4 MATERIALS AND METHODS

4.1 Introduction

The aim of this chapter is to describe the details of the materials and methods used for recording comparative data on morphology, geographical distribution, anatomy and phytochemistry.

4.2 Field studies

The study is based on extensive field work in South Africa. Close to 30 sites were visited in the Eastern Cape, KwaZulu-Natal, Mpumalanga, Gauteng, Free State and North West to observe and collect *Hypoxis* plants. From literature and herbarium sheets, sites with several species were determined and intensive field studies were carried out at these localities. These sites include Mkambati Nature Reserve, Mateku and Stutterheim in the Eastern Cape; Reservoir Hills and Byrne Valley in KwaZulu-Natal; Melville Koppies in Gauteng and Sentinel Peak in the Free State. As *Hypoxis* is plentiful in grasslands in the summer rainfall region of South Africa and is easily accessible, opportunistic collecting was undertaken during general field trips organised by the KwaZulu-Natal Herbarium. In addition, three sites close to Durban, at the coast of KwaZulu-Natal were visited at different seasons each year for three years to record phenology, growth patterns and ecology of the common species *H. hemerocallidea* and *H. angustifolia*. Plants were photographed in the field to record habit, rhizome shape and colour, leaf colour, inflorescence features and mode of fruit dehiscence. Where possible, four plants of a species were collected from a population; two were selected and pressed as herbarium vouchers and lodged at the KwaZulu-Natal Herbarium (NH), Durban, and the remaining two were grown in black plastic bags in open conditions at the NH. Species with short delicate leaves, like *H. flanaganii* and *H. parvula*, were grown in seedling trays for better protection from adverse weather conditions. Vegetative and floral characters were surveyed from observations of plants in the field and those kept in cultivation. Further, plant parts of select species from each trip were preserved in formalin-acetic-acid (FAA) for dissection and anatomical studies.

4.3 Herbarium studies

The study was conducted at the KwaZulu-Natal Herbarium (NH), South African National Biodiversity Institute. Material was obtained on loan from 15 southern African Herbaria [BLFU,

KEI, UNIN, WIND, BOL, J, NBG (including SAM), PRE, PRU, BEWS, GRA, NU, NH, Umtamvuna Nature Reserve Herbarium and Ward Herbarium] and 11 herbaria in tropical Africa, Europe and the United Kingdom namely B, BM, BR, EA, GAB, K, LMA, MAL, SRGH, TCD, Z including ZT. Once there was an understanding of species and their diagnostic characters from the study of type specimens and protolgues, non type specimens were reclassified into respective species. A total of about 4000 herbarium specimens were examined. Specimens were also studied and data on macromophology and distribution were acquired through visits to PRE, EA, K, B and POZ. During these visits, specimens of *Hypoxis* from the rest of Africa were studied with the following aims:

- to compare southern African taxa with those in tropical Africa
- to note the southern African species that extend northwards into tropical Africa
- to evaluate variation in taxa occurring in both southern and tropical Africa

4.3.1 Morphological data

Measurements were made of rhizome, pseudostems, leaves, inflorescences and flower parts. Number of veins on leaves and whether veins were prominent or subtle were noted. Using mostly specimens collected by the author and housed at NH, flowers were reconstituted by boiling for one minute in a microwave oven and characters of the stamens and pistil were determined. Anther tip split or entire and style to stigma ratios were recorded to verify their diagnostic value as Nel (1914) placed great emphasis on these characters, as well as on leaf venation.

Macromorphological data obtained from living and herbarium materials were used to compile descriptions for species and the genus. Leaf hairs were studied under an Olympus SZ40 Stereomicroscope at a magnification of 6.7x to 40x. In specimens where hair characters were obscure due to pressing, hairs were scrapped off leaves, placed on a slide and studied under an Olympus CH-2 compound microscope at a magnification of 200x to 400x. Hair characters were also evaluated from scanning electron micrographs, the details of which are provided under anatomical studies.

Throughout the thesis, authors of plant names follow Brummit & Powell (1992) and acronyms of herbaria are according to Holmgren *et al.* (1990). Citation of herbarium specimens in the formal taxonomic treatment is according to the conventions of the SANBI in-house journal, *Bothalia*. Their format is based on the Degree Reference System proposed by Edwards & Leistner (1971). In a citation, where a collector's number is lacking, the herbarium number is recorded, in the absence

of which the date of collection is cited. Terminology applied to morphology is mainly according to Stearn (1995), but reference is also made to Dahlgren *et al.* (1985) and Kubitzki (1998). Since plant parts namely rhizome and inflorescence in *Hypoxis* do not strictly match the definitions provided by the literature, the structures are described in detail in Chapters 5 and 7 and appropriate terminology for the genus is suggested.

4.3.2 Distribution data

Once species were determined, the quarter degree square was established for specimens with complete locality details that were not previously georeferenced by the loaning herbarium. For each species, all the grid data was entered into a MS Word document in the format compatible with the MAPPIT Programme (SANBI, Pretoria). Maps were generated using MAPPIT and these were used for checking and editing. Grid data and maps were then supplied to the Data Section of SANBI, Pretoria for overlay of point data over southern African biomes following Rutherford & Mucina (2006).

4.4 Anatomical studies

4.4.1 Leaves

Leaf samples of 20 species of *Hypoxis* were examined by means of light microscopy. Duplicates were included for common taxa to help assess variation. Samples were prepared from plants collected in the wild and fixed in formalin-acetic acid-alcohol (FAA) in the ratio of 1:1:18 (Bridson & Forman 1989). Locality details of samples are recorded in Table 4.1.

Suitable portions of the leaf material were selected about midway up the length of leaves and passed through an alcohol series to dehydrate them. Epidermal leaf scrapes of ad- and abaxial surfaces of all species were prepared using a razor blade. Scrapes were stained with toluidine blue. The glycol methacrylate (GMA) embedding technique for LM (Feder & O'Brien 1968) was used for the study of internal leaf structure. One and a half micrometer (µm) thin sections were cut on a Jung RM2045 ultramicrotome. Sections were stained with the periodic acid-Schiff (PAS) reaction and counterstained with toluidine blue (Feder & O'Brien 1968). Sections were studied on an Olympus BH-2 photomicroscope with an Olympus DP71 digital camera. For SEM studies, leaf material of species (Table 4.2) preserved in FAA was dehydrated through an alcohol series, critical point dried, mounted on stubs, sputter-coated with gold, viewed and photographed using a Jeol JSM 840 SEM at the National Herbarium, Pretoria. For species not available in FAA, leaf samples were selected off herbarium sheets, mainly from the NH Collection. Samples were mounted

Table 4.1.—Specimen details of FAA-preserved collections used in anatomical study of leaves. Vouchers in NH

Species	**Collector**	**No.**
H. acuminata	Singh	286
H. acuminata	Singh & Baijnath	314
H. angustifolia var. *buchananii*	Singh	535
H. angustifolia var. *buchananii*	Singh	583
H. angustifolia var. *buchananii*	Singh	814
H. argentea var. *argentea*	Singh & Baijnath	325
H. argentea var. *argentea*	Singh	626
H. argentea var. *sericea*	Singh	259
H. argentea var. *sericea*	Singh	301
H. colchicifolia	Singh	481
H. colchicifolia	Singh & Govender	435
H. costata	Singh	300
H. costata	Singh	803
H. filiformis	Singh	528
H. filiformis	Singh & Baijnath	418
H. filiformis	Singh & Govender	559
H. flanaganii	Singh	807
H. galpinii	Singh & Baijnath	334
H. hemerocallidea	Singh & Baijnath	227
H. hemerocallidea	Singh, Baijnath & Govender	262
H. interjecta	Singh	280
H. longifolia	Singh	290
H. membranacea	Singh	826
H. multiceps	Singh	279
H. obliqua	Singh	531
H. obtusa	Singh	277
H. obtusa	Singh & Baijnath	337
H. obtusa	Singh, Baijnath & Govender	283
H. parvifolia	Singh	470
H. parvula var. *parvula*	Singh	556
H. rigidula var. *pilosissima*	Singh & Baijnath	318
H. rigidula var. *pilosissima*	Singh, Baijnath & Govender	263
H. rigidula var. *rigidula*	Singh	278
H. rigidula var. *rigidula*	Singh	282
H. rigidula var. *rigidula*	Singh	328
H. rigidula var. *rigidula*	Singh	329
H. rigidula var. *rigidula*	Singh & Baijnath	317
H. rigidula var. *rigidula*	Singh & Baijnath	335
H. sobolifera var. *sobolifera*	Singh	816
H. stellipilis	Singh	621

Table 4.2.—Specimens used in SEM studies of leaf surfaces

Species	Collector	Number	Vouchers housed at
H. angustifolia var. *buchananii*	Singh	303	NH
H. angustifolia var. *buchananii*	Singh	535	NH
H. angustifolia var. *buchananii*	Singh	583	NH
H. argentea var. *sericea*	Singh	295	NH
H. argentea var. *sericea*	Singh	301	NH
H. colchicifolia	Singh	481	NH
H. costata	Singh	300	NH
H. filiformis	Singh	289	NH
H. filiformis	Singh & Baijnath	418	NH
H. filiformis	Singh	462	NH
H. filiformis	Singh	471	NH
H. flanaganii	Singh	628	NH
H. galpinii	Singh & Baijnath	334	NH
H. hemerocallidea	Singh & Baijnath	262	NH
H. hemerocallidea	Singh & Baijnath	321	NH
H. membranacea	Menne	Sn	ex cult.
H. multiceps	Singh	279	NH
H. multiceps	Singh & Baijnath	322	NH
H. multiceps	Singh	615	NH
H. obtusa	Singh	330	NH
H. obtusa	Singh & Govender	563	NH
cf. *H. obtusa x rigidula*	Singh	331	NH
H. parvifolia	Singh	470	NH
H. parvifolia	Burgoyne	7672	PRE
H. parvula var. *parvula*	Singh	465	NH
H. rigidula var rigidula	Singh & Baijnath	317	NH
H. rigidula var. pilossisima	Singh & Baijnath	318	NH
H. rigidula var. *rigidula*	Singh & Govender	435	NH
H. sobolifera var. *sobolifera*	Singh	233	NH
H. sobolifera var. *sobolifera*	Singh	502	NH
H. stellipilis	Singh	621	NH

directly on stubs using double sided adhesive tape and sputter-coated with gold to about 20 nm thickness with a Polaron Sputter Coater and were examined on a JEOL-JSM-840 SEM at the Electron MicroscopeUnit of the University of Pretoria, Pretoria. Scans were photographed using the PC programme Orion Version 6. For both FAA-preserved and herbarium material, leaf samples were removed from about midway the length of the leaves and reduced to roughly 5 x 5 mm sections before mounting on stubs. Both adaxial and abaxial surfaces were mounted for study.

4.4.2 Pollen

Pollen samples representing ten species were obtained from specimens at the KwaZulu-Natal Herbarium for SEM observations. Taxa and specimens are listed in Table 4.3. Pollen grains were

Table 4.3.—Specimen details of collections used in pollen studies. Vouchers in NH, except *Moss 7982* in J

Species	Collector	Number
H. acuminata	*Singh*	*638*
H. angustifolia var. *angustifolia*	*Singh*	*594*
H. colchicifolia	*Singh*	*464a*
H. filiformis	*Singh*	*397*
H. galpinii	*Singh*	*640*
H. gerrardii	*Haygarth*	*76*
H. hemerocallidea	*Singh*	*734*
H. kraussiana	*Moss*	*7982*
H. multiceps	*Singh*	*642*
H. parvula var. *parvula*	*Singh*	*308*

mounted directly on stubs using double sided adhesive tape and sputter-coated with gold to about 20 nm thickness with a Polaron E5200C Sputter Coater. Coated pollen grains were examined on a JEOL-JSM-840 SEM. Scans were photographed using the PC programme Orion Version 6.

4.4.3 Seeds

The materials and methods used in the study of seeds are outlined in a manuscript by Singh & Van Wyk (submitted) (Appendix 1.5). The specimens selected for study are listed in a table included in the manuscript.

