A search for taxonomically informative characters in the large genus *Heliophila* L. (Brassicaceae / Cruciferae)

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By

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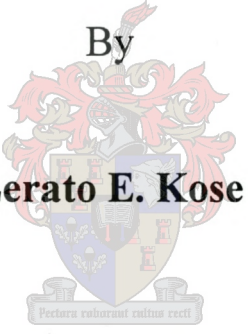

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DECLARATION

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 university for a degree.

Signature: Date:

SUMMARY

Several authors regard the subdivision of the large genus *Heliophila* as unsatisfactory and in need of a detailed taxonomic study. Previous studies on this genus were based exclusively on gross external morphological characters. The present study investigates patterns of variation in *Heliophila* in order to identify taxonomically informative characters that could be used in the subdivision of this large genus. The study differs from previous studies in *Heliophila* because, in addition to macro-morphology, it employs micro- morphological (SEM) and palynological evidence to elucidate the subdivision of *Heliophila*. The study emanates from a taxonomic revision of *Heliophila* proposed by Sonder (1860), in which he subdivided the genus into six sections, based on the variation in fruit characters. Subsequent authors ignored the sections, regarding the generic subdivision as insufficiently supported, hence unsatisfactory.

The results of cluster analysis, which are based on all the characters examined in the study (overall variation), propose the subdivision of *Heliophila* into three main clades. Micro-morphological characters of fruits, seeds, and leaves are consistently found to be more congruent with the phenogram than macro-morphological characters of the same organs. This suggests that micro-morphological characters are taxonomically informative in *Heliophila* and should prove very important in a future phylogenetic classification of the genus. Palynological characters were found to be of limited taxonomic importance in the subdivision of the genus.

Keywords: *Heliophila*, Brassicaceae, phenetic approach, macro-morphology, micro-morphology and palynology.

OPSOMMING

Verskeie outeurs beskou die onderverdeling van die groot genus *Heliophila* as onbevredigend, en meen dat dit 'n gedetaileerde taksonomies studie benodig. Vorige studies op hierdie genus het slegs op ekstern morfologiese kenmerke gekonsentreer. In die huidige studie word patrone van variasie in *Heliophila* ondersoek met die oog op 'n moontlike onderverdeling van die genus, en taksonomies betekenisvolle kenmerke wat in hierdie verband gebruik kan word, word geidentifiseer. Die huidige stud'e verskil van vorige studies daarin dat, benewens makro-morfologiese kenmerke, dit ook mikro-morfologiese tegnieke (SEM) en palinologiese kenmerke gebruik om 'n sinvolle subverdeling van *Heliophila* te probeer vind. Die huidige studie spruit uit 'n taksonomiese hersiening van *Heliophila* deur Sonder (1860), waarin hy voorstel dat die genus in ses seksies verdeel word op grond van variasie in vrug kenmerke. Hierdie generiese onderverdeling en die voorgestelde seksies is deur latere outeurs as onbevredigend beskou, en is meestal in die literatuur geignoreer.

Die resultate van fenetiese analise, wat op alle ingeslote kenmerke gebaseer is (algehele variasie), stel voor dat *Heliophila* in drie hoof groepe verdeel moet word. Taksonomies belangrike kenmerke wat hierdie onderverdeling ondersteun sluit in blaartipe, variasie in blaar-oppervlakke (SEM), variasie in die aard van die saadhuid (SEM) en variasie in vrug-oppervlakke (SEM). Palinologiese en makro-morfologiese kenmerke was van geringe waarde in die onderverdeling van die genus.

Sleutelwoorde: *Heliophila*, Brassicaceae, fenetiese benadering, makro-morfologie, mikro-morfologie and palinologie.

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TABLE OF CONTENTS

Page No.

A LIST OF APPENDICES

CHAPTER 2: TAXONOMIC REVIEW

2.1 General background

The angiosperm is the most conspicuous and successful of all plant groups in terms of number, and diversity in form and structure. About 300 families are recognised, including about 275 000 species in 12 650 genera (Angiosperm Phylogeny Group, 1998). Angiosperm reproduces far more rapidly than any other plant groups, and represents the most dominant group of land plants (Gifford & Foster, 1989). Through different centuries, several attempts were made to propose a classification for the angiosperms, and these leave taxonomists with many classification systems (Judd *et al.*, 1999). Some of these systems were controversial and this controversial question is still not fully resolved. However, with systematics rapidly advancing from a morphological to molecular based data sets, a series of angiosperm phylogenies have been produced, many of which challenge traditional views. There are conflicting trees on the phylogeny of angiosperms generated with different genes. However, there are fundamental similarities in terms of the monophyly of monocots and tricolpates, and the unresolved non-monocot paleoherbs magnoliids (Judd *et al.*, 1999).

In the past, there have been considerable disagreement and controversies concerning the order into which the Brassicaceae should be placed, as summarised in Table 2.1 (Bhattacharyya & Johri, 1998). According to recent published cladistic analyses, the family Brassicaceae belongs to the order Brassicales, included in the Eurosids II clade of the tricolpates (Judd *et al.*, 1999). The Brassicales is more closely related to Sapindales and Malvales, although it has been traditionally considered close to Violales (Takhtajan 1987 & Thorne 1992). Brassicales is characterized by the presence of glucosinolates (mustard oil glucosides), which contain sulphur. The presence of glucosinolates (and myrosin cells) is synapomorphic for members of the Brassicales, and thus taxonomically important in the phylogeny of the order. The only other taxon that contains these compounds is *Drypetes* (Rodman *et al.*, 1993).

Table 2.1 A summary of the historical order classification of the Brassicaceae (Bhattacharyya & Johri, 1998).

Author	Order
Rendle 1925, Lawrence 1951, Melchior 1964	Parietales
Bentham & Hooker 1865	Rhoeadales
Hutchinson 1969	Cruciales
Hutchisnson 1973	Brassicales
Cronquist 1981, Dahlgren 1983, Takhtajan 1987	Capparales

Brassicaceae is the largest family in the order Brassicales, and consists of about 350 genera and 3000 species. The genera are distributed mainly in the temperate (or cold) and warm-temperate parts of the northern hemisphere, although some are cosmopolitan. The greatest concentration of genera and species occurs in the area from the periphery of the Mediterranean Sea extending to central Asia. There is a lesser but substantial centre of diversity in western North America. The family is sparingly represented in the southern hemisphere by 34 genera (20 exotic) and 153 species (37 exotic), with centres in temperate South America, southern Africa and Australia. It is represented by six indigenous genera in South Africa of which *Heliophila* is the largest (Heywood, 1978). Members of the Brassicaceae are partial to dry climates, but some occur in moist regions or habitats, and there are even some submerged aquatics (Cronquist, 1981). The largest genera include *Draba* L. (350 spp.), *Erysimum* L. (180), *Cardamine* L. (170), *Lepidium* L. (170), *Arabis* L. (170), *Alyssum* L. (150), *Lesquerella* S. Watson (90), *Heliophila* (71), *Thlaspi* L. (70), *Rorippa* Scop. (70) and *Hesperis* L. (60) (Judd *et al.*, 1999).

Brassicaceae is regarded as a natural family characterised by features such as tetradynamous stamens, which are fixed, and apparently a very efficient contrivance for successful pollination (Hutchinson, 1969). The literature is unclear whether the Brassicaceae is derived from Papaveraceae or Capparaceae. The Brassicaceae resemble Papaveraceae in being dominant in the northern hemisphere and especially in the Mediterranean region, and in being generally herbaceous with scattered stipulate leaves (Rendle, 1959). Studies by different taxonomists suggest that the Brassicaceae originated from a Capparaceous ancestor (Cronquist, 1981). The gynoecium of the Brassicaceae is unique in forming a silique with a partition or false septum. The species in which the partition fails to develop closely resemble some members of the Capparaceae. Therefore, based on gynoecial morphology and anatomy, the Brassicaceae seems to be derived from Capparaceous

ancestors (Lawrance, 1951). The morphology of gynoecium of the Brassicaceae has occasioned much controversy and is still not settled to the satisfaction of all concerned (Cronquist, 1988). Chemical composition supports the view that the Brassicaceae is derived from the Capparaceae, since isothiocynates are reported in both the Capparaceae and Brassicaceae (Harborne and Turner 1984). Molecular studies based on rRNA, rbcL and atpB also suggest that Brassicaceae originated from Capparaceae (Chase *et al.*, 1993 & Judd *et al.*, 1999). Brassicaceae differs from the Papaveraceae in the chemical composition of their endospermous seeds, although there are a few resemblances in androecial and gynoecial features, and the tetramerous perianth (Bhattacharyya and Johri, 1998).

Brassicaceae is morphologically distinct and diagnosed by four-merous flowers that are arranged in the form of a cross, hence the name Cruciferae. Other characters include a unique gynoecium with an elongated gynophore, six stamens arranged in two whorls of four and two, seeds with curved or folded embryos, lack of endosperm, vessels with vestured pits, and protein-rich, unspecialised to vacuolar cisternae of the endoplasmic reticulum (Cronquist, 1981). Brassicaceae also produces glucosinolates (mustard oil glucosides), which contain sulphur. This explains the pungency of most species (Rodman *et al.*, 1993). Flowers of Brassicaceae are frequently white, yellow, or pale to deep purple, and are pollinated by nectar gathering bees, flies, butterflies, moths, and beetles (Judd *et al.*, 1999). The fruit is very characteristic of the family and is dehiscent in a majority of species. The fruit is known as a siliqua, or when scarcely longer than broad, a silicula. Fruit types and seed arrangement vary greatly, so that these characters are used extensively in the classification of the family at tribal, generic and species level (Rendle, 1959).

Brassicaceae is one of the better-defined and readily recognisable large families of flowering plants. Despite this, the genera are ill-defined and frequently confluent (Cronquist, 1981). Various attempts have been made to produce a natural subdivision of the family into tribes, using fruit characters, embryo features (distribution of myrosine cells in the embryo) and nectar glands. The first extensive, modern treatment of the Brassicaceae by Schulz (1936), divided the family into 19 tribes. However, there are conflicting ideas on the tribal delimitation of the family, and several authors argue that most of the tribes are far from being natural. As a result, various modifications have been suggested. Al-Shehbaz (1984) and Rollins (1993) placed the Brassicaceae genera into poorly defined tribes based on fruit morphology, calyx aestivation, flower colour and symmetry, stigma form, number of seeds per locule, type of embryo folding and indumentum (Judd *et al.*, 1999). Only four tribes are regarded as natural, namely Brassicaeae, Lepideae, Pringleae and

Chamireae, and only two tribes, Chamireae and Heliophileae, are present in the Cape Flora (Heywood, 1978). The endemic tribe Heliophileae, as defined by Schulz (1936), includes *Cycloptychis* E. Mey. ex Sond., *Thlaspeocarpa* C.A. Smith and *Carponema* DC. (now known as *Heliophila*). The first two genera contain one or two species each, and the large genus *Heliophila* includes 71 species (Bean, 1990). In the most recent revision of the Brassicaceae in southern Africa by Marais (1970), the genera are arranged according to Schulz's (1936) classification. However, Marais (1970) made no formal use of the groupings proposed by Schulz (1936).

2.2 The genus *Heliophila*

The name *Heliophila* was derived from the Greek **helios**, the sun and **philos**, loving. *Heliophila* occurs in South Africa, Namibia, Lesotho and the southern part of Zimbabwe (Riley, 1963). Most species are restricted to the winter-rainfall area of the Western and Northern Cape Provinces, occurring from Namaqualand to Saldanha Bay and inland to Montagu, Laingsburg and Calvinia. About 24 species occur in the Cape Peninsula (Goldblatt & Manning, 2000). Many species have restricted distribution areas and some show luxuriant growth after fire. Most *Heliophila* species are usually recognised by the intense blue of their flowers (Figure 2.2), although some species display shades of pink, yellow and white flowers. The flowering season of the majority of *Heliophila* species is between August and February. The shimmering drifts of blue seen in the veld are hard to capture in the garden but several species are suitable for rockery pockets. *Heliophila* is represented by both shrubby and annual species and the latter can easily be raised from seeds (Manning & Goldblatt, 1996). Almost all species of *Heliophila* develop a slim, laterally flattened, two-chambered siliqua, with one or more ribs. The only exception is *H. esterhuyseniae* Marais, which develops a smooth, dark, woody siliqua that are convex on both walls, so that the external shape resembles that of a pea pod (Bean, 1990).

The most comprehensive studies on *Heliophila* include that of Sonder (1860) and Marais (1970). Sonder (1860) used fruit characters in keys to South African genera and species of the Brassicaceae. He subdivided *Heliophila* species into six sections (Table 2.2), but commented that, the sections are distinguished by minute and unsatisfactory characters. Adamson and Salter (1950) in the Flora of the Cape Peninsula, expanded on the work of Sonder (1860) by including seed characters in keys to genera and species, and commented that the species were much in need of detailed study. The most recent study is that of Marais (1970) in which fruit types were also used in keys to the genera and species of the Brassicaceae. However, Marais (1970) did not recognize the subdivisions of *Heliophila* proposed by Sonder (1860) and merged the species into a massive genus

deprived of an internal structure (Marais, 1970). This left uncertainties and controversies regarding the phylogenetic affinities among *Heliophila* species.

Table 2.2 Sections of *Heliophila* proposed by Sonder (1860).

