

The Systematics of *Hypodontium*

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Submitted in partial fulfilment for the requirements for the degree Masters of Science (Systematics and Biodiversity Science) in the Department of Botany, University of Cape Town.

March 2002

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ACKNOWLEDGEMENTS

I am grateful to a number of people and organisations that helped and supported me throughout my studies.

Firstly, I would like to thank SABONET (Southern Africa BOTanical diversity NETwork) for their financial support, without which the study would not have been possible.

I thank Dr. Terry Hedderson, my supervisor, for his guidance, support and encouragement and for his help in securing loan material from different herbaria.

I also thank Terry Trinder-Smith (Curator) of Bolus Herbarium for assistance in the herbarium and with herbarium specimens and equipment.

Thank you to the curators of Pretoria Herbarium (NBG) and British Museum Natural History (BM) for loaning me herbarium material for study.

I am also grateful to people at the Stellenbosch sequencing unit for running the sequences.

Thank you to all my colleagues in the Molecular Systematics Lab., UCT Botany Department, especially Tracy Nowell, for the in the lab. I also thank my classmates Elizabeth Mwafongo, Lineekela Kandjengo and Lerato Kose (University of Stellenbosch) for their support and discussions throughout the study period.

Thank you to my parents, brothers and sisters and my friends for the support and encouragement.

ABSTRACT

Hypodontium is a moss genus currently comprising two species, *H. dregei* and *H. pomiforme*, endemic to Southern Africa, occurring in Zimbabwe and the eastern and southern parts of the Flora of Southern Africa areas. It was originally assigned to the family Calymperaceae, but was recently moved to Pottiaceae. The two families do not differ absolutely and *Hypodontium* possesses combinations of characters that could place it in either. This study used molecular DNA sequence data from two chloroplast gene regions, *trnL-trnF* and *rps4*, to investigate the family status of the genus. The results obtained showed that it is not a member of either Calymperaceae or Pottiaceae, and supports the erection of a new family to accommodate *Hypodontium*. *Hypodontium pomiforme* occurs as two distinct morphological forms, and it has been suggested that these two forms might constitute two separate species. This study used both morphological (cluster analysis and Canonical Discriminant Functions Analysis) and molecular (nuclear ITS sequences) data to test this hypothesis. Results of both methods support the idea of significant difference between the two forms. Three species are therefore proposed for the genus *Hypodontium*, with *H. humilopapillosum* Manyanga & Hedderson described as new. A key is provided for the three species along with brief descriptions and a distribution map for each.

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CHAPTER 1

GENERAL INTRODUCTION

Hypodontium C. Müll. is a genus of haplolepideous (Vitt et al. 1998) mosses currently assigned to the tribe Pottiaceae where it is placed in the tribe Hyophileae, subfamily Pottioideae (Zander 1993). Two *Hypodontium* species are currently recognised, *H. dregei* (Hornsch) C. Müll. and *H. pomiforme* (Hook) C. Müll. Both are endemic to southern Africa, being known from forests and woodlands of Zimbabwe and the eastern and southern parts of the Flora of southern Africa areas (Magill 1981). Both species grow in dense tufts or cushions and occur as epiphytes or on rocks (saxicolous), or occasionally on shallow soils over rocks (terricolous). The large plants are dioicous, and glaucous-green to yellow-green. The leaves are in-curved when dry and spreading when wet, occasionally with unistratose margins, involute above the mid leaf or only at the apex. The costa has dorsal and ventral stereid bands. The operculum is rostrate and the calyptra is cucullate.

The taxonomic history of the genus *Hypodontium*.

The classification of *Hypodontium* has altered considerably prior to its current placement. Both species of the genus were described well before the genus *Hypodontium* was established. Hooker in 1818 described the present day *Hypodontium pomiforme* as *Weissia pomiforme* and in 1841 Hornschuch (cited in Sim 1926) described the current *Hypodontium dregei* as *Syrrhopodon dregei*. When Muller (1849) published the first volume of his synopsis, both species were described as members of *Syrrhopodon*, family Syrrhopodontaceae, tribe Pottioideae (Reese et al. 1986). In 1897 Kindberg classified *Syrrhopodon* (including the present day *Hypodontium*) in the Weissiaceae (=Pottiaceae) separating it from Calymperaceae, which included only *Calymperes*. It was Muller's (1899) analysis of the family Weissiaceae that provoked recognition of *Hypodontium* C. Müll. as distinct from its parent genus *Syrrhopodon*. He placed it in the family Calymperaceae, as did

Brotherus (1924) and later authors. Reese et al. (1986) suggested the genus was wrongly placed in Calymperaceae, and suggested a position in Pottiaceae, a move made formally by Reese and Zander (1988).

