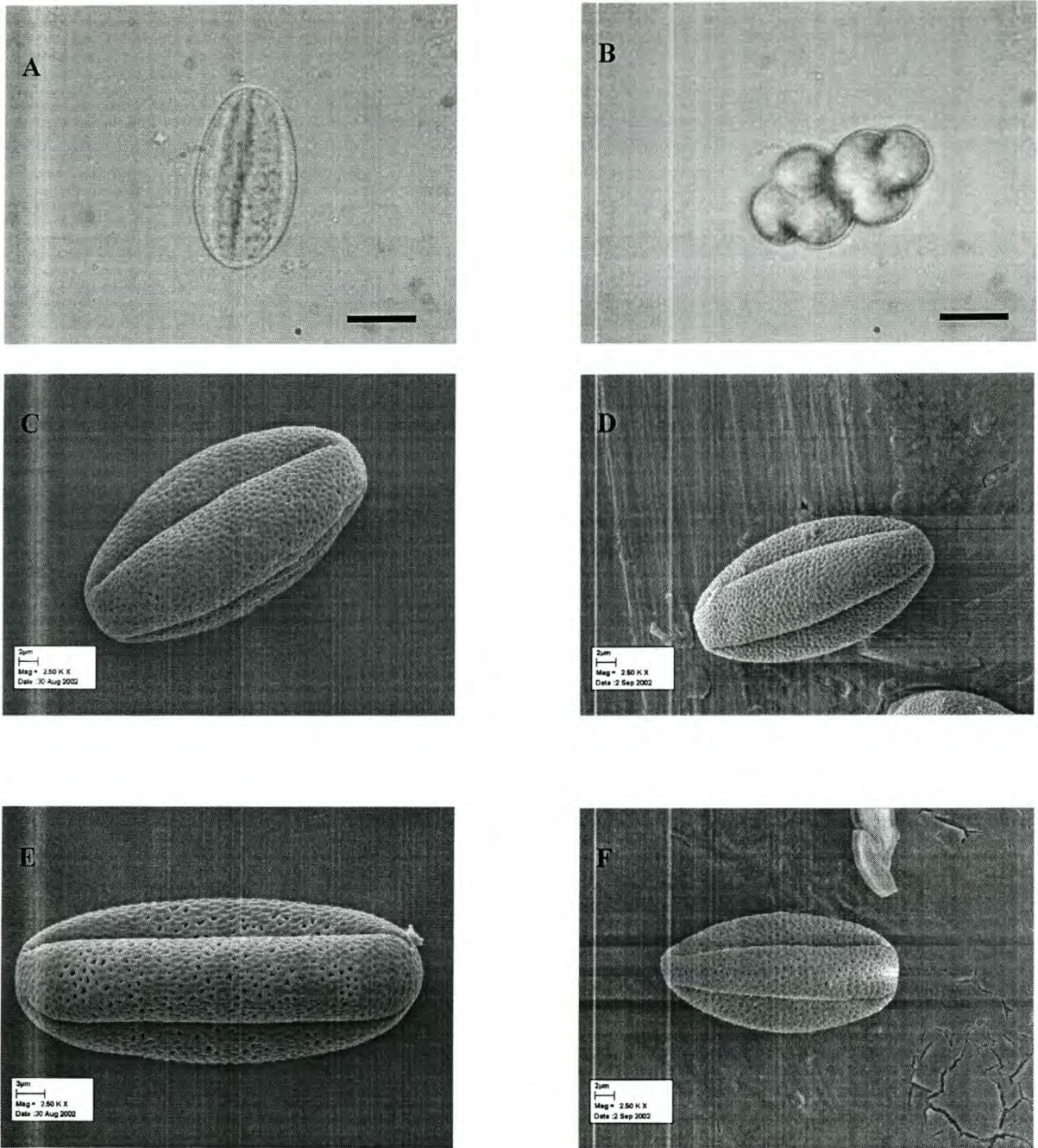


Figure 4.3 (E–I) *Heliophila* pollen grains, subtype B<sub>2</sub>cont. SEM (E) mesocolpium of the pollen grain of *H. diffusa* var. *diffusa*. (Pillans s.n.), (G) mesocolpium of the pollen grain of *H. diffusa* var. *diffusa*. (Pillans s.n.), (G) mesocolpium of the pollen grain of *H. pendula* (Lewis s.n.). TEM: - (H) wall section of the pollen grain of *H. meyeri* var. *meyeri* (Pillans 4033) showing tectum (t), columellae (c), foot layer (f), endexine (e) and intine (i). Scale bar H = 1  $\mu\text{m}$ .

Pollen type B <sub>3</sub>	Tectum micro-reticulate to reticulate, heterobrochate, with few supratectal spinules/granulae scattered all over grains (Figure 4.4)
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## LM

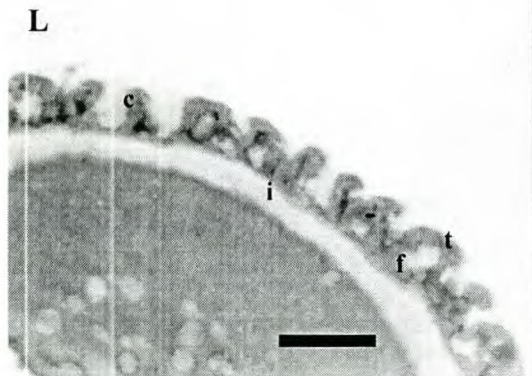
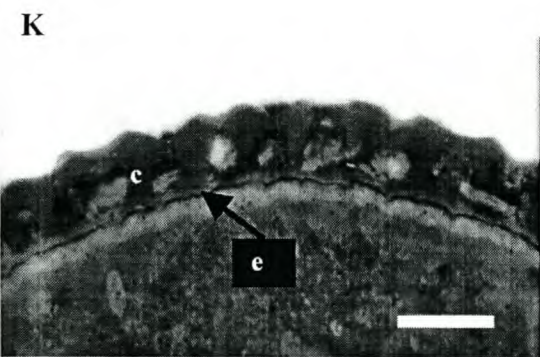
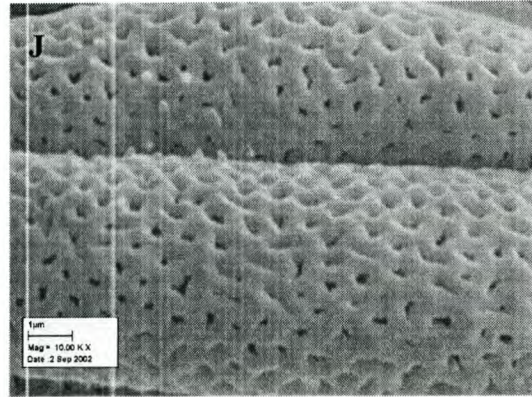
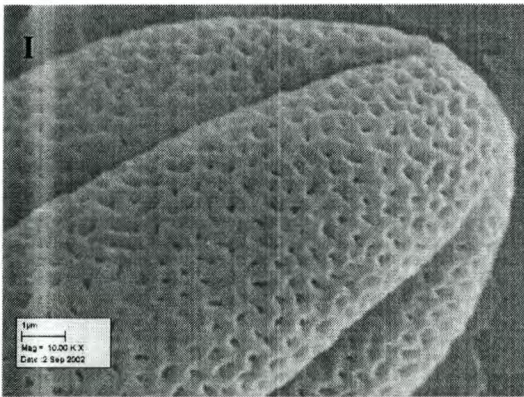
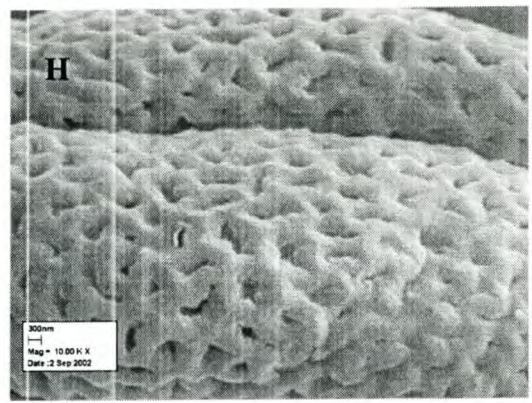
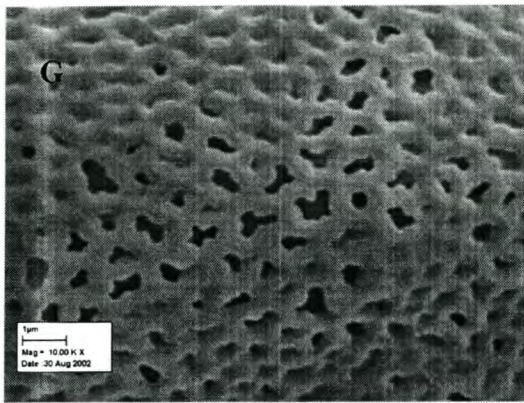
Pollen grains measure 22.50—37.50 (-45)  $\mu\text{m}$  in polar axis and 13.75—25.00 (-30)  $\mu\text{m}$  in equatorial diameter. The shape of the pollen grains ranges from subprolate to perprolate, with the majority being euprolate, elliptic in equatorial view (Figure 4.4A, B–F) and rounded in polar view (Figure 4.4B).



Figures 4.4 (A—F) *Heliophila* pollen grains, subtype B<sub>3</sub>. LM: - (A) equatorial view of the pollen grain of *H. cedarbergensis* (Esterhuysen 35055), (B) polar view of the pollen grains of *H. cedarbergensis* (Esterhuysen 35055). SEM: - (C) pollen grain of *H. tulbaghensis* (Esterhuysen 9080), (D) pollen grain of *H. dregeana* (Leighton 1263), (E) pollen grain of *H. rimicola* (Erasmus 131), (F) pollen grain of *H. nubigena* (Esterhuysen 14043), Scale bars A & B = 10 µm.

## SEM

**Pollen** semitectate (Figures 4.4 C—J). **Tectum** micro-reticulate to reticulate, heterobrochate with sparsely scattered supratectal spinules or granules. **Lumina** mostly angular and less than 1 µm in diameter. (Figures 4.4 G—J). **Muri** smooth, 0.3—0.5 µm thick. Colpi thin long furrows, parallel to the polar axis and stretches along almost the entire length of the grain. The colpi are fairly sunken (Figures 4.4 C—F).



Figures 4.4 (G—L) *Heliophila* pollen grains, subtype B<sub>3</sub>. SEM: - (G) mesocolpium of the pollen grain of *H. tulbaghensis* (Esterhuysen 9080), (H) mesocolpium of the pollen grain of *H. tricuspidata* (Oliver 11181), (I) mesocolpium of the pollen grain of *H. cedarbergensis* (Esterhuysen 35055), (J) mesocolpium of the pollen grain of *H. nubigena* (Esterhuysen 14043). TEM: - (K) wall section of pollen grain of *H. tulbaghensis* (Esterhuysen 9080) showing columellae (c) and endexine (e). (L) Wall section of pollen grain of *H. nubigena* (Esterhuysen 14043) showing tectum (t), columellae (c), foot layer (f), and intine (i). Scale bars K & L = 1 µm.

**TEM (Figures 4.4 K & L)**

**Exine:** 0.7—0.8  $\mu\text{m}$  thick, consisting of comparatively thick ectexine (supratectal spines, tectum, columellae, foot layer), a much thinner endexine and thin intine. **Supratectal spines** triangular, apices acute. **Tectum** 0.14—0.20  $\mu\text{m}$  thick, slightly thicker than foot layer, discontinuous, outer and inner surfaces smooth to undulating. **Columellae** well-developed, almost half the total thickness of the exine, simpli-columellate; columellae 0.35  $\mu\text{m}$  in height, erect, cylindrical in cross section; intercolumnellar spaces empty or partially filled with a dark-stained substance. **Foot layer:** 0.12—0.14  $\mu\text{m}$  thick, uneven and continuous. **Endexine:** not always prominent, 0.035  $\mu\text{m}$  thick, continuous, black in colour. **Intine:** 0.14—0.20  $\mu\text{m}$ , continuous, even to somewhat undulate.

Table 4.1: Size (in  $\mu\text{m}$ ), shape and pollen type (PT) of the *Heliophila* taxa examined. <sup>a</sup> Shapes classified according to Walker and Doyle (1975). Taxa arranged according to Marais (1970).

TAXON	TAXON	Polar axis	Equatorial diameter	P/E ratio	Pollen shape <sup>a</sup>	PT
	No.	Range (Mean)	Range (Mean)	Range (Mean)		
<i>H. amplexicaulis</i>	1.1	31.25 – 37.25 (34.50)	18.75 – 22.50 (20.63)	1.47 – 1.87 (1.68)	Euprolate	A
<i>H. obibensis</i>	2.1	-	-	-	-	-
<i>H. gariepina</i>	3.1	37.50 – 42.50 (39.54)	23.75 – 27.50 (25.33)	1.36 – 1.63 (1.56)	Euprolate	A
<i>H. namaquana</i>	4.1	37.50 – 47.50 (43.21)	21.25 – 27.50 (24.50)	1.45 – 2.00 (1.77)	Euprolate to Perprolate	A
<i>H. bulbostyla</i>	5.1	33.33 – 39.96 (37.13)	23.31 – 26.64 (24.92)	1.40 – 1.57 (1.49)	Euprolate	A
<i>H. adpressa</i>	6.1	40.00 – 52.50 (45.25)	25.00 – 30.00 (28.04)	1.42 – 2.00 (1.62)	Euprolate to Perprolate	A
<i>H. pectinata</i>	7.1	27.50 – 42.50 (32.54)	20.00 – 27.50 (23.63)	1.20 – 1.65 (1.39)	Subprolate to Euprolate	A
<i>H. pubescens</i>	8.1	41.25 – 47.50 (44.58)	22.50 – 28.75 (25.63)	1.52 – 1.94 (1.74)	Euprolate	A
<i>H. collina</i>	9.1	37.50 – 45.00 (39.71)	21.25 – 27.50 (23.71)	1.50 – 1.89 (1.68)	Euprolate	A
<i>H. laciniata</i>	10.1	35.00 – 45.00 (40.38)	20.00 – 25.00 (21.67)	1.65 – 2.12 (1.87)	Euprolate to Perprolate	A
<i>H. deserticola</i>	11.1	35.00 – 42.50 (37.79)	18.75 – 23.75 (20.92)	1.56 – 2.00 (1.81)	Euprolate to Perprolate	A
<i>H. seselifolia</i> var. <i>seselifolia</i>	12.1	40.00 – 43.75 (41.46)	22.50 – 27.50 (25.79)	1.42 – 1.78 (1.61)	Euprolate	A