Section	Diagnostic features	Species included
1. LEPTORMUS DC.	Pods linear, moniliform; the beading oval. Herbs	*H. dissecta* Thunb., *H. longifolia* DC., *H. sonchifolia* DC., *H. fistulosa* Sond., *H. caledonica* Sond., *H. pubescens* Burch., *H. affinis* Sond., *H. eckloniana* Sond.
2. ORMISCUS DC.	Pods linear, moniliform; beadings orbicular. Herbs	*H. amplexicaulis* L.f., *H. pusilla* L.f., *H. monticola* Sond., *H. trifida* Thunb., *H. concatenata* Sond., *H. rivalis* Burch., *H. pendula* Willd., *H. variabilis* Burch., *H. coronopia* L., *H. dentifera* Sond.
3. SELENOCARPEA DC.	Pods oval or sub-orbicular. Herbs	*H. diffusa* DC., *H. peltaria* DC., *H. flacca* Sond.
4. ORTHOSELIS DC.	Pod linear, with straight margins or somewhat torulose. Herbs or shrubs	*H. macrostylis* E.Mey., *H. latisiliqua* E.Mey., *H. meyeri* Sond., *H. viminalis* E. Mey., *H. tenuifolia* Sond., *H. seselifolia* Burch., *H. pectinata* Burch., *H. refracta* Sond., *H. crithmifolia* Willd., *H. chamaemelifolia* Burch., *H. foeniculacea* R.Br., *H. gracilis* Sond., *H. trifurca* Burch., *H. stricta* Sond., *H. linearis* DC., *H. divaricata* Herb., *H. graminea* DC., *H. pilosa* Lam., *H. cornuta* Sond, *H. abrotanifolia* Herb., *H. brassicaefolia* E. & Z., *H. reticulata* E. & Z., *H. scoparia* Burch., *H. brachycarpa* Meisn., *H. dregeana* Sond., *H. virgata* Burch., *H. glauca* Burch., *H. callosa* DC., *H. elata* Sond., *H. stylosa* Burch., *H. rigidiuscula*

		Sond., *H. fascicularis* Herb., *H. suavissima* Burch., *H. subulata* Burch., *H. succulenta* Herb., *H. linearis* DC.
5. PACHYSTYLUM DC.	Pubescent suffrutices. Pods linear, tipped with a short and thickened style	*H. incana* Ait., *H. arenaria* Sond.
6. LANCEOLARIA DC.	Glabrous shrubs, with lanceolate pods	*H. florulenta* Sond., *H. macrosperma* Burch.

Heliophila africana *Heliophila digitata* *Heliophila refracta*

Figure 2.2 Pictures of some *Heliophila* species (*H. africana* (L.) Marais, *H. digitata* L.f., *H. refracta* Sond.) showing different flower colours (from Manning & Goldblatt, 1996).

CHAPTER 3: MATERIALS AND METHODS

3.1 Taxon sampling

Herbarium specimens, representing 18 of the 71 recognised species of *Heliophila*, were selected and requested on loan from the Compton herbarium (NBG). The selection was done based on specimen availability, species distribution and maximum morphological variation. Firstly, 24 species were eliminated because of limited number of specimens available (less than five specimens per species). A further 11 species were eliminated due to their far (not locally available), unclear or limited distribution. Finally, 18 of the remaining 36 species were selected, targeting maximum representation of the following characters:

- Leaf morphological variation (Appendix 1)
- Fruit morphological variation (Appendix 2)
- Growth forms
    - Perennials and annuals (based on Goldblatt & Manning, 2000)
    - Herbs and shrubs (based on Goldblatt & Manning, 2000)

The 18 selected species were *H. amplexicaulis* L.f., *H. namaquana* Bolus, *H. pectinata* Burch ex. DC., *H. deserticola* Schultr., *H. crithmifolia* Willd., *H. pusilla* L.f. var *laceolata* (Adamson) Marais, *H. diffusa* (Thunb.) DC. var *flacca* (Sond.) Marais, *H. pendula* Willd, *H. acuminata* (Eckl. & Zeyher) Steud, *H. digitata* L.f., *H. callosa* (L.f.) DC., *H. cinerea* Marais, *H. cornuta* Sond. var *squamata* (Schultr.) Marais, *H. linearis* (Thunb.) DC. var *linearis*, *H. macra* Schultr., *H. lacinata* Marais, *H. suavissima* Burch.ex DC. and *H. subulata* Burch. These species represent a substantial range of morphological variation in the genus. A total of five to ten specimens were studied per species (Table 3.1).

Table 3.1 A list of the herbarium specimens of *Heliophila* from NBG examined for macro-morphological study. Specimens marked with * were also used in SEM analyses. Specimens marked with s.n. do not have collector's number.

		Specimens used for SEM studies			
TAXON	**COLLECTOR & COLLECTOR'S NUMBER**	**Fruits**	**Seeds**	**Leaves**	**Pollen**
H. crithmifolia	Burger & Louw 182	*			*
	Thompson M.F. 2322				
	Oliver E.G.H. 9580				
	Perry & Snijman 2198				
	Perry P.L. & Snijman D. 2212				
	Barker W.F. 6528				
	Fellingham A. 1181		*		
	Perry & Snijman 2134				
	Vlok J. 1048				
	Cloete I. & Haselau W. 127			*	
H. deserticola	Perold S.M. 1575	*			
	Steiner K. 651				
	Reid C. 1572				
	Cloete I. & Haselau W. 59				
	Goldblatt P. 2765		*		*
	Barker W.F. 8293			*	
	Carrick P. 11				
	Barker W.F. 8328				
	Middlemost A.G. 1653				
H. amplexicaulis	v.d.Merwe J.J.M. 188				
	Compton R.H. 15911				
	Compton R.H. 11048	*			
	Bean P.A. & Viviers M. 2535			*	*

	v.d. Merwe J.J. M. 188				
	Compton R.H 5725				
	Barker W.F. 9347				
	v.d. Merwe M. 162				
	Van Wyk C.M. 1243				
	Goldblatt P. 6153		*		
H. pectinata	Thompson M.F. 2465				
	Marais W. 1416				
	Oliver E.G.H 3485	*			
	51 Hankeom W.J. 1085		*		
	Morley M. 424				*
	Oliver E.G.H 9651				
	Marais W. 1424				
	Compton R.H. 5628			*	
	Compton R.H. 8058				
	Compton R.H. 2800				
	Compton R.H. 7351				
H. namaquana	Cruz O. 201				
	Van Rooyen M.W., Steyn H.M. & de villiers A.J. 15				
	Bean P.A. 1345				
	Thompson M.F. 2382			*	
	Thompson M.F. 346				*
	Marais W. 1410	*	*		
	Perry P.L. & Snijman W. 2257				
	Van Rooyen M.W., Steyn H.M. & de villiers A.J. 493				
H. suavisima	Rycroft H.B. 1649				
	Müller D. 521			*	*
	Compton R.H. 19647				
	Compton R.H. 8639				
	Müller D.B. 30				

	Marsh J.A. 396				
	Bohnen P. 6494	*			
	Olivier M.C. 87				
	Bohnen P. 8291				
	Fellingham A. 158		*		
H. macra	Esterhuysen E. 4942				
	Bolus H. 8518				
	Bond P. 756				*
	Guthrie F. 4118		*		
	Paterson-Jones J.C. 238				
	Cowling R.M. 1824			*	
	Maquire B. 854				
	Barker W.F. 10854				
	Vlok J.H.J. 2071	*			
H. subulata	Fourcade H.G 6125				
	Barker W.F. 2378				
	Esterhuysen E.E. 33,598		*	*	
	Olivier M.C. 1112				
	Bohmen P. 7607				
	Joffe H. 866				*
	Morley M. 170				
	Fourcade H.G 1673				
	Esterhuysen E.E. 33598 duplicate				
	Compton R.H. 23544	*			
H. lacinata	Compton R.H. 22090		*		
	Van Berkel N.J. 375				
	Compton R.H. 22090 duplicate				
	Le Roux A. 2959	*			
	Thompson M.F. 1053				
	Thompson M.F. 1293			*	*
H. linearis	Bohnen P. 4421				
	Vlok J.H.J. 1949	*		*	

	Walters I.B. 1149				
	Walters I.B. 2521				
	Compton R.H. 9104		*		
	Schurach M.C. 293				*
	Martin B. 112				
	Keet T.D. 8				
	Jordan P.G. 18931				
	Taylor H.C. 3986				
H. pendula	Oliver E.G.H. 5033				
	Lloyd J.W. 1118				
	Salter T.M. 9014	*	*		
	Compton R.H. 19583				
	Esterhuysen E. 15887			*	
	Barker W.F. 6096				
	Bayliss R.D.A. 4763				
	Laidler D.F. 545				
	Boucher C. 5165				*
	Oliver E.G.H. 9524				
H. diffusa	Richardson D.M. 116	*			*
	Kerfoot O. 5334				
	Kruger J. F. 1752				
	Boucher C. 4687				
	Bolus H. 17968		*		
	Compton R.H. 15339				
	Parker R. H. 4247			*	
	Compton R.H. 13916				
	Compton R.H. 13673				
	Compton R.H. 11788				
	Compton R.H. 16179				
H. pusilla	Compton R.H. 11750	*			
	Phillips E.P. s.n.				
	Steyn M. 338			*	

	Compton R.H. 14977				
	Barker W.F. 4107				
	Compton R.H. 15978				
	Martin B. 815				
	Thompson M.F. 2324				*
	Van Zyl L. 3487		*		
	Gubb A.A. 86				
H. callosa	Thode J. 5911				
	Taylor H.C. 7325				
	Bolus H. 3265				
	Horrocks H. 90			*	
	Kerfoot O. 5682	*	*		
	Bayliss R.D. 2457				
	Horrocks H. 90				
	Gillett J.B. 3450				*
H. digitata	Steyn M. 570			*	
	Whitehead V.B. s.n.				
	Maquire B. 92				*
	Compton R.H. 15073	*			
	Plowes D.C.H. s.n.				
	Schlechter R. 4944				
	Barker W.F. 781		*		
	Kies P. 214				
	Steiner K. 1550				
	Bohnen P. 7112				
H. cornuta	Bean P.a. & Trinder-Smith 2686			*	
	Oliver E.G.H. 9712				*
	Walters I.B. 635				
	Walgate M. 1062	*	*		
	Maquire B. 251				
	Compton R.H. 18399				
	Compton R.H. 19922				

	Taylor H.C. 11805				
	Fellingham A. 1216				
	Vlok J.H.J. 437				
H. cinerea	Compton R.H. 12302A				
	Compton R.H. 12302B			*	
	Compton R.H. 6020	*	*		
	Compton R.H. 6020duplicate				
	Taylor H.C. 6519				*
H. acuminata	Maquire B. 214	*			*
	Snijman D. 1101				
	Thompson M.F. 2665		*		
	Cloete I. & Haselau W. 89				
	Oliver E.G.H. 4723			*	
	Kuun B. 11232				
	Lewis G.F. 5241				
	Compton R.H. 14983				
	Bolus L. 24098				
	Barker W.F. 9361				

3.2 Methods of data collection

3.2.1 Macro-morphology

A wide range of macro-morphological characters of the fruits, seeds and leaves were identified, measured and compared among the selected *Heliophila* species, using a ruler and stereo microscope. A list of the characters used for assessment of macro-morphological variation is given in Table 4.2.

3.2.2 Micro-morphology

Fruits, seeds and leaves were carefully removed from herbarium specimens and mounted onto brass stubs using nail polish as glue. The samples were sputter coated with a gold-palladium layer and examined using an ABT-60 automatic scanning electron microscope (SEM). The wall structures were studied, and micrographs taken at fixed magnifications to enable comparison between different species. The specimens and characters used are listed in Table 3.1 and Table 4.2 respectively

3.2.3 Palynology

Anthers with pollen grains were removed from flower buds of herbarium specimens using a dissecting microscope. At first, unacetolysed pollen grains were used. The pollen grains were suspended in ethanol and left in a dust free area so that the ethanol could evaporate. The samples were placed onto brass stubs using double-sided tape as glue, sputter-coated with a gold-palladium layer and viewed using an SEM. It was found that the pollen grains were dirty and covered with pollen kid. This forced the use of acetolysed material instead. According to Nilsson & Praglowski (1992), it is possible to study the surface features of pollen grains by transferring them directly from living plants or herbarium specimens onto stubs with no processing other than coating. However, the results obtained are rarely satisfactory and can be improved upon enormously by preparation techniques (such as acetolysis) that remove such surface material as the oily pollen coat.

Pollen samples were acetolysed according to Radford *et al.*, 1974 (Appendix 3). The samples were thoroughly washed, first with distilled water, and then ethanol. The pollen-ethanol mixture was air-dried on SEM stubs, sputter-coated with a gold-palladium layer and viewed using an SEM. The wall structure of pollen grains was studied and electron micrographs taken at fixed magnifications in order to enable comparison between the different taxa. Measurements of polar axes and equatorial planes were made using a computerised morphometric unit. It was unfortunately found that most of pollen grains were damaged by the acetolysis method, despite its great advantage of removing surface material (such as oily pollen coat).