The genus *Hypodontium* was put in the family Calymperaceae based on the following characteristics. Members of the genus have 16 short and incurved triangular, deep orange, smooth or weakly vericulose peristomatic teeth. The upper basal cells of at least one of the outer perichaetal leaves are sharply differentiated and inflated-quadrate. The cauline leaves are lanceolate, bordered by elongate cells with thick walls and the apex is broadly acute to rounded. The leaf margins are plane to incurved and often spinose. However, the gross appearance of the plants, their restriction to temperate-arid southern Africa, their propensity for terrestrial (non-epiphytic) habitat, lack of gemmae on leaf tips and sheathing perichaetal leaves, all argue against inclusion in Calymperaceae (Reese et al. 1986).

Zander (1993) rationalised the transfer of the genus into the family Pottiaceae from Calymperaceae on the grounds that its members "...lack many of the central characteristics of genera of Calymperaceae, e.g. the campanulate calyptra of *Calymperes*, a stem central strand, the intramarginal bands of elongated cells, and the enlarged but short-rectangular to quadrate basal cells of *Calymperes*, *Mithyridium* and *Syrrophodon*". According to him, none of the earlier described characteristics of *Hypodontium* that were used to place it in the family Calymperaceae are unique to that family. The cells of pottiaceous cancellinae, in addition to lacking external pores, are generally clearly papillose distally, a condition that does not occur in Calymperaceae. The distal cells of the cancellinae in *Hypodontium* are papillose, among its various other pottiaceous qualities, and this is why it was transferred to Pottiaceae (Reese and Zander, 1988).

There has thus been considerable debate as to whether *Hypodontium* is best placed in Calymperaceae or Pottiaceae. Both families are of worldwide occurrence, and have been considered as allied but distinct (Reese and Zander, 1988). Pottiaceae is a large, heterogeneous and possibly polyphyletic

family (Reese 1987 cited in Reese and Zander, 1988) while Calymperaceae is smaller and more homogeneous. There is no single morphological characteristic in which Pottiaceae and Calymperaceae differ absolutely (Reese and Zander, 1988), hence the problem of classifying *Hypodontium* into either of the two families.

However, La Farge et al. (2000), using *rbcL*, *rps4* and *trnL-trnF* molecular data, showed that Calymperaceae and Octoblepharaceae are cladistically distant from Pottiaceae and together form a clade that is more closely related to families of Dicranales than to Pottiaceae. They divided Dicranidae into three monophyletic groups, Dicranales, Pottiales and Grimmiales. Calymperaceae is included in the Dicranales while Pottiaceae is in the Pottiales. More recent molecular studies have also shown that the two families are not closely related. (Goffinet et al. 2001, Hedderson, unpublished).

The current placement of the genus *Hypodontium* under the family Pottiaceae is also being questioned. There is no consistency in the characters that will be used to assign *Hypodontium* to a particular family. There are suggestions that *Hypodontium* might be placed in a family of its own "...if there were some distinctive autopomorphy or compelling combinations of unusual characters." (Zander, 1993). Reese (1987) suggested that Pottiaceae are ancestors to Calymperaceae and that *Hypodontium* is intermediate between the two and deserving of family status. Recent molecular studies using *rps4* gene region sequence data (Hedderson personal communication) have produced phylogenies with *Hypodontium* closer to *Fissidens* and Dicranaceae than to Calymperaceae and Pottiaceae but not nested in any existing family. This supports the idea of a family status for the genus, but the low support values found for the *rps4* data shed little light on its precise affinities. Since there is no consistency in the morphological characteristics used to classify *Hypodontium* in the Pottiaceae or Calymperaceae, there is a need for additional DNA sequence data that might help resolve the family status of the genus. DNA provides an abundance of easily retrieved and circumscribed characters that give few problems in making initial homology hypotheses. This

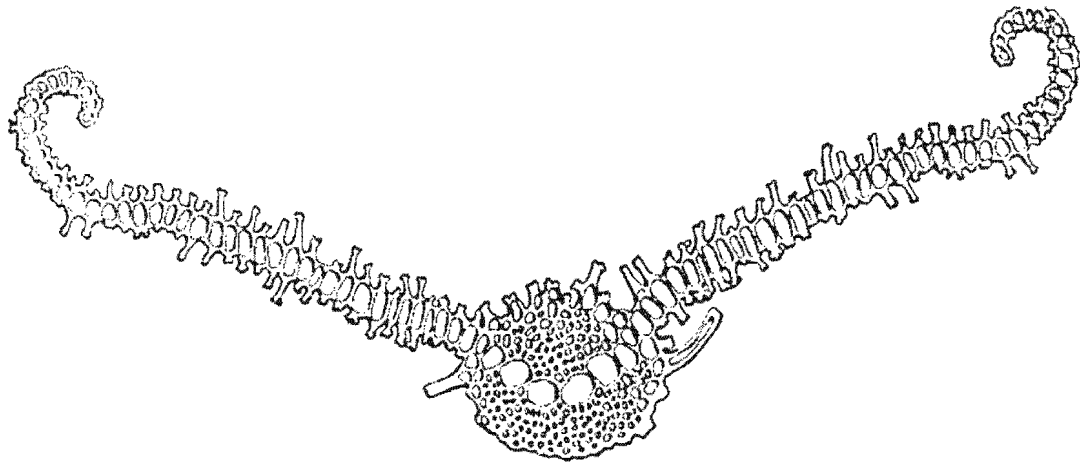
study uses DNA molecular data to clarify the family placement of *Hypodontium*.