TAXON	TAXON	Polar axis	Equatorial diameter	P/E ratio	Pollen shape <sup>a</sup>	PT
	No.	Range (Mean)	Range (Mean)	Range (Mean)		
<i>H. seselifolia</i> var. <i>nigellifolia</i>	13.1	37.50 – 45.00 (40.42)	22.50 – 30.00 (25.88)	1.36 – 1.80 (1.57)	Euprolate	A
<i>H. seselifolia</i> var. <i>marlothii</i>	14.1	35.00 – 40.00 (37.04)	21.25 – 27.50 (23.75)	1.36 – 1.72 (1.57)	Euprolate	A
<i>H. variabilis</i>	15.1	31.64 – 33.33 (32.52)	21.65 – 24.98 (22.73)	1.33 – 1.54 (1.43)	Subprolate to Euprolate	A
<i>H. minima</i>	16.1	28.75 – 35.00 (31.67)	17.50 – 22.50 (19.63)	1.33 – 2.00 (1.62)	Subprolate to Perprolate	A
<i>H. crithmifolia</i>	17.1	37.50 – 42.50 (39.96)	20.00 – 22.50 (20.75)	1.76 – 2.13 (1.93)	Euprolate to Perprolate	A
<i>H. trifurca</i>	18.1	32.50 – 41.25 (37.33)	20.00 – 25.00 (22.42)	1.40 – 1.88 (1.67)	Euprolate	A
<i>H. latisiliqua</i> var. <i>latisiliqua</i>	19.1	27.50 – 33.75 (30.46)	16.25 – 22.50 (19.63)	1.38 – 1.86 (1.56)	Euprolate	A
<i>H. latisiliqua</i> var. <i>macrostylis</i>	20.1	33.75 – 42.50 (39.17)	18.75 – 25.00 (20.46)	1.53 – 2.13 (1.92)	Euprolate to Perprolate	A
<i>H. pinnata</i>	21.1	32.50 – 42.50 (37.42)	20.00 – 25.00 (22.08)	1.44 – 2.07 (1.70)	Euprolate to Perprolate	A
<i>H. pusilla</i> var. <i>pusilla</i>	22.1	32.50 – 40.00 (35.46)	20.00 – 25.00 (22.63)	1.40 – 1.88 (1.57)	Euprolate	A
<i>H. pusilla</i> var. <i>macrosperma</i>	23.1	37.50 – 42.50 (39.92)	20.00 – 22.50 (21.75)	1.67 – 2.13 (1.84)	Euprolate to Perprolate	A
<i>H. pusilla</i> var. <i>setacea</i>	24.1	25.00 – 27.50 (26.75)	17.50 – 22.50 (19.75)	1.18 – 1.57 (1.36)	Subprolate to Euprolate	A

TAXON	TAXON	Polar axis	Equatorial diameter	P/E ratio	Pollen shape <sup>a</sup>	PT
	No.	Range (Mean)	Range (Mean)	Range (Mean)		
<i>H. pusilla</i> var. <i>lanceolata</i>	25.1	32.50 – 37.50 (34.38)	21.25 – 25.00 (23.17)	1.25 – 1.58 (1.49)	Subprolate to Euprolate	A
<i>H. patens</i>	26.1	-	-	-	-	A
<i>H. diffusa</i> var. <i>diffusa</i>	27.1	35.00 – 42.50 (37.92)	27.50 – 32.50 (30.21)	1.07 – 1.45 (1.26)	Subprolate to Euprolate	B <sub>2</sub>
<i>H. diffusa</i> var. <i>diffusa</i>	27.2	36.50 – 42.00 (37.01)	27.50 – 33.00 (30.42)	1.05 – 1.55 (1.30)	Subprolate to Euprolate	B <sub>2</sub>
<i>H. diffusa</i> var. <i>flacca</i>	28.1	26.25 – 36.25 (31.88)	17.50 – 20.00 (19.42)	1.40 – 1.87 (1.64)	Euprolate	B <sub>2</sub>
<i>H. pendula</i>	29.1	32.50 – 40.00 (36.08)	20.00 – 23.75 (21.79)	1.53 – 1.78 (1.66)	Euprolate	B <sub>2</sub>
<i>H. meyeri</i> var. <i>meyeri</i>	30.1	32.50 – 40.00 (37.08)	18.75 – 23.75 (21.63)	1.58 – 1.88 (1.72)	Euprolate	B <sub>2</sub>
<i>H. meyeri</i> var. <i>minor</i>	31.1	35.00 – 40.00 (37.33)	20.00 – 22.50 (21.00)	1.65 – 1.88 (1.78)	Euprolate	B <sub>2</sub>
<i>H. coronopifolia</i>	32.1	28.31 - 33.33 (30.14)	19.98 – 23.31 (21.26)	1.21 – 1.58 (1.42)	Subprolate to Euprolate	A
<i>H. concatenata</i>	33.1	-	-	-	-	A
<i>H. tabularis</i>	34.1	-	-	-	-	-
<i>H. acuminata.</i>	35.1	30.00 – 43.75 (35.42)	20.00 – 25.00 (22.08)	1.30 – 2.00 (1.61)	Subprolate to Perprolate	A
<i>H. macowaniana</i>	36.1	37.50 – 50.00 (41.29)	17.50 – 23.75 (20.54)	1.84 – 2.25 (2.01)	Euprolate to Perprolate	A
<i>H. promontorii</i>	37.1	26.64 – 31.64 (29.03)	18.32 – 23.31 (20.70)	1.29 – 1.64 (1.41)	Subprolate to Euprolate	A



TAXON	TAXON	Polar axis	Equatorial diameter	P/E ratio	Pollen shape <sup>a</sup>	PT
	No.	Range (Mean)	Range (Mean)	Range (Mean)		
<i>H. digitata</i>	38.1	37.50 – 47.50 (40.92)	23.75 – 30.00 (25.46)	1.50 – 1.80 (1.61)	Euprolate	A
<i>H. digitata</i>	38.2	36.50 – 46.50 (41.54)	22.50 – 31.32 (26.40)	1.54 – 1.84 (1.64)	Euprolate	A
<i>H. refracta</i>	39.1	37.50 – 47.50 (41.96)	20.00 – 27.50 (22.58)	1.54 – 2.13 (1.87)	Euprolate to Perprolate	A
<i>H. schulzii</i>	40.1	36.63 – 46.62 (41.24)	19.98 – 26.64 (23.25)	1.50 – 2.16 (1.78)	Euprolate to Perprolate	A
<i>H. lacteal</i>	41.1	29.97 – 36.63 (33.61)	19.98 – 23.31 (21.76)	1.43 – 1.69 (1.55)	Euprolate	A
<i>H. linoides</i>	42.1	39.96 – 44.96 (41.87)	18.32 – 21.65 (20.31)	1.85 – 2.18 (2.06)	Euprolate to Perprolate	A
<i>H. remotiflora</i>	43.1	35.00 – 40.00 (38.08)	22.50 – 27.50 (25.33)	1.36 – 1.72 (1.51)	Euprolate	A
<i>H. leptophylla</i>	44.1	-	-	-	-	-
<i>H. arenosa</i>	45.1	40.00 – 47.50 (44.08)	23.75 – 27.50 (26.61)	1.54 – 1.90 (1.68)	Euprolate	A
<i>H. arenaria</i> var. <i>arenaria</i>	46.1	34.97 – 39.96 (37.19)	19.98 – 23.31 (21.31)	1.57 – 2.00 (1.75)	Euprolate – Perprolate	A
<i>H. arenaria</i> var. <i>acocksii</i>	47.1	33.33 – 41.63 (38.41)	21.65 – 26.64 (23.89)	1.36 – 1.85 (1.61)	Euprolate	A
<i>H. arenaria</i> var. <i>glabrescens</i>	48.1	26.64 – 33.33 (30.14)	16.65 – 23.31 (18.68)	1.31 – 1.80 (1.62)	Subprolate to Euprolate	A
<i>H. arenaria</i> var. <i>agtertuinensis</i>	49.1	33.33 – 39.96 (36.27)	16.65 – 21.65 (19.62)	1.62 – 2.10 (1.85)	Euprolate to Perprolate	A
<i>H. descurva</i>	50.1	-	-	-		A

TAXON	TAXON	Polar axis	Equatorial diameter	P/E ratio	Pollen shape <sup>a</sup>	PT
	No.	Range (Mean)	Range (Mean)	Range (Mean)		
<i>H. affinis</i>	51.1	26.64 – 33.33 (29.08)	18.32 – 23.31 (20.62)	1.23 – 1.63 (1.42)	Subprolate to Euprolate	A
<i>H. africana</i>	52.1	28.31 – 34.97 (31.52)	19.98 – 23.31 (21.76)	1.29 – 1.75 (1.45)	Subprolate to Euprolate	A
<i>H. brassicaefolia</i>	53.1	26.64 – 31.64 (29.58)	16.65 – 19.98 (18.95)	1.42 – 1.73 (1.56)	Euprolate	A
<i>H. cinerea</i>	54.1	29.97 – 41.63 (37.93)	19.98 – 24.98 (22.26)	1.47 – 1.92 (1.71)	Euprolate	A
<i>H. cuneata</i>	55.1	34.97 – 41.63 (37.52)	19.98 – 23.31 (21.98)	1.57 – 2.00 (1.71)	Euprolate to Perprolate	A
<i>H. linearis</i> var. <i>linearis</i>	56.1	34.97 – 39.96 (37.41)	19.98 – 23.31 (21.59)	1.50 – 2.00 (1.74)	Euprolate to Perprolate	A
<i>H. linearis</i> var. <i>linearifolia</i>	57.1	29.97 – 34.97 (32.91)	19.98 – 25.81 (21.95)	1.29 – 1.75 (1.51)	Subprolate to Euprolate	A
<i>H. linearis</i> var. <i>reticulata</i>	58.1	36.63 – 41.43 (38.74)	16.65 - - 21.65 (19.76)	1.69 – 2.40 (1.97)	Euprolate to Perprolate	A
<i>H. cornuta</i> var. <i>cornuta</i>	59.1	29.97 – 34.97 (32.61)	19.98 – 23.31 (21.59)	1.29 – 1.67 (1.51)	Subprolate to Euprolate	A
<i>H. cornuta</i> var. <i>squamata</i>	60.1	33.33 – 38.30 (36.52)	19.98 – 23.31 (21.70)	1.50 – 2.00 (1.69)	Euprolate to Perprolate	A
<i>H. elata</i> var. <i>elata</i>	61.1	31.25 – 41.45 (36.71)	20.00 – 25.00 (21.04)	1.50 – 2.00 (1.75)	Euprolate to Perprolate	A
<i>H. elata</i> var. <i>pillansii</i>	62.1	26.25 – 35.00 (30.25)	22.50 – 23.75 (21.54)	1.22 – 1.63 (1.41)	Subprolate to Euprolate	A
<i>H. sabulata</i>	63.1	27.50 – 35.00 (31.67)	17.50 – 22.50 (20.17)	1.28 – 1.86 (1.58)	Subprolate to Euprolate	A

TAXON	TAXON	Polar axis	Equatorial diameter	P/E ratio	Pollen shape <sup>a</sup>	PT
	No.	Range (Mean)	Range (Mean)	Range (Mean)		
<i>H. suavissima</i>	64.1	36.25 – 45.00 (41.38)	21.25 – 27.50 (23.88)	1.45 – 2.00 (1.74)	Euprolate to Perprolate	A
<i>H. carnosa</i>	65.1	35.00 – 46.25 (39.92)	20.00 – 28.75 (24.33)	1.50 – 2.00 (1.65)	Euprolate to Perprolate	A
<i>H. rigidiuscula</i>	66.1	27.50 – 35.00 (32.83)	15.00 – 20.00 (18.00)	1.60 – 2.08 (1.83)	Euprolate to Perprolate	A
<i>H. katbergensis</i>	67.1	-	-	-		-
<i>H. eximia</i>	68.1	35.00 – 45.00 (40.04)	18.75 – 25.00 (21.96)	1.65 – 2.25 (1.83)	Euprolate to Perprolate	A
<i>H. scandens</i>	69.1	26.25 – 31.25 (29.21)	13.75 – 18.75 (15.88)	1.60 – 1.92 (1.82)	Euprolate	B <sub>1</sub>
<i>H. glauca</i>	70.1	27.50 – 35.00 (30.21)	17.50 – 21.25 (19.29)	1.29 – 1.86 (1.57)	Subprolate to Euprolate	B <sub>1</sub>
<i>H. brachycarpa</i>	71.1	15.00 – 17.50 (16.75)	11.25 – 13.75 (12.50)	1.20 – 1.56 (1.34)	Subprolate to Euprolate	B <sub>1</sub>
<i>H. macrosperma</i>	72.1	30.00 – 32.50 (31.17)	16.25 – 21.25 (18.88)	1.50 – 1.86 (1.66)	Euprolate	B <sub>3</sub>
<i>H. filicaulis</i>	73.1	-	-	-		-
<i>H. tulbaghensis</i>	74.1	30.00 – 37.50 (32.75)	17.75 – 25.00 (20.25)	1.30 – 1.93 (1.63)	Subprolate to Euprolate	B <sub>3</sub>
<i>H. rimicola</i>	75.1	40.00 – 45.00 (42.08)	20.00 – 22.50 (21.46)	1.78 – 2.13 (1.97)	Euprolate to Perprolate	B <sub>3</sub>
<i>H. esterhuyseniae</i>	76.1	-	-	-		B <sub>3</sub>
<i>H. dregeana</i>	77.1	31.25 – 37.50 (34.63)	20.00 – 25.00 (21.71)	1.35 – 1.88 (1.60)	Euprolate	B <sub>3</sub>