3.3 Methods of data handling

3.3.1 Character coding

Qualitative characters from macro- and micro-morphological as well as palynological data were coded for phenetic analysis by investigating character variation within the selected *Heliophila* species. The observed variation was then partitioned into discrete characters and their component states (Table 4.2). The different taxa were coded for the presence or absence of such characters (1 for presence and 0 for absence). The data were prepared for analysis by creating a data file matrix in the STATISTICA 5.0 software package (STATISTICA, Statsoft 1984-1995). Measurements for quantitative characters were standardized before analysis using natural log (Log_{10}) transformation. This was done in order to eliminate the distorting effects of different scales of measurements on the output results.

3.3.2 Data capture

Macro-morphological data was collected from all the herbarium specimens representing the 18 selected *Heliophila* species (Table 3.1). The gathered data were then summarised per species for the cluster analysis (Appendix 4). However, some of the specimens were not used as Operational Taxonomic Units (OTU's) in the cluster analysis, because some characters were missing on them. In some cases fruits were immature hence difficult to study. Such OTU's were excluded from the analysis, because the missing data would have been restrictive.

3.3.3 Phenetic analyses

Numerical taxonomy was introduced by Sneath and Sokal (1962), in order to facilitate ordering at/around the species level. The phenetic approach is one of two methods of numerical taxonomy, in which a tree diagram (phenogram) is constructed by considering the phenotypic similarities of the individuals without trying to reconstruct the evolutionary history that led to the phenotypes. All characters of all the individuals (Operational Taxonomic Units) are compared to one another, and degrees of similarity and dissimilarity are calculated based on the total evidence and expressed in the form of cluster diagrams (Stuessy, 1990).

Cluster analysis (CA) was carried out to assess and analyse the resemblances between the *Heliophila* species. The entire data set, consisting of the macro- and micro-morphological and palynological data (Table 4.2), was used in the analysis. The STATISTICA computer program was used to perform the analyses. Cluster analyses do not assume any *a priori* grouping of objects or variables, but assess and examine similarities between them. This technique provides a visually displayed simplification of the variation pattern by analysing it and constructing a hierarchical classification based on this pattern. The percentage of similarity or dissimilarity links particular clusters together (STATISTICA, Statsoft 1984-1995). City-block (Manhattan) distance, which is the average difference across dimension, was used as the distance measure, because the data set contained mixed (qualitative and quantitative) data. The Amalgamation linkage rule of unweighted pair-group method using arithmetic averages (UPGMA) was employed, resulting in a phenogram depicting similarity between the OTU's (Figure 4.9). The UPGMA model is based on joining an OTU to existing clusters based on their average (mean) distance to the members of that cluster (Quicke, 1993).

3.3.4 Pollen data set

According to Morton & Kincaid (1995), pollen morphologists routinely measure polar (P) and equatorial (E) axes and place pollen into the size classes defined by Erdtman (1971), Walker and Doyle (1975), and Nilsson and Praglowski (1992). This grouping of pollen into globally arbitrary classes may not correspond to statistically significant differences among the taxa within a data set (Morton & Kincaid, 1995). As a result, pollen variability was determined using single classification analysis of variance (ANOVA). The ANOVA was performed for pollen grain size, in terms of polar diameter (P), equatorial diameter (E) and P/E ratio. The measurements were entered into the STATISTICA software package, with P, E, and P/E in separate columns, and standardized using natural log transformation.

The pollen variability was further tested using Discriminant function analysis (DFA) in STATISTICA. The technique is used to determine the variables that discriminate between naturally occurring groups. Therefore, suspected distinct groups of individuals are identified *a priori* (STATISTICA, Statsoft 1984-1995). The *Heliophila* species were coded into three groups based on the phenetic results given in Figure 4.9. Species coded into group 1 are those occurring in clade 1, namely *H. crithmifolia*, *H. deserticola*, *H. pendula*, *H. lacinata*, *H. pectinata*, *H. diffusa* and *H. digitata*. Group 2 species are those occurring in clade 2, namely *H. amplexicaulis*, *H. pusilla*, *H. namaquana*, *H. acuminata* and *H. cornuta*. Group 3 species are those occurring in clade 3, namely *H. suavissima*, *H. macra*, *H. callosa*, *H. subulata*, *H. linearis* and *H. cinerea*. A scatter plot was computed to investigate whether there are significant differences in pollen grain size between different groups.

CHAPTER 4: RESULTS

4.1 Morphology

Morphological data are helpful at all levels of the taxonomic hierarchy from the variety to the division. Although morphology may be regarded as old-fashioned by some authors, it is still the foundation for solving taxonomic problems. In most instances, it provides the best mirror of genetic and evolutionary relationships and gives clues to the way in which the plants have adapted to their environment (Stuessy, 1990). Morphological features have the advantage of being easily seen, and hence their variability has been much more appreciated than for other kinds of characters. This is especially true with herbarium material, on which most taxonomic work is usually based (Davis and Heywood, 1963).

4.1.1 Macro-morphological variation

Fruits

There is a wide variation in fruit size and margin shape. The fruit is a pod-like structure normally with constrictions between the seeds. *H. diffusa* (Figure 4.1 A) has short fruits (10 mm long) that contain few (one to three) seeds per fruit, whereas most species have long (15-71 mm) fruits containing many (7-42) seeds per fruit. There is also variation among the long, constricted fruits. For example, in *H. callosa* the fruits are broad, whereas *H. acuminata* (Figure 4.1 B), *H. pusilla*, *H. digitata*, *H. amplexiculis*, *H. pectinata*, *H. namaquana* and *H. cornuta* have narrow fruits. However, *H. callosa* (Figure 4.1 C) and *H. digitata* have entire margins (no constriction between seeds).

Seeds

There is limited variation between the seeds of the *Heliophila* species studied. The seeds are very small, 0.8-5 mm long and 0.5-3 mm wide. The seeds vary mainly in shape and colour. Three main shapes were identified, namely oval, obovate and orbicular. The oval shape is displayed in *H. digitata*, *H. cornuta*, *H. acuminata*, *H. pectinata* and *H. namaquana* whereas the seeds of *H. amplexicaulis*, *H. macra*, *H. lacinata*, *H. linearis*, *H. diffusa*, *H. pusilla* and *H. callosa* are obovate. The seeds of *H. crithmifolia*, *H. deserticola*, *H. suavissima*, *H. subulata*, *H. pendula* and *H. cinerea* are orbicular. The seeds were either light brown or dark brown in colour. Six species namely: *H. crithmifolia*, *H. deserticolar*, *H. macra*, *H. subulata*, *H. linearis* and *H. cornuta*, have light brown seeds, whereas the rest of the species have dark brown seeds.

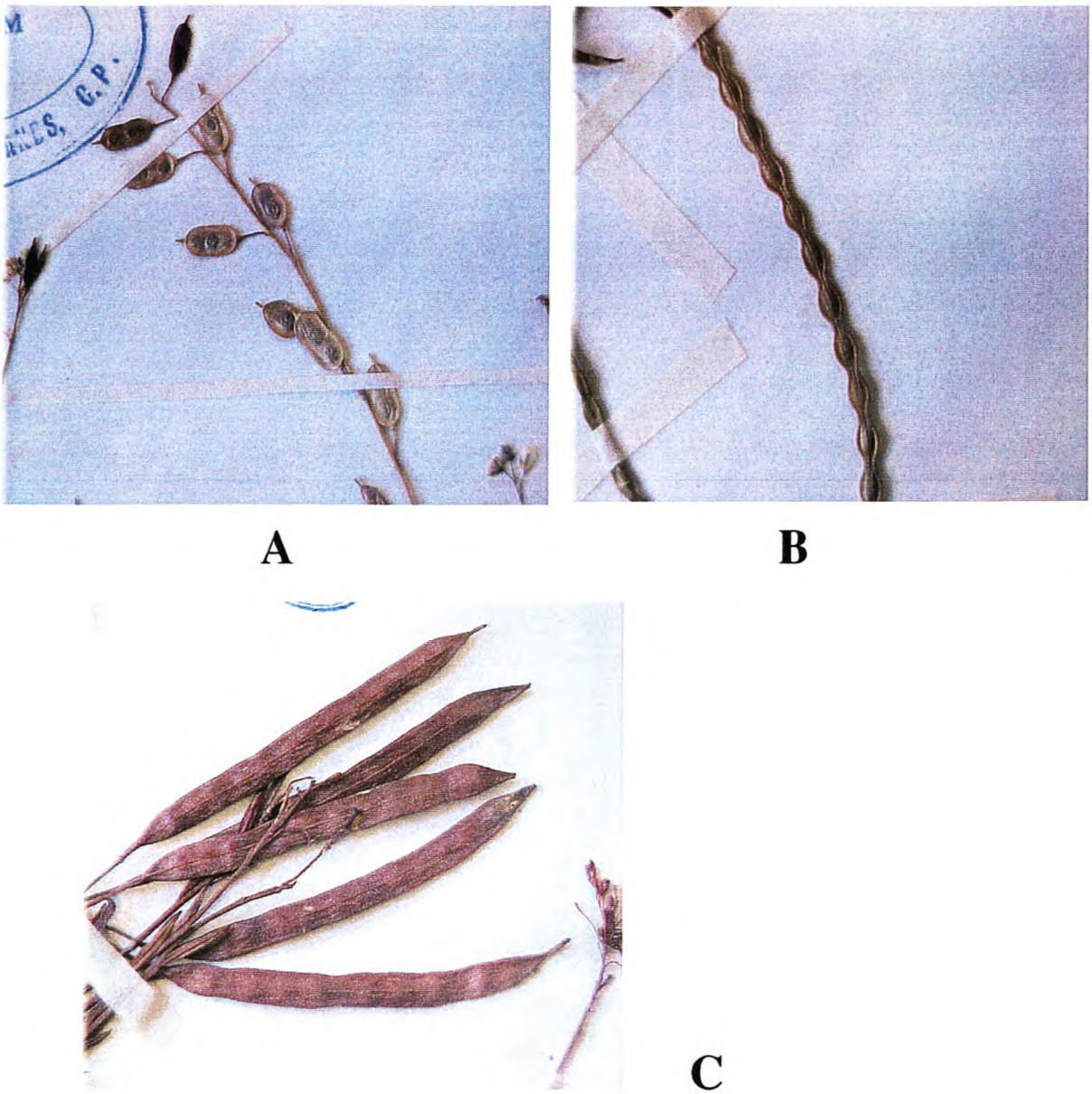


Figure 4.1 Morphological variations of the fruits of *Heliophila*. A: short and few-seeded fruits of *H. diffusa* (Compton R.H. 13673), B: narrow, long, constricted and multi-seeded fruits of *H. acuminata* (Thompson M.F. 2665) and C: the broad fruits having entire margin of *H. callosa* (Thode J. 5911).

Leaves

There is a wide variation in *Heliophila* leaf morphology. Most species, including *H. pectinata*, *H. deserticola*, *H. crithmifolia* and *H. lacinata* (Figure 4.2 A & B) have compound (imparipinnate) leaves with narrow leaflets. Other species such as *H. digitata* and *H. acuminata*, display both lobed and simple leaves. Simple leaves normally occur distally along the stem, whereas lobed leaves occur basally on the stem. A variation in the number of lobes present per leaf also occurs, ranging

from three to seven lobes within different species. In most species, leaves are evenly distributed along the stem, but in *H. lacinata* they are basally-clustured to form a 'basket' (Figure 4.2 A). *H. amplexicaulis* was found to be unique in having simple and broad (4.5 mm wide) leaves, whereas *H. cinerea* have fleshy, hairy leaves (Figure 4.2 C). *H. subulata*, *H. cornuta*, *H. pusilla* and *H. namaquana* have simple, narrow (0.5-1 mm wide) leaves.

A

B

C

Figure 4.2 Morphological variation of the leaves of *Heliophila*. A: imparipinnate leaves of *H. lacinata* (Le Roux A. 2959), B: basally clustered leaves of *H. lacinata* (Le Roux A. 2959) and C: simple, broad, fleshy and hairy leaves of *H. cinerea* (Compton R.H. 12302A).

4.1.2 Micro-morphology (SEM)

Fruits

The fruit surfaces of most species are characterised by small, epicuticular was particles and these are predominant in *H. linearis*, *H. digitata*, *H. cornuta* and *H. acuminata* (Figure 4.3 A). *H. namaquana*, is unique in being hairy (Figure 4.3 B). Fruits of *H. pusilla*, *H. diffusa*, *H. suavissima*, *H. pectina*, *H. macra* (Figure 4.3 C) and *H. amplexiculis*, have numerous and sunken stomata. However, *H. lacinata*, *H. pendula*, *H. callosa*, *H. crithmifolia* (Figure 4.3 D), *H. deserticola*, *H. subulata* and *H. cinerea*, the stomata are surrounded by prominent epidermal cells that appear like dark spots.