The species of *Hypodontium*

Currently two species of *Hypodontium* are recognised, and separated mainly by leaf morphology. *H. dregei* has leaf margins that are involute from mid-leaf to apex, with a hyaline border rarely reaching mid-leaf. The dorsal junction of lamina and costa has large spines above the base, and laminal cells have massive papillae. *H. pomiforme* has leaf margins that are plane, or involute only at the apex. The dorsal costal surface is smooth above and papillose below, and leaf cells usually have low, stout papillae. The costa of *H. pomiforme*, in cross section (Figure 1b), is strongly convex ventrally, while that of *H. dregei* (Figure 1a) is flat to weakly convex ventrally but very prominent dorsally. Cancellinar cells of *H. dregei* are quadrate to short rectangular and bear pores typical of Calymperaceae while the cancellinar cells of *H. pomiforme* are narrowly rectangular and bear vertically elongate, slit-like pores unlike those of any Calymperaceae (Reese, 1987). *H. pomiforme* is a large, stout, yellow-green plant growing mainly on rock and rarely on bark while *H. dregei* is smaller, dark green to glaucous-green and is frequently collected on trees.

Whilst *H. dregei* seems relatively stenotypic, *Hypodontium pomiforme*, as currently circumscribed, comprises two distinct morphological forms (see Chapter 3) based mainly on leaf structure. The genus has never been revised, and the status of these morphological forms is unclear. As part of the revision of *Hypodontium*, both molecular and morphological data are used in this project to evaluate their status.

The decision to use both molecular and morphological data is justified because both approaches have distinct advantages in systematics. Each is able to address questions and problems that cannot be addressed by the other while at the same time both methods can provide answers to the same questions. Because the organisms under study have a single history,



(Zander 1993)

a



b

(Magill 1981)

Figure 1. Mid-leaf sections of (a) *H. dregei* and (b) *H. pomiforme*.

systematic studies of any set of genetically determined characters should be congruent with other such studies based on different sets of characters in the same organism. Congruency between molecular and morphological studies is strong evidence that the underlying historical pattern has been discovered (Hillis, 1987).

Objectives

The main objectives of this study are:

- (1) To use *rps4* and *trnL-trnF* molecular data to establish the family status of the genus *Hypodontium*.
- (2) To use both morphological and molecular (nuclear ITS sequences) data to test the hypothesis that the two morphological forms of *Hypodontium pomiforme* constitute two different species.
- (3) To use molecular data to examine the phylogeographic structure of *Hypodontium pomiforme* in the South Western Cape.
- (4) To provide a taxonomic revision of the genus *Hypodontium*.

CHAPTER 2

PHYLOGENETIC POSITION OF HYPODONTIUM

Introduction

As Zander (1993) puts it " At present the genera of the Pottiaceae are in some cases neither 'natural' nor instructive of phylogenetic relationships and many present generic definitions seem to cut across natural lines". *Hypodontium* seems to be one genus that not only cuts across natural generic lines, but also across natural family lines. Reese (1987), in explaining the phylogenetic relationships of *Syrrhopodon* (Calymperaceae), suggested that *Hypodontium* is intermediate between Pottiaceae and Calymperaceae and considered it representative of ancestors of *Syrrhopodon*. The few phylogenetic reconstructions that have included sequences of *Hypodontium* (Hedderson, unpublished) suggest that the genus is distantly related to the other members the Pottiaceae in which it is placed and to those of the Calymperaceae in which it used to be placed. There is therefore a need for phylogenetic studies that will show the family position of *Hypodontium*.

The aim of this chapter is to use the chloroplast DNA (cpDNA) regions *trnL-trnF* and small ribosomal protein (*rps4*) gene data to determine the phylogenetic relationship of *Hypodontium*.

Utility of the sequences used

TrnL-trnF region and (small ribosomal protein) *rps4* gene are both part of the circular chloroplast genome whose utility in phylogenetic reconstruction has been discussed in various publications (e.g. Chapters in Soltis et al. 1992, Böhle et al. 1996, Soltis and Soltis, 1998). The advantages of the chloroplast genome in phylogenetic inference include its small size, the fact that genes are single copy and that the genome is conservative and evolves slowly at the nucleotide sequence level (Soltis and Soltis, 1998).