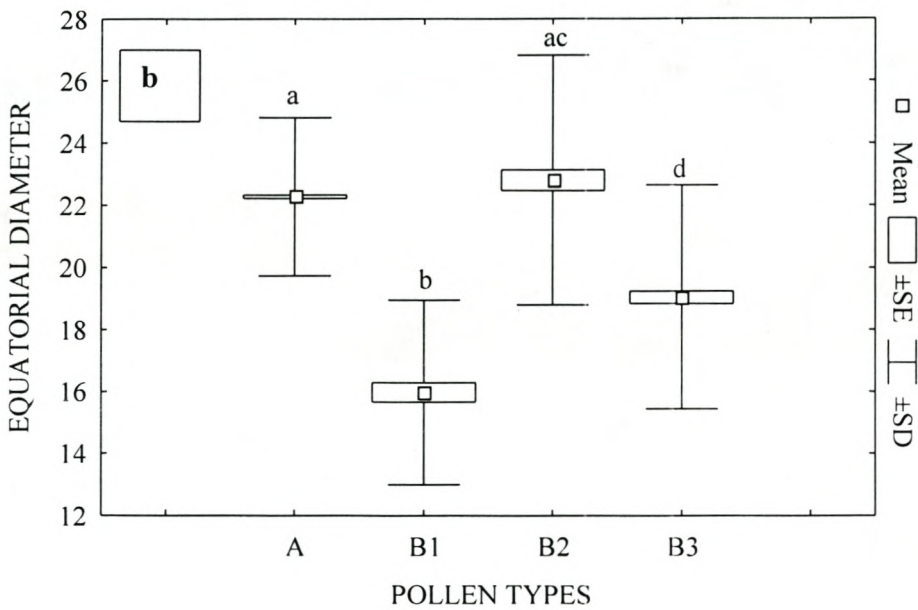
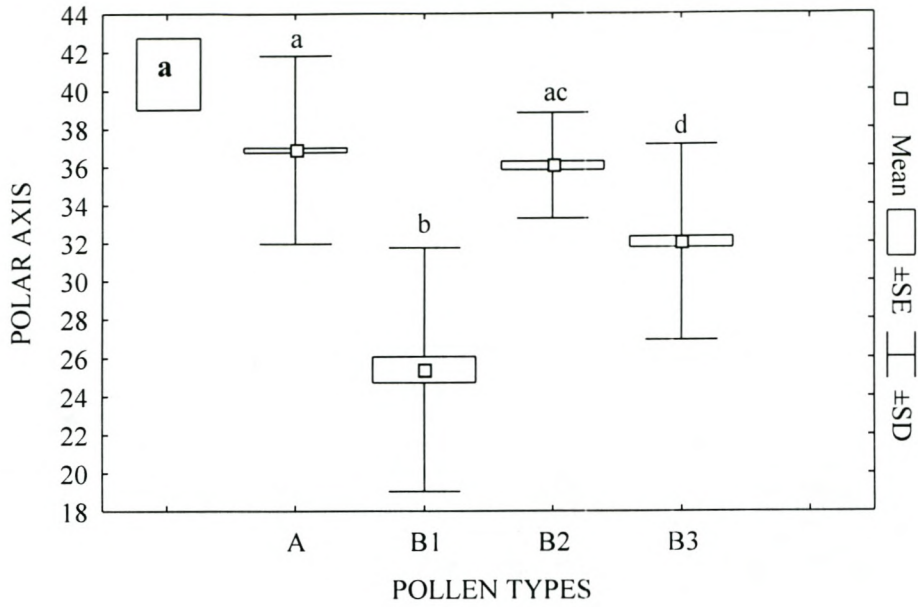
TAXON	TAXON	Polar axis	Equatorial diameter	P/E ratio	Pollen shape <sup>a</sup>	PT
	No.	Range (Mean)	Range (Mean)	Range (Mean)		
<i>H. tricuspidata</i>	78.1	30.00 – 37.50 (34.08)	23.75 – 30.00 (26.58)	1.09 – 1.50 (1.29)	Subprolate to Euprolate	B <sub>3</sub>
<i>H. cedarbergensis</i>	79.1	25.00 – 30.00 (28.17)	15.00 – 20.00 (17.25)	1.38 – 2.00 (1.64)	Euprolate to Perprolate	B <sub>3</sub>
<i>H. scoparia</i> var. <i>scoparia</i>	80.1	25.00 – 32.50 (26.46)	15.00 – 20.00 (17.58)	1.25 – 1.67 (1.51)	Subprolate to Euprolate	B <sub>3</sub>
<i>H. scoparia</i> var. <i>aspera</i>	81.1	22.50 – 27.50 (25.79)	12.50 – 15.00 (13.33)	1.67 – 2.10 (1.94)	Euprolate to Perprolate	B <sub>3</sub>
<i>H. callosa</i>	82.1	25.00 – 32.50 (30.08)	13.75 – 17.50 (15.58)	1.67 – 2.18 (1.94)	Euprolate to Perprolate	B <sub>3</sub>
<i>H. nubigena</i>	83.1	35.00 – 41.25 (38.46)	15.00 – 20.00 (17.38)	2.00 – 2.54 (2.22)	Perprolate	B <sub>3</sub>
<i>H. alpina</i>	84.1	37.50 – 45.00 (41.96)	20.00 – 25.00 (22.13)	1.67 – 2.13 (1.90)	Euprolate to Perprolate	A
<i>H. elongata</i>	85.1	40.00 – 46.25 (43.38)	20.00 – 27.50 (24.25)	1.60 – 2.31 (1.80)	Euprolate to Perprolate	A
<i>H. elongata</i>	85.2	40.00 – 45.00 (42.83)	25.00 – 27.50 (25.58)	1.59 – 1.80 (1.69)	Euprolate	A
<i>H. macra</i>	86.1	26.25 – 30.00 (28.88)	17.50 – 22.50 (19.46)	1.33 – 1.64 (1.49)	Subprolate to Euprolate	B <sub>3</sub>
<i>H. ramosissima</i>	87.1	-	-	-	-	B <sub>3</sub>

TAXON No. = Taxon number; P/E ratio = Polar/Equatorial ratio; PT = Pollen type

### 4.1.3 Analysis of Variance (ANOVA) Test

The assessment of pollen variability between the two main pollen type groups and among the four groups/subgroups of pollen grains types of *Heliophila* taxa were carried out using ANOVA. The ANOVA determines if evidence exists for statistical heterogeneity between and among the means of two or more groups. If the sample size is large (greater than 30) ANOVA is used to test for heterogeneity between two samples and if the sample is less than 30, the T– test is preferred. In this study, the sample size was larger than 30. The analysis was performed separately for polar axis and equatorial diameter measurements.

The results of one-way analysis of variance between the means of the two major pollen types show that there are significant differences both in terms of the polar axis measurements and the equatorial diameter (both having  $P = 0.001$ , 1 degree of freedom). The results among the means of the pollen types and subtypes indicate that there are significant differences ( $P = 0.001$ , 3 degrees of freedom) among the groups or subgroups for both polar axis measurements and equatorial diameter measurements. The ANOVA test only tells us whether or not there is a difference in the mean of the groups, but does not indicate where the differences lie. In order to determine where the differences occur, the Scheffé test was performed. The results indicate that there are significant differences between type A and subtype B<sub>1</sub> ( $P = 0.001$ ), between type A and subtype B<sub>3</sub> ( $P = 0.001$ ), between subtype B<sub>1</sub> and subtype B<sub>2</sub> ( $P = 0.001$ ), and finally between subtype B<sub>2</sub> and subtype B<sub>3</sub> ( $P = 0.001$ ) and that there is no significant difference between type A and subtype B<sub>2</sub> ( $P = 0.2647$ ) in terms of polar axis. Figure 4.5a shows the relationships of the pollen types in terms of polar axis. A similar picture is obtained for the equatorial diameter (Figure 4.5b) where there are also no significant differences between type A and subtype B<sub>2</sub> ( $P = 0.1847$ ), but significant differences between the other combinations, each combination having a P-value of 0.001.



Figures 4.5 Box whisker plots of polar and equatorial measurements of some selected *Heliophila* pollen grains showing mean, standard error and standard deviation a) Pollen grain type's verses polar axis measurements and b) Pollen grain type's verses equatorial diameter measurements.

## 4.2 Phenetic Analyses Results

Cluster analysis was only performed after the pollen grain types were identified and compared with the Marais' (1970) classification (Table 4.1) in order to determine which morphological characters support the suggested groupings as proposed by the tectums of the pollen grains. Most of the taxa with subtype B<sub>1</sub> and B<sub>2</sub> pollen form clearly delimited groups. The cluster analysis was based on morphological characters using the vegetative and reproductive characters only. Pollen morphology was excluded in the analysis, as it could have influenced the clustering of the taxa. Two major clusters are formed at a linkage distance of about 26.5 and several subclusters follow (Figure 4.6). These groups correlate well with some of the morphological features of the taxa. The first cluster (Cluster A, Figure 4.6) stretches from *H. amplexicaulis* to *H. meyeri* and all of these taxa, except *H. cuneata*, *H. elata* and *H. cornuta*, are herbaceous (Fig 4.2A, C, D, and F) and all, except *H. cuneata*, *H. linearis*, *H. cinerea*, *H. elata*, *H. cornuta* and *H. alpina*, are annuals. The taxa have both glabrous or hairy, simple and compound leaves with entire or lobed margins occurring separately or in the same individual (Fig 4.2 F). The leaves are sometimes subtended by minute stipules and sometimes the stipules are absent. The petals are without appendages and the ovary without a stipe.

The second cluster (Cluster B, Figure 4.6) stretches from *H. sabulata* to *H. eximia*, and the included plants are all perennials comprising both shrubs (Fig. 4.2B) and herbs. This group is also well supported by morphological characters like glabrous, simple leaves with entire margins, except for *H. nubigena* and *H. cedarbergensis*, which have hairy leaves and *H. macrosperma* which has compound leaves (Fig. 4.2B). The leaves are always subtended by minute stipules and two of the petals have appendages.

Cluster A is subdivided into two subclusters (A1 and A2) at a linkage distance of 23 (Figure 4.6) and are well supported by leaf type, fruit shape and the venation of the fruits. The first subcluster (A1) comprising species from *H. amplexicaulis* to *H. cuneata* has simple leaves with entire margins and fruits with one or three veins. The second subcluster (A2), comprising species from *H. pectinata* to *H. meyeri*, have either simple or compound leaves in separate species. Margins of simple leaves are lobed, (except for *H. minima* and *H. trifurca*) and the fruits have only one vein. Subcluster A1 is further subdivided into two subclusters, which are well supported by morphological characters. The first subcluster (A1a) comprising species from *H. amplexicaulis* to *H. cornuta* that have petals that are 2.5—11.5 mm long, few to many ovules (2—40 -56) per ovary, linear fruits, that are 7.0—65.0 (- 80) mm long, with both entire and moniliform margins and only one vein. Seeds are always narrowly margined.

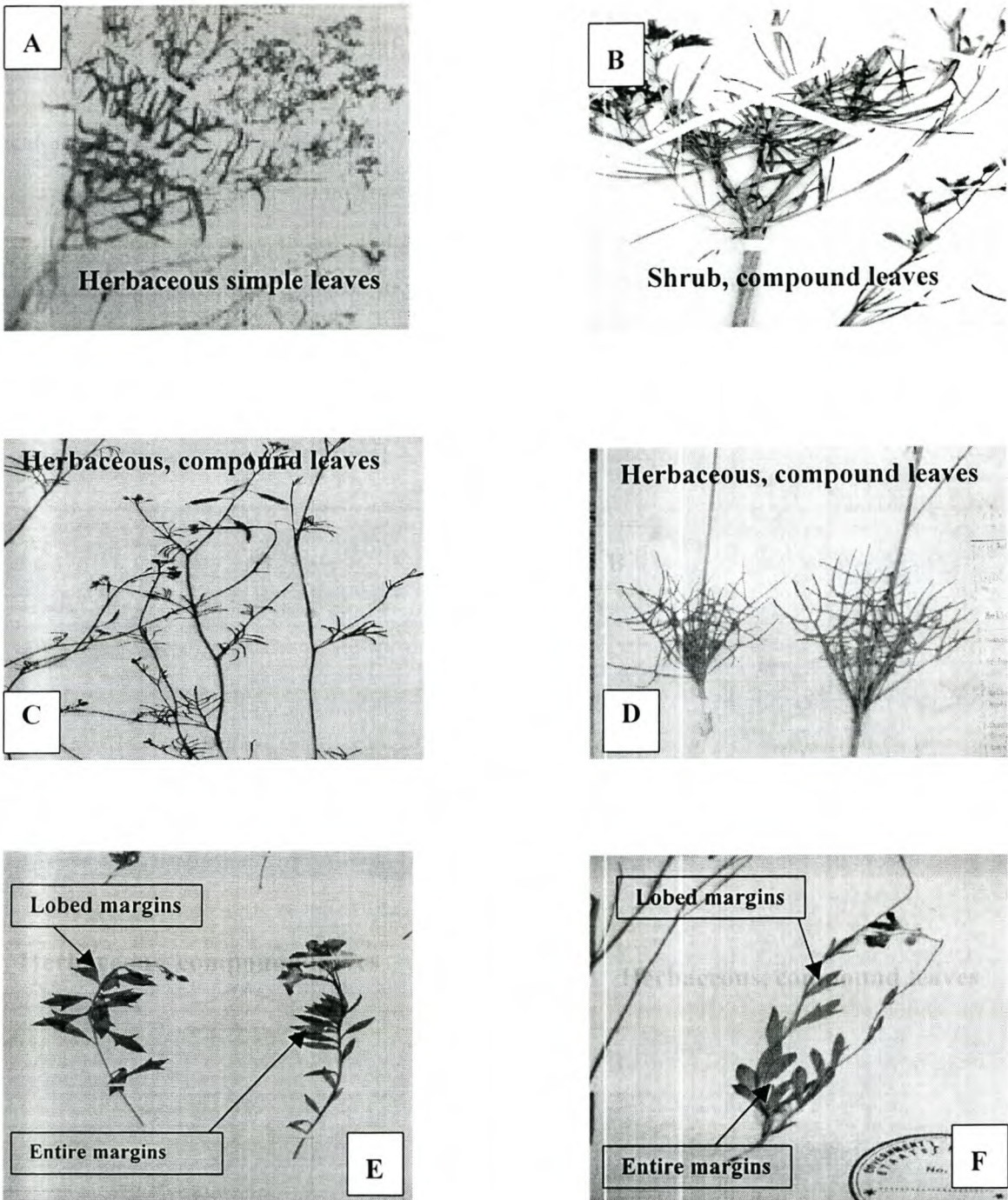


Figure 4.2 (A – F) *Heliophila* specimens showing habit, leaf type and margins. (A) *H. amplexicaulis* (Compton, 11048) annual herb, simple leaves with entire margins, (B) *H. macrosperma* (Bayliss 2257) shrubby plant with compound leaves. (C) *H. diffusa* var. *diffusa* (Pillans s.n.) annual herbs with compound leaves. (D) *H. laciniata* (Le-Reux 2959) annual herb with compound leaves. (E) *H. tricuspidata* (Oliver 11181) showing leaves with entire and lobed margins on separate branches. (F) *H. africana* (Fellingham 1145 NBG) showing leaves with entire and lobed margins on the same branch.



Subcluster (A1b) comprising species from *H. linoides* to *H. cuneata* that have petals that are 4.0—16.5 mm long, many ovules (20—52) per ovary, linear fruits, that are 25.0—110.0 mm long, with entire margins and three veins. Seeds are always winged. Cluster A2 is also subdivided into two subclusters A2a and A2b and are also well supported by morphological characters. The first (A2a), from species *H. pectinata* to *H. alpina*, includes species with petals measuring 1.5—7.0 mm in length, filaments without appendages, few ovules (4—16) per ovary, fruits 8.0—25.0 mm long and seeds mostly narrowly margined or immarginate. The second (A2b), from species *H. laciniata* to *H. meyeri*, includes species with petals measuring 1.9—12.0 mm in length, the two short filaments has appendages, few to many ovules (1—60 -80) per ovary, and seeds that are winged or with narrow margins. Subcluster A2b is further subdivided into two subclusters A2ba and A2bb, which are again well supported by morphological characters. The first (A2ba), comprising species from *H. laciniata* to *H. schulzii*, is well supported by characters like petals that are 3.0—12.0 mm in length, many ovules (20—60 -80) per ovary, smooth filaments, fruits that are 10.0—60.0 (-80) mm long. However, the variable characters are the presence or absence of minute stipules, seed shape and seed margins. The second subcluster (A2bb), comprising species from *H. diffusa* to *H. meyeri*, is also morphologically well supported in that all species are herbs with pinnate leaves that are subtended by two minute stipules, small petals that are 1.9—9.0 mm long, few ovules (1—18) per ovary, all filaments papillate near the base, fruits smaller, 3.0—25.0 (-50) mm in length and seeds that are mostly suborbicular with winged margins. Species of all the subclusters in cluster A have pollen grains with a psilate or psilate micro-perforate tectum with suprategal spinules. The only exception is subcluster A2bb comprising *H. diffusa*, *H. pendula* and *H. meyeri*. These three species form a separate subcluster at a linkage distance of 21, and these have a reticulate tectum (pollen type B<sub>2</sub>; Figure 4.6).