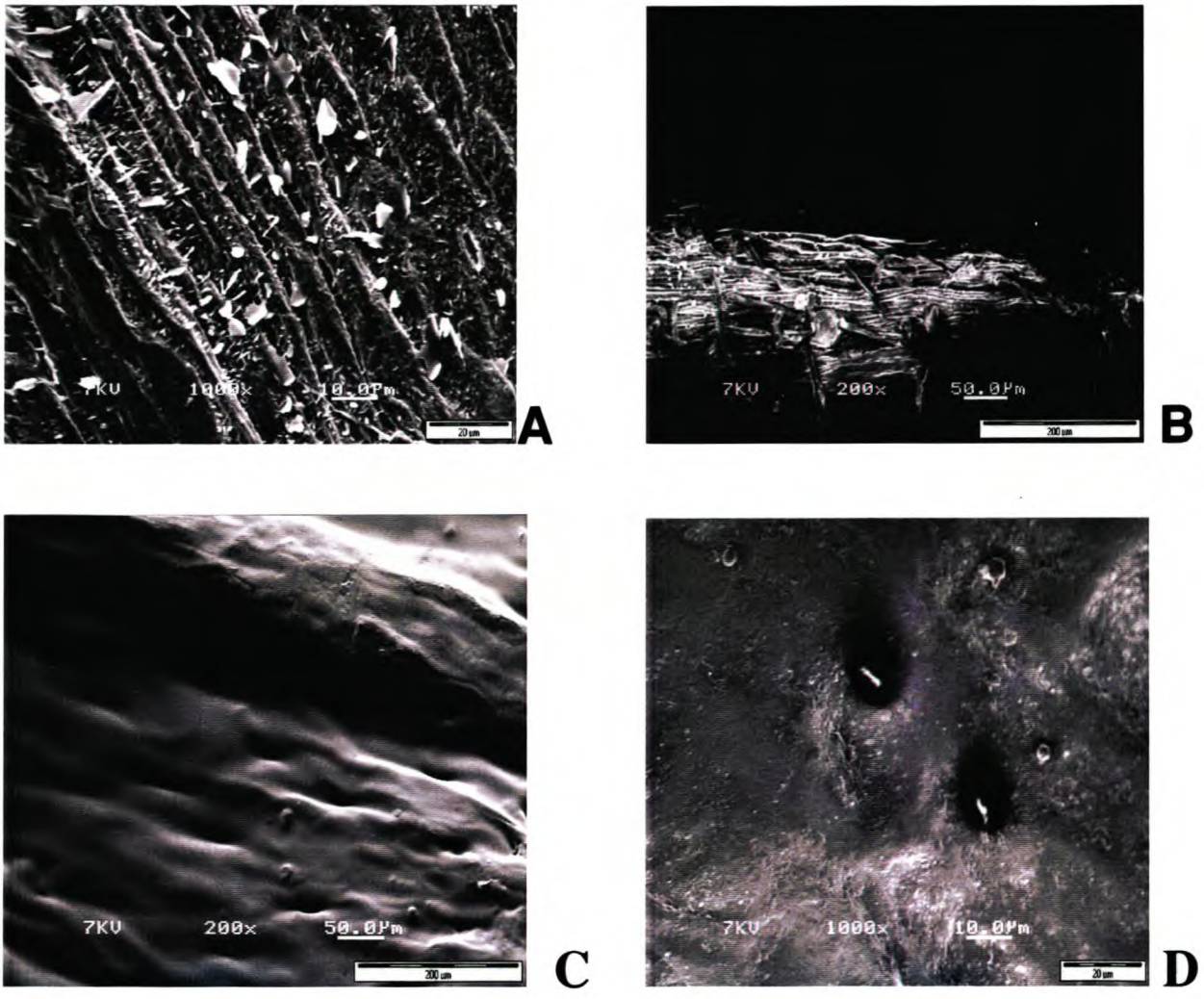
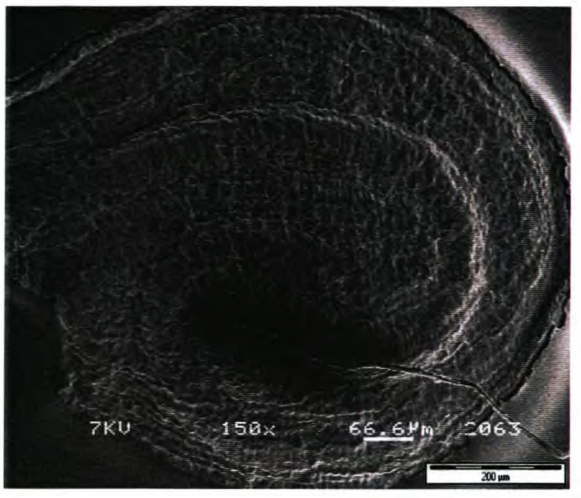


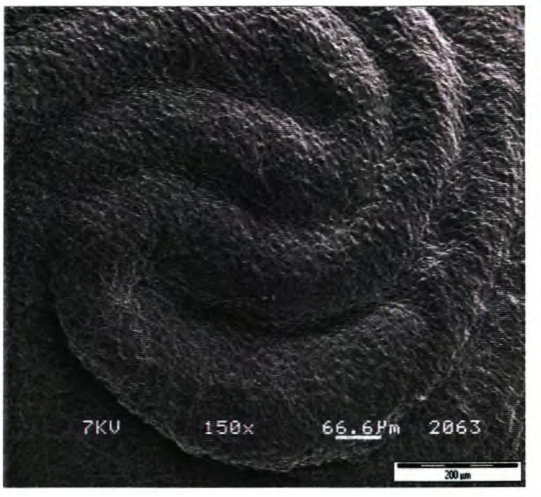
Figure 4.3 SEM micrographs of fruits of *Heliophila* species. A: small, epicuticular wax on the fruits of *H. acuminata* (Maquire B. 214), B: numerous trichomes on the fruits of *H. namaquana* (Marais W. 1410), C: a smooth surface with numerous, sunken stomata on the fruits of *H. macra* (Volk J.H.J. 2071) and D; prominent epidermal cells that appear like dark spots on the fruits of *H. crithmifolia* (Burger & Louw 182).

Seeds

The seeds of most *Heliophila* species have a spongy and papillose coat, which is characterised by distinct spiralled to rounded patterns (Figure 4.4). No significant macro-scale differences in seed coat pattering were obvious between the different species. However, at a higher magnification, significantly different sculptural patterns were clearly visible.



A



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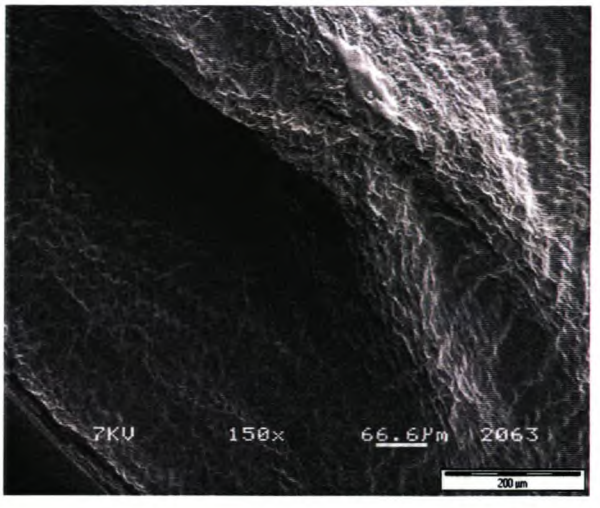


C

Figure 4.4 SEM micrographs of the seeds of *Heliophila* species showing different structures and patterns of seed coats at low magnification. A: *H. callosa* (Kerfoot O. 5682), B: *H. linearis* (Compton R.H. 9104) and C: *H. deserticola* (Goldblatt P. 2765).

At high magnification, seed coats of the *Heliophila* species have distinctive patterns, as there is variation in the shapes and patterns of the epidermal cells (Figure 4.5). *H. crithmifolia*, *H. subulata*, *H. callosa* (Figure 4.5 A), and *H. namaquana* have almost square epidermal cells with a smooth

surface. *H. pusilla*, *H. macra*, *H. deserticola*, *H. lacinata*, *H. linearis*, *H. pectinata*, *H. suavissima* and *H. pendula* (Figure 4.5 B) have polygonous or almost rounded epidermal cells (B). The seed coats of *H. digitata*, *H. acuminata* (Figure 4.5 C) and *H. cornuta* appear almost striate. The epidermal cells are elongated and rectangular (Figure 4.5 C). The seed coats of *H. diffusa*, *H. cinerea* and *H. amplexicaulis* (Figure 4.5 D) are characterised by granulated, polygonous epidermal cells.

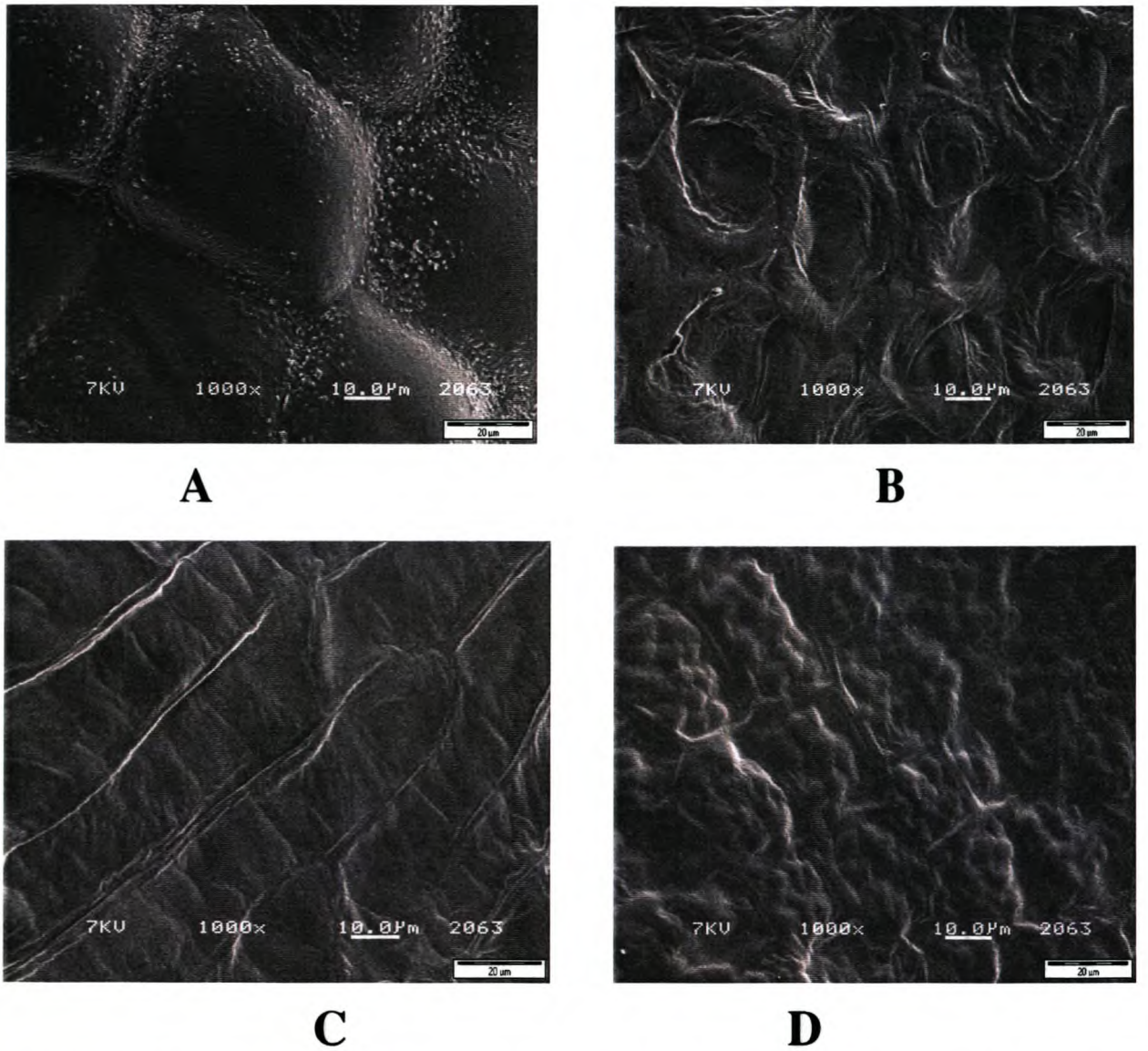
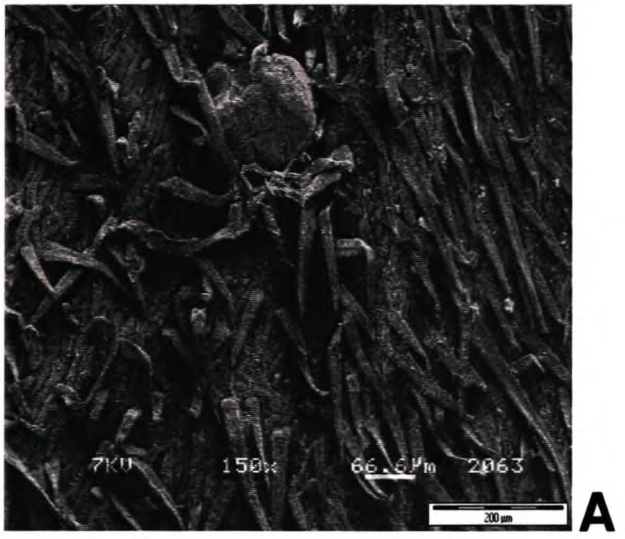


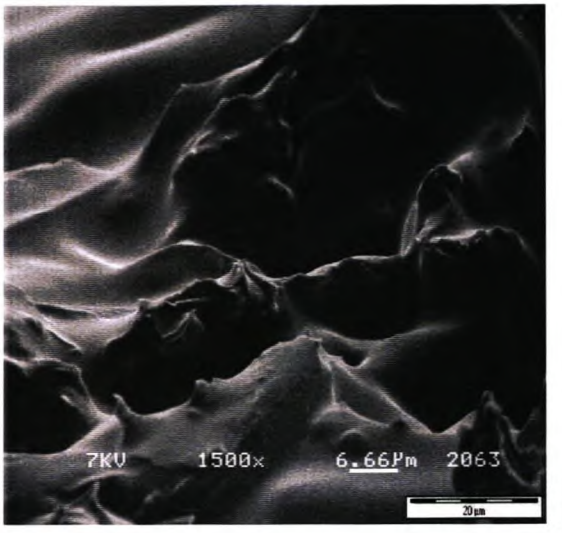
Figure 4.5 SEM micrographs of the seeds of *Heliophila* species at high magnification. A: almost square epidermal cells with a smooth surface in *H. callosa* (Kerfoot O. 5682), B: polygonous or almost rounded epidermal cells in *H. pendula* (Salter T.M 9014), C: elongated and rectangular epidermal cells in *H. acuminata* (Thompson M.F. 2665) and D: the granulated, polygonous epidermal cells in *H. amplexicaulis* (Goldblatt P. 6153).