4.5 Phytochemical studies

Chemical analysis of selected species of *Hypoxis* was undertaken in the Botany Department at the Rand Afrikaans University (now University of Johannesburg), in collaboration with Professors B-E Van Wyk and A. Viljoen. Chemical compounds in rhizomes of 14 species of *Hypoxis* were accessed using thin layer chromography (TLC) and high performance liquid chromatography (HPLC). Five samples of *H. hemerocallidea* and two for *H. rigidula* var *rigidula* were included to assess variation among populations. Taxa used in the study are listed in Table 4.4. Rhizomes were removed from plants in cultivation and sliced into cubes. Samples were dried in an oven at 40^0C over 48 hours until completely dried. About 0.5 g of rhizome of each species was weighed and ground with a mortar and pestle with some acid washed sand. Powdered samples were transferred into test tubes. To this 20 ml of 96% ethanol was added. Some of the ethanol was used to rinse out the mortar. Test tubes were covered with cotton wool and placed in a water bath at 40^0C for two and half hours. Samples were filtered. The ethanol in the filtrate was evaporated in a Rotavapor. Extracts were washed with methanol and stored in vials overnight. Vials were weighed. Pressure was used to dry the methanol. An equivalent volume of Me was added (5 g = 0.5 ml MeOH *u*l).

The solvent was spotted onto a silica plate and allowed to reach two thirds the plate. The TLC for hypoxoside comprised ethyl acetate: water: methanol (100: 16,5: 13,5). For the phytosterols, the TLC was made up of toluene: ethyl acetate (G:4). Plates were dried in an oven at 80^0C and sprayed with 5% ethanol- H_2SO_4, then 1% EtOH-Vanillin. Spray solution was prepared by dissolving 0.5 g vanillin in 50 ml 37% H_2SO_4.

The HPLC system used was a Column Phenomonex IB-Sil (C15,5µmm, 250 x 4.6 mm). The solvent system consisted of 30–60% linear gradient of methanol in 1% acetic acid-water and run for 20 minutes. Detection: diode array (A: 280±40nm; B: 330±70nm).

Table 4.4.—Details of specimens used in phytochemical studies. Vouchers except *McMaster 40* in NH

Sample Number	**Taxon**	**Collector and number**	**Mass of rhizome (g)**	**Mass of vial (g)**
1	*H. hemerocallidea*	Singh 261	0.5453	4.6090
2	*H. hemerocallidea*	Singh 262	0.5031	4.6527
3	*H. hemerocallidea*	Singh & Baijnath 321	0.5197	4.7096
4	*H. hemerocallidea*	Baijnath s.n.	0.5235	4.6640
5	*H. hemerocallidea*	Singh & Baijnath 265	0.5135	4.7588
6	*H. rigidula* var. *pilosissima*	Singh 326	0.5176	4.6161
7	*H. rigidula* var. *rigidula*	Singh 317	0.5637	4.7381
8	*H. colchicifolia*	Singh 264	0.5466	4.6658
9	*H. galpinii*	Singh 273	0.4987	4.7432
10	*H. obtusa*	Singh 277	0.5695	4.5852
11	*H. setosa*	Singh 233	0.5419	4.6658
12	*H. costata*	Singh 304	0.5342	4.7432
13	*H. multiceps*	Singh s.n.	0.5406	4.5852
14	*H. parvula* var. *parvula*	Singh 308	0.1158	4.5968
15	*H. membranacea*	McMaster 40 (ex cult.)	0.2778	4.6547
16	*H. angustifolia*	Singh 303	0.5083	4.7146
17	*H. argentea* var. *argentea*	Singh 348	0.4214	4.6247
18	*H. acuminata*	Singh 341	0.5680	4.6530
19	*H. filiformis*	Singh 289	0.1385	4.6760
20	*H. rigidula* var. *rigidula*	Singh 337	0.5501	4.6179

5 VEGETATIVE MORPHOLOGY

5.1 Introduction

Members of the Hypoxidaceae display taxonomically significant diversity in their gross morphology. They are variable in growth form, leaves, stamens and pistils and these provide diagnostic characters for recognising genera. Many character states found in *Hypoxis* are also present in other genera and there is no one character that is unique to the genus as is the case for *Empodium* or *Pauridia* (Table 2.4). The genus *Hypoxis* is therefore defined by a combination of characters that include geophytic plants with hairy leaves and scapes, yellow (seldom white) star-shaped flowers with tepals free to the base, anthers basifixed with latrorse dehiscence, ovary trilocular and fruit a pyxis (circumscissile capsule, the top coming off as a lid) with occasional loculicidal dehiscence. Morphologically, *Hypoxis* is most similar to *Rhodohypoxis* but differs from it in its yellow flowers and free tepals. In *Rhodohypoxis*, flowers are white, pink or red and tepals fuse at the base to form a tube.

Hypoxis species are often difficult to differentiate and classify due to the subtle differences in morphological characters. There is a great degree of uniformity in the floral morphology among species and differences in flowers are seen only in size of tepals, shape of anther tips and stigma type. However, even these characters show an overlap in the range among closely related species making it difficult to use them for species recognition. There is therefore much reliance on vegetative morphology for demarcating species within the genus.

Much of the present study is based on morphological data mainly for the purpose of presenting a practical key to identifying species of *Hypoxis* in southern Africa. This chapter presents a summary of the vegetative morphology and defines the terminology used to describe structures. It provides comments on the combination of characters used by previous authors to demarcate species of *Hypoxis* in Africa. For each plant part, the overall structure is described, reference is made to its use in earlier classifications and the diagnostic value is indicated. Finally, the chapter summarises the states of characters that are later combined with floral characters (discussed in Chapter 7) and used in the drawing up of character sets for use in a phenetic analysis in the future (Chapter 10) and the formal taxonomic treatment (Chapter 12).

5.2 Combination of characters used in previous studies on African *Hypoxis*

Characters used by previous workers form a useful basis for assessing their diagnostic value for separating species in *Hypoxis*. This section traces the development in selecting characters for keys presented by various works on the genus in Africa. Baker (1878b) used leaf shape and texture, inflorescence type and flower size to classify 31 species of *Hypoxis* (excluding what is now *Rhodohypoxis*) in Africa. In Flora Capensis that covers mainly the Western Cape, Eastern Cape and KwaZulu-Natal, the latter two provinces having the greatest diversity of species of *Hypoxis* in southern Africa, Baker (1896) recognised 29 species in the genus based on a combination of characters. He employed 'corm' size, leaf shape, texture and width, hairiness of leaves, inflorescence type, pedicel length, flower number, tepal size and hairiness of ovary. In his key, Baker (1896) did not strictly provide couplets with well-defined contrasting characters and it is therefore difficult to apply his key. It is understandable that such a key was inevitable due to the lack of distinct characters and the overlap in range of characters. Nevertheless, the key was used for many years to identify *Hypoxis* species in the region. For the Flora of Tropical Africa region, Baker (1898) recognised 16 species and applied characters similar to those used in his key (1878b) to global species, using single characters throughout the couplets. In this key, he also used relative terms like 'short' and 'long' and 'minute' and 'large' that are difficult to interpret.

Nel (1914) took the approach of classifying 83 species of *Hypoxis* in Africa into sections. In his key to sections he applied a few characters which included anther tips entire or split, leaf width correlated with number of veins, vein thickness, inflorescence type and ratio of style to stigma. Within the sections, Nel applied characters relating to the leaf, inflorescence, flower, gynoecium and androecium. He used lengths of leaves, inflorescences, pedicels, tepals and stamens, as well as the ratio of style to stigma lengths at various points in the keys. Nel further employed texture, hairness, venation of leaves, number of flowers and, bract shape and width in his keys to species.

Common characters used by Baker (1878b, 1896, 1898) and Nel (1914) include leaf shape, texture, width and hairiness; inflorescence type, pedicel length, flower number and tepal size. Baker placed great taxonomic significance on leaf and inflorescence characters while Nel emphasised three characters, namely anther tips entire or split, ratio of style to stigma and number of leaf veins. Discussion on taxonomically significant characters offered by the inflorescence, stamen and pistil structures are given in Chapter 7 on floral and fruiting morphology. Leaf characters including venation, offer reliable attributes for separating species of *Hypoxis*, and these are discussed later in this Chapter.

Anyone attempting to draw up a key to *Hypoxis* is familiar with the failure experienced in finding well-defined differentiating characters. The first key on *Hypoxis* to offer a comprehensive combination of characters for separating species was that of Nordal *et al*. (1985). These authors were also the first to use seed surface characters in their key to species in Africa. Seed characters were used as early as 1923 by Brackett to separate the American species. Nordal *et al*. (1985) also found leaf width, leaf hair density, colour of hairs, flower number, capsule width and pedicel length to be useful for demarcating species in the flora of Tropical East Africa region. Recent treatments of *Hypoxis* in African floras were provided by Zimudzi (1996); Wiland-Szymańska (2001); Nordal & Zimudzi (2001) and Wiland-Szymańska & Nordal (2006), where the approach continued to provide more characters including seed surface features for comparison. Two contributions, both appearing in 2001 by Wiland-Szymańska and Nordal & Zimudzi offer workable keys for identifying tropical African species of *Hypoxis*. Wiland-Szymańska (2001) concluded that for the Central African species of *Hypoxis*, seed surface and leaf indumentum characters were most useful for species delimitation. The advantage of this treatment is that it offers insightful discussion on the diagnostic value of the characters. Nordal & Zimudzi (2001), on the other hand, did not weight particular characters but used a range of characters from growth form, leaf, inflorescence, flower and seed. They provided sufficient detail of characters in their key and this makes interpretation straightforward. The most recent key by Wiland-Szymańska & Nordal (2006) to 15 species of *Hypoxis* in the Flora of Tropical Africa region combines the characters of pseudostem, leaf shape, dimensions and hairiness, seed ornamentation, inflorescence type and tunic form. The key offers comparisons that are mostly easily observable and is workable for the species in the area. The only difficulty with their key is that three sets of couplets offer only seed characters and if plants are collected early in the season, they lack seeds and this poses a problem. Nevertheless, the taxonomy of *Hypoxis* in Africa is no doubt reaching a point of being resolved through the contributions made over especially the past 22 years.

5.3 Taxonomic significance of vegetative morphology

5.3.1 Growth form

Hypoxis are perennial geophytes that generally have synanthous leaves and a life cycle of growth, storage, flowering and dormancy as defined for geophytes by Dafni *et al*. (1981). The genus is predominant in the summer rainfall region in southern Africa, the underground stem (rhizome) thus allowing the plants to perennate in the dry winter months, a time when fire is also a recurring feature. During this study, repeat visits were made to grassland sites in and around Durban to observe flowering in *Hypoxis*. It is concluded that flowering and fruiting take place every year,

possibly due to favourable habitats and sufficient reserves in the rhizomes. The genus also has a long season of growth from September to April, following the spring rains. During the growing season, *Hypoxis* plants continue to develop new leaves and inflorescences, and flowering among most species takes place between November and January.

Plants of *Hypoxis* range from robust to delicate and this relates to length and width of rhizomes, leaves and inflorescences. The terms robust, medium-sized and delicate are relative and are applied with difficulty in a key. However, they may be used in combination with other characters. Relative size of plants has been used by Compton (1976) in his key to *Hypoxis* for the Flora of Swaziland. He describes plants as small or robust and defines small plants as those less than 100 mm tall and robust plants being more than 100 mm tall. In general, for the southern African species, using height to determine robustness does work, but poses problems for hysteranthous species when height varies over the season.

In *Hypoxis*, aerial stems are lacking; the leaves arise directly from the rhizome meristem in principally three ranks referred to as trifarious. In a few species, for example *H. argentea*, *H. hemerocallidea*, *H. obliqua*, *H. obtusa*, *H. stellipilis* and *H. villosa* the ranking of leaves is more noticeable than in the rest of the species. However, in some populations of *H. hemerocallidea* and *H. obtusa*, plants were noted to have leaves that are not neatly stacked in three ranks. In this case, type and distribution of leaf hairs are useful in recognising the species. In some species, the bases of leaves wrap around each other tightly to form a column called a false stem (pseudostem). Above the pseudostem, the leaves radiate upwards and outwards. This is discussed in more detail under 5.3.6.2 on leaf arrangement.