In Cluster B two subclusters are formed at a linkage distance of 21.5 (Figure 4.6), the first (B1) from species *H. sabulata* to *H. nubigena* and the second (B2) from species *H. rigidiuscula* to *H. eximia*. All taxa in subcluster B1 are shrubs, with filaments lacking appendages, few to many ovules per ovary, the ovary with or without a stipe and seeds with narrow or winged margins. In contrast, the taxa in subcluster B2 are all perennial herbs with filaments with or without appendages, few ovules (6—20) per ovary, the ovary with a stipe, fruits that are 30.0—70.0 mm in length and seeds with winged margins. Subcluster B1 is further subdivided into two subclusters, B1a and B1b. Subcluster B1a comprises species from *H. sabulata* to *H. scoparia*, which has glabrous leaves, whereas Subcluster B1b consisting of a single species *H. nubigena*, has hairy leaves. Subcluster B1a is further divided into two subclusters, B1aa, which start from

species *H. sabulata* to *H. carnososa* and the second B1ab from species *H. scandens* to *H. scoparia*. Subcluster B1aa has taxa with petals that are 6.0—13.5 mm long, many ovules (14—42) per ovary, ovary without a stipe, fruits 20.0—80.0 mm in length and without a stipe, and the seeds with winged margins. Subcluster B1ab is well supported by species having simple glabrous leaves, petals that are 5.5—15.0 mm in length, few ovules (2—18 -36) per ovary, ovary with a stipe, fruits that are 5.0—50.0 mm in length, fruit with a stipe and seeds with narrow or immarginate margins. Subclusters B2 and B1aa have a psilate tectum type with supratectal spinules, whereas cluster B1b and B1ab have reticulate to micro-reticulate tectum types.

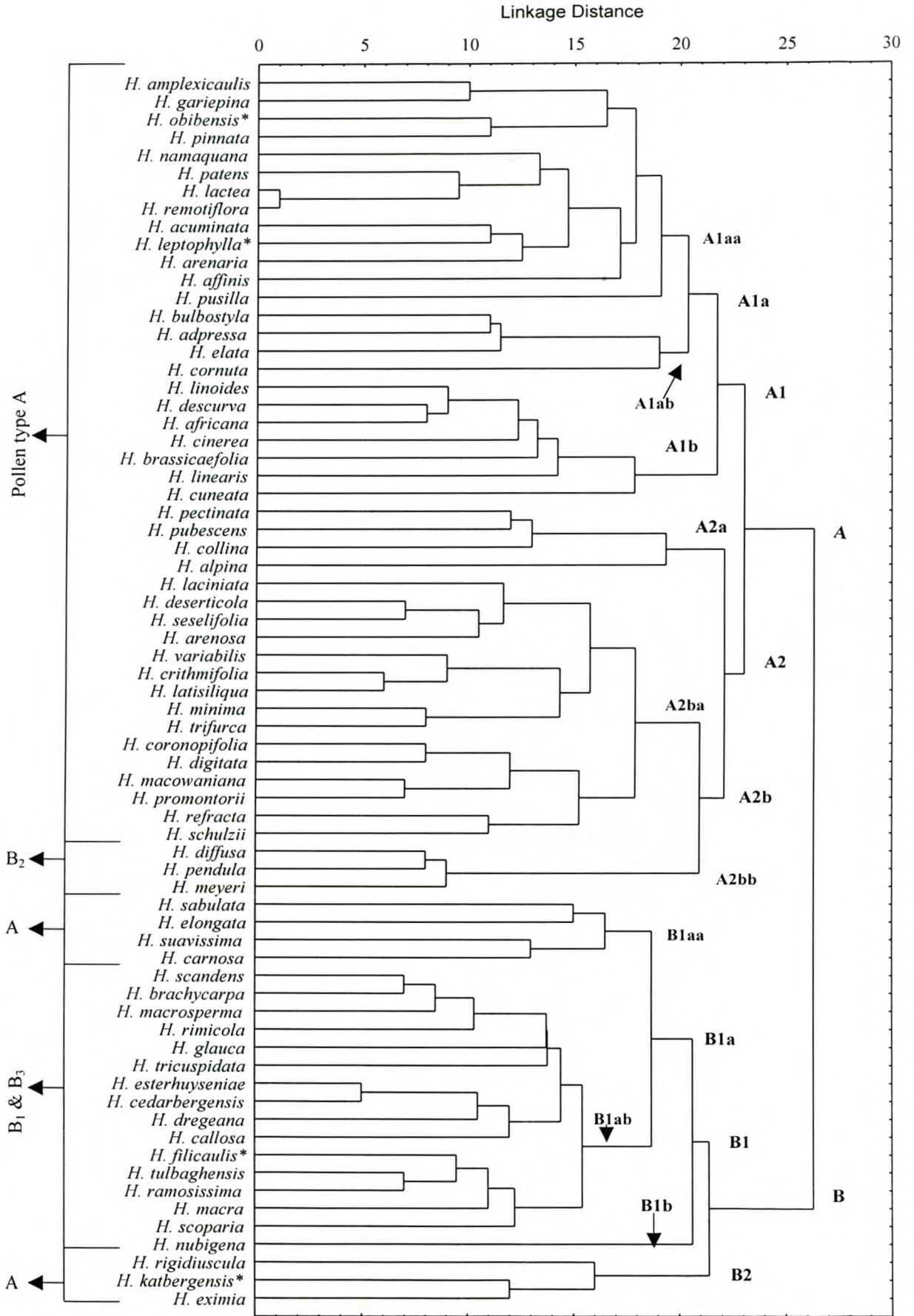


Fig. 4.6 A phenogram depicting the overall similarity between 69 *Heliophila* species based on morphological data with a cophenetic value of 0.72. \* Pollen types unknown

## CHAPTER FIVE

## DISCUSSION

### 5.1 Introduction

Palynological data have, in many previous studies, proved successful in resolving taxonomic problems at various taxonomic levels (Stuessy, 1990). Although there are many examples of palynology resolving taxonomic problems in the literature, only a few are highlighted here. Abu-Asab and Cantino (1989) used pollen data to understand the relationship between the genus *Trichostema* L. (Lamiaceae) and the monotypic genera *Amethystea* L. and *Cardioteucris* C.V. Wu. At the same time they used palynology to evaluate the morphological sectional classification of *Trichostema* by Lewis (1945). The pollen morphology revealed closer relationship between *Trichostema* and the genera *Amethystea* and *Cardioteucris* than to *Teucrium* L. as was suggested by Lewis (1945). Palynological data, however, support the sectional classification of *Trichostema* as was suggested by Lewis (1945).

For the family Euphorbiaceae, Levin (1992) suggested, with regard to leaf morphology and palynological evidence, that the genus *Paradrypetes* Kuhlmann should be placed in its own subtribe, the Paradrypetinae in subfamily Oldfieldioideae rather than in subfamily Phyllanthoideae. The four equatorial apertures in the pollen grains of *Paradrypetes* are brevicolporate, and are almost identical to those of the pollen grains of the subfamily Oldfieldioideae, with four or more brevicolporate to por(or)ate apertures. In contrast, the pollen grains of Phyllanthoideae are tricolpate.

Ferguson and Stirton (1993) used pollen data to provide additional evidence to support the view that genera *Bowdichia* Knuth and *Panurea* Spruce ex Benth (Leguminosae: Papilionoideae: Sophoreae) have very close relatives in the tribe Sophoreae. *Bowdichia* and *Panurea* were found to be the only genera in the tribe Sophoreae with psilate pollen grains. From these examples, it is clear that pollen data can provide significant taxonomic information, which can help in solving some of the taxonomic problems currently still confusing botanists.

The present study suggests that palynology does contribute substantially towards a better understanding of the interrelationships between and among members of the genus *Heliophila*. Although palynology alone cannot form a basis for a new classification, several taxonomic deductions can be made from the present study. Some of these are discussed in some detail below under separate headings or subheadings.

## 5.2 Pollen data

Pollen data as an additional source of evidence can play a significant role in resolving taxonomic problems in the classification of taxa. Results from the SEM (revealing external features) tend to be more useful at lower taxonomic levels (Radford *et al.* 1974), whereas TEM data (revealing internal features) are often more significant at higher taxonomic levels (Stuessy, 1990). Despite the obvious taxonomic value of pollen data, certain cautions must be kept in mind in any palynological studies. These include aspects such as the basic structure of the exine, which is the net product of various selective pressures (Dreyer, 1996).

### 5.2.1 Exine sculpture and structure

The *Heliophila* taxa examined in the present study are divided into two main groups on the basis of the exine sculpture and structure as it appears under the SEM and TEM respectively. The tectum of the first group is psilate or psilate micro-perforate with suprategal spinules, named pollen type A. The tectum of the second group is micro-reticulate to reticulate with or without suprategal spinules, and named pollen type B. The group with type B pollen can be divided into three subgroups based on the presence and position of suprategal spinules and the shape of the lumina. The first subgroup comprises *H. scandens*, *H. glauca* and *H. brachycarpa* and has pollen type B<sub>1</sub>, with circular lumina without suprategal spinules. The second subgroup comprises *H. diffusa* var. *diffusa*, *H. diffusa* var. *flacca*, *H. meyeri* var. *meyeri*, *H. meyeri* var. *minor* and *H. pendula* and has pollen of the type B<sub>2</sub> with angular lumina and suprategal spinules concentrated near the colpal regions. Finally the third subgroup, which is the largest of the three subgroups, comprises *H. tulbaghensis*, *H. rimicola*, *H. esterhuyseniae*, *H. dregeana*, *H. tricuspadata*, *H. cedarbergensis*, *H. scoparia* var. *scoparia*, *H. scoparia* var. *aspera*, *H. callosa*, *H. nubigena*, *H. macra* and *H. ramosissima* and has pollen of the type B<sub>3</sub>, which has angular lumina with suprategal spinules sparsely scattered around the tectum of the pollen grains (Fig 4.3 G—J).

No previous studies on the ultrastructure of the pollen grain wall of any *Heliophila* taxa could be traced. The present study shows a homogenous structure of the sexine, nexine and intine for all the taxa examined. All specimens have a thick, continuous footlayer and a thin continuous endexine. However, the differences are in the thickness of the exine, the nature of the tectum, the presence of a dark stained substance in the spaces between columellae. The taxa with a psilate or psilate micro-perforate tectum (pollen type A) have a continuous tectum and is thus tectate, whereas those with micro-reticulate to reticulate tectum (pollen grain type B) have a discontinuous tectum and can be described as semitectate. The exine of the *Heliophila* grains

with pollen type A is thicker than those with pollen type B. The spaces between the columellae of most of the specimens with pollen type A are filled with a dark stained substance, while these spaces in the pollen grains with a reticulate tectum are only partially filled or completely without this dark substance. The ultrastructure of the exine of the *Heliophila* taxa is generally in accordance with the two main divisions of pollen type A and pollen type B, as based on the exine sculpture.

However, the subdivision of the pollen sculpture was complicated by the uninterrupted continuum display of most of the above-mentioned sculptural characters. The demarcation of the subtypes in pollen type B was very difficult and done somewhat arbitrarily. The subtypes within pollen type B may therefore be a fairly artificial arrangement, which requires additional studies, preferably based on molecular techniques.

### 5.2.2 Pollen grain size

The size range of Walker and Doyle (1975) was used in this study. The size of the pollen grains of the *Heliophila* taxa studied are between 15.00—52.50  $\mu\text{m}$  in polar view, which according to Walker and Doyle (1975) ranges from their small to medium size classes. The two main pollen types A and B comprise small grains, which range from 15.00—24.00  $\mu\text{m}$  in polar view and medium grains range from 25.00—52.50  $\mu\text{m}$  in polar view (Table 4.1). In contrast, the psilate or psilate micro-perforate pollen with suprategal spinules (Pollen grain type A) seems to have larger grains than the micro-reticulate to reticulate pollen (pollen grain type B). For example, *H. adpressa*, which has pollen of the A type, has the largest pollen grains (average of polar axis: 45.25  $\mu\text{m}$ ), whereas *H. brachycarpa*, with pollen grain type B, has the smallest pollen grains (average of polar axis: 16.75  $\mu\text{m}$ ). Both in terms of polar axis dimension and equatorial axis, pollen grains with type A sculpturing can be regarded as having medium size pollen grains (31.75—42.00  $\mu\text{m}$ ). For both measurements pollen grains with type B sculpturing show a variation in size. Pollen grains, with pollen type B<sub>1</sub> sculpturing, can be regarded as small (17.00—32.00  $\mu\text{m}$ ), pollen type B<sub>2</sub> sculpturing as medium sized and pollen type B<sub>3</sub> sculpturing as between small and medium sized grains (Figure 4.5). Dreyer (1996) cited various studies, which indicated that higher ploidy levels are often associated with an increase in grain size, but since no chromosome numbers are available for *Heliophila*, polyploidy versus pollen grain size could not be verified at this stage.

An analysis of variance (ANOVA) test was performed to see if there are significant differences between the two main pollen types and among the main type and subtypes respectively. The ANOVA results show that there are significant differences between the means of the two main pollen types both in terms of polar axis measurements and equatorial axis measurements, each having a P-value of 0.001 and 1 degree of freedom. The ANOVA results also indicated significant differences among the means of the subtypes both in terms of polar axis measurements ( $P = 0.001$ , 3 degrees of freedom) and equatorial axis measurements ( $P = 0.001$ , 3 degrees of freedom; Figure 4.5). In all sets of measurements, the P-value is smaller than 0.05, therefore there is evidence of statistical heterogeneity between and among pollen grain measurements of the different pollen types or subtypes.

The Scheffé test results indicate that there are significant differences among the means of the different pollen grain types or subtypes, except between pollen type A and pollen subtype B<sub>2</sub> ( $P = 0.2647$  and  $P = 0.184676$  in terms of polar axis measurements and equatorial axis measurements respectively). It is important to note that all taxa with pollen type A (except for *H. cornuta*, *H. elata*, *H. elongata*, *H. suavissima* and *H. carnososa*) and all taxa with pollen subtype B<sub>2</sub> are herbaceous plants, whereas taxa with pollen subtypes B<sub>1</sub> and B<sub>3</sub> are shrubs (Table 5.1). From these results, it seems difficult to classify the taxa based on the pollen grain types and pollen grain sizes alone, since there are no significant differences in the means between pollen type A, which has a psilate or psilate micro-perforate tectum and pollen type B<sub>2</sub>, which has a micro-reticulate to reticulate tectum. However, it seems that herbaceous taxa, whether having a psilate or psilate micro-perforate tectum or micro-reticulate tectum, have larger pollen grains than the shrubby ones. Pollen grain size seems to be of taxonomic significance in classifying *Heliophila* taxa only when used in conjunction with other data sources such as plant habit and life form.