Leaves

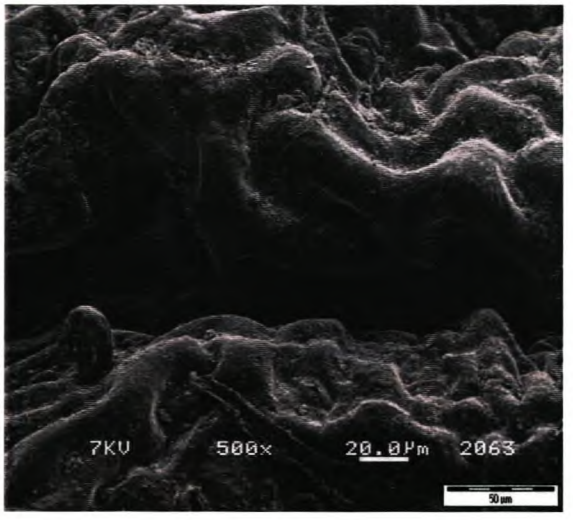
The leaf surface of most species is muriculate, and it is especially obvious in *H. linearis*, *H. suavissima* (Figure 4.6 C) and *H. pectinata*. Three species, *H. cinerea* (Figure 4.6 A), *H. digitata* and *H. crithmifolia*, have trichomes, whereas the rest of the species are glabrous. The majority of species, including, *H. pectinata*, *H. namaquana* (Figure 4.6 B), *H. acuminata*, *H. macra*, *H. pusilla*, *H. pendula*, *H. diffusa*, *H. macra*, *H. callosa*, *H. cornuta*, *H. lacinata* and *H. amplexicaulis* have numerous and large stomata on the abaxial side.



A



B



C

Figure 4.6 SEM micrographs of abaxial leaf surfaces of *Heliophila* species. A: numerous trichomes in *H. cinerea* (Compton R.H. 12302B), B: large, numerous pores in *H. namaquana* (Thompson M.F. 2382) and C: the muriculate surface in *H. suavissima* (Muller D. 521).

4.2 Palynology

Palynology provides a multitude of characters with taxonomic significance, and palynological data have proven to be useful at all levels of the taxonomic hierarchy (Radford *et al.*, 1974). Results from SEM (revealing external features) tend to be more useful at lower taxonomic levels (Radford *et al.*, 1974). Although pollen grains are small and the features only observable with compound and electron microscopes, the usefulness of palynology has become obvious and is now routinely incorporated in most systematic and evolutionary studies (Keating, 1979). For taxonomic purposes, most emphasis is placed on the comparative features of the apertures and the wall structure (Stuessy, 1990).

4.2.1 Description of pollen types

The descriptive terminology of Punt *et al.* (1994) is used in this study. The main delimiting characters for pollen types found in the genus include features such as the exine structure and surface patterning of pollen grains. Based on these features, two distinct pollen (tectum) types are recognized. All the species have tricolpate pollen grains, and the description of each pollen type is given, followed by notes on its representation among the investigated taxa.

Micro-rugulate-spinate

Pollen grains are relatively large, ca. 22-40 μm in equatorial and 10-29 μm in polar diameter. The rugulate-reticulate tectum is covered with small, sharp supratectal spinules. The colpus membrane is smooth. This pollen type is present in *H. cinerea* (Figure 4.7-A & B), *H. cornuta*, *H. linearis*, *H. subulata*, *H. suavissima*, *H. acuminata*, *H. namaquana*, *H. pusilla*, *H. amplexicualis*, *H. lacinata*, *H. diffusa*, *H. pectinata*, *H. pendula*, *H. deserticola* and *H. crithmifolia*.

Rugulate-reticulate

Pollen grains are relatively small and the size ranges from ca. 15-22 μm polar diameter and 12-19 μm in equatorial diameter. The tectum is reticulate and the lumina range from rounded to angular. The tectal elements form an irregular network. The polar diameter is shorter than the equatorial diameter, resulting in an oblate grain shape. This pollen type is present in *H. digitata* (Figure 4.7 C), *H. callosa* and *H. macra* (Figure 4.7 D).

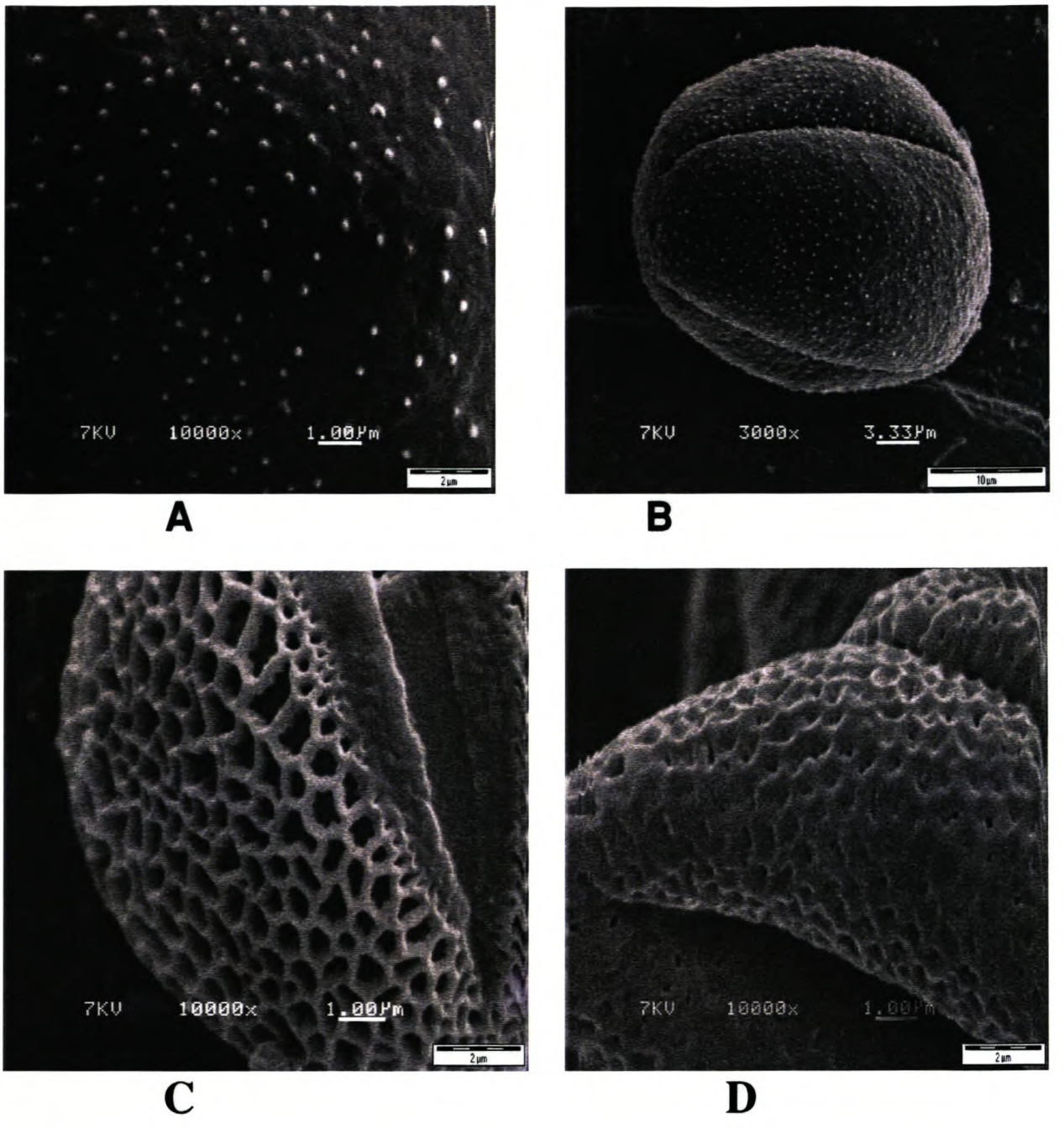


Figure 4.7 SEM micrographs of pollen in *Heliophila* species. A & B: micro-rugulate-spinate lumina with small, sharp supratectal spinules in *H. cinerea* (Taylor H.C. 6519), C & D: rugulate-reticulate lumina with irregular network of tectal elements in *H. digitata* (Maquire B. 92) and *H. macra* (Bond P. 756) respectively.

4.2.2 Analysis of variance

The assessment of pollen variability among the 18 *Heliophila* species was carried out using ANOVA. The ANOVA determines if evidence exists for statistical heterogeneity between the means of the subordinate taxa, with respect to a particular variable. If ANOVA is significantly different (probability of less than 0.05), then means for that variable are statistically significantly different (Morton & Kincaid, 1995). The analysis was performed separately for polar diameter, equatorial diameter and P/E ratio, and the results are given in Table 4.1 below.

Table 4.1 Pollen data set. One-way analysis of variance (ANOVA) performed for mean polar diameter, equatorial diameter and polar/equatorial ratio for the 18 *Heliophila* species. Measurements for polar and equatorial diameters are in μm.

Taxon	**Polar diameter**	**Equatorial diameter**	**Polar/Equatorial ratio**
H.crithmifolia	39.41	20.79	1.896
H. deserticola	26.05	18.22	1.430
H. amplexicaulis	34.01	28.87	1.178
H. pectinata	32.50	24.28	1.339
H. namaquana	24.13	13.45	1.794
H. suavissima	26.02	16.46	1.581
H. macra	20.45	18.34	1.115
H. subulata	32.54	20.15	1.615
H. lacinata	30.45	17.96	1.695
H. linearis	39.71	17.71	2.24
H. pendula	26.85	13.51	1.987
H. diffusa	22.14	10.56	2.097
H. pusilla	24.91	15.84	1.572
H. callosa	21.97	12.59	1.745
H. digitata	15.05	16.44	0.915
H. cornuta	26.82	18.23	1.471
H. cinerea	33.06	21.67	2.098
H. acuminata	26.22	15.76	1.664

P-values	**0.91480**	**0.91483**	**0.86407**
Degrees of freedom	**2**	**2**	**2**

4.2.3 Discriminant Function Analysis

Pollen variability among *Heliophila* species was further tested using discriminant function analysis in STATISTICA. This technique was used to determine whether pollen size discriminates between species of different clades. The results are shown in Figure 4.8.

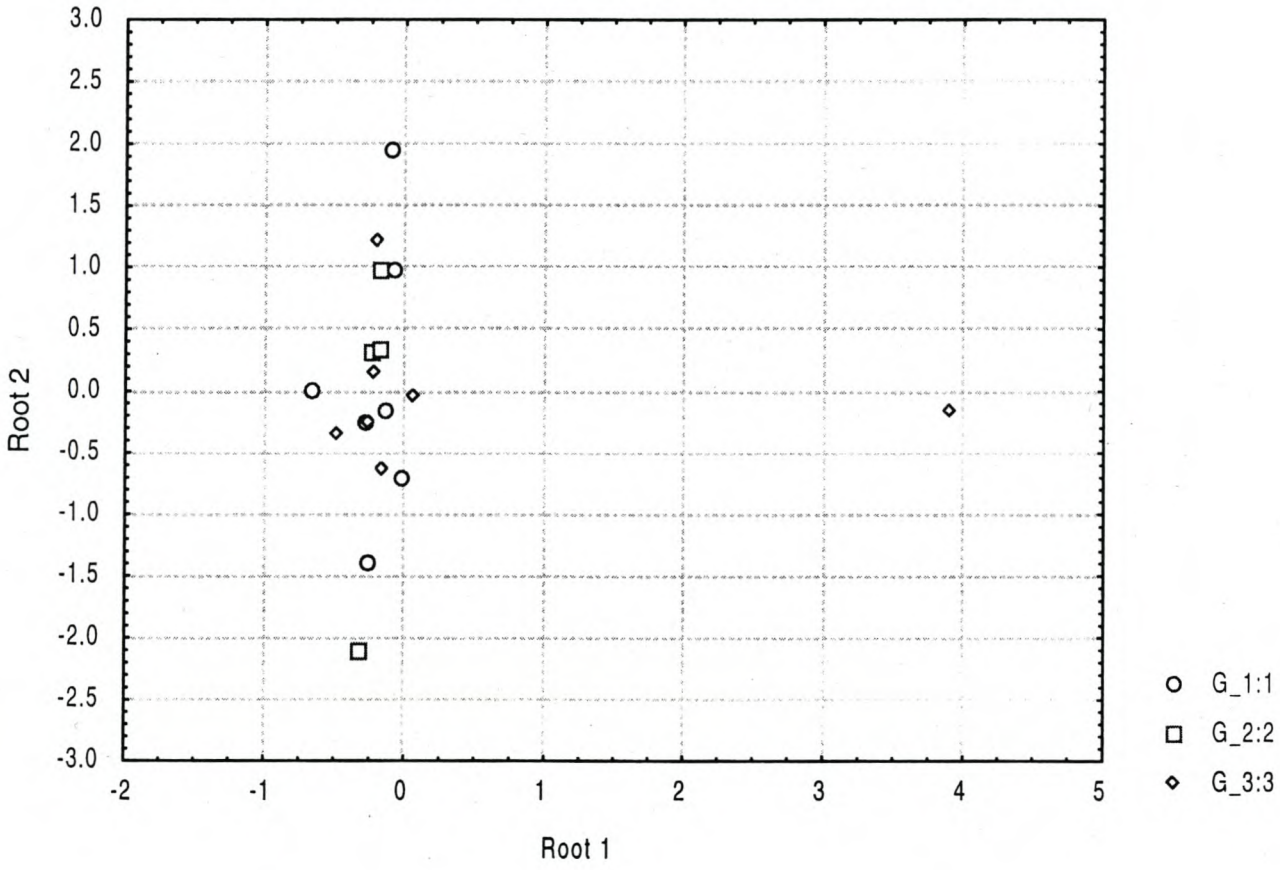


Figure 4.8 A Scatter-plot of *Heliophila* species classified according to pollen sizes. The groups (1-3) correspond to those in section 4.3 (P = 0.9004086).