Another character that offers confirmatory evidence for classifying species in *Hypoxis* is whether the plants grow solitary or in clumps, the latter resulting from branching of the rhizomes (see 5.3.3). Branching occurs in *H. acuminata*, *H. angustifolia* var. *buchananii*, *H. costata*, *H. galpinii*, *H. multiceps* and *H. sobolifera* and gives rise to many rhizomes and shoots on one plant. Plants of these species may, however, also be found growing singly. In *H. angustifolia* var. *buchananii* and *H. sobolifera*, branching clones are more prevalent in comparison to solitary rhizomes.

5.3.2 Roots

Hypoxis has contractile roots that pull the rhizome firmly downwards into the ground. The roots occur just below the region where new reserves were added in the growing season (Figure 5.1). Root scars from the previous seasons roots can be seen on the lower portion of rhizome. Roots vary from few in the delicate species to about 30 in the robust species. Except for differences in number and size of roots which are proportional to the size of the rhizome, there are no morphological differences in root structure and therefore they are of little diagnostic value.

Figure 5.1.—Solitary rhizome showing roots in A, *H. hemerocallidea*; B, *H. angustifolia* var. *angustifolia*.

5.3.3 Rhizome

5.3.3.1 Structure and terminology

The storage organ in *Hypoxis* is an underground stem that is fleshy with a thin rind. It is an orthotropic (vertical) structure where reserves are added at the apex each season while the opposite end withers gradually upwards. Both the terms ‘corm’ and ‘rhizome’ have been used interchangeably to describe the underground stems in *Hypoxis*. Bell & Tomlinson (1980) use the term ‘rhizome’ to indicate vegetative extension over or within the subtrate by axis elongation and include organs which may be distinguished more precisely as stolons, offsets, or suckers and which may intergrade with tubers and corms. These authors consider rhizomes to be underground or

aerial as well as horizontal or vertical (ascending, descending or both). They point out that although it is useful to make a morphological distinction between a 'rhizome' being a thickened axis, a 'corm' a squat upright axis and a 'stolon' an extended axis, there is no precise cicumscription in terms of clone spread. According to Tillich (1998) a rhizome is a creeping shoot (normally underground, but may be aerial) with a storage function as well as for vegetative propagation through branching which could be monopodial or sympodial. He describes very thick rhizomes as 'pachycaul rhizomes' and those that grow vertically downwards as 'positively geotropic rhizomes'. For a corm, Tillich (1998) offers the definition of it being a vertical subterranean shoot with only a few swollen internodes, bears a terminal inflorescence and after flowering, shrivels and decays and is replaced by one or a few new corms that develop in an axillary position. In following the definitions by Bell & Tomlinson (1980) and Tillich (1998), it is concluded that in *Curculigo*, *Molineria*, *Hypoxis*, *Hypoxidia* and *Rhodohypoxis*, the underground stem survives for decades by new reserves being added seasonally to the proximal end while it disintegrates slowly at the distal end; therefore a more appropriate term for the axia in these genera is a 'fleshy vertical rhizome'. Corms are considered annual perennating organs that are replaced after flowering and are present in *Empodium*, *Pauridia*, *Saniella* (Burtt 2000) and *Spiloxene*. In this study, the term rhizome is used for *Hypoxis* following the definition by Tillich (1998). In the thesis when reference is made to the structure from earlier works, the term applied by the authors is used in inverted commas.

Rhizomes in *Hypoxis* usually grow singly (Figure 5.1) and give rise to one aerial shoot at the apical meristem. However, in a few southern African species, branching of the rhizome through the development of lateral buds occurs. Wood (1976) recognised two types of branching in *Hypoxis*, the first where lateral buds develop from a 'corm', each bud remaining attached to the original 'corm' forming a 'plate' of rhizome material from which many aerial shoots arise. In the second type, being found in *H. angustifolia*, lateral buds develop into slender 'rhizome-like' portions at the end of which a 'corm' develops. In this type, the 'rhizome-like' portion thickens with deposition of food reserves, and the parent rhizome becomes twisted. Heideman (1979) also recorded two types of branching in *Hypoxis*, slightly different to that of Wood (1976). In Heideman's description, in the first type the apical meristem splits into three to five parts and a shoot arises from each part. In the second type, one large rhizome gives rise to short runners each ending in a rhizome that produces a shoot and this agrees with the first type described by Wood (1976).

Observations made during this study confirm that there are two types of branching in rhizomes of *Hypoxis*. The first is lateral branching where a rhizome gives rise to lateral rhizomes (Figure

5.2A) that are attached to the parent rhizome by reduced stolon-like structures (Figure 5.2B). Each lateral rhizome ends in an erect shoot with scapes. In this case the laterally produced scapes are attached to rhizomes without an associated thickened portion distally. Proliferation by lateral rhizomes gives rise to a number of aerial shoots per plant forming tussocks (Figure 5.3A & B).

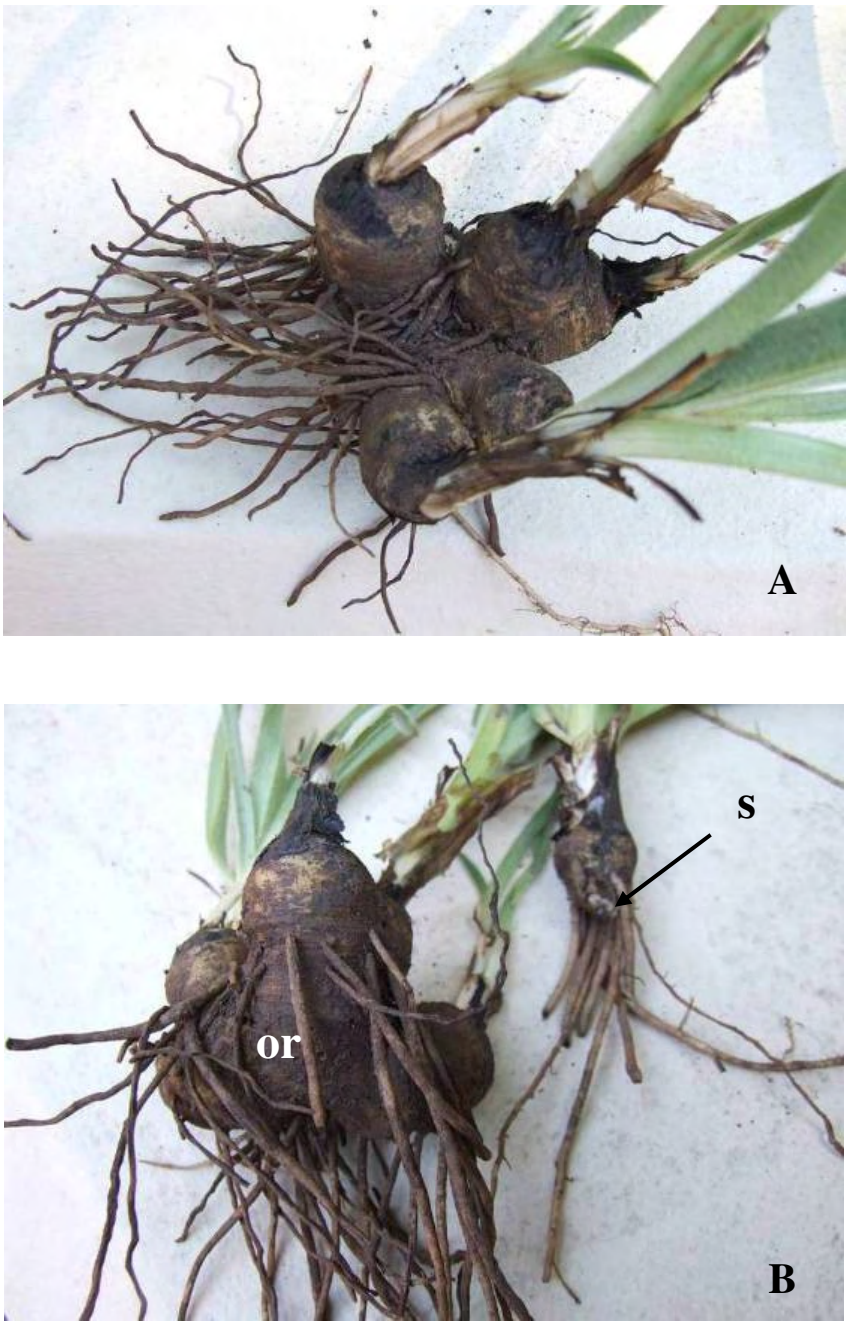

Figure 5.2.—Branching rhizome in *H. sobolifera* var. *sobolifera*. A, four lateral rhizomes produced from an original rhizome; B, original rhizome (or) and reduced stolon-like structure (s).

Figure 5.3.—Rhizomes forming tussocks in A, *H. multiceps*; B, *H. ludwigii;* C, *H. zeyheri*

This occurs in *H. acuminata*, *H. angustifolia* var. *buchananii*, *H. costata*, *H. ludwigii*, *H. galpinii*, *H. multiceps* and *H. sobolifera*. The reduced stolons (Figure 5.2B) are hardly differentiated from the lateral rhizomes. Over a number of years, the original rhizome (Figure 5.2B) which also lengthens by addition of new reserves each year and is usually longer than the lateral rhizomes may become twisted as described by Wood (1976). This definition combines both branching types described by Wood (1976). Twisting of rhizomes occurs more often in species with narrow, elongated rhizomes, namely *H. angustifolia* var. *buchananii* and *H. sobolifera*. These species are suitable for cultivation as they are easy to propagate vegetatively by separation of rhizomes. They also offer a number of flowers per tussock and are ideal for garden beds. In most *Hypoxis* species, damage to the apex of the rhizome induces this type of proliferation, where a number of rhizomes develop around the apex (Figure 5.4 A,B). This happens in the wild through, for example, trampling by cattle or other antelope, and the same damage can be applied in cultivation as a means of increasing number of rhizomes as the rhizomes can be removed and planted out as individual plants.

Figure 5.4.—Rhizomes forming tussocks possibly due to damage to apex in A, *H. rigidula*; B, *H. hemerocallidea*.

In the second type of branching, referred to as vegetative fragmentation, the apical meristem of the rhizome splits into three to five parts (Figure 5.5A) and each of these gives rise to an aerial shoot as described by Heideman (1979). As reserves are added each year to the apex of each fragment rhizome, the rhizomes separate further apart from each other but remain attached to the basal portion of the original rhizome which disintegrates over time (Figure 5.5B). This type of branching has been observed in most robust species like *H. colchicifolia*, *H. galpinii*, *H. hemerocallidea*, *H. obtusa* and *H. rigidula* but is not common.

In both types of branching there is always part of the original rhizome to which the newer rhizomes are attached. The presence of lateral branching is valuable in confirming whether a species belongs to *H. acuminata*, *H. costata*, *H. galpinii*, *H. ludwigii*, *H. multiceps, H. sobolifera* or *H. angustifolia* var. *buchananii*. The second type of branching appears to be due to ecological and biological factors. It is not constant for a species and is therefore unreliable for species identification.

Figure 5.5.—Split in apical meristem in *H. hemerocallidea*. A, splits into three parts (one lying behind the two shown) each giving rise to a rhizome that bears a shoot; B, split deepens but segments are held together by the basal portion of the original rhizome that disintegrates slowly.

5.3.3.2 Shape

Rhizomes are generally subglobose, oblong or turbinate in shape (Figure 5.6A–E) and range from 10 to 100 mm in length and 7 to 60 mm wide. Baker (1896) used size of rhizomes to separate *H. filiformis* from *H. kraussiana*, both of which have subterete leaves. However, due to the overlap in the range of size in rhizomes between the species, the character is not suitable. Leaf hair characters, flower size and filament shape are more useful for separating these species. Size and shape of rhizomes are useful mostly in separating the robust species from delicate species in *Hypoxis* and can be used to confirm a species when used in combination with other characters. Rhizomes in a few species branch through reduced stolons (Figure 5.6E) as discussed in 5.3.3.1.