Pollen grain size in *Heliophila* also does not correlate with petal size, since some taxa with larger petals have small pollen grains and vice versa. It appears that most of the perennial taxa have smaller pollen grains together with long petals (6.5—16.5 mm long), whereas most annual taxa have larger pollen grains and short petals (2.0—10.0 mm, rarely exceeding 10.0 mm in length). It is not known whether taxa with smaller pollen grains produce a large quantity of grains or not compared to those producing larger pollen grains.

Flowers of all *Heliophila* taxa are tetradynamous, with two short stamens and four long ones. There is a need to test if there is any correlation between pollen grain size and filament length, as is the case with *Oxalis* taxa (Dreyer, 1996).

### 5.2.3 Pollen grain shape

According to the shape classification of Walker and Doyle (1975) the shape of the pollen grains of all specimens examined during the present study can be described as prolate. The shape can further be divided into subprolate, euprolate and perprolate and all these shape classes are found in each of the pollen types or subtypes. Euprolate pollen grains are most common and are represented in all the pollen types and subtypes. However, subtype B<sub>3</sub> is most frequently represented by perprolate pollen grains. It is difficult to divide or classify taxa on the basis the pollen grains shape and therefore is of minor taxonomic significance in terms of the subdivision of the genus *Heliophila*.

### 5.2.4 Apertures

All pollen grains of *Heliophila* taxa are tricolpate. Thus aperture number does not contribute any data of taxonomic importance that can be used in the subdivision of the genus.

## 5.3 Cluster analysis

Groupings done using palynological features such as pollen grain size and pollen grain shape demonstrate that it is very difficult to distinguish different groups. One way to understand a large group of organisms is to subdivide them into recognisable categories and then sort them out and understand the array that exists (Sokal and Sneath, 1963). The different subgroups formed can help to display this range of variation and might even contribute to a better understanding of the taxonomy of the taxon. In the present study a statistical analysis of unweighted, numerical coded data, including morphological data alone and obtained by using cluster analysis methods, correlates remarkably well with the groups as proposed by pollen grain types. Cluster analysis was only done after the pollen grain types were identified and then compared to the classification of Marais, 1970 (Table 4.1). This was done in order to determine which other morphological characters support the suggested groupings as proposed by the pollen grain tectums.

The result of the cluster analysis also proposes a hypothesis for the subdivision of *Heliophila* into groups and/or subgroups. Two main clusters (groups) of OTUs labelled Cluster A and Cluster B are identified at a linkage distance of 26.5 (Fig 4.6). These groups correlate well with some of the morphological features of the taxa such as habit, life form, and leaf type.

Plant habit seems to play a major role in the construction of the phenogram. Both herbaceous and shrubby plant habits are found in the two major clusters A and B. The majority of the taxa in cluster A are annual herbs with psilate pollen grains (Pollen type A). The only exceptions are *H.*



*cornuta*, *H. elata* and *H. cuneata*, which are shrubs and *H. linearis* and *H. cinerea*, which are perennial herbs. Subcluster A2bb is the only separate group consisting of annual herbaceous taxa with micro-reticulate to reticulate pollen grains (Pollen type B<sub>2</sub>). All species in Cluster B are perennials, which are either herbs or shrubs sharing the three types of pollen grains namely pollen types A, B<sub>1</sub> and B<sub>3</sub>. Subcluster B2 consists only of perennial herbs with psilate pollen grains, whereas subcluster B1 consists of shrubs with both psilate to psilate micro-perforate and micro-reticulate to reticulate pollen grains. When this subcluster is further divided into two clusters, B1aa and B1ab, it is found that taxa in subcluster B1aa have psilate pollen grains, whereas the remaining subclusters B1ab and B1b consist of shrubs with micro-reticulate to reticulate tectums. However, subcluster B1ab includes taxa with two different pollen subtypes, type B<sub>1</sub> and B<sub>3</sub>.

Plant habit and life form can thus play a significant role in the subgeneric delimitation within *Heliophila*. The subdivisions based on plant habit and life form are almost completely congruent with the distribution of the pollen grain types.

Leaf type and leaf margins are in a way congruent with the subclusters proposed by the morphological cluster analysis. Some subclusters have only simple leaves with entire margins such as subcluster A1b and cluster B, whereas others consist of species with compound leaves only such as subclusters A2a and A2bb (Fig 4.6). However, there are some taxa that consist of species with both simple and compound leaves, occurring in separate individuals. Examples include *H. acuminata*, *H. sabulata*, *H. carnosa*, *H. minima*, *H. trifurca* and *H. bulbostyla*, just to mention a few. The leaf margins also vary among the taxa of *Heliophila*. For example, subclusters A1 and cluster B consist of taxa, which have leaves with entire margins, except for *H. elongata* (Fig 4.6). However, there are some taxa in which leaves with both entire and lobed margins are formed by the same individual, but on different branches such as *H. tricuspidata* (Fig. 4.2 E) or along the same branch such as *H. africana* (Fig. 4.2 F). There is also a variation in the number of lobes present per leaf, ranging from three to seven within different taxa. It is therefore difficult to classify the taxa of *Heliophila* based on the leaf type and margins alone. These characters may, however, play a significant role when used in conjunction with other characters.

Petal size also seems to vary depending on the plant habit and pollen grain types. The petals of annual herbs with psilate pollen grains are smaller than the petals of shrubs, which have micro-reticulate to reticulate pollen grains. All taxa with micro-reticulate pollen grains have

appendages on two of their petals. For example, all taxa in subcluster B (except for *H. rigidiuscula*) and all taxa in subcluster A2bb display appendages on two of their petals.

Appendages and indumentum of filaments can also play a role in the classification of the taxa within the genus *Heliophila*. All the taxa in subcluster B1 have filaments without appendages and all are shrubby plants. The only subcluster that has filaments papillated near the base, is A2bb comprising *H. diffusa*, *H. pendula* and *H. meyeri*. These are the only herbaceous taxa with a micro-reticulate to reticulate pollen grain type. Therefore, the filament indumentum is of taxonomic significance when it comes to the identification of the three species mentioned above.

The presence of the stipe on the ovary is also taxonomically significant. The ovaries of all annual herbaceous taxa (except for *H. brassicaefolia*) lack stipes, whereas the majority of shrubby taxa with a micro-reticulate pollen grain type do have stipes. Exceptions are *H. scandens*, *H. glauca*, *H. dregeana* and *H. nubigena*. All perennial herbs in subcluster B2 do have stipes. The ovule number per ovary can also be of taxonomic importance when it comes to the identification of taxa within the genus *Heliophila*. The majority of taxa with micro-reticulate to reticulate pollen grains have a limited number of ovules per ovary ranging from 1 to 18. The only exception is *H. scoparia*, which may have up to 36 ovules per ovary. The majority of taxa with psilate pollen grains usually have a high number of ovules per ovary, ranging from 20 to 52 and rarely going down below 20.

The size of the fruits and shape of the margins of the fruits is somehow congruent with some of the subclusters proposed by the cluster analysis. The fruits of shrubby taxa with reticulate pollen grains are much wider, containing few seeds per fruit and the margins are entire. Exceptions here are *H. dregeana*, *H. filicaulis*, *H. tulbaghensis* and *H. ramosissima*, which have fruits with moniliform margins. In contrast, the fruits of the majority of taxa with psilate pollen grains are long and narrow, containing many seeds per fruit and the margins are either entire or moniliform (Appendix IV). Therefore the size of the fruits can play a significant role in the classification of the genus *Heliophila*, while it is difficult to classify the genus based on the type of fruit margins as both habits and pollen types have fruits with either entire or moniliform margins.

The subdivision of *Heliophila* as proposed by the cluster analysis is almost congruent with the pollen grain types based on tectum structure. Both types of pollen grains occur in the two main clusters (A and B; Fig. 4.6). The subclusters formed by the cluster analysis are also congruent with the two pollen grain tectum types. Most of the subclusters in Cluster A have psilate or

psilate micro-perforate pollen grains (type A). However, in cluster A, only subcluster A2bb (Fig. 4.6), comprising *H. diffusa*, *H. pendula* and *H. meyeri*, has reticulate pollen grains (pollen grain subtype B<sub>2</sub>). The subclusters in cluster B are also congruent with the main pollen grain types. Subclusters B2 and B1aa have pollen type A with a psilate or psilate micro-perforate tectum with suprategal spinules, whereas subcluster B1ab has micro-reticulate to reticulate pollen grains consisting the two subtypes B<sub>1</sub> and B<sub>3</sub>. However, within this cluster, the two pollen grain subtypes do not form recognised clusters.

#### **5.4 Proposed Groups based on palynological and some selected morphological data**

According to the palynological data and morphological data used in cluster analysis, *Heliophila* taxa can be divided into several subgroups. With regard to habit linked to life form and palynological evidence, *Heliophila* taxa can be divided into two main groups, which again can be subdivided into six subgroups (Table 5.1). However, other morphological features such as leaf type, reproductive parts (androecium and gynoecium), fruit venations and margins can also play an important role in the classification of the genus and identification of taxa. The two main pollen types are found in both of the two groups. From the exposition of the groups as proposed by pollen types and plant habit linked to life form (Table 5.1), it is clear that pollen type A is dominant pollen type in herbaceous taxa whereas pollen type B represents the dominant pollen type in shrubby taxa. However, as stated before, boundaries between the different types of exine sculpturing in pollen type B are not sharp. On the basis of pollen data alone, the placing of the taxa with type B pollen in a particular group becomes quite difficult.

The present study demonstrates the difficulties met when classification based on only one set of data such as pollen morphology (including pollen size, pollen shape and exine sculpturing) is used. However, when plant habit and life form are used together with palynological evidence, the genus *Heliophila* can be subdivided into groups that are well understood. Therefore, integration of data from different sources is required for a better understanding and classification of the genus *Heliophila*. A multidisciplinary approach including as many micro- and macro-morphological data, molecular data and karyological data would still yield better results.

Table 5.1. Grouping of *Heliophila* taxa according to habit linked to life form as well as pollen types (SG = Subgroup, PT = Pollen type)

Group	SG	Plant habit	PT	Taxa
I	a	Annual herbs	A	<i>H. amplexicaulis</i> , <i>H. gariepina</i> , <i>H. pinnata</i> , <i>H. namaquana</i> , <i>H. patens</i> , <i>H. lactea</i> , <i>H. remotiflora</i> , <i>H. acuminata</i> , <i>H. leptophylla</i> , <i>H. arenaria</i> var. <i>arenaria</i> , <i>H. arenaria</i> var. <i>acocksii</i> , <i>H. arenaria</i> var. <i>glabrescens</i> , <i>H. arenaria</i> var. <i>agtertuinensis</i> , <i>H. affinis</i> , <i>H. pusilla</i> var. <i>pusilla</i> , <i>H. pusilla</i> var. <i>macrosperma</i> , <i>H. pusilla</i> var. <i>setacea</i> , <i>H. pusilla</i> var. <i>lanceolata</i> , <i>H. bulbostyla</i> , <i>H. adpressa</i> , <i>H. linoides</i> , <i>H. descurva</i> , <i>H. africana</i> , <i>H. brassicaefolia</i> , <i>H. pectinata</i> , <i>H. pubescens</i> , <i>H. collina</i> , <i>H. laciniata</i> , <i>H. deserticola</i> , <i>H. seselifolia</i> var. <i>seselifolia</i> , <i>H. seselifolia</i> var. <i>nigellifolia</i> , <i>H. seselifolia</i> var. <i>marlothii</i> , <i>H. arenosa</i> , <i>H. variabilis</i> , <i>H. crithmifolia</i> , <i>H. latisiliqua</i> var. <i>latisiliqua</i> , <i>H. latisiliqua</i> var. <i>macrosperma</i> , <i>H. minima</i> , <i>H. trifurca</i> , <i>H. coronopifolia</i> , <i>H. digitata</i> , <i>H. macowaniana</i> , <i>H. promontorii</i> , <i>H. refracta</i> and <i>H. schulzii</i> .
	b	Annual herbs	B <sub>2</sub>	<i>H. diffusa</i> var. <i>diffusa</i> , <i>H. diffusa</i> var. <i>flacca</i> , <i>H. meyeri</i> var. <i>meyeri</i> , <i>H. meyeri</i> var. <i>minor</i> and <i>H. pendula</i>
	c	Perennial herbs	A	<i>H. cinerea</i> , <i>H. linearis</i> var. <i>linearis</i> , <i>H. linearis</i> var. <i>linearifolia</i> , <i>H. linearis</i> var. <i>reticulata</i> , <i>H. alpina</i> , <i>H. rigidiuscula</i> , <i>H. katbergensis</i> and <i>H. eximia</i>
II	a	Shrubs	A	<i>H. cornuta</i> var. <i>cornuta</i> , <i>H. cornuta</i> var. <i>squamata</i> , <i>H. elata</i> var. <i>elata</i> , <i>H. elata</i> var. <i>pillansii</i> , <i>H. sabulata</i> , <i>H. elongata</i> , <i>H. suavisissima</i> and <i>H. carnosa</i> .
	B	Shrubs	B <sub>1</sub>	<i>H. scandens</i> , <i>H. glauca</i> and <i>H. brachycarpa</i>
	C	Shrubs	B <sub>3</sub>	<i>H. tulbaghensis</i> , <i>H. rimicola</i> , <i>H. esterhuyseniae</i> , <i>H. dregeana</i> , <i>H. tricuspidata</i> , <i>H. cedarbergensis</i> , <i>H. scoparia</i> var. <i>scoparia</i> , <i>H. scoparia</i> var. <i>aspera</i> , <i>H. callosa</i> , <i>H. nubigena</i> , <i>H. macra</i> and <i>H. ramosissima</i>

This subdivision of the genus *Heliophila* based on plant habit, life form and palynological evidence, supports the classification of the genus into infrageneric units as proposed by De Candolle (1821) and revised by Sonder (1860). De Candolle (1821) divided the *Heliophila* taxa into eight sections namely sections *Carponema*, *Leptormus*, *Ormiscus*, *Selenocarpaea*, *Orthoselis*, *Pachystylem*, *Lanceolaria* and *Carpopodium* (Appendix I). Sonder (1860) divided the genus into six sections using the same divisions, but excluding *Carponema* and *Carpopodium* (Appendix II). Both De Candolle (1821) and Sonder (1860) used features such as plant habit, life form and fruit morphology to subdivide the genus into sections. Although Marais (1970) merged the species under a massive genus without internal structures, he did arrange the taxa in an order (or sequence) that supports the groupings based on habit and life form. This arrangement is also supported by pollen type and the proposed subdivisions compares well with the arrangement made by Marais (1970; Table 4.1). Pollen data do provide additional evidence to support the view that the *Heliophila* is a heterogeneous genus and should be divided into infrageneric units.