4.3 Phenetic analyses

The degree of similarity between the *Heliophila* species was assessed using cluster analysis. The technique considers similar species as clusters. The formation of those clusters is produced by overall similarity between the species as a function of their individual similarity in each of the characters in which they are compared. The clusters may not include fixed characters, but are recognized by the possession of a particular minimum number of characters in common (Sneath and Sokal, 1973). The cluster analysis was performed on a combination of macro- and micro-morphological as well as palynological data. There is variation in the number of characters identified and compared for the fruit, seed, leaf and pollen (Table 4.2). The leaf dominates the analysis since it has the highest number of characters (30), whereas pollen has the lowest number of characters (5).

Table 4.2 A list of macro- and micro- morphological and palynological characters used in the assessment of variation between *Heliophila* species.

A. Qualitative characters

Character	Character description	Character States	List No.
Fruits	Margin shape	Entire	1
		Irregularly Lobed	2
		Crenate	3
		Crenulate	4
		Dentate	5
	Margin thickness	Thin	6
		Thick	7
		Very thick	8
	Apex shape	Mucronate	9
		Cuspidate	10
		Aristate	11
	Fruit shape	Linear	12
		Acicular	13
		Oval	14
	Fruit surface (SEM)	Hooked papillae	15
		Sunken stomata	16
		Prominent epidermal cells	17
		Trichomes	18
Seeds	Shape	Orbicular	19
		Obovate	20
		Oval	21
	Colour	Light brown	22
		Dark brown	23
	Pattern of papillae (SEM)	Square, thickened cells	24

		Round, sunken cells	25
		Long, rectangular cells	26
		Small, thickened cells	27
Leaves	Margin shape	Entire	28
		Pinnately-lobed	29
		Mixed (entire & lobed)	30
	Margin thickness	Thin	31
		Thick	32
		Very thick	33
	Leaf shape	Linear	34
		Lanceolate	35
		Imparipinnate	36
		Acicular	37
	Apex shape	Mucronate	38
		Acuminate	39
		Cuspidate	40
		Acute	41
	Base shape	Rounded	42
		Truncate	43
	Indumentum	Glabrous	44
		Sparsely hairy	45
		Densely hairy	46
	Position of leaves on stem	Interspaced throughout stem	47
		Restricted to lower half of stem	48
		Basally clustered	49
	Arrangement of leaves	Alternate	50
		Opposite	51
		Whorled	52
	Leaf surface (SEM)	Muriculate	53
		Trichomes	54
		Stomata	55
Pollen	Pollen type (SEM)	Micro-rugulate-spinate	56
		Rugulate-reticulate	57

B. Quantitative characters

Character	Character description	List No.
Fruits	Fruit length	58
	Fruit width	59
	Number of seeds per fruit	60
Seeds	Seed length	61
	Seed width	62
Leaves	Leaf length	63
	Leaf width	64
Pollen	Polar diameter	65
	Equatorial diameter	66
	Polar/equatorial ratio	67

Fruit, seed and leaf measurements are in millimetres, whereas pollen measurements are in micro-metres.

The results of the cluster analysis are regarded as the core of the study, since they are based on the overall variation of all the characters examined in the study, and the results are given in Figure 4.9.

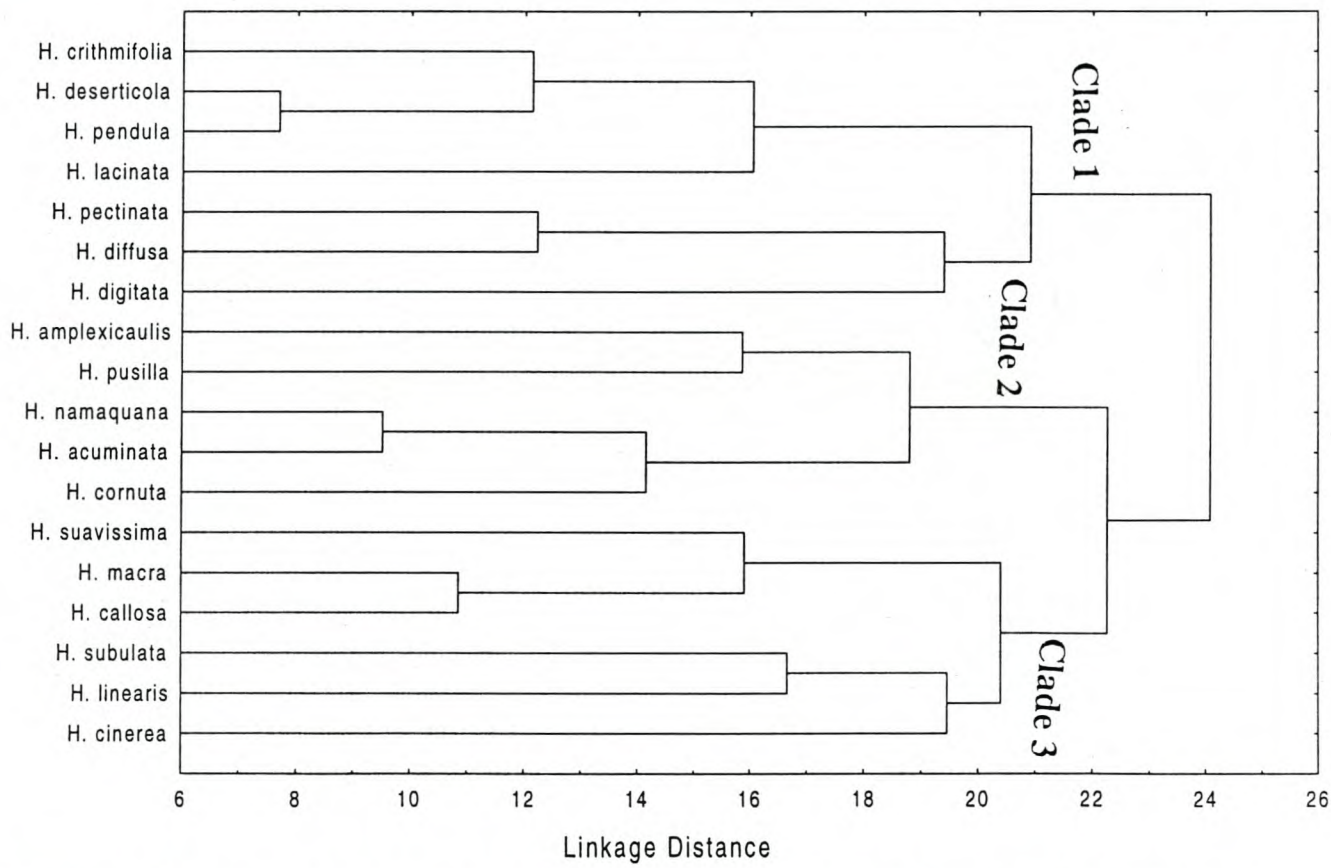


Figure 4.9 A phenogram depicting the overall similarity between the different *Heliophila* species, based on macro- and micro- morphological as well as palynological data. Cluster analysis was performed using unweighted pair-group average and Manhattan linkage distance.

CHAPTER 5: DISCUSSIONS AND CONCLUSIONS

It is crucial to note that the main aim of the present study was to identify informative characters that could aid in a subgeneric classification of the large genus *Heliophila*, and not to propose such a classification per se.

A relatively small, yet variable, set of both taxa and potentially informative characters were selected for this purpose. These are summarised in Table 3.1 and Table 4.2 respectively. As reflected in Table 4.2, 67 potentially informative characters were identified and analysed across all the selected taxa. From Table 4.2 it is, however, immediately apparent that there was unequal representation of characters. A total of 30 leaf characters (including both micro- and macro-morphology) were included, representing almost half of the total number of characters. The rest of the character set comprises of 21 fruit characters, 11 seed characters, and only five pollen characters. Three of the five pollen characters are based on pollen grain dimensions, a feature known to be taxonomically relatively uninformative (Dreyer 1996).

The unequal character representation obviously had a marked influence on the resulting phenogram (Figure 4.9). All characters were included in the analyses bearing equal weights. It is therefore not surprising that leaf and fruit characters underpin the main clades in the phenogram (Figure 4.9). Although two distinctly different pollen types were found among the 18 selected species, pollen type had limited influence on the phenogram structure. It is therefore important to stress that this figure does not present a template for a new classification, but rather a tool to evaluate character variation between the selected *Heliophila* species.

Overall phenogram structure

The results of the cluster analysis, which are based on a combination of macro- and micro-morphological and palynological data (overall variation), produced three primary clades (groups of OTU's), labelled 1, 2 and 3 (Figure 4.9). Species occurring in clade 1 are *H. crithmifolia*, *deserticola H. pendula*, *H. lacinata*, *H. pectinata*, *H. diffusa* and *H. digitata*. Species occurring in clade 2 are *H. amplexicaulis*, *H. pusilla*, *H. namaquana*, H. *acuminata* and *H. cornuta*. Species occurring in clade 3 are *H. suavissima*, *H. macra*, *H. callosa*, *H. subulata H. linearis*, and *H. cinerea*. There are no fixed characters separating the species of the three clades. However, species in the different clades possess certain common characters. Species occurring in clade 1 are

characterised by imparipinnate (compound) leaves, which have numerous trichomes and stomata predominant on the abaxial side, prominent epidermal cells in the fruit that appear like dark spots and seed coats with almost rounded epidermal cells. Species occurring in clade 2 are characterised by simple leaves, which have numerous stomata, especially on the abaxial side. Species occurring in clade 3 are characterised by simple leaves, which have muricated surfaces. The phenogram, therefore, suggests that both leaf type and micro-morphology of fruit, seed and leaf could be of considerable future use in the classification of *Heliophila*. The individual contributions of different characters are discussed in more details under separate headings below.

Fruit characters

The variation in fruit size and fruits margin shape is not congruent with the subdivision proposed in the cluster analysis. However, the subdivision of *Heliophila* proposed by variation in fruit surfaces (SEM) is to some extend congruent with that produced in the cluster analyses. For example, *H. crithmifolia*, *H. deserticola*, *H. lacinata* and *H. pendula*, which are characterised by epidermal cells that appear like dark spots, occur in clade 1 in the phenogram (Figure 4.9). *H. pusilla* and *H. amplexicaulis*, which are characterised by numerous and sunken stomata, occur in clade 2.

Seed characters

The subdivision of *Heliophila* proposed by seed variation in terms of shape and colour is not congruent with the groups proposed by the cluster analysis. The subdivision of *Heliophila* proposed by the variation of seed coat pattern (SEM) is to some extend congruent with the results of cluster analysis, which is based on the total evidence. *H. lacinata*, *H. pendula*, *H. deserticola* and *H. pectinata*, which are characterised by polygonous or almost rounded epidermal cells, occur in clade 1 of the phenogram (Figure 4.9). The rest of the species that are characterised by the granulated, almost square, elongated and rectangular epidermal cells are randomly scattered throughout the phenogram.

Leaf characters

The subdivision of *Heliophila* proposed by leaf type variation is to some extend congruent with the supraspecific groups proposed in the cluster analysis, and displays two main groups. The first group consists of species characterised by having imparipinnate leaves, and these are found in clade 1 of the phenogram (Figure 4.9). The second group, which consists of species characterised by simple leaves, occur in clades 2 and 3. However, there is a large variation in the types of leaves within a single species (in some species both imparipinnate and simple leaves were found on the same

specimen/species). There is limited congruence between the subdivision proposed by leaf micro-morphology (SEM) and the cluster analyses. In clade 3, only two species, *H. linearis* and *H. suavissima*, are characterised by a muriculate leaf surface, whereas in clade 1, leaf surface patterns vary between *H. crithmifolia* and *H. digitata*, which have prominent trichomes, and *H. pectinata*, *H. pendula* and *H. diffusa*, which are characterised by numerous, large stomata on the abaxial side (Figure 4.9).