5.3.3.3 Sap

Rhizomes in *Hypoxis* are fleshy and mucilaginous and when sliced, they exude a slimy juice that turns black through oxidation. In the robust species, *H. colchicifolia*, *H. galpinii*, *H. hemerocallidea* (Figure 5.7A) and *H. rigidula*, the flesh of rhizomes is yellow to deep orange internally. Robust species have large rhizomes and firm leaves. Rhizomes of species with a small stature and soft-textured leaves are white internally (Figure 5.7C). These species include

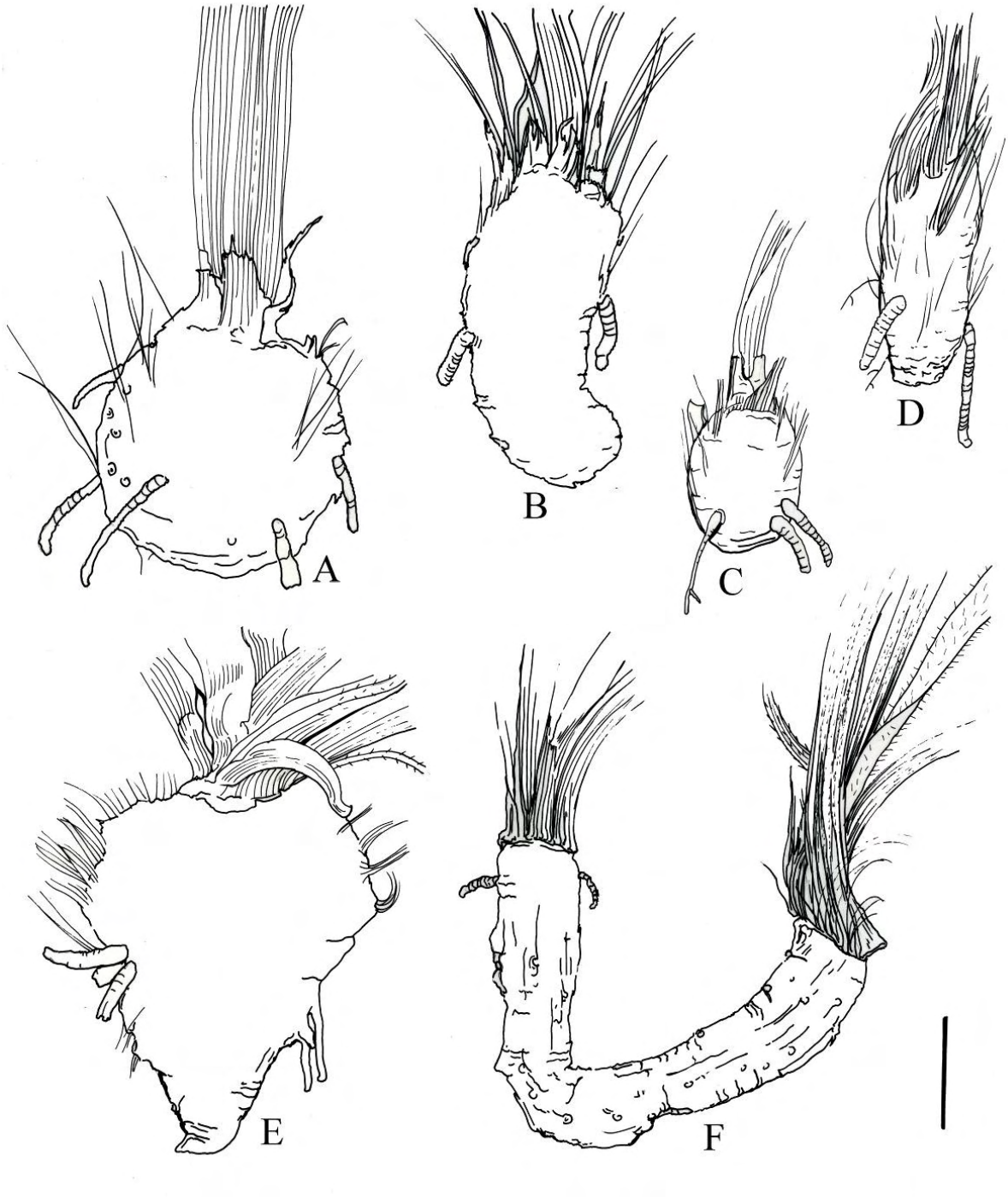

Figure 5.6.—Rhizome shapes in *Hypoxis* A, C, subglobose in *H. rigidula* var. *rigidula*, *Singh 328* (NH) and *H. filiformis, Singh 823* (NH); B, D, oblong in *H. rigidula* var. *pilosissima*, *Singh 326* (NH) and *H. parvula* var. *parvula*, *Singh 308* (NH); E, turbinate in *H. hemerocallidea*, *Singh 649* (NH); F, stoloniferous in *H. sobolifera* var. *sobolifera*, *Singh & Baijnath 233* (NH). Scale bars: A,B, 30 mm; C,D, E,F, 10 mm. A.J. Beaumont.

H. angustifolia, *H. argentea*, *H. filiformis*, *H. flanaganii*, *H. floccosa*, *H. gerrardii*, *H. membranacea, H. parvifolia*, *H. parvula* and *H. tetramera*. Species that fit morphologically in between the robust and the small-statured species (see formal treatment in Chapter 12), namely *H. acuminata*, *H. longifolia*, *H. costata*, *H. interjecta*, *H. muticeps*, *H. obliqua*, *H. sobolifera*, *H. stellipilis*, *H. villosa* and *H. zeyheri* produce rhizomes that are cream or light yellow internally (Figure 5.7B). The yellow-orange colour in robust species is associated with the high amounts of hypoxoside in rhizomes and this is elaborated upon in Chapter 8. Colour of sap, when combined with size of rhizome adds confirmatory value to identifications.

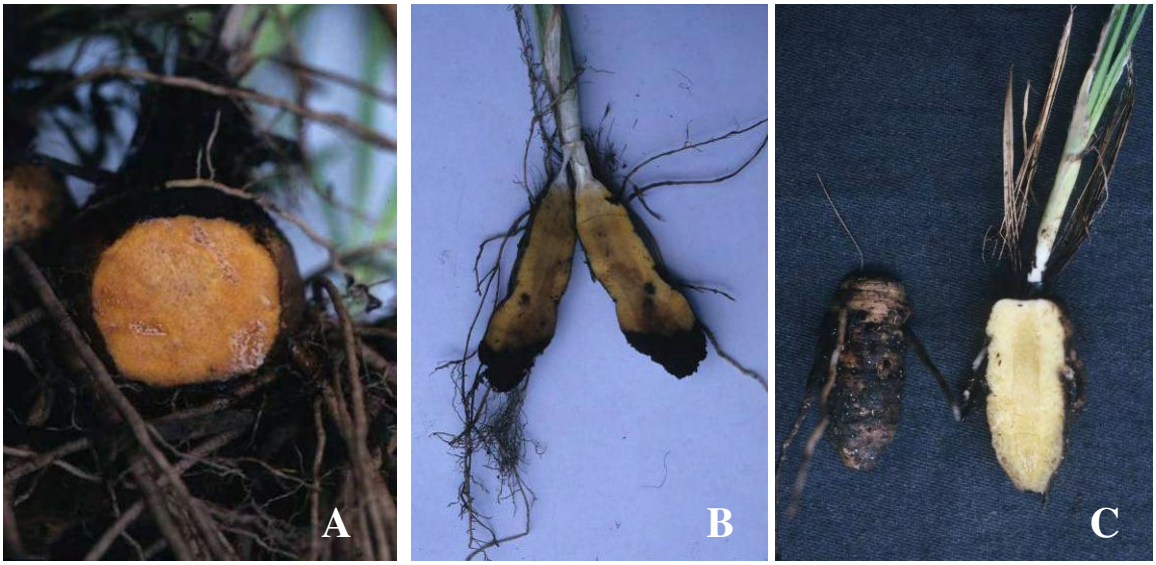

Figure 5.7.—Rhizomes in robust, medium and small-statured *Hypoxis* showing internal colouration. A, *H. hemerocallidea* with orange colouration; B, *H. sobolifera* var. *sobolifera* with yellow colouration; C, *H. angustifolia* var. *angustifolia* with white colouration.

5.3.4 Tunic

In *Hypoxis*, a tunic is present at the leaf bases. It occurs as a white or brown membranous covering that wraps around the leaf bases in species with soft-textured leaves. In the majority of species, the tunic is formed by the persistent veins that remain from the previous season's leaves and is visible as stiff fibres. Presence of a tunic has not been used much for demarcating individual species except in Wiland-Szymańska (2001) where it is used to separate species with slender leaves and narrow scapes. Wiland-Szymańska (2001) separates *H. filiformis* from *H. canaliculata* on its membranous tunic and slightly narrower leaves. *H. canaliculata* is considered to have a fibrous, stiff tunic. Among the southern African material of *H. filiformis*, specimens were found to have both types of tunics, the fibrous tunic being of varied mass. Some specimens show only a membranous tunic [*McLean 11a* (NH), *Williams 1135* (NH)] which could be due to the fibrous

tunic being left behind with the rhizome in the field. Specimens with rhizomes and only few fibrous strands [*Singh 397, 462* (NH), *Ngwenya 420* (NH)] were collected in fields that were recently burned and tunics in these may be reduced due to burning. Examples of specimens of *H. filiformis* that have distinct fibrous tunics of varying masses include *Ross 5*; *Nicholas & Button 1981*, *Singh 443*, *Ngwenya 1468* (all at NH). Wiland-Szymańska & Nordal (2006) used the red colour of tunics in *H. obtusa*, a species widespread in subsaharan Africa to separate it from black tunics of five tropical species.

In summary, the type of tunic is only useful in separating species with membranous leaves from the generally robust species with firm leaves. In southern African species, membranous tunics are associated with membranous leaves while fibrous tunics are found in species with firm to rigid leaves.

5.3.5 Cataphylls

In *Hypoxis*, the outermost leaves are reduced to cataphylls. One to three cataphylls occur in the genus and they vary in length on a plant but are $^{1}/_{3}$ to $^{1}/_{10}$ the length of leaves. They have the same shape as the leaves, either oblong-ovate or linear and are also hairy. Cataphylls wrap around the newly forming leaves, offering them protection. In robust species with distinct three ranks, for example *H. hemerocallidea* and *H. obtusa*, three cataphylls are found to correlate with the position of the ranks and these spread outwards. In robust species with pseudostems like *H. colchicifolia* and *H. rigidula*, two cataphylls wrap tightly around the pseudostem, one larger than other. Cataphylls in the soft-leaved species are usually membranous and fragment as the leaves mature. Most herbarium specimens lack cataphylls as they would have deteriorated with leafing and flowering. Cataphylls differ only in texture but this feature can be assessed in the leaves. They are also similar in related species and are unsuitable for separating taxa.

5.3.6 Leaves

In identifying species of *Hypoxis*, there has been much reliance on leaf characters. Cues to diagnostic value of leaf characters in the genus were pointed out by earlier authors. Baker (1878b) presented a short description of leaves in the genera of Hypoxidaceae and described *Hypoxis* as having sessile, linear or lanceolate and rarely subterete leaves. In the key in this publication, Baker used leaf shape, texture and width to separate species. In the key to species in Flora Capensis, Baker (1896) used the same leaf features but added to it characters of venation and hairiness. Nel (1914) provided discussion on leaf characters in *Hypoxis* and emphasised shape, venation and

hairiness. Heideman (1987) used the same leaf characters but also included leaf shape in cross section. Recent taxonomic works on *Hypoxis* in Tropical Africa (Nordal & Zimudzi 2001; Wiland-Szymańska & Adamski 2001 and Wiland-Szymańska & Nordal 2006) give prominence to leaf characters.

Most certainly, leaves offer the largest number of characters that are useful for species identification in *Hypoxis*. Leaf characters of diagnostic importance include arrangement at the base, shape, dimension, texture and leaf indumentum. Leaf length to width ratios are of taxonomic value in separating species into groups (see Singh 2004, Appendix 2.4). They are also useful in demarcating taxa of small stature with corymbose inflorescences. In a few species, bases of leaves wrap around each other to form a false stem (pseudostem) and this is easily distinguished from those where leaves spread upwards and outwards from base. Texture of leaves vary from firm and rigid to soft and flaccid and this is useful in grouping species. Leaf indumentum characters like density, distribution and position relative to the leaf surface and type of hairs are of importance in recognising species. Leaves in the genus may be sparsely, moderately or densely pubescent depending upon the species and a few varieties are recognised based on hair density and distribution. Leaves turn yellow-brown towards the end of the growing season and are marcescent, and depend on external factors like wind for slow obliteration and fire for defoliation. If grasslands are not subjected to burning, the relic of old fibrous leaves remain around the newly forming leaves.