## 5.5 Pollination

An understanding of the evolution of angiosperms is often closely related to an understanding of their pollination mechanisms and evolution thereof (Crepet 1979 as cited by Dreyer 1996). No pollination studies could be traced for any *Heliophila* taxa, but several systematists have studied and written about the pollination of the family Brassicaceae. Knuth (1908) and Hicky and King (1981) both indicated that the flowers of Cruciferae (Brassicaceae) are homogamous, but they differ in the way they suggest that Brassicaceae species are pollinated. According to Knuth (1908) all taxa of Brassicaceae are entomophilous (pollinated by insects), while Hicky and King (1981) as cited on the website <http://www.vancouver.wsu.edu/fac/robson/c/natrs301/dicots/mustard.htm> indicated that Brassicaceae taxa are both anemophilous (wind pollinated) and entomophilous, but that entomophily is more common. Insects are attracted by the petals, which are usually yellow or white, and rarely violet, blue or red (Knuth 1908). Hicky and King (1981) also indicated that pollination is accomplished by nectar secretion into the bases of the pouched sepals, which attract insects and promote cross-pollination. Self-pollination does, however, occur often.

In most of the perennial *Heliophila* taxa, the pollen grains are smaller in size, than the pollen grains of the annuals. The pollen grain size may correlate well with the distance the pollen grains of each life form have to be dispersed to still affect successful pollination. An assumption may

be made that most shrubby taxa are perennials, and are large plants and grow further apart. Hence they require smaller pollen grains to be dispersed over longer distances. In contrast, annual herbs are usually smaller plants, growing near to each other and have bigger pollen grains. This may be more advantageous since pollen grains have to be dispersed over shorter distances. However, according to Levin and Kerster (1974) as cited by Proctor *et al.* (1996), most pollen grains are deposited near their source and that the quantity of pollen grains deposited decreases with distance. This is true for both wind and insect pollinated species, although it is most obvious for wind-pollinated species (Levin and Kerster, 1974). The distance of pollination also depends on the length of the visit (how far the pollinator has to travel from one plant to the other), the condition of the individual flowers and the behaviour of the visitor (Proctor *et al.*, 1996).

An annual has a short period of time to complete its life cycle, and thus only has the opportunity to acquire limited metabolic reserves during this short time. So what the annuals here seem to do is to not waste their limited energy reserves on making large flowers with large petals, but rather to reserve their energy resources to make large pollen grains. And this is clever, because at the end of the day it is crucial for the annual plant to make sure that it achieves successful pollination and thus makes seeds before they die down at the end of the growing season. The perennial plants in contrast, have more resources available because they live longer. They seem to dedicate more metabolic energy to making larger flowers. This would give them a competitive advantage in terms of attracting pollinators. The smaller pollen grains may not all survive, and affect successful pollination, but it is not a train smash, because the plant does not die at the end of the growing season – it could try again next year, without “killing the species” if it does not succeed this year.

## **5.6 Evolution**

It is a well-known phenomenon that morphological features, and especially vegetative characters, are rather susceptible to environmentally induced changes. In contrast, palynological features are known to be much more conservative and stable in an evolutionary sense. According to Kuprianova (1969) as cited by Dreyer (1996), pollen is more resilient against environmentally induced changes because of its involvement in the reproductive system of the plant.

Evolutionary trends in pollen wall architecture are a potential source of phylogenetic information. Muller (1970) proposes an evolutionary progression of exine structure from columella-derived structures (mostly with reticulate architecture) to tectal structures (mostly

spinose or verrunose suprategal structures) to combined structure such as found in the spinose exine of the Compositae. However, Walker (1976) warns that sculpturing represents a more or less reversible character, which should be interpreted in terms of the individual correlations observed within a given taxon. I therefore regard the micro-reticulate to reticulate tectum to represent the primitive state in this genus. With regard to the genus *Heliophila*, a reticulate tectum is also associated with many other primitive characters. Morphologically, many of the taxa in the genus *Heliophila* with reticulate pollen are associated with a shrubby habit, a perennial life form, simple leaves, the presence of stipules and reduced number of ovules as compared to those with psilate tectums with suprategal spinules. Various systematists, such as Hutchinson (1959), suggested that shrubs and perennials (especially woody plants), simple leaves, the presence of stipules and a reduction in number of ovules in the ovary represent the primitive state. The habit is also a character of primary and fundamental importance in the more primitive groups, although recently there has been some controversy regarding the habit of the most primitive living angiosperm being woody or herbaceous (Subrahmanyam, 1995). I regard the pollen grain type with a psilate or psilate micro-perforate tectum with suprategal spinules to be the advanced type.

However, these suggestions should be considered with care, because similar pollen structures can develop independently in unrelated taxa merely to improve the functionality of their grains. The problems of relationships, convergence and parallelism must therefore be conceded when studying the patterns of pollen morphological variation. Convergence is generally brought about by taxa growing in similar climatic and habitats, having similar methods of pollination and dispersal.

The evolution of the taxa of *Heliophila* should be evaluated in association with the biogeographical patterns of the Cape. The Cape Region is well known for its variations in climatic and abiotic conditions. The majority of *Heliophila* taxa are endemic to the Cape Region with its diverse climatic and abiotic conditions. The evolutionary hypothesis proposed here must be regarded as a speculative view until additional data on the pollen of the related genera and even related families are available. Phylogenetic studies of the genus *Heliophila* are therefore required to determine their true significance of pollen morphology in the systematics of the genus. Once a robust molecular phylogeny for *Heliophila* is in place, these hypotheses can be tested.

## CHAPTER SIX

## CONCLUSIONS

Palynological data alone cannot form the basis of a classification however, it can contribute to the systematics of the genus *Heliophila*. Palynology reveals that the genus *Heliophila* is a heterogeneous taxon and it also supports the idea of a subdivision into infrageneric groupings or sections, as was proposed by De Candolle (1821) and revised by Sonder (1860). The structural and sculptural features of the exine proved important in the demarcation of the pollen types and two distinctively different pollen types were identified namely, grains with the psilate or psilate micro-perforate and grains with the micro-reticulate or reticulate type of tectum. Although there are significant differences in pollen grain size between and among the pollen types, pollen grain size does not appear to be of taxonomic importance in the subdivision of the genus *Heliophila*. However, the pollen grains of the taxa with the psilate or psilate micro-perforate pollen grains with suprategal spines, which comprise herbaceous taxa, seem to be larger than the micro-reticulate to reticulate pollen grains, which occur in the shrubby taxa. Therefore, pollen grain size can be of taxonomic significance when used in conjunction with other data sources. All taxa have prolate pollen grains with tricolpate apertures and thus shape and apertures are of no significant importance in the subdivision of the genus. The most important contribution of the pollen morphology to the taxonomy of the genus is the delimitation of the genus into two major groups based on the sculpture and structure of the pollen tectum. To a larger extent, the proposed groupings of the taxa based on tectum types are well supported by plant habit, life form, leaf-type and reproductive parts (androecium and gynoecium). There is a need for further studies in terms of morphology, karyology and molecular work in order to have a comprehensive taxonomic revision of the genus *Heliophila*. Molecular work could give insight as to whether the genus *Heliophila* is monophyletic or not and can provide a better understanding of the phylogenetic relationships between the taxa. The present study forms a part of a multidisciplinary approach in understanding the systematics of the genus. These results will be integrated with results from other data sources in future.



## CHAPTER SEVEN                      REFERENCES

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**APPENDIX I:**Sections and species of *Heliophila* as proposed by De Candolle (1821).

SECTION	TAXA
SECTION I: <b>CARPONEMA</b>	<i>H. filiformis</i> L.f.
SECTION II: <b>LEPTORMUS</b>	<i>H. dissecta</i> Thunb., <i>H. tenela</i> Banks., <i>H. tenuisiliqua</i> DC., <i>H. longifolia</i> DC., and <i>H. sonchifolia</i> DC.,
SECTION III: <b>ORMISCUS</b>	<i>H. amplexicaulis</i> L.f., <i>H. rivalis</i> Burch., <i>H. variabilis</i> Burch., <i>H. pendula</i> Willd., <i>H. trifida</i> Thunb., <i>H. pusilla</i> L.f., <i>H. lepidiodes</i> Link., and <i>H. seselifolia</i> Burch.
SECTION IV: <b>SELENOCARPAEA</b>	<i>H. diffusa</i> DC., and <i>H. peltaria</i> DC.
SECTION V: <b>ORTHOSELIS</b>	
SUB SECTION 1	<i>H. pilosa</i> Lam., <i>H. digitata</i> L.f., <i>H. incisa</i> DC., <i>H. divaricata</i> DC., <i>H. coronopifolia</i> L., <i>H. trifurca</i> Burch., <i>H. pectinata</i> Burch., <i>H. faeniculacea</i> Brown, <i>H. chamaemelifolia</i> Burch., and <i>H. crithmifolia</i> Willd.
SUB SECTION 2	<i>H. abrotanifolia</i> DC., <i>H. glauca</i> Burch., <i>H. fascicularis</i> DC., <i>H. suavissima</i> Burch., <i>H. subulata</i> Burch., <i>H. platysiliqua</i> Brown, <i>H. linearifolia</i> Burch., <i>H. stylosa</i> Burch., <i>H. virgata</i> Burch., and <i>H. scoparia</i> Burch.
SECTION VI: <b>PACHYSTYLUM</b>	<i>H. incana</i> Ait h.
SECTION VII: <b>LANCEOLARIA</b>	<i>H. macrosperma</i> Burch.,
SECTION VIII: <b>CARPOPODIUM</b>	<i>H. cleomoides</i> DC.,
NOT PLACED IN ANY SECTION	<i>H. liniflora</i> DC., <i>H. molluginea</i> DC., <i>H. heterophylla</i> Thunb., <i>H. lyrata</i> Thunb., <i>H. callosa</i> DC., <i>H. linearis</i> DC., <i>H. graminea</i> DC., and <i>H. elongata</i> DC.

## APPENDIX II

Sections and species of *Heliophila* as proposed by Sonder (1860)

Section	Diagnostic Features	Species included
1. LEPTORMUS DC.	Pods linear, moniliform; the beading oval. Herbs.	<i>H. dissecta</i> Thunb., <i>H. longifolia</i> DC., <i>H. sonchifolia</i> DC., <i>H. fistulosa</i> Sond., <i>H. caledonica</i> Sond., <i>H. pubescens</i> Burch., <i>H. affinis</i> Sond., <i>H. eckloniana</i> Sond.
2. ORMISCUS DC.	Pods linear, moniliform; beading orbicular. Herbs.	<i>H. amplexicaulis</i> L.f., <i>H. pusilla</i> L. f., <i>H. monticola</i> Sond., <i>H. trifida</i> Thunb., <i>H. concatenata</i> Sond., <i>H. rivalis</i> Burch., <i>H. pendula</i> Willd., <i>H. variabilis</i> Burch., <i>H. coronopia</i> L., <i>H. dentifera</i> Sond.
3. SELENOCARPEA DC.	Pods oval or sub-orbicular. Herbs	<i>H. diffusa</i> DC., <i>H. peltaria</i> DC., <i>H. flacca</i> Sond.
4. ORTHOSELIS DC.	Pod linear, with straight margins or somewhat torulose. Herbs of shrubs	<i>H. macrostylis</i> E. Mey., <i>H. latisiliqua</i> E. Mey., <i>H. meyeri</i> Sond., <i>H. viminalis</i> E. Mey., <i>H. tenuifolia</i> Sond., <i>H. seselifolia</i> Burch., <i>H. pectinata</i> Burch., <i>H. refracta</i> Sond., <i>H. crithmifolia</i> Willd., <i>H. chamaemelifolia</i> Burch., <i>H. foeniculaceae</i> R.Br., <i>H. gracilis</i> Sond., <i>H. trifurca</i> Burch., <i>H. stricta</i> Sond., <i>H. linearis</i> DC., <i>H. divaricata</i> Herb., <i>H. graminea</i> DC., <i>H. pilosa</i> Lam., <i>H. cornuta</i> Sond., <i>H. abrotanifolia</i> Herb., <i>H. brassicaefolia</i> E. & Z., <i>H. reticulata</i> E. & Z., <i>H. scoparia</i> Burch., <i>H. brachycarpa</i> Meisn., <i>H. dregeana</i> Sond., <i>H. virgata</i> Burch., <i>H. glauca</i> Burch., <i>H. callosa</i> DC., <i>H. elata</i> Sond., <i>H. stylosa</i> Burch., <i>H. rigidiuscula</i> Sond., <i>H. fascicularis</i> Herb., <i>H. suavissima</i> Burch., <i>H. subulata</i> Burch., <i>H. succulenta</i> Herb., <i>H. linearis</i> DC.
5. PACHYSTYLUM DC.	Pubescent suffrutices. Pods linear, tipped with a short and thickened style	<i>H. incana</i> Ait., <i>H. arenaria</i> Sond.
6. LANCEOLARIA DC.	Glabrous shrubs, with lanceolate pods	<i>H. florulenta</i> Sond., <i>H. macrosperma</i> Burch.

**APPENDIX III:** Data Matrix of 72 vegetative and reproductive characters (excluding palynological characters) used in the cluster analysis of 69 *Heliophila* taxa

TAXON	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
<i>H. amplexicaulis</i>	1	0	1	0	0	1	0	0	1	0	0	1	0	1	0	0	0	1	1	0	0	1	1	0
<i>H. obibensis</i>	1	0	1	0	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1	0	1	0
<i>H. gariiepina</i>	1	0	1	0	0	1	0	0	1	0	0	1	0	1	0	0	0	1	0	0	0	1	1	0
<i>H. namaquana</i>	1	0	1	0	0	1	0	1	0	0	0	0	1	1	0	0	0	1	0	0	1	1	1	0
<i>H. bulbostyla</i>	1	0	1	0	0	1	1	1	0	1	0	0	0	1	1	0	0	1	0	1	1	0	1	0
<i>H. adpressa</i>	1	0	1	0	0	1	1	1	0	1	0	0	0	1	1	0	0	1	0	1	1	0	0	0
<i>H. pectinata</i>	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	1	0
<i>H. pubescens</i>	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	1	0
<i>H. collina</i>	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	1	1
<i>H. laciniata</i>	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	1	0	1	0
<i>H. deserticola</i>	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	1	1	0
<i>H. seselifolia</i>	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	1	1	0
<i>H. variabilis</i>	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	0	1	0	1	0	1	0	1	0
<i>H. minima</i>	1	0	1	0	0	1	1	1	0	0	0	0	1	1	0	0	0	0	0	1	1	0	1	0
<i>H. crithmifolia</i>	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	1	0
<i>H. trifurca</i>	1	0	1	0	0	1	1	1	0	0	0	0	0	1	0	0	0	1	0	0	1	1	1	0
<i>H. latisiliqua</i>	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	1	0
<i>H. pinnata</i>	1	0	1	0	0	1	1	1	0	1	0	0	0	1	0	0	0	0	1	0	1	0	1	0
<i>H. pusilla</i>	1	0	1	0	0	0	1	1	1	1	1	0	0	1	0	0	0	1	0	0	0	1	0	0
<i>H. patens</i>	1	0	1	0	0	1	0	1	0	1	0	0	0	1	0	0	0	1	0	0	0	1	1	0
<i>H. diffusa</i>	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	1	0	1	0
<i>H. pendula</i>	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	1	0	0	0
<i>H. meyeri</i>	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	1	0	1	0
<i>H. coronopifolia</i>	1	0	1	0	0	1	1	0	0	0	0	0	0	1	1	1	0	1	1	0	1	1	1	0
<i>H. acuminata</i>	1	0	1	0	0	1	1	1	1	0	0	0	0	1	1	0	0	1	1	0	0	1	1	0
<i>H. macowaniana</i>	1	0	1	0	0	1	1	0	0	0	0	0	0	1	1	1	0	1	1	0	1	0	0	0
<i>H. promontorii</i>	1	0	1	0	0	1	1	0	0	0	0	0	0	1	1	1	0	0	1	0	1	0	1	0
<i>H. digitata</i>	1	0	1	0	0	1	1	0	0	0	0	0	0	0	1	1	0	0	1	0	0	1	1	0
<i>H. refracta</i>	1	0	1	0	0	1	1	0	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0
<i>H. schulzii</i>	1	0	1	0	0	0	1	0	0	0	0	0	0	1	1	1	0	0	0	1	1	0	1	1
<i>H. lactea</i>	1	0	1	0	0	1	1	1	0	1	0	0	0	1	0	0	0	1	0	0	0	1	1	0
<i>H. linoides</i>	1	0	1	0	0	1	0	1	0	0	0	0	0	1	1	1	0	1	0	0	1	1	1	0
<i>H. remotiflora</i>	1	0	1	0	0	1	0	1	0	1	0	0	0	1	0	0	0	1	0	0	0	1	1	0
<i>H. leptophylla</i>	1	0	1	0	0	1	1	1	0	0	0	0	1	1	0	0	0	1	0	0	0	1	1	0
<i>H. arenosa</i>	1	0	1	0	0	0	1	0	0	0	0	0	0	0	1	1	0	1	0	0	0	1	1	0