Summary (micro- and macro-morphology)

As discussed above, it was expected (and found) that leaf and fruit features would dominate the structure of the phenogram due to their higher number of included characters. Despite this, an interesting pattern emerged when the distribution of individual sets of characters were compared to the phenogram. Micro-morphological characters of fruits, seeds, and leaves are consistently found to be more congruent with the phenogram than macro-morphological characters of the same organs. This suggests that micro-morphological characters should prove very important in a future phylogenetic classification of the genus.

Pollen characters

As discussed above, only five pollen characters were included in the analyses, namely three size characters and two pollen sculptural characters. The three pollen grain dimension characters did not contribute significantly to the phenogram. The ANOVA results indicate that there is no significant difference between the *Heliophila* species in terms of polar diameter ($P = 0.914803$), equatorial diameter ($P = 0.91483$) and P/E ratio ($P = 0.86407$). In all the three sets of measurements, the P value is greater than 0.05, therefore, there is no evidence for statistical heterogeneity between pollen grain sizes of the different species. However, the majority of pollen grains were damaged by the acetolysis procedure, hence affecting pollen grain measurements. The results of the discriminant function analysis in Figure 4.8 show that species of different clades do not form distinct, intact groups (species of different groups overlap). This indicates that there are no significant differences among the species of different groups in terms of polar diameter, equatorial diameter and P/E ratio ($P = 0.9004086$). Therefore, pollen grain dimensions are not particularly good taxonomic markers for future phylogenetic analyses of the genus. Pollen grain dimension are also not congruent with the subdivision of *Heliophila* proposed in the cluster analysis (Figure 4.9).

The fact that only two pollen morphological characters (tectum type) were included in the analysis probably masks the potential phylogenetic importance of pollen wall characters in *Heliophila*. The subdivision of *Heliophila* proposed by variation in pollen types is not congruent with that produced in the cluster analysis. Species displaying the two different pollen types are randomly scattered across all three the clades in the phenogram. Numerous previous authors have highlighted the phylogenetic importance of pollen characters (notably tectum type) at different levels of the taxonomic hierarchy (Humphries, 1993; Ferguson & Skvarla, 1991; Chesselet & Linder, 1993; Johansson, 1992). Johansson (1992) found 22 different pollen types within the genus *Psychotria* L., and suggested that these are likely to be informative in the phylogenetic classification of the genus. He was, however, reluctant to propose such a new classification on pollen data alone. Similarly, a subgeneric split in *Heliophila* cannot be proposed based only on different pollen types, but suggest that variation in pollen types should form an integral part of any future phylogenetic classification of this genus.

Although morphology and palynology alone cannot form the basis of a classification, numerous taxonomic deductions can be made from the results. The following conclusions were reached at the end of the study, providing answers to the key questions.

- Fruit, seed and leaf surface characters (SEM) promise to be of taxonomic importance in the subdivision of *Heliophila*.
- Macro-morphology of fruit, seed and leaf has limited taxonomic importance in the subdivision of *Heliophila*.
- Structural and sculptural features of the exine proved significantly important in the demarcation of pollen types. Two distinctively different pollen types were identified, which show no transitional zones. It is recommended that, the study of pollen grains in *Heliophila* be expanded upon by including more species.
- Pollen size does not prove to be taxonomically significant for subdivision of *Heliophila*, although micro-rugulate-spinate pollen grains are larger than rugulate-reticulate pollen grains.
- Pollen size characters were proven uninformative both in the phenetic analysis presented here, and possibly in future phylogenetic analyses of the genus.

The results of this study provide a framework for the assessment of taxonomically informative characters of fruits, seeds, leaves and pollen, for possible subdivision of *Heliophila*. The use of the stereo and scanning electron microscopes has greatly facilitated the study of the detailed structure of these organs. Due to time limitation, the study only focused on macro- and micro-morphology and palynology. Furthermore, a limited number of species (18) were examined in the study. As a result, the resultant patterns of variation in selected *Heliophila* species cannot be adequately compared with the subdivision of *Heliophila* proposed by Sonder in 1860, which was based on a larger number of species. Therefore, the classification hypothesis set forward in the present study is seen as speculative and should be tested through comparison with data from other study fields. Evidence from other features should be integrated to draw real taxonomic conclusions and improve on the proposed subdivision of *Heliophila*. Despite the potential taxonomic value of morphological and palynological data, the true affinities within *Heliophila* will only be completely resolved through a comprehensive, detailed taxonomic study. It is therefore, recommended that the search should be expanded, by examining a larger number of *Heliophila* species, more characters and employing other modern techniques, such as molecular systematics. Detailed evidence from additional modern techniques could help reach a better understanding of the species variation and a resolution of the subdivision within *Heliophila*.

CHAPTER 6: REFERENCES

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Appendix 1: South African *Heliophila* species grouped into nine informal sections, based upon leaf morphology and habitat, derived from W. Marais (1970), in Flora of Southern Africa 13: 17-77 and from the Bolus collection.

Section 1

Leaf simple, cauline, lanceolate, apex often acuminate and base broadest.

H. amplexicaulis, *H. gariepina*, *H. tulbaghensis*, *H. rimicola*, *H. esterhunseniae*, *H. dregeana*, *H. scoparia*, and *H. callosa.*

Section 2

Leaf simple (or almost so) and linear.

H. obibensis, *H. namaquana*, *H. bulbostyla*, *H. adpressa*, *H. minima*, *H. trifurca*, *H. pinnata*, *H. patens*, *H. coronopifolia*, *H. tabularis*, *H. acuminata*, *H. macowaniana*, *H. promontorii*, *H. digitata*, *H. refracta*, *H. lacteal*, *H. linoides*, *H. remotiflora*, *H. leptophylla*, *H. arenaria*, *H. descurva*, *H. affinis*, *H. africana*, *H. linearis*, *H. cornuta*, *H. elata*, *H. subulata*, *H. suavissima*, *H. carnosa*, *H. rigidiuscula*, *H. filicaulis*, *H. tulbaghensis*, *H. scoparia*, *H. elongata*, *H. macra*, *H. ramosissima*, *H. pugioniformis*, *H. venusta* and *H. dissecta.*

Section 3

Leaf simple, broad, entire, but not lanceolate.

H. acuminata, *H. africana*, *H. brassicaefolia*, *H. cinerea*, *H. cuneata*, *H. linearis*, *H. cornuta*, *H. subulata*, *H. suavissima*, *H. rigidiuscula*, *H. katbergensis*, *H. scandensis*, *H. glauca*, *H. brachycarpa*, *H. tulbaghensis*, *H. rimicola*, *H. esterhunseniae*, *H. cedargergensis*, *H. scoparia*, *H. callosa*, *H. nubigena*, *H. elongata*, *H. macra*, *H. sarcostyla* and *H. dissecta.*

Section 4

Leaf simple, broad and lobed.

H. bulbostyla, *H. digitata*, *H. africana*, *H. cuneata*, *H. eximia* and *H. tricuspidata*.

Section 5

Leaf compound, linear, cauline and habitat delicate.

H. pectinata, H. variabilis, H. minima, H. pinnata, H. pusilla, H. diffusa, pendula, H. meyeri, H. concatenata, H. acuminata, H. macowaniana, H. promontorii, H. digitata, H. refracta, H. schulzii, H. lacteal, H. arenosa, H. arenaria, H. alpina and *H. viminalis*.

Section 6

Leaf compound, linear, cauline and habitat sturdey.

H. bulbostyla, H. crithmifolia, H. trifurca, H. diffusa, H. concatenata, H. acuminata, H. macowaniana, H. digitata, H. schulzii, H. lacteal, H. arenaria, H. elata, H. subulata and *H. alpina.*

Section 7

Leaf compound, linear, radical, or mostly crowded near the base.

H. pubescens, H. collina, H. laciniata, H. deserticola, H. seselifolia, H. variabilis, H. minima, H. subulata and *H. carnosa*.

Section 8

Leaf compound, linear, radical and cauline.

H. pubescens, H. collina, H. deserticola, H. variabilis, H. crithmifolia, H. trifurca, H. thunbergii (latisiliqua), H. diffusa, H. meyeri, H. coronopifolia, H. concatenata, H. tabularis, H. arenosa, H. subulata, H. suavissima, H. carnosa and *H. dissecta.*

Section 9

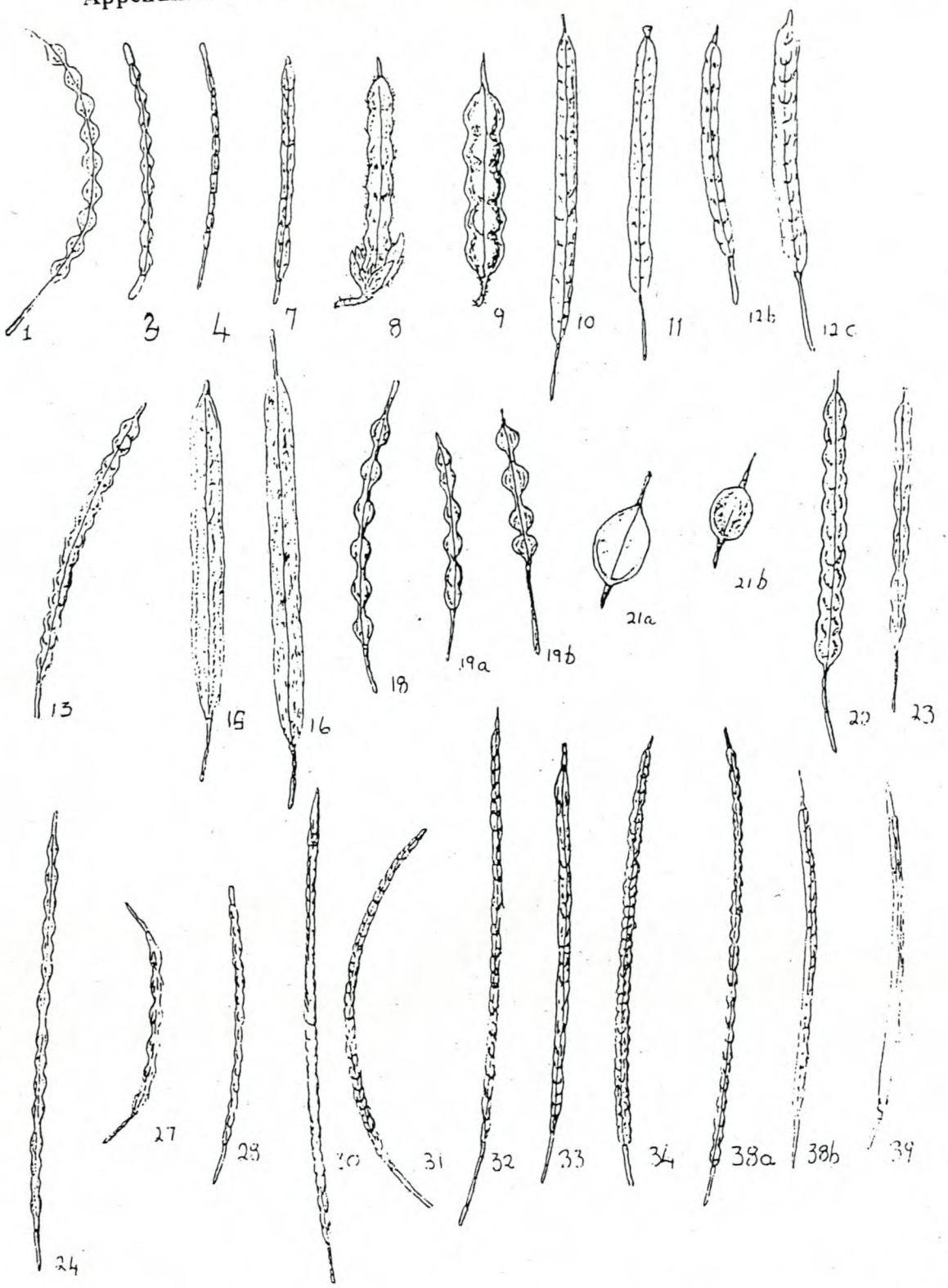
Leaf compound and broader than linear.