5.3.6.1 Maturation

Members of *Hypoxis* are acaulescent, deciduous herbs and the new flush of leaves arise directly from the apex of the rhizome. Leaves in the genus usually develop with the onset of the new growing season each year, around August-September in southern Africa. This is followed closely by the production of flowers. Most species in the genus are synanthous producing a few leaves at the start of the growing season and continues to produce leaves and flowers simultaneously during the growing season. A few species display hysteranthy where flowers are produced first and then the leaves start to emerge. The term hysteranthy is used as many of the flowers in these plants have passed anthesis by the time the emerging leaves become noticeable (Figure 5.8A). Different degrees of hysteranthy are noted within and among species. It occurs most frequently in *H. multiceps* and *H. interjecta* and occasionally in *H. galpinii* and *H. obtusa*. Due to hysteranthy in *H. multiceps* [*Heideman 98* (J); *Wood 91* (NU); *Stalmans 611* (PRE); *Young A126* (PRE)] and *H. interjecta*, flowers develop with the onset of the growing season (August) and by the time the

leaves reach maturity (December), the flowers have been pollinated, and have set fruit and seeds. Towards the end of the growing season, the plants are often with old scapes or without a trace of scapes and flowers (Figure 5.8B). Another feature unique to these species is the pseudopetiolate leaves at the end of the growing season (see 5.3.6.3). The varying facies in *H. multiceps* and *H. interjecta* confuse their identification. Hysteranthy being common and pronounced in these two species can be used to separate them from the rest of the species. Since the two species are similar in their two to five-flowered corymbose inflorescences, leaf characters are necessary for their demarcation. The first few emerging leaves are useful as they are hairy in *H. multiceps* but glabrous in *H. interjecta*. *H. galpinii* and *H. obtusa* are generally synanthous, however if they produce flowers first, then they can be easily separated from *H. multiceps* and *H. interjecta* by their scapes (at least one on a plant) with usually five or more flowers in a raceme and on the type of leaf indumentum explained in 5.3.9.

5.3.6.2 Arrangement

Leaves in *Hypoxis* are arranged in a basal rosette or the bases clasped together to form a false stem (pseudostem). Pseudostems are cylindrical and may form a well-defined column or may be subtle. They are column-like, 80–200 mm tall and diagnostic in *H. colchicifolia*, *H. galpinii*, *H. rigidula* (Figure 5.9A–D) and *H. longifolia*; species with rigid leaves that arise upwards and outwards above the pseudostems. By using the presence of a well-defined pseudostem, these species are easily separated from other robust species like *H. hemerocallidea* (Figure 5.10A) and *H. obtusa* (Figure 5.10B) in which the leaves spread upwards and outwards from base (false stem absent). In the small to medium-statured species, *H. argentea* (Figure 5.11A), *H. sobolifera* (Figure 5.11B, see also 5.7B), *H. angustifolia* (Figure 5.19A) and *H. nivea* (Figure 5.19D), pseudostems are subtle and are concealed by the flexible, arching leaves in these species.

Heideman (1987) begins her key to *Hypoxis* taxa by separating species with pseudostems from those that lack a pseudostem. Wood (1976) used it to separate the robust *H. colchicifolia* from the medium-sized *H. interjecta* which she grouped together on the basis that their leaves being glabrous. No authors working on southern African species prior to Wood (1976) and Heideman (1987) appear to have used this character. Singh (2004) illustrated the states of leaves clasped into a pseudostem in comparison to leaves spreading upwards and outwards from base, and used the character in grouping species. Wiland-Szymańska & Nordal (2006) used the presence of a pseudostem in *H. rigidula*, a species widespread in sub-Saharan Africa to separate it from the remaining 14 species in the Flora of Tropical Africa region. These authors placed *H. galpinii*

Figure 5.8.—Hysteranthy and facies in *H. multiceps*. A, plants showing most flowers pollinated by the time new leaves are noticeable; B, plants in post fruiting with leaves mature.

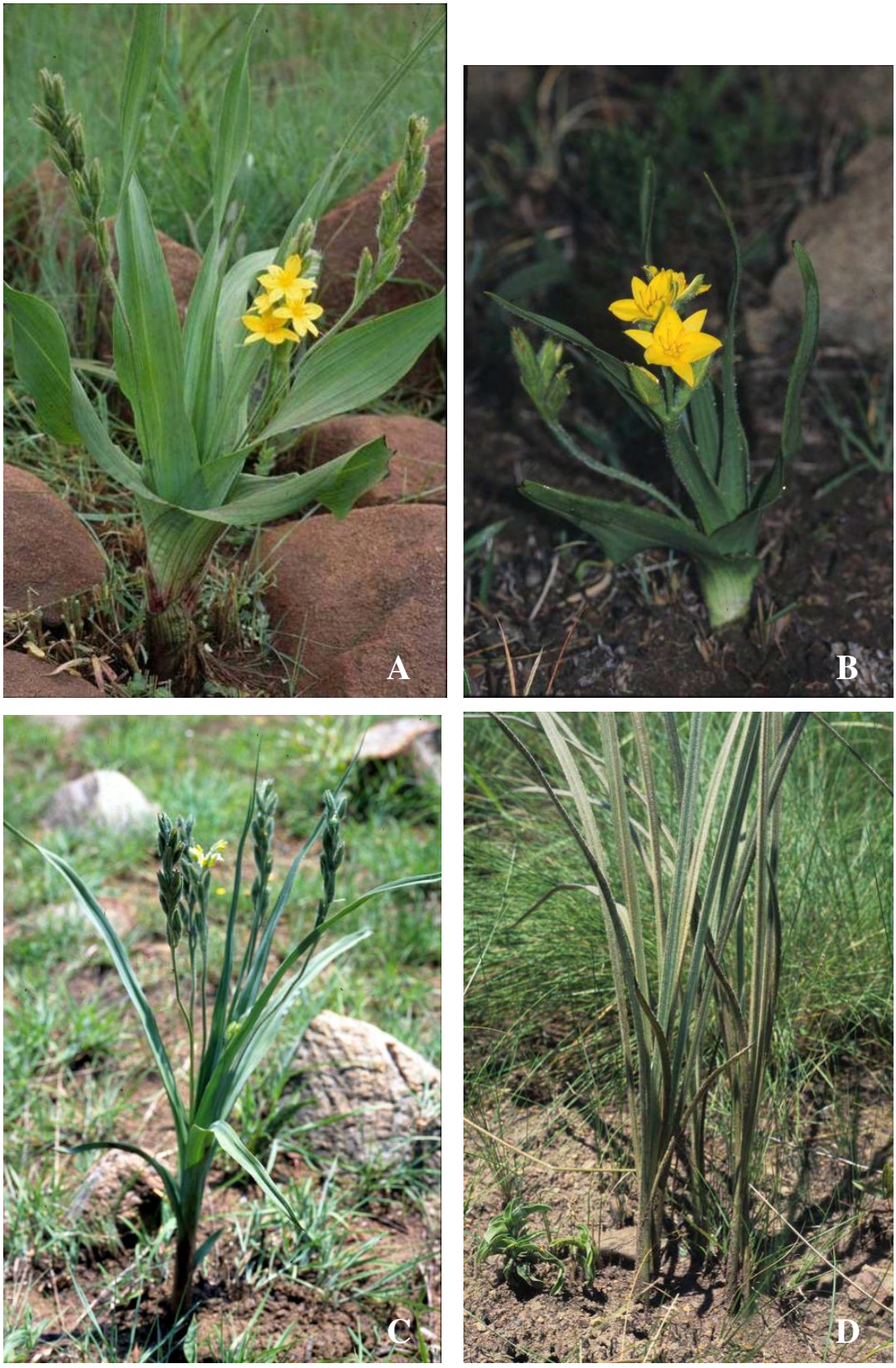

Figure 5.9.—Pseudostem as a distinct column. A, *H. colchicifolia*; B, *H. galpinii*; C, *H. rigidula* var. *rigidula*; D, *H. rigidula* var. *pilosissima*.

Figure 5.10.—Leaves do not wrap at the base into a pseudostem but spread outwards and upwards from the apex of the rhizome in robust species. A, *H. hemerocallidea*; B, *H. obtusa*.

Figure 5.11.—Subtle pseudostems. A, *H. argentea*, a small-statured species; B, *H. sobolifera*, medium-sized species, obscured by the overlapping leaves (see also Figure 5.7B).

among species that lack a distinct pseudostem. In southern Africa, however, *H. galpinii* (Figure 5.8B) has a distinct pseudostem similar to that in its closest relatives *H. colchicifolia* and *H. rigidula*.

The presence or absence of pseudostems is a valuable character for identification of species in southern Africa. The advantage of the character is that it is easily observable and unambiguous in the few species in which it is present.

5.3.6.3 Shape

In *Hypoxis*, leaves are simple, entire and keeled at the midrib with tips acuminate. They are folded together along the length towards the base (conduplicate). Leaf shapes were broadly classified into lanceolate and linear, and used in earlier treatments of *Hypoxis*. Baker (1878b, 1896) placed much emphasis on leaf shape in his keys, often using it as the only character in separating groups of species. However, he only used the character after he separated species on flower size or inflorescence type. Nel (1914) used leaf shape in combination with leaf indumentum and leaf venation. It was also used by Wood (1976) in combination with other leaf characters. Heideman (1987) used many leaf characters except leaf shape. However, she used the outline of leaves in cross section to separate closely related species.

Leaf shape in *Hypoxis* is variable and leaves may appear lanceolate, linear, filiform and ovate in outline (Figure 12A–E). Due to their gradual elongation over the growing season, leaves in the genus start to taper and the shape of mature leaves is different from those at the start of the season. Therefore, the use of leaf shape to separate species is limiting. However, leaf shape is extremely valuable in separating species with distinct shapes. For example, leaves in *H. filiformis* (Figure 5.13A), *H. tetramera*, *H. kraussiana* and *H. longifolia* (Figure 5.13B) are linear and less than 5 mm wide and on these characters they can be separated from the rest of the species. Also, leaves of *H. colchicifolia* are broadly lanceolate (Figure 5.9A, 5.16A) and can be easily separated from the linear leaves of *H. angustifolia* and the filiform leaves in *H. filiformis*.

Generally, leaf shape is not constant in *Hypoxis* and species display different degrees of variation in leaf shape. Leaves of *H. costata*, *H. multiceps* and *H. interjecta* are short and ovate (Figure 5.8A) at the start of the growing season and elongate in post flowering (Figures 5.8B, 5.14A,B, 5.20B), appearing lanceolate later in the season. Mature leaves of *H. costata* exhibit a range in shape between ovate-lanceolate (Figure 5.15B) and linear (Figure 5.15A). The one