TAXON	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
<i>H. amplexicaulis</i>	1	0	0	1	1	0	1	1	1	1	0	0	1	1	0	1	0	0	0	0	0	0	1	0
<i>H. obibensis</i>	0	0	0	1	0	0	1	0	0	0	1	0	1	1	0	0	1	0	0	0	0	0	1	0
<i>H. gariiepina</i>	0	0	0	0	0	0	1	1	0	1	0	0	1	1	0	0	1	0	0	1	0	0	0	0
<i>H. namaquana</i>	0	0	0	1	1	0	1	0	0	1	0	0	1	1	0	1	0	0	0	0	1	0	0	0
<i>H. bulbostyla</i>	0	0	0	1	0	0	0	1	1	1	0	0	0	0	0	1	1	0	0	1	0	0	0	0
<i>H. adpressa</i>	0	1	0	1	1	1	1	0	0	1	0	0	0	0	0	0	1	0	0	1	0	0	0	0
<i>H. pectinata</i>	0	0	0	1	1	0	0	1	0	0	0	1	1	1	0	0	1	0	0	0	1	0	0	0
<i>H. pubescens</i>	1	0	0	1	0	0	0	0	0	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0
<i>H. collina</i>	0	0	0	1	1	0	0	1	0	1	0	0	1	1	0	0	1	0	0	0	0	0	1	0
<i>H. laciniata</i>	0	0	0	1	1	0	1	0	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	0
<i>H. deserticola</i>	0	0	0	1	0	0	1	1	0	0	0	1	0	0	0	1	1	0	0	1	0	0	0	0
<i>H. seselifolia</i>	0	0	0	1	0	0	1	1	0	1	1	1	1	1	0	1	0	0	0	0	0	0	1	0
<i>H. variabilis</i>	0	0	0	1	0	0	1	1	0	0	0	1	0	1	0	1	0	0	0	1	0	0	0	0
<i>H. minima</i>	0	0	0	1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0
<i>H. crithmifolia</i>	0	0	0	1	0	0	1	1	0	0	0	1	0	0	0	1	0	0	0	0	0	0	1	0
<i>H. trifurca</i>	0	0	0	1	1	0	1	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	1	0
<i>H. latisiliqua</i>	0	0	0	1	0	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	1	0	0	0
<i>H. pinnata</i>	0	0	0	1	0	0	1	0	0	0	0	1	1	1	0	1	0	0	0	0	0	0	1	0
<i>H. pusilla</i>	1	0	0	1	0	0	1	0	0	0	0	1	1	1	0	1	0	0	1	1	0	0	1	0
<i>H. patens</i>	0	0	0	1	0	0	1	0	1	1	0	0	1	1	0	1	0	0	0	0	0	0	0	0
<i>H. diffusa</i>	0	0	0	1	0	0	1	1	1	0	1	0	1	1	0	1	0	1	0	0	1	0	0	0
<i>H. pendula</i>	1	0	0	1	0	0	1	1	1	0	1	0	1	1	0	1	0	1	0	0	1	0	1	0
<i>H. meyeri</i>	0	0	0	1	0	0	1	0	1	0	1	0	0	1	0	1	0	1	0	1	0	0	1	0
<i>H. coronopifolia</i>	0	0	0	1	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0
<i>H. acuminata</i>	0	0	0	1	0	0	1	1	0	0	0	1	1	1	0	1	0	0	0	1	0	0	0	0
<i>H. macowaniana</i>	1	0	0	1	1	0	1	1	0	0	0	1	0	1	0	1	0	0	0	1	0	0	0	0
<i>H. promontorii</i>	0	0	0	1	1	0	1	1	0	0	0	1	1	1	0	1	0	0	0	1	0	0	0	0
<i>H. digitata</i>	0	0	0	1	1	1	1	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0
<i>H. refracta</i>	0	1	0	1	1	1	1	1	0	0	1	0	0	0	0	1	0	0	1	1	0	0	0	0
<i>H. schulzii</i>	0	0	0	1	1	1	1	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0
<i>H. lactea</i>	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0
<i>H. linoides</i>	0	0	0	1	1	1	1	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0
<i>H. remotiflora</i>	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0
<i>H. leptophylla</i>	0	0	0	0	0	0	1	0	0	1	0	0	1	1	0	1	0	0	0	1	0	0	0	0
<i>H. arenosa</i>	0	0	0	1	0	0	0	1	0	1	0	0	0	1	0	1	0	0	0	1	0	0	0	0

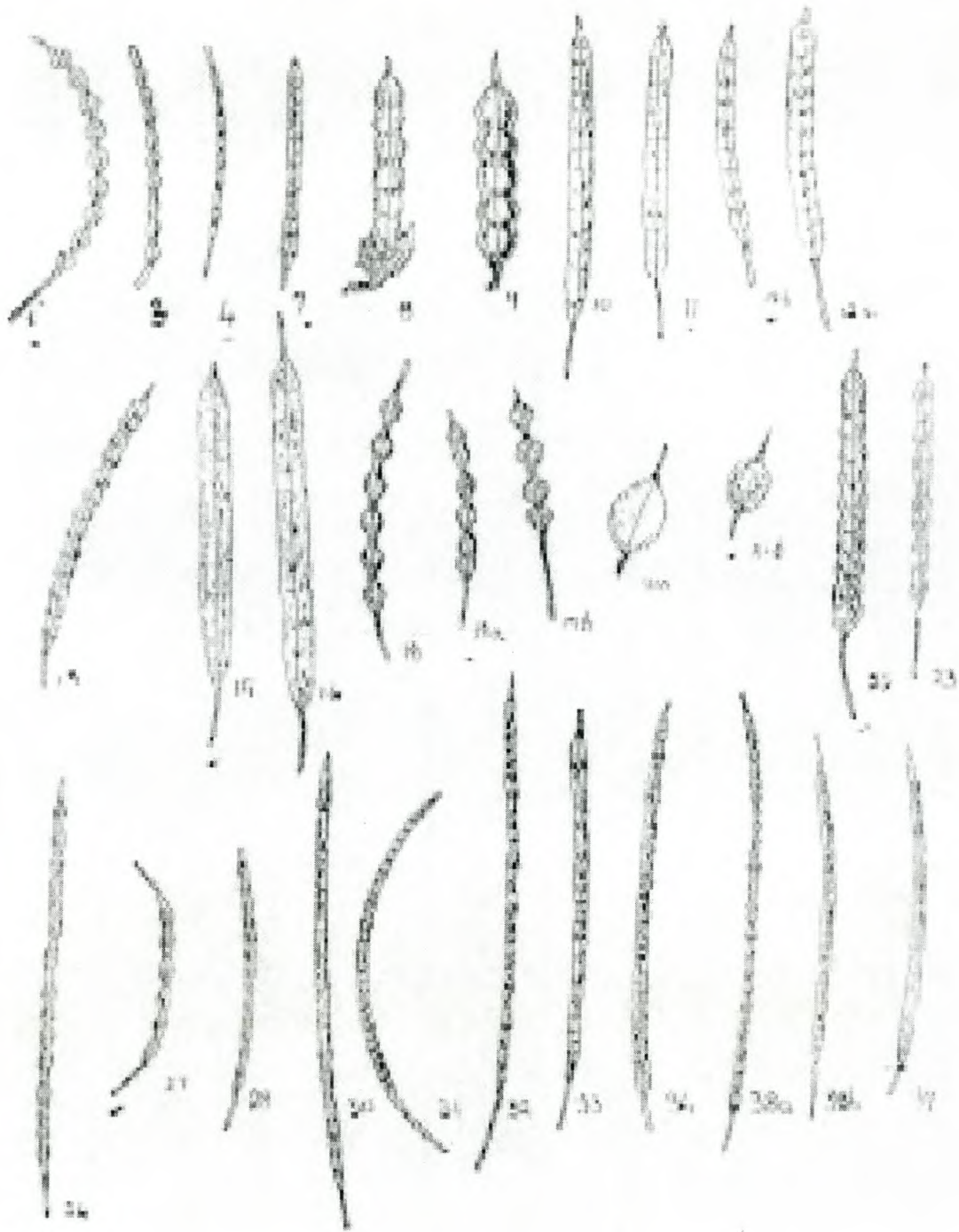
TAXON	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
<i>H. amplexicaulis</i>	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	0	0	0	1	0	0
<i>H. obibensis</i>	1	1	0	0	0	0	0	1	0	0	0	1	1	0	0	1	1	0	0	0	0	1	0	0
<i>H. gariiepina</i>	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	0	0	1	0	0
<i>H. namaquana</i>	0	0	1	0	0	0	0	0	0	0	1	0	1	1	0	0	1	1	0	0	0	0	0	1
<i>H. bulbostyla</i>	0	1	1	0	0	0	0	0	0	0	1	1	1	0	0	0	1	0	0	0	0	1	0	1
<i>H. adpressa</i>	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	1	0	0
<i>H. pectinata</i>	1	1	1	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	1	0	1	0	0
<i>H. pubescens</i>	1	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	1	0	0
<i>H. collina</i>	1	0	1	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	1	0	0	0	1
<i>H. laciniata</i>	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	1	0	0	1	0	0	0	1	0
<i>H. deserticola</i>	0	1	1	0	0	0	0	1	0	0	1	0	1	0	0	1	0	0	1	0	0	0	1	0
<i>H. seselifolia</i>	0	1	1	0	0	0	0	1	0	0	1	0	1	0	0	1	0	0	1	0	0	0	1	0
<i>H. variabilis</i>	0	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	1	0
<i>H. minima</i>	0	1	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	0	1	0
<i>H. crithmifolia</i>	0	1	1	0	0	0	0	1	0	0	1	0	1	0	0	1	0	1	0	0	0	0	1	0
<i>H. trifurca</i>	0	1	1	0	0	0	0	1	0	0	1	0	1	0	0	1	0	1	0	0	0	0	1	0
<i>H. latisiliqua</i>	1	1	1	0	0	0	0	1	0	0	1	0	1	0	0	0	0	1	0	0	0	0	1	0
<i>H. pinnata</i>	1	1	1	0	0	0	0	1	0	0	0	1	1	0	0	0	1	1	0	0	0	1	0	0
<i>H. pusilla</i>	1	1	1	0	0	0	0	1	0	0	1	1	1	1	0	0	1	1	0	0	0	1	0	0
<i>H. patens</i>	1	1	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	1	0	0	0	1	0	0
<i>H. diffusa</i>	1	0	0	0	1	0	1	0	1	0	1	0	1	0	0	1	0	1	0	0	0	0	1	0
<i>H. pendula</i>	1	1	0	0	0	0	1	0	1	0	0	1	1	0	0	0	0	1	0	0	0	0	1	0
<i>H. meyeri</i>	1	0	0	0	0	0	0	0	1	0	1	1	1	0	0	0	0	1	0	0	0	0	1	0
<i>H. coronopifolia</i>	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	1	0	0
<i>H. acuminata</i>	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	1	0	0
<i>H. macowaniana</i>	0	1	1	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	1	0	0
<i>H. promontorii</i>	0	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0
<i>H. digitata</i>	0	1	1	1	0	0	0	0	0	0	1	0	1	1	0	0	1	1	0	0	0	1	0	0
<i>H. refracta</i>	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1	0	0
<i>H. schulzii</i>	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1	0	0
<i>H. lactea</i>	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1	0	0
<i>H. linoides</i>	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	1	0
<i>H. remotiflora</i>	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	0	0	0	1	0	0
<i>H. leptophylla</i>	0	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	1	0	0
<i>H. arenosa</i>	0	0	1	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	1	0	0	0	0	0

TAXON	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
<i>H. arenaria</i>	1	0	1	0	0	1	1	1	0	0	0	0	0	1	1	1	0	1	1	0	0	1	1	0
<i>H. descurva</i>	1	0	1	0	0	1	0	1	0	0	0	0	0	1	1	0	0	0	1	0	1	1	1	0
<i>H. affinis</i>	1	0	1	0	0	1	0	0	0	1	0	0	1	1	0	0	0	1	0	0	0	1	1	0
<i>H. africana</i>	1	0	1	0	0	1	0	1	1	0	0	0	0	1	1	0	0	0	1	0	0	1	1	0
<i>H. brassicaefolia</i>	1	0	1	0	0	1	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	1	1	0
<i>H. cinerea</i>	1	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	1	0
<i>H. cuneata</i>	0	1	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	1	0	0
<i>H. linearis</i>	1	0	0	1	0	1	0	1	1	0	0	1	0	1	1	0	0	1	1	0	0	1	1	0
<i>H. cornuta</i>	0	1	0	1	0	1	0	0	0	1	0	1	0	1	0	0	0	0	1	0	0	1	1	0
<i>H. elata</i>	0	1	0	1	0	1	1	1	0	1	0	0	0	1	1	0	0	1	1	0	1	0	1	0
<i>H. sabulata</i>	1	1	1	1	0	1	1	0	1	1	0	0	0	1	0	0	0	1	0	0	1	0	1	0
<i>H. suavissima</i>	0	1	0	1	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1	0	1	0
<i>H. carnosa</i>	0	1	0	1	0	1	1	0	0	1	0	0	0	1	1	0	0	1	0	0	1	0	1	0
<i>H. rigidiuscula</i>	1	0	0	1	0	1	0	1	1	0	0	0	0	1	0	0	0	1	0	0	1	0	1	0
<i>H. katbergensis</i>	1	0	0	1	0	1	0	0	0	0	1	0	0	1	0	0	0	1	0	0	1	0	1	0
<i>H. eximia</i>	1	0	0	1	1	1	1	0	0	0	0	0	0	1	0	0	0	1	0	0	0	1	1	0
<i>H. scandens</i>	0	1	0	1	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1	0	1	0
<i>H. glauca</i>	0	1	0	1	1	1	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	1	1	0
<i>H. brachycarpa</i>	0	1	0	1	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1	0	1	0
<i>H. macrosperma</i>	0	1	0	1	0	0	1	1	0	0	0	1	0	1	0	0	0	1	0	0	1	0	1	0
<i>H. filicaulis</i>	0	1	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	1	0	0	1	0	1	0
<i>H. tulbaghensis</i>	0	1	0	1	0	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1	0	1	0
<i>H. rimicola</i>	0	1	0	1	0	1	0	1	0	0	0	1	0	1	0	0	0	1	0	0	1	0	0	1
<i>H. esterhuyseniae</i>	0	1	0	1	0	1	0	1	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1
<i>H. dregeana</i>	0	1	0	1	0	1	0	0	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1
<i>H. tricuspidata</i>	0	1	0	1	0	1	0	0	1	0	1	0	0	1	1	0	0	1	0	0	1	0	1	0
<i>H. cedarbergensis</i>	0	1	0	1	0	1	0	0	1	0	1	0	0	1	0	0	0	1	1	0	1	0	0	1
<i>H. scoparia</i>	0	1	0	1	0	1	0	1	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0
<i>H. callosa</i>	0	1	0	1	0	1	0	0	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0
<i>H. nubigena</i>	0	1	0	1	1	1	0	0	0	0	1	0	0	1	0	0	0	0	1	0	0	1	1	0
<i>H. alpina</i>	1	0	0	1	0	0	1	0	0	0	0	0	0	0	1	1	0	0	1	0	1	0	1	0
<i>H. elongata</i>	0	1	1	1	0	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	0	1	1
<i>H. macra</i>	0	1	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	1	0	0	1	1	1	0
<i>H. ramosissima</i>	0	1	0	1	1	1	0	1	0	0	0	1	0	1	0	0	0	1	0	0	0	1	1	0