H. crithmifolia, H. thunbergii (latisiliqua), H. pusilla, H. diffusa, H. eximia and *H. macrosperma.*

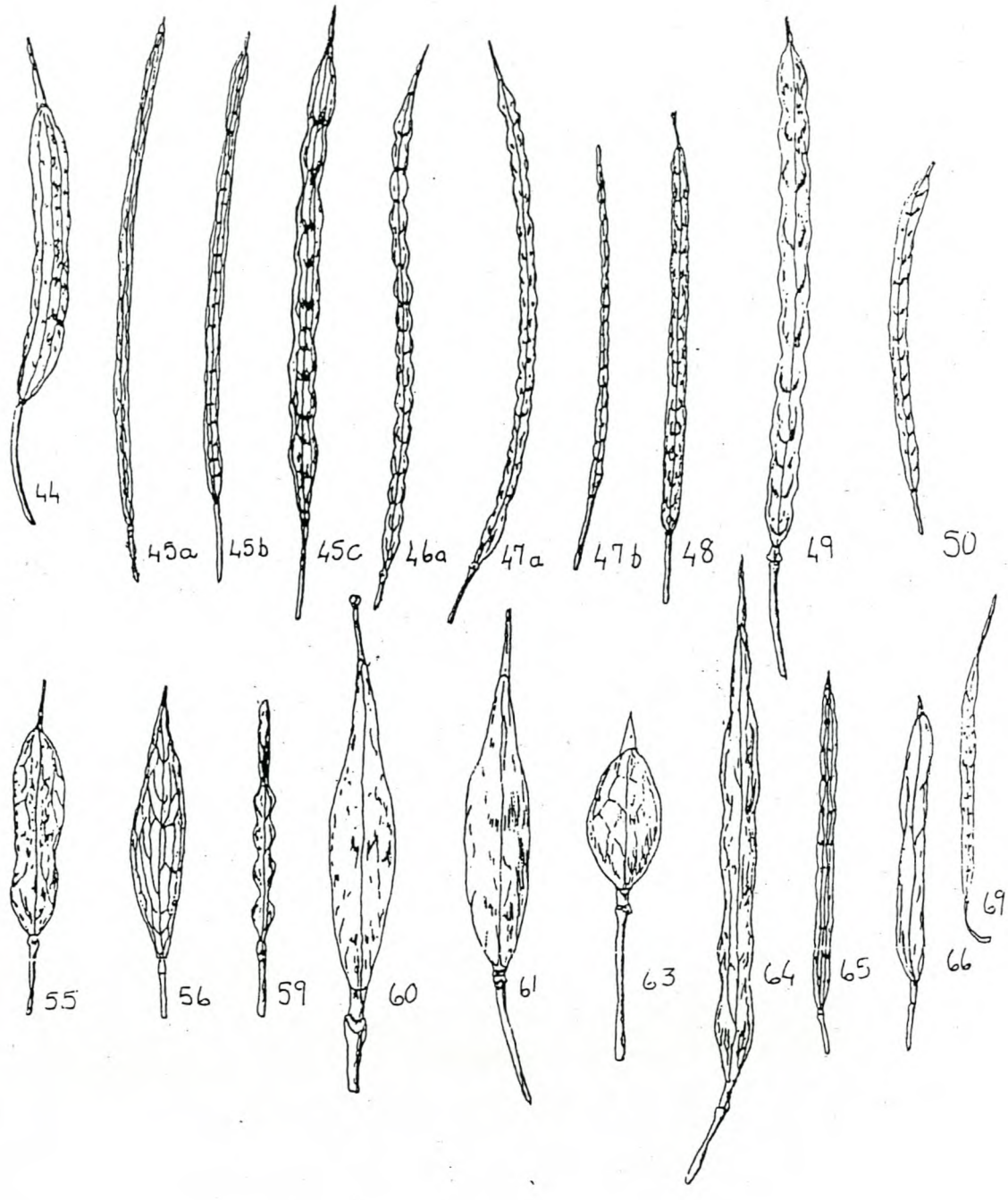
NB:

Names underlined appear in more than one section.

Appendix 2: Fruit variation in *Heliophila* species (from Compton herbarium)



1. amplexicaulis L.f.
3. gariepina Schltr.
4. namaquana Bol.
7. pectinata Burch. ex DC.
8. pubescens Burch. ex Sond.
9. collina O.E. Schulz
10. laciniata Marais
11. deserticola Schltr.
12b. seselifolia Burch ex DC. var. nigellifolia (Schltr.) Marais
12c. seselifolia Burch. ex DC. var. marlothii (Schulz) Marais
13. variabilis Burch. ex DC.
15. crithmifolia Willd.
16. trifurca Burch. ex DC.
18. pinnata L.f.
19a. pusilla L.f. var. pusilla
19b. pusilla L.f. var. macrosperma Marais
21a. diffusa (Thunb.) DC. var. diffusa
21b. diffusa (Thunb.) DC. var. flacca (Sond.) Marais
22. pendula Willd.
23. meyeri Sond.
32 Schulzii Marais
33. lactea Schltr.
34. linoides Schltr.
38a. arenaria Sond. var. arenaria
38b. arenaria Sond. var. acocksii M
39. descurva Schltr.



44. cuneata Marais

45a. linearis (Thunb.) DC.
var. linearis

45b. linearis (Thunb.) DC.
var linearifolia (Burch. ex DC) Marais

45c. linearis (Thunb) DC.
var. reticulata (Eckl. & Zeyh.) Marais

46a. cornuta Sond.
var. cornuta

47a. elata Sond.
var. elata

47b. elata Sond.
var. pillansii Marais

49. suavissima Burch. ex DC.

50. carnosa (Thunb.) Steud.

55. glauca Burch. ex DC.

56. brachycarpa Meisn.

59. tulbaghensis Schinz

60. rimicola Marais

61. esterhuyseniae Marais

63. tricuspidata Schltr.

64. cedarbergensis Marais

65. scoparia Burch. ex DC.

66. callosa (L.f.) DC.

69. elongata (Thunb.) DC.

Appendix 3: Acetolysis procedure (modified from Radford *et al.*, 1974)

- Pick out anthers under a dissecting microscope and place them in a centrifuge tube.
- Suspend the anthers in glacial acetic acid, centrifuge and decant.
- Crush the anthers against the wall of the tube with a glass rod.
- Prepare acetolysis mixture by adding one part of concentrated sulphuric acid to nine parts acetic anhydride.
- Add 5ml of the acetolysis mixture to each tube, and heat in a water bath (100^0C) for 4 minutes, stirring a few times with a glass rod.
- Place the tubes into a cold water bath to cool.
- Centrifuge tubes and decant.
- Add concentrated acetic acid to the tubes, centrifuge and decant.
- Add distilled water to the tubes, centrifuge and decant (repeat three times).
- Add 95% ethanol, centrifuge and decant.

NB. Centrifuging was done at 1500 revolutions per minute for 3 minutes.

Appendix 4: Data matrix of 67 morphological (macro- and micro- morphological) and palynological characters used in the cluster analysis of 18 *Heliophila* species.

0 CASE NAME	1 C1	2 C2	3 C3	4 C4	5 C5	6 C6	7 C7	8 C8	9 C9	10 C10	11 C11	12 C12
H. crithmifolia	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000
H.deserticola	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000
H.amplexicaulis	0.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000
H. pectinata	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000
H. namaquana	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000
H. suavissima	0.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	1.000
H. macra	0.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	1.000
H. subulata	0.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	1.000
H. lacinata	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000
H. linearis	0.000	1.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000
H. pendula	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000
H. diffusa	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000
H. pusilla	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	1.000	0.000
H. callosa	0.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	1.000
H. digitata	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000
H. cornuta	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000
H. cinerea	1.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000
H. acuminata	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000

0 CASE NAME	13 C13	14 C14	15 C15	16 C16	17 C17	18 C18	19 C19	20 C20	21 C21	22 C22	23 C23	24 C24
H. crithmifolia	0.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000
H.deserticola	0.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000
H.amplexicaulis	1.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000
H. pectinata	1.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000
H. namaquana	1.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	1.000
H. suavissima	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000
H. macra	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000

0 CASE NAME	13 C13	14 C14	15 C15	16 C16	17 C17	18 C18	19 C19	20 C20	21 C21	22 C22	23 C23	24 C24
H. subulata	0.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000
H. lacinata	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000
H. linearis	0.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000
H. pendula	0.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000
H. diffusa	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000
H. pusilla	1.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000
H. callosa	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	1.000
H. digitata	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000
H. cornuta	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000
H. cinerea	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000
H. acuminata	1.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000

0 CASE NAME	25 C25	26 C26	27 C27	28 C28	29 C29	30 C30	31 C31	32 C32	33 C33	34 C34	35 C35	36 C36
H. crithmifolia	0.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000
H.deserticola	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000
H.amplexicaulis	0.000	0.000	1.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000
H. pectinata	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000
H. namaquana	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000
H. suavissima	1.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000
H. macra	1.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000
H. subulata	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000
H. lacinata	1.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000
H. linearis	1.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000
H. pendula	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000
H. diffusa	0.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000
H. pusilla	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	0.000
H. callosa	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000
H. digitata	0.000	1.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	0.000	0.000	1.000
H. cornuta	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000
H. cinerea	0.000	0.000	0.000	1.000	0.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000
H. acuminata	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000

0 CASE NAME	37 C37	38 C38	39 C39	40 C340	41 C41	42 C42	43 C43	44 C44	45 C45	46 C46	47 C47	48 C48
H. crithmifolia	0.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000	1.000	0.000
H.deserticola	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000
H.amplexicaulis	0.000	0.000	0.000	0.000	1.000	0.000	1.000	1.000	0.000	0.000	1.000	0.000
H. pectinata	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000
H. namaquana	0.000	0.000	0.000	0.000	1.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000
H. suavissima	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000
H. macra	0.000	0.000	0.000	0.000	1.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000
H. subulata	1.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	1.000
H. lacinata	0.000	0.000	0.000	1.000	0.000	1.000	0.000	1.000	0.000	0.000	0.000	0.000
H. linearis	1.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000
H. pendula	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000
H. diffusa	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000
H. pusilla	1.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000
H. callosa	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000
H. digitata	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000
H. cornuta	1.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	0.000	1.000	0.000
H. cinerea	1.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	0.000	1.000	1.000	0.000
H. acuminata	0.000	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	0.000

0 CASE NAME	49 C49	50 C50	51 C51	52 C52	53 C53	54 C54	55 C55	56 C56	57 C57	58 C58	59 C59	60 C60
H. crithmifolia	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	56.000	3.500	10.000
H.deserticola	0.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	30.000	2.000	19.000
H.amplexicaulis	0.000	0.000	1.000	0.000	0.000	0.000	1.000	1.000	0.000	19.000	1.000	8.000
H. pectinata	0.000	1.000	0.000	0.000	1.000	0.000	1.000	1.000	0.000	19.000	1.000	14.000
H. namaquana	0.000	1.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	21.000	1.000	10.000
H. suavissima	0.000	0.000	0.000	1.000	1.000	0.000	0.000	1.000	0.000	61.000	3.000	15.000
H. macra	0.000	1.000	0.000	0.000	0.000	0.000	1.000	0.000	1.000	40.000	2.000	8.000
H. subulata	0.000	1.000	0.000	0.000	0.000	0.000	0.000	1.000	0.000	57.000	2.000	17.000
H. lacinata	1.000	0.000	1.000	0.000	0.000	0.000	1.000	1.000	0.000	32.000	2.000	11.000
H. linearis	0.000	1.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	49.000	2.000	14.000
H. pendula	0.000	1.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	28.000	2.000	13.000
H. diffusa	0.000	1.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	10.000	3.500	2.000

0 CASE NAME	49 C49	50 C50	51 C51	52 C52	53 C53	54 C54	55 C55	56 C56	57 C57	58 C58	59 C59	60 C60
H. pusilla	0.000	1.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	15.000	1.000	7.000
H. callosa	0.000	0.000	0.000	1.000	0.000	0.000	1.000	0.000	1.000	71.000	6.000	7.000
H. digitata	0.000	1.000	0.000	0.000	0.000	1.000	0.000	0.000	1.000	47.000	.500	42.000
H. cornuta	0.000	1.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	32.000	1.000	21.000
H. cinerea	0.000	1.000	0.000	0.000	0.000	1.000	0.000	1.000	0.000	41.000	1.500	10.000
H. acuminata	0.000	1.000	0.000	0.000	0.000	0.000	1.000	1.000	0.000	41.000	1.000	14.000

0 CASE NAME	61 C61	62 C62	63 C63	64 C64	65 C65	66 C66	67 C67
H. crithmifolia	1.200	1.200	20.000	18.000	39.410	20.790	1.896
H.deserticola	1.800	2.000	14.000	18.000	26.050	18.220	1.430
H.amplexicaulis	1.200	1.000	25.000	4.500	34.010	28.870	1.178
H. pectinata	1.500	1.000	17.000	19.000	32.500	24.280	1.339
H. namaquana	1.000	.500	14.000	.500	24.130	13.450	1.794
H. suavissima	2.000	2.000	45.000	1.000	26.020	16.460	1.581
H. macra	1.500	1.000	28.000	1.000	20.450	18.340	1.115
H. subulata	2.000	2.000	20.000	.800	32.540	20.150	1.615
H. lacinata	1.500	1.000	34.000	63.000	30.450	17.960	1.695
H. linearis	1.000	1.500	67.000	1.500	39.710	17.710	2.240
H. pendula	2.000	2.000	13.000	24.000	26.850	13.510	1.987
H. diffusa	3.000	2.500	16.000	15.000	22.140	10.560	2.097
H. pusilla	1.000	.800	14.000	.800	24.910	15.840	.196
H. callosa	5.000	3.000	33.000	4.000	21.970	12.590	1.745
H. digitata	1.000	.500	25.000	26.000	15.050	16.440	.915
H. cornuta	.800	.500	29.000	1.000	26.820	18.230	1.471
H. cinerea	2.000	1.500	35.000	8.500	33.060	21.670	2.098
H. acuminata	1.000	.500	30.000	2.000	26.220	15.760	.221