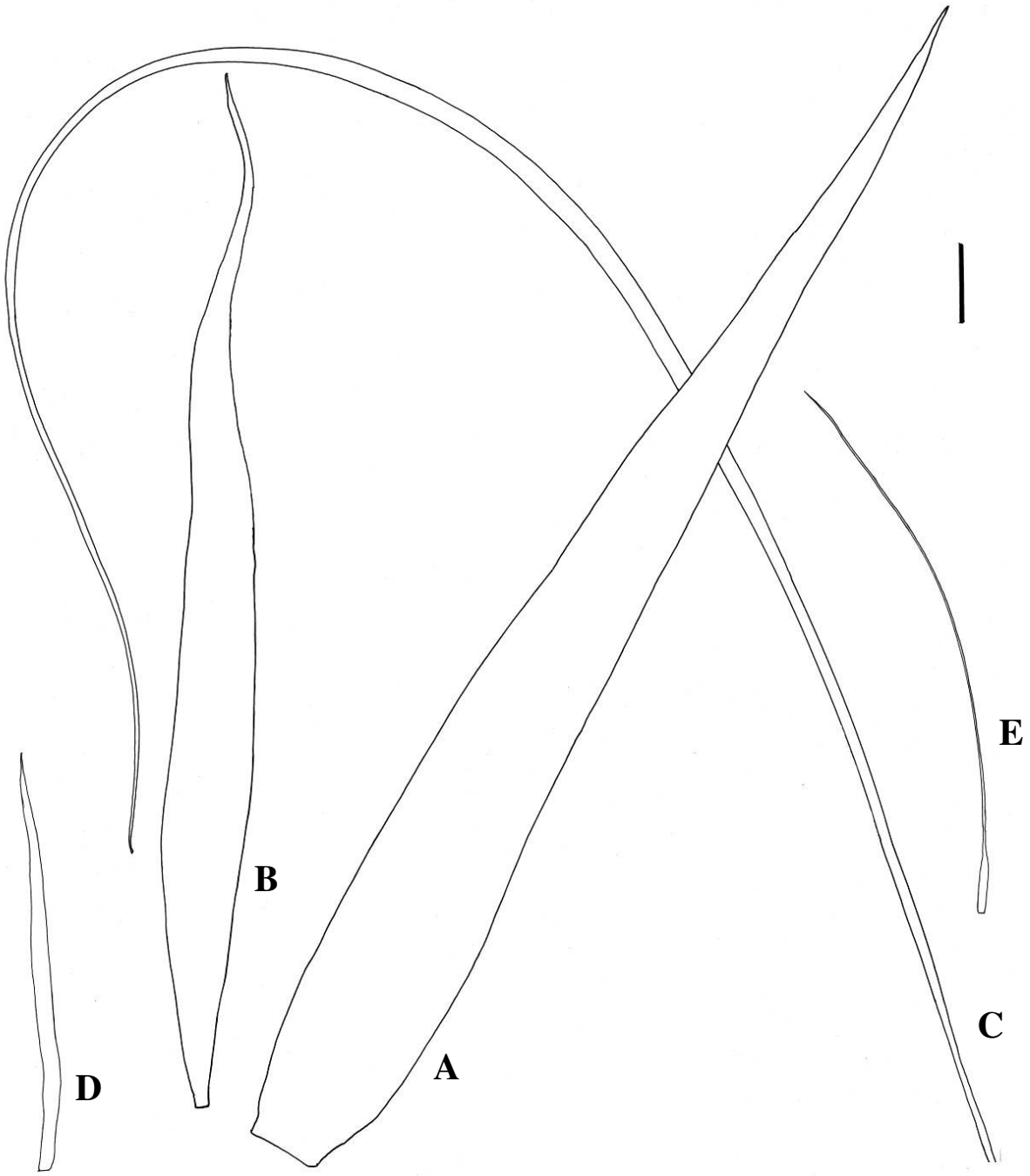

Figure 5.12.—Leaf shapes in *Hypoxis*. A, lanceolate, *H. colchicifolia*, *Singh 294* (NH); B, lanceolate-linear, *H. obtusa*, *Singh 439* (NH); C, linear (long), *H. rigidula*, *Singh 328* (NH); D, linear (short), *H. nivea*, *Singh 874* (NH); E, filiform, *H. filiformis*, *Singh 433* (NH). Scale bar: A–E, 30 mm. A.J. Beaumont.

Figure 5.13.—Leaf shapes. A, filiform, *H. filiformis* (Steenskampberg, Mpumalanga); B, linear, *H. longifolia* (Port Edward, KwaZulu-Natal).

Figure 5.14.—Elongate, pseudopetiolate leaves. A, *H. multiceps*; B, *H. interjecta*.

extreme in range includes plants with broad, stout leaves (Figure 5.15B), while the other extreme includes plants with narrow leaves (Figure 5.15A). The linear leaves are associated with shading caused by tall unburned grass. In some plants of *H. multiceps* (Figure 5.14A) and *H. interjecta* (Figure 5.14B), the bases of leaves narrow to form a pseudopetiole in post flowering and this has also been noticed when plants are growing in between tall grass, usually in unburnt vegetation. Pseudopetioles in *H. multiceps* are observable in specimens *Codd 5912* (PRE); *Coetzee 279* (PRU); *Eckhardt 319* (PRU); *Heideman 116* (J); *Nombekela 117* (NH); *Reid 584* (PRE); *Singh 322*, *609* (NH). *H. interjecta* specimens that show pseudopetioles include *Gilliland s.n.* (J); *Singh 613* (NH); *Smit 877* (PRE); *Strey 3956* (PRE). All specimens cited were collected late in the season between January and July, the start of the growing season being around September. Heideman (1979) gives an account of the stages of seasonal growth pattern in *H. multiceps* and *H. interjecta* which are similar for both species. Pseudopetioles are very likely to be present in *H. costata* as well, but do not appear to be represented in any of the investigated herbarium material.

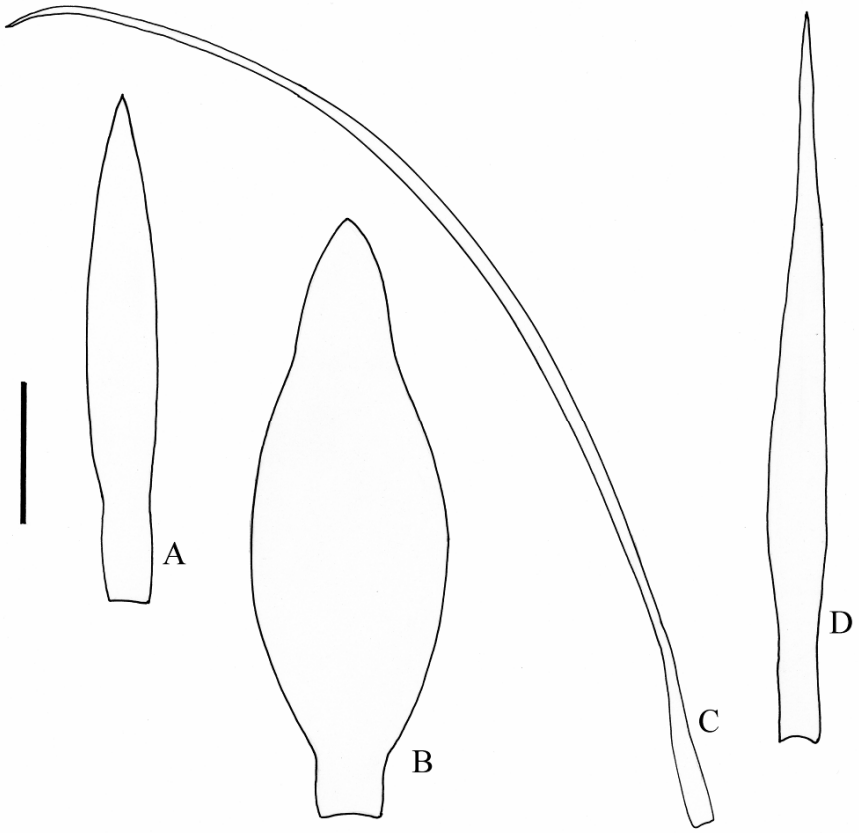

Figure 5.15.—Variable leaf shapes. A, linear, *H. costata Singh 455* (NH); B, ovate-lanceolate, *H. costata Singh 300* (NH); C, linear, *H. gerrardii Singh 448* (NH); D, lanceolate, *H. gerrardii Singh 419* (NH). Scale bars: A, B, 10 mm; C, D, 20 mm. Artist: A.J. Beaumont.

5.3.6.4 Colour

Usually leaves of *Hypoxis* are green on both surfaces and bases are lighter in colour tending towards white. Variation in leaf colouration is useful for recognising some species, mainly live material. In species with small plants like *H. angustifolia*, *H. filiformis*, *H. membranacea*, *H. parvula* and *H. tetramera*, leaf bases are membranous or at least along the margins. Leaf bases may be white (Figure 5.9B) or purple or red-coloured as in *H. colchicifolia* (Figure 5.16). In populations of *H. obtusa* (Figure 5.17), *H. argentea*, *H. angustifolia*, *H. costata* and *H. sobolifera*, leaf bases may be red-tinged. In plants of *H. argentea*, *H. angustifolia* and *H. sobolifera* with red-tinged leaf bases, the scape, pedicel and tepal midribs are also tinged with red. It should be noted that the purple or red colouration is not constant for all populations of these species. However, its presence in these species is helpful in identifying them.

In *H. stellipilis*, leaf colouration is very diagnostic for the species. Leaves are dark green on the upper surface and white on the lower surface (clearly discolourous) (Figure 5.18). The white lower surface is due to the white, stellate hairs that intertwined to form a dense covering. This colouration is constant and unique to the species.

Figure 5.16.—Purple colouration in *H. colchicifolia*. A, all leaf bases purple; B, close up of lower leaf bases.

Figure 5.17.—Purple-red-coloured leaf bases in *H. obtusa*.

Figure 5.18.—Leaves of *H. stellipilis*, green and almost glabrous above, white below formed by tomentose layer of stellate hairs.

5.3.6.5 Length and width

Leaf dimensions are variable among species, ranging from 50 to 600 mm in length and 1.5 to 85 mm in width across the broadest section. The longest leaves in the genus are found in *H. rigidula* reaching 600 mm and the shortest of about 70 mm long is found in *H. floccosa* and *H. flanaganii*. The broadest leaves are found in *H. colchicifolia*, up to 110 mm wide and the narrowest in *H. tetramera* and *H. filiformis* (Figure 5.12E) usually about 2–3 mm.

Baker (1896) used differences in leaf width to separate *H. obtusa* and *H. latifolia* (= *H. colchicifolia*), both of which he considered to have lanceolate or oblong-lanceolate leaves and racemose inflorescences. Based on width of leaves, Baker (1878b) recognised two varieties of *H. angustifolia* Lam. namely var. *angustifolia* and var. *buchananii*. He separated var. *buchananii* in having larger membranous leaves and pedicels about twice as long in comparison to the typical variety. In Flora Capensis, Baker (1896) indicated that this is a shade grown variety with thin textured leaves and long slender pedicels. Nel (1914) used leaf length or width in his keys to species. In his key to sections, he used the ratio of leaf length to width. Both leaf length or width works in Nel's keys only because he first used floral characters and this grouped species with very varied leaf dimensions together, making the use of the character suitable. Species described by Nel as new on the basis of leaf shape and length, namely *H. cordata*, *H. elliptica* and *H. oblonga* as well as *H. distachya* and *H. gilgiana* have been reduced to synonymy (Singh 2007, Appendix 1.2). Wood (1976) applied ranges in leaf length and width at points in her key but in combination with inflorescence type and flower characters. Heideman (1987) also found it useful to use these characters, again mostly in combination with other leaf and floral features.

Leaf dimensions are valuable in recognising species as well as grouping related species of *Hypoxis*. The character works well for identification of species when used in combination with leaf shape. Species with narrow, linear leaves less than 5 mm wide are easily recognised from those with broad oblong-lanceolate leaves. Both leaf length and width are unsuited for the identification of individual plants in *H. multiceps*, *H. costata* and *H. interjecta* as they display a huge range over the growing season. As seen for leaf shape, the group of species with linear leaves including *H. angustifolia* var. *buchananii*, *H. sobolifera*, *H. stellipilis*, *H. villosa*, *H. zeyheri*, leaf dimensions are similar and therefore unsuitable for separating species. Nevertheless, they confirm a close relationship among these species.

5.3.6.6 Venation

Leaf venation is variable among species in *Hypoxis* and is a good character for recognising some species. In most species, one to four veins close to each margin are thickened and raised on the upper surface while the rest are flush with the leaf surface. Only in a few species, almost all veins are of even thickness and raised on the upper surface.

Nel (1914) emphasised leaf venation characters in demarcating formal sections in *Hypoxis*. He considered total number of veins per leaf, number of thickened veins and whether veins are of equal thickness to be valuable characters for recognising sections. He recorded that the veins in the leaves of some species are noticeably unevenly thickened and described with illustrations the venation patterns covering eight sections in detail from leaf cross sections. Nel used the higher number of veins in Sections *Villosae*, *Orbiculatae* and *Nyassicae* to distinguish them from Sections *Angustifoliae* and *Argenteae*, and Section *Subspicatae* from Section *Recurvatae*. In his key to sections, he used equally or unequally thickened veins to separate Section *Obtusae* from Section *Rigidulae*. *Obtusae* was separated from *Rigidulae* in having uniformly thickened veins while the latter section is defined as having one to three thickened veins on each half of the leaf. Nel used number of veins only once in separating southern African taxa namely *H. obtusa* from his newly described *H. patula*, but the character was used in combination with hairiness and hair type.