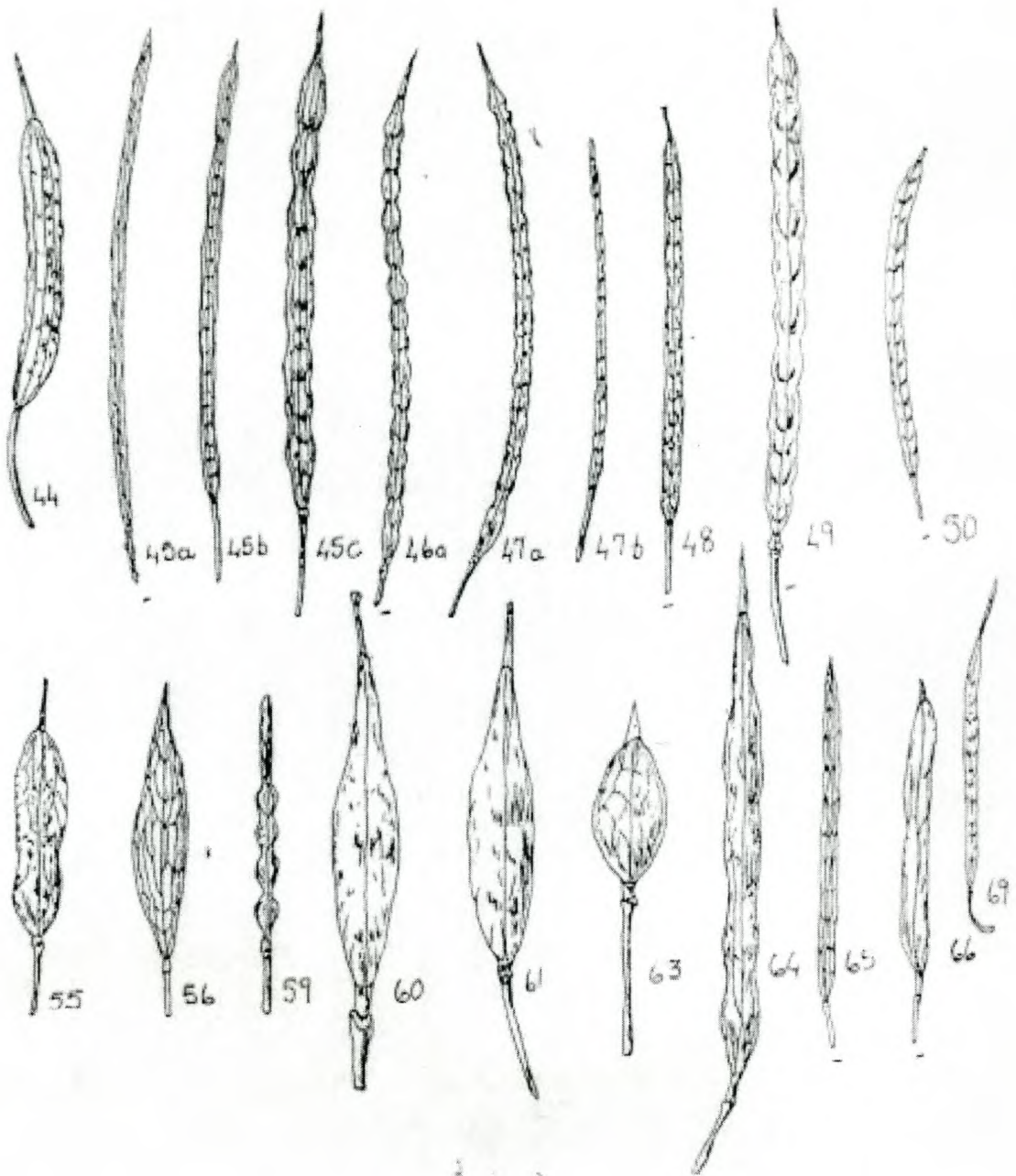
TAXON	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48
<i>H. arenaria</i>	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0
<i>H. descurva</i>	0	0	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0
<i>H. affinis</i>	0	0	0	1	0	1	1	1	0	0	1	0	1	1	0	0	1	0	0	1	0	0	0	0
<i>H. africana</i>	0	0	0	1	1	1	1	0	0	1	0	0	0	0	1	1	0	0	0	1	0	0	0	0
<i>H. brassicaefolia</i>	0	0	0	1	0	1	1	0	0	1	0	0	0	0	0	1	0	0	1	0	1	0	0	1
<i>H. cinerea</i>	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	1	0	0	0	0
<i>H. cuneata</i>	0	1	0	1	0	0	1	1	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0	0
<i>H. linearis</i>	1	0	0	1	1	1	1	0	0	1	0	0	0	0	0	1	0	0	1	0	1	0	0	0
<i>H. cornuta</i>	0	0	0	1	1	0	1	0	0	1	0	0	0	0	0	1	0	0	0	1	0	0	0	0
<i>H. elata</i>	0	0	0	1	1	0	1	0	1	1	0	0	0	0	0	1	1	0	0	1	0	0	0	0
<i>H. sabulata</i>	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0
<i>H. suavissima</i>	0	0	0	1	0	1	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0
<i>H. carnosa</i>	0	0	0	1	0	1	1	1	1	0	1	0	0	0	0	0	1	0	1	0	0	0	1	0
<i>H. rigidiuscula</i>	0	0	0	1	0	0	1	0	0	1	0	0	0	0	1	1	0	0	0	0	1	0	0	1
<i>H. katbergensis</i>	0	0	0	0	0	0	1	0	0	0	1	0	1	1	0	1	0	0	0	0	0	0	0	1
<i>H. eximia</i>	0	0	0	1	0	1	1	1	0	0	1	0	1	1	0	0	1	0	0	0	1	0	0	0
<i>H. scandens</i>	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0	0
<i>H. glauca</i>	0	0	0	1	1	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
<i>H. brachycarpa</i>	0	0	1	0	0	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1
<i>H. macrosperma</i>	0	0	0	1	0	1	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1
<i>H. filicaulis</i>	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1
<i>H. tulbaghensis</i>	0	0	0	1	1	0	1	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	1
<i>H. rimicola</i>	0	0	0	1	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1
<i>H. esterhuyseniae</i>	0	0	0	1	0	1	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1
<i>H. dregeana</i>	1	0	0	1	0	1	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	0
<i>H. tricuspidata</i>	0	0	0	1	0	0	1	1	0	0	1	0	1	1	0	0	1	0	0	0	1	1	0	1
<i>H. cedarbergensis</i>	0	0	0	1	0	1	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	1
<i>H. scoparia</i>	0	1	1	1	1	0	1	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	1
<i>H. callosa</i>	1	0	0	1	0	1	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1
<i>H. nubigena</i>	0	1	0	1	0	1	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0
<i>H. alpina</i>	0	1	0	1	1	0	1	1	1	0	1	0	1	1	0	0	1	0	0	0	0	0	0	0
<i>H. elongata</i>	0	0	0	1	1	0	0	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0
<i>H. macra</i>	0	0	0	1	1	0	1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	1	1
<i>H. ramosissima</i>	0	0	0	1	1	0	1	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	1

TAXON	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72
<i>H. arenaria</i>	1	0	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	1	1	1	0	1
<i>H. descurva</i>	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	0	0	0	0	0
<i>H. affinis</i>	1	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0
<i>H. africana</i>	0	1	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0
<i>H. brassicaefolia</i>	1	1	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0
<i>H. cinerea</i>	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0
<i>H. cuneata</i>	1	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	1	0	0	0	0	1	0
<i>H. linearis</i>	0	0	1	0	0	0	0	0	0	0	1	0	0	0	1	0	1	1	1	0	0	0	1	0
<i>H. cornuta</i>	0	0	1	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	1	0
<i>H. elata</i>	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	0	0	0	1	0	0
<i>H. sabulata</i>	0	0	1	0	0	0	0	0	0	0	1	0	1	1	0	0	1	0	0	0	0	0	1	0
<i>H. suavissima</i>	0	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	1	0	1	0	0	0	1	0
<i>H. carnosa</i>	0	1	1	0	0	0	0	0	0	0	1	0	1	0	0	1	1	1	0	0	0	0	1	0
<i>H. rigidiuscula</i>	1	1	0	0	0	0	0	1	0	1	1	0	1	0	0	1	1	1	0	0	0	0	1	0
<i>H. katbergensis</i>	1	0	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	1	0
<i>H. eximia</i>	1	1	0	0	0	0	0	0	1	0	1	0	1	0	0	1	1	1	0	0	0	0	1	0
<i>H. scandens</i>	1	1	0	0	0	0	1	0	0	1	1	0	1	0	0	1	0	1	0	0	0	1	0	0
<i>H. glauca</i>	1	1	0	0	0	0	0	1	0	0	1	0	1	0	0	1	0	1	0	0	0	1	0	0
<i>H. brachycarpa</i>	1	1	0	0	0	1	1	0	0	1	1	0	1	0	0	1	0	1	0	0	0	1	0	0
<i>H. macrosperma</i>	1	0	0	0	0	1	1	0	0	1	1	0	1	0	0	1	0	1	0	0	0	1	0	0
<i>H. filicaulis</i>	1	0	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1	0	1	0	0
<i>H. tulbaghensis</i>	1	0	1	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	1	1	0	0
<i>H. rimicola</i>	1	0	1	0	0	0	0	0	0	1	1	0	1	0	0	1	0	1	0	0	0	1	0	0
<i>H. esterhuyseniae</i>	1	0	0	0	0	1	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	1	0	0
<i>H. dregeana</i>	1	1	1	0	0	0	0	0	0	1	0	1	1	0	0	0	0	1	0	0	0	1	0	0
<i>H. tricuspidata</i>	1	1	1	0	0	0	0	0	0	1	1	0	1	0	0	1	0	1	0	0	0	1	0	0
<i>H. cedarbergensis</i>	1	1	0	0	0	1	0	0	0	1	1	0	1	0	0	0	0	0	0	1	0	1	0	0
<i>H. scoparia</i>	1	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	1	0	1	0	0	1	0	0
<i>H. callosa</i>	1	0	0	0	0	0	0	1	0	1	1	0	1	0	0	0	1	1	0	0	0	0	1	0
<i>H. nubigena</i>	1	1	0	0	1	0	1	0	0	1	1	0	1	0	0	1	1	1	0	0	0	0	0	1
<i>H. alpina</i>	1	0	0	0	0	0	0	1	1	1	1	0	1	0	0	1	1	0	0	0	0	1	0	0
<i>H. elongata</i>	0	0	1	0	0	0	0	0	0	0	1	0	1	0	0	0	1	1	0	0	0	1	0	0
<i>H. macra</i>	0	1	1	0	0	0	0	0	0	1	1	0	1	0	0	0	1	0	0	0	0	1	0	0
<i>H. ramosissima</i>	1	0	1	0	0	0	0	0	0	1	0	1	1	0	0	0	1	0	0	0	0	1	0	0





- |   |  |  |
|---|--|--|
| 1. <i>H. amplexicaulis</i>                          | 13. <i>H. variabilis</i>                       | 24. <i>H. coronopifolia</i>                  |
| 2. <i>H. gariepina</i>                              | 15. <i>H. crithmifolia</i>                     | 27. <i>H. acuminata</i>                      |
| 4. <i>H. namaquana</i>                              | 16. <i>H. trifurca</i>                         | 28. <i>H. macowaniana</i>                    |
| 7. <i>H. pectinata</i>                              | 18. <i>H. pinnata</i>                          | 30. <i>H. digitata</i>                       |
| 8. <i>H. pubescens</i>                              | 19a. <i>H. pusilla</i> var. <i>pusilla</i>     | 31. <i>H. refracta</i>                       |
| 9. <i>H. collina</i>                                | 19b. <i>H. pusilla</i> var. <i>macrosperma</i> | 38b. <i>H. arenaria</i> var. <i>acocksii</i> |
| 10. <i>H. laciniata</i>                             | 21a. <i>H. diffusa</i> var. <i>diffusa</i>     | 32. <i>H. Schulzii</i>                       |
| 11. <i>H. deserticola</i>                           | 21b. <i>H. diffusa</i> var. <i>flacca</i>      | 33. <i>H. lactea</i>                         |
| 12b. <i>H. seselifolia</i> var. <i>nigellifolia</i> | 22. <i>H. pendula</i>                          | 34. <i>H. linoides</i>                       |
| 12c. <i>H. seselifolia</i> var. <i>marlothii</i>    | 23. <i>H. meyeri</i>                           | 38a. <i>H. arenaria</i> var. <i>arenaria</i> |



44. *H. cuneata*

45a. *H. linearis* var. *linearis*

45b. *H. linearis* var. *linearifolia*

45c. *H. linearis* var. *reticulata*

46a. *H. cornuta* var. *cornuta*

47a. *H. elata* var. *elata*

47b. *H. elata* var. *pillansii*

48. *H. sabulata*

49. *H. suavissima*

50. *H. carnosa*

55. *H. glauca*

56. *H. brachycarpa*

59. *H. tulbaghensis*

60. *H. rimicola*

61. *H. esterhuyseniae*

63. *H. tricuspidata*

64. *H. cedarbergensis*

65. *H. scoparia*

66. *H. callosa*

69. *H. elongata*