The number of veins was found to vary slightly within a species depending on width of leaves. However, number of prominent veins is useful in combination with other leaf characters like leaf shape and indumentum. Number of prominent veins is useful in separating closely related species like *H. colchicifolia*, where all veins are of about the same thickness and equally spaced while in *H. galpinii* only three or four veins close to each margin are thickened. Similarly, veins in *H. obtusa* are prominent and are of almost equal thickness (but not as thick as in *H. colchicifolia*) in comparison to its related species, *H. hemerocallidea* which has veins flush with the leaf surface except two or three veins near each margin are raised on the upper surface. Leaves of *H. multiceps* and *H. costata* are strongly veined and veins are of even thickness in both, except in *H. costata* in which one or two veins close to the margins are of greater thickness and yellow. The character can be used in combination with hair type to separate these species. Number of prominent veins is also useful in recognising species if working with fragments of a leaf.

5.3.6.7 Texture

Previous authors (Brackett 1923; Baker 1878b, 1896, 1898; Nel 1914) used leaf texture to separate species. The states of leaves rigid or flaccid were used. In the Flora Capensis treatment, Baker (1896) applied the terms firm, moderately firm and membranous to separate species with corymbose inflorescences and these terms being relative are applied with difficulty, unless well-understood for the genus.

Texture of leaves in *Hypoxis* varies from soft and membranous to rigid. *H. angustifolia* var. *buchananii*, *H. membranacea*, *H. nivea* and *H. parvula* (Figures 5.19A–D) have thin-texture, almost membranous, flaccid leaves and can be easily separated from the remaining taxa. When dried, the leaves appear membranous. The membranous texture of leaves is associated with semi-shade conditions in cliff forests or provided by rocks and tall vegetation when in open grasslands. This character when combined with the fragile, lax inflorescences in these species is useful for confirming identifications. The remaining species have firm to rigid leaves (Figures 5.9A–D, 5.10A–B, 5.16A, 5.20A–C) and this is correlated with their occurrence in grasslands, most frequently in full sun. Leaves in *H. multiceps*, *H. costata* and *H. intejecta* are short, broad and firm, and are held almost erect in comparison to the soft-textured species. Robust species with rigid leaves include *H. colchicifolia*, *H. galpinii*, *H. rigidula*, *H. obtusa*, *H. multiceps*, *H. costata* and *H. interjecta*. In these species, leaves twist with age (Figures 5.9A, 5.20A–C). As implied by its name, the leaves of *H. rigidula* (Figure 5.9C–D) are rigid. They are also long and slender, and are held erect to about midway where they all bend in one direction or the outer leaves bend backwards on itself. Species with a small stature that have usually rigid, sometimes firm leaves include *H. argentea* (Figure 5.11A), *H. filiformis* (Figure 5.12A) and *H. gerrardii*. There are species that have firm leaves that are not as rigid as those mentioned above and these include *H. hemerocallidea*, *H. flanaganii*, *H. floccosa*, *H. parvifolia*, *H. stellipilis*, *H. sobolifera* and *H. villosa*. In these species, leaves are flexible and are recurved in the first four mentioned species and erect in the latter that have small leaves. Leaf texture is an important diagnostic character and can be used in combination with venation pattern and inflorescence type to identify species in *Hypoxis*.

Figure 5.19.—Soft-textured leaves. A, *H. angustifolia* var. *buchananii* (KwaZulu-Natal, Stainbank Nature Reserve); B, *H. membranacea* (Eastern Cape, Lambazi); C, *H. parvula* var. *parvula* (KwaZulu-Natal, Noodsberg); D, *H. nivea* (KwaZulu-Natal, Umtamvuna).

Figure 5.20.—Rigid leaves showing twist with age. A, *H. obliqua* (Highmoor State Forest Reserve, KwaZulu-Natal); B, *H. costata* (Stutterheim, Eastern Cape); C, *H. obtusa* (Mbidlana, Eastern Cape).

5.3.9 Leaf indumentum

The leaf surface in *Hypoxis* is characterised by a sparse or dense indumentum of non-glandular hairs, usually more dense on the lower surface. Hairs in the genus are non secretory, single, bifurcate or stellate (three to ten arms), and the angle of arms relative to the leaf in horizontal plane may be patent, ascending or appressed (see definitions in Hewson 1988). Differences in distribution of hairs on leaves, density, number and position of arms provide taxonomic characters for separation of species. Hair characters are discussed below and its occurrence in the southern African species of *Hypoxis* is summarised in Table 5.1.

Leaf indumentum was used in many keys to *Hypoxis* species, initially described very coarsely and more recently in greater detail with the aid of Scanning Electron Microscopy techniques. In the first treatment to species in Africa, Baker (1878b) used mainly leaf and inflorescence characters and placed no emphasis on leaf indumentum. In his treatment in Flora Capensis, Baker (1896) used

differences in distribution of hairs on leaf surfaces in his key to species. However, Baker (1896) did not find the character very useful in separating the species in Tropical Africa. Nel (1914) used distribution, density, type, colouration and position of leaf indumentum to separate species in Africa. Wood (1976) considered leaf indumentum to be valuable for separating species, and used characters similar to those selected by Nel. Heideman (1987) also applied hair characters to species in the Witwatersrand but less frequently than Wood (1976). Wiland-Szymańska's (2001) key includes distribution of hairs on leaf blades in identifying species in Central Africa. Nordal and Zimudzi (2001) used hair characters similar to those used by Nel in their key to *Hypoxis* in the Flora Zambesiaca region, but applied a number of them in combination with other characters. Wiland-Szymańska & Nordal (2006) found hair type as well as density to be diagnostic for demarcating some species found in the Flora of Tropical east Africa region. Leaf indumentum no doubt offers a suite of characters that are of taxonomic importance in recognising species in *Hypoxis*.

5.3.9.1 Distribution and density of hairs

Hairs may be sparsely scattered in leaves of *Hypoxis* or they may cover the entire leaf surface or may be concentrated only along the veins, margins and midribs (Figure 5.21A–D). The leaves of *H. colchicifolia* and *H. interjecta* are subglabrous and if hairs are present, they are very sparse and distributed mainly on the leaf bases and along margins. The subglabrous state of leaves in *H. colchicifolia* is distinctive and is used to separate it from the closely related species, *H. galpinii* and *H. rigidula* that have bifurcate and stellate hairs in varying density. Subglabrous leaves in *H. interjecta* are easily separated from *H. multiceps* and *H. costata*, species with which it has a close affinity and these have hairs noticeable to the naked eye.

In *H. obtusa* (Figure 5.22D,E) and *H. costata*, hairs form a distinct white band along margins and midribs and this ciliation is unique to these two species. However, apart from other vegetative and floral characters, the species can be separated by the short, appressed hairs in *H. obtusa* and the long, ascending hairs in *H. costata*. In a few species, the distribution of hairs is distinctly dense on the lower surface of leaves. For example, in *H. stellipilis* (Figure 5.7C,D), a species named after its star-shaped hairs (see Figure 6.8D), the hairs form a white tomentose layer on the lower surface of the leaf, while the upper surface is sparsely clothed with hairs. In *H. argentea* var. *argentea* and *H. villosa*, a similar distribution is observed, but in these species, the hairs form a silky white covering over the lower surface as well as along the margins and midribs and the hairs are appressed with arms lying parallel to the length of the leaves. Two varieties are recognised in

Figure 5.21.—Distribution of hairs on leaves. A, *H. rigidula* var. *rigidula* (ad- and abaxial surfaces), x 1; B, *H. sobolifera* var. *sobolifera* (abaxial surface), x 5; C, *H. hemerocallidea* (adaxial surface), x 0.5; D, *H. membranacea* (abaxial surface), x 20.

H. argentea, *H. rigidula* and *H. sobolifera* based on density of leaf hairs. In *H. argentea* var. *sericea*, hairs are sparsely scattered on the lower surface of the leaf while in *H. argentea* var. *argentea*, hairs are dense on the lower surface giving the leaf a sericeous appearance. The typical varieties of *H. rigidula* and *H. sobolifera* are sparsely hairy on both leaf surfaces and hairs are spaced apart from each other, while in the second varieties in both species, hairs are dense, ascending and overlapping giving leaves a furry appearance.

Newly formed leaves early in the growing season appear more hairy (Figure 5.23A,B) than when they mature and this is due to their distribution across an expanded area in the mature leaves (Figure 5.23C). In a few specimens of *H. hemerocallidea* and *H. sobolifera*, it has been observed that as leaves age and reach marcescens, hairs deteriorate on the lamina, but remain on the margins and midrib.

5.3.9.2 Hair type

Based on number of arms, hairs in *Hypoxis* are classified into simple, bifurcate or stellate types (Figure 5.22 C,J,G,K,L,Q,R). Usually a combination of hair types is present in a species with one type being more predominant. Simple hairs are rare and are present in four species, namely *H. filiformis*, *H. membranacea*, *H. nivea* and *H. parvula*. Such hairs have never been found to occur on their own but in combination with bifurcate and stellate hairs in these three species. Bifurcate and stellate hairs occur widely in the genus, often intermingled with each other (Table 5.1).

In *H. membranacea*, hairs develop on pustules that are observable as dark green dots on the upper surface of the leaf when held against the light (Figure 5.21D). This state is not found in any other species and in the absence of inflorescences and flowers, it can be used to separate the species from *H. angustifolia*, *H. nivea* and *H. parvula* which also have membranous leaves. The use of this character is however, restricted to live material as the feature is lost in dried specimens.

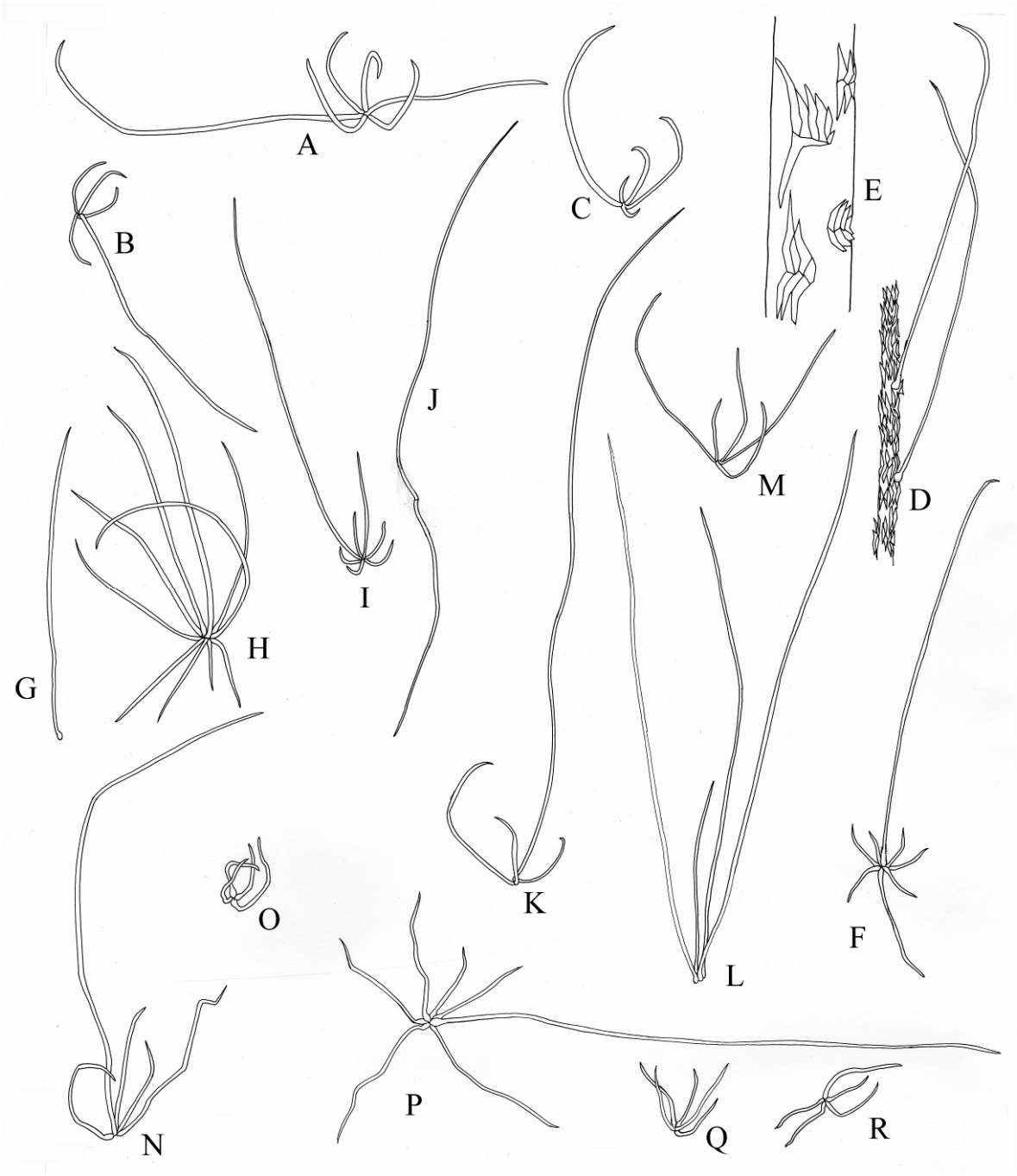

Figure 5.22.—Hair types found in *Hypoxis* A, scape, *H. galpinii Singh 632* (NH), B, tepal surface, abaxial *H. galpinii Singh 632* (NH); C, leaf margin, *H. galpinii Singh 632* (NH); D, E, leaf margin, *H. obtusa Singh 563* (NH); F, tepal surface, abaxial, *H. obtusa Singh 563* (NH); G, bract, *H. sobolifera* var. *sobolifera Singh 233* (NH); H, pedicel, *H. sobolifera* var. *sobolifera Singh 233* (NH); I, tepal surface, abaxial and scape, *H. costata Singh 455* (NH); J, leaf lamina, *H. costata Singh 455* (NH); K, L, leaf lamina, *H. membranacea Singh 826* (NH); M, leaf base; *H. membranacea Singh 826* (NH); N, O, scape, *H. multiceps Singh 642* (NH); P, pedicel, *H. multiceps Singh 642* (NH); Q, R, leaf lamina *H. multiceps Singh 642* (NH).

Figure 5.23.—Leaf hairiness in *H. hemerocallidea*. A, newly formed leaves in young plants with hairy leaves; B, developing leaves hairy; C, mature leaves with hairs beocming spread over a larger surface and less noticelable.

Table 5.1.—Summary of hair characters in southern African species of *Hypoxis*

Taxon	**Distribution**	**Density**	**Type**	**Shape of arms**	**Position of arms**	**Colour of hairs**
acuminata	both surfaces, more dense on lower surface	sparse, dense on margins and midrib	bifurcate or interspersed with stellate	filiform, held in U or V	ascending or patent	white
angustifolia var. *angustifolia*	both surfaces, mainly along veins and margins	sparse	bifurcate or stellate	filiform	ascending	white
angustifolia var. *buchananii*	both surfaces, mainly along veins and margins	sparse	bifurcate or stellate	filiform	ascending	white
argentea var. *argentea*	both surfaces, forming silky covering on lower surface	dense	bifurcate or stellate	filiform	appressed or ascending	white or golden
argentea var. *sericea*	both surfaces, more dense on lower surface	sparse	bifurcate or stellate	filiform	appressed or ascending	white or golden
colchicifolia	absent, if present, then few noticeable in young leaves	sparse	bifurcate or stellate	acicular	ascending	white
costata	mainly veins, margins, midribs	sparse to dense	bifurcate interspersed with stellate	filiform	ascending or appressed	white
filiformis	mainly veins, margins, midribs	sparse	bifurcate or stellate interspersed with simple	filiform	appressed or patent	white
flanaganii	abaxial or both surfaces, margins, midrib	sparse or dense	bifurcate or stellate	filiform	ascending	white, turning brown on drying
floccosa	abaxial or both surfaces, margins, midrib	sparse or dense	bifurcate or stellate	filiform	ascending	white, turning brown on drying
galpinii	along margins, midrib	sparse	bifurcate or stellate	acicular	ascending	white or brown
gerarrdii	abaxial or both surfaces	sparse or dense	bifurcate	filiform, held in U or V	ascending	golden brown
hemerocallidea	evenly on lamina	sparse	bifurcate interspersed with stellate	filiform, held U or V	ascending	white
interjecta	absent, if present, then few noticeable in young leaves	sparse	stellate	acicular	acsending	white
kraussiana	one or both surfaces, margins, midrib	dense	bifurcate interspersed with stellate	acicular, held in a U or V	ascending or patent	white
longifolia	margins, midrib, leaf bases, intercosta	sparse	stellate	acicular	appressed	white or yellow
ludwigii	margins, midrib	dense	stellate	acicular	ascending or appressed	white
membranacea	both surfaces, arises from pustules	sparse	stellate interspersed with simple	filiform	patent	white

Table 5.1.—cont.

Taxon	**Distribution**	**Density**	**Type**	**Shape of arms**	**Position of arms**	**Colour of hairs**
multiceps	even on both surfaces	sparse or dense	stellate	acicular	ascending	yellow to brown
nivea	both surfaces	sparse	bifurcate interspersed with stellate	filiform	ascending	white
obliqua	both, lining midrib and margins on abaxial surface, scattered on veins on upper surface	dense or sparse	stellate	acicular	appressed or acsending	white
obtusa	margins and midrib	dense forming a white band	stellate	acicular	appressed or acsending	white
parvifolia	both surfaces, most on lower surface	sparse or dense	bifurcate or stellate	acicular, held in a U or V	patent	white, yellow or brown
parvula	both surfaces	sparse	bifurcate or stellate interspersed with simple	filiform	ascending	white
rigidula var. *pilosissima*	covering leaf surfaces	dense	bifurcate, stellate	acicular	ascending	white
rigidula var. *rigidula*	lamina, margins, midrib	sparse on lamina, dense on margins and midrib	bifurcate, stellate	acicular or filiform	ascending or appressed	white
sobolifera var. *pannosa*	covering leaf surfaces	dense	stellate interspersed with bifurcate	filiform	ascending	red-brown
sobolifera var. *sobolifera*	abaxial or both surfaces margins, midrib	sparse	stellate or bifurcate	filiform	ascending	white or brown
stellipilis	abaxial surface, light on adaxial surface	dense, forming layer	Stellate	acicular	appressed	white
tetramera	margins and midrib, blade	sparse	bifurcate or stellate	filiform	ascending	white
uniflorata	both surfaces, midrib and veins	sparse, dense on midrib and veins	bifurcate or stellate	filiform	ascending to patent	white
villosa	abaxial or both surfaces, margins and midrib	sparse or dense on margins and midrib	stellate with occasional bifurcate	filiform	appressed or ascending	white
zeyheri	absent, if present only along margins and midrib	sparse	bifurcate or stellate	acicular	ascending or appressed	white or lightly brown

5.3.9.3 Shape of hair arms

The arms of bifurcate and stellate hairs vary in length with one (or two in stellate hairs) more strongly developed than the rest (Figure 5.22C,K,L,Q,R). Based on the shapes of arms, hairs can be categorised into two types in *Hypoxis*. Arms of hairs may be short and acicular (stiff) [Figure 5.24A] or arms long and filiform (lax) [Figure 5.24B]. The acicular arms may be patent, ascending or appressed while filiform arms are mostly ascending and this has an effect on leaf texture. In *H. muticeps*, *H. kraussiana* and *H. parvifolia*, acicular hairs give the leaves a scabrous feel while filiform hairs of *H. argentea* and *H. villosa* create a soft silky covering on the lower surface.

Figure 5.24.—Shape of hair arms in *Hypoxis*. A, U-shaped patent hairs in *H. argentea* var. *argentea*; B, long, filiform arms in *H. angustifolia*.

5.3.9.4 Position of arms relative to the leaf surface

Arms of hairs in *Hypoxis* are patent, ascending or appressed. Most species have ascending hairs. Only two species, namely *H. kraussiana* and *H. parvifolia* have distinctly patent hairs and a few species have appressed hairs [*H. stellipilis*, *H. villosa*]. Leaves of *H. kraussiana* and *H. parvifolia* are distinctive in that they have predominantly bifurcate hairs on blades in a 'U' or 'V' outline (see Figure 6.6C). In *H. angustifolia*, *H. argentea*, *H. acuminata*, *H. gerrardii* and *H. hemerocallidea*, arms may also be held in a 'U', but in these species the arms are filiform (see Figure 6.7B) and have a soft texture. Ascending hairs are associated with long, filiform arms while patent and most appressed hairs are acicular (Table 5.1). In *H. argentea* and *H. villosa*, arms bend at the hair base to lie parallel to the length of the leaf and ascend at the tips, and this aids in recognising these species. The combination of arms being short, acicular and patent or ascending in *H. kraussiana*, *H. parvifolia* and *H. multiceps* give their leaves a scabrous texture. In *H. rigidula* var. *pilosissima* and *H. sobolifera* var. *pannosa*, the dense hairs are ascending giving the leaves a soft, furry texture and this character is used to separate them from the typical varieties. In *H. stellipilis*, hairs are stellate with arms acicular and appressed.

5.3.9.5 Colour of hairs

Hairs in *Hypoxis* are white in the majority of the species, but in a few species they may turn yellow or reddish-brown on drying. In *H. multiceps*, *H. costata* and *H. kraussiana*, hairs on leaves and inflorescences are white or yellow. Often, the inflorescence hairs in these species are yellow and are useful for recognising the species. *H. argentea* can be distinguished by its long silky hairs that appear white or golden yellow giving the leaves a sericeous effect. In *H. sobolifera*, two varieties are recognised based on the density and colour of hairs. *H. sobolifera* var. *pannosa* differs from the typical variety in having a dense indumentum on both surfaces of the leaf and in hairs being red-brown. In the typical variety, hairs are scattered over both surfaces, occur in tufts and are white or pale brown (See Singh *et al*. 2007). In all species, if the previous season's leaves remain on the specimen, the hairs on these leaves turn grey-white when dried even in species with yellow and red-brown hairs, but this is particularly noticeable in *H. sobolifera*.

5.3.9.6 Difficulty in using leaf hair characters in *Hypoxis*

Leaf hairs offer stable characters for identifying species of *Hypoxis*. However, there are a few difficulties that are worth noting. As in seed characters, hairs are minute and their characters are assessed with difficulty using a hand lens in the field. Pressing of specimens for the herbarium also obscures the position of arms relative to the leaves and it becomes problematic to evaluate the

character. Newly formed leaves in the growing season are more hairy due to hairs being spread over a smaller area than when they mature. As leaves age, hairs disintegrate leaving the blades glabrous in a few species. This is seen in *H. costata*, *H. hemerocallidea* and *H. sobolifera*. However, in these species, hairs remain on the margins and midrib of the leaves. These changes in distribution of hairs therefore need be noted in plants at various stages of their growth. A combination of leaf hair characters is useful to distinguish some species, for example *H. obtusa* and *H. ludwigii* that have acicular and appressed hairs forming a distinct white band along margins and midribs.

5.3.9.7 Possible function of leaf hairs

Nel (1914) regarded hairiness in *Hypoxis* plants as a protection to reduce excessive transpiration. More recent studies report that leaf hairs are effective in reducing water loss (Ehleringer *et al*. 1976) and in reflecting solar radiation (Johnson 1975). In general, *Hypoxis* species with moderately firm leaves, growing in well drained grasslands, in full sun have leaves with a dense indumentum (*H. sobolifera*, *H. stellipilis* and *H. rigidula* var. *pilosissima*), at least on the lower surface, while those species in semi-shade and wet areas have leaves only lightly hairy, for example *H. flanaganii*, *H. filiformis*, *H. parvula* and *H. nivea*. However, species with almost glabrous leaves like *H. colchicifolia* and *H. interjecta* and those with rigid leaves and varying degrees of hairiness (e.g. *H. rigidula*, *H. galpinii, H. costata*) were found growing sympatrically under the same conditions. It is possible that together with a waxy epidermal layer, the hairs play a role in water retention and reflecting solar radiation while this may not be necessary in species that are adapted to semi-shade and damp conditions.

5.4 Conclusions

This assessment indicates that vegetative characters, especially those of the leaves, are valuable in demarcating species in *Hypoxis*. Rhizome, tunic and pseudostem characters may be used to confirm the identity of a species. Structurally, the star-shaped flowers are fairly uniform and vegetative characters are therefore important for demarcating species. In this chapter, the characters of diagnostic value in the genus were described and illustrated. Due to an overlap in ranges of character states among species and the developmental change in appearance of plants over the growing season, it is concluded that a combination of characters is necessary for reliably identifying infraspecific taxa.