The *trnL-trnF* region is relatively short and easy to amplify across a wide taxonomic range because universal primers designed by Taberlet et al. (1991) are placed in highly conserved tRNA genes (Bayer and Starr, 1998). It can be used in phylogenetic reconstruction at species, genus and family level (Soltis and Soltis, 1998), providing resolution similar to that obtained from longer gene sequences (*rbcL* and *ndhF*), but requiring much less labour to generate data (Bayer and Starr, 1998). It is a non-coding sequence, which sometimes displays higher mutation rates than coding regions, often with numerous large indels (Gielly and Taberlet, 1996), and this increases resolution, permitting assessment at intrageneric and even intraspecific levels.

Rps4 exhibits little size variation and is easy to amplify using primers developed by Nadot et al. (1994) which have a wide applicability (Soltis and Soltis, 1998): thus it also enables a wide range of things to be compared. The combination of *rps4* and *trnL-trnF*, at times with *rbcL*, has been useful in examining the relationship of orders, families and suborders within the arthrodontous mosses (Cox and Hedderson, 1999), examining relationships among haplolepidious mosses (La Farge et al. 2000), among diplolepidious-alternate mosses (Cox et al. 2000) and among Hypnobryalean pleurocarpous mosses (De Luna et al. 2000).

Materials and Methods

The chloroplast gene (cpDNA) region *trnL-trnF* was sequenced for recently collected *Hypodontium dregei* and *Hypodontium pomiforme* specimens. The sequences produced were combined with *rps4* gene region sequences and used to establish the phylogenetic relationship of *Hypodontium* with the other genera within and outside Pottiaceae and Calymperaceae. Dr. T. Hedderson provided some *rps4* gene (cpDNA) region sequences from his previous studies. Sequences for other haplolepidious moss species in other genera and families were obtained from the Genbank.

Selection of the sequences

The sequences used in this analysis and their accession numbers are given in Table 1. Based on previous results (La Farge et al 2000, Hedderson unpublished) an effort was made to include all of the major groups found within the haplolepidaceous mosses. All families were included, except Ptychomitriaceae, Seligeriaceae, Bryoxiphiaceae, Bruchiaceae, Schistostegaceae, Eustichiaceae, Viridivelleraceae, Ephemeraeae, Serportortellaceae and Bryobartramiaceae (Classification as by Buck and Goffinet, 2000), for which no material was available. Since good representation of the Pottiaceae was necessary, a number of taxa were included for which only *rps4* data are available (*Barbula*, *Cinclidotus fontinaloides*, *Cinclidotus mucronatus* and *Astomum*).

DNA extraction, amplification, cleaning and sequencing

The DNA extraction and amplification of target DNA regions for *Hypodontium* were performed as outlined for the ITS region outlined in chapter 4 but using the primers, *trn c* and *trn f* to amplify *trnL-trnF* (Tabertlet et al. 1991). *Rps4* sequences were available from Dr T. Hedderson.

Sequence assembly, alignment and analysis

The forward (5' – 3') and reverse (3' – 5') sequences for *Hypodontium trnL-trnF* were assembled and checked for inaccurate base calling using SeqMan II (LaserGene System software DNA Star, inc.). The consensus sequences for the region were saved for the analysis. *Rps4* and *trnL-trnF* gene regions for each species used were combined in SeqEdit (LaserGene System software DNA Star, inc.). The combined sequences were aligned in Megalign on an apple Macintosh. Alignment of the sequences was first done automatically using Clustal and adjusted manually. The alignment was exported as a NEXUS file.

Table 1. List of taxa used in the analysis with Genbank accession numbers for sequences from Genbank. (Classification according to Buck and Goffinet, 2000)

TAXON	GENBANK ACCESSION NUMBER	
	<i>TrnL-trnF</i>	<i>Rps4</i>
Family Pottiaceae		
<i>Hypodontium dregei</i>	Unpublished	Unpublished
<i>Hypodontium pomiforme</i>	Unpublished	Unpublished
<i>Splachnobryum obtusum</i>	AF 229915	Unpublished
<i>Astomum ludovicianum</i>	Unpublished	Unpublished
<i>Tortula obtusissima</i>	AF231180	Unpublished
<i>Barbula unguiculata</i>	Unpublished	Unpublished
<i>Goniomitrium acuminatum</i>	AF 229914	Unpublished
<i>Timmiella crassinervis</i>	AF 231173	AF222902
Family Clinclidontaceae		
<i>Cinclidotus mucronatus</i>	Unpublished	Unpublished
<i>Cinclidotus fontinaloides</i>	Unpublished	Unpublished
Family Calymperaceae		
<i>Calymperes palisoti</i>	AF 231153	Unpublished
<i>Calymperes afzelii</i>	AF 231152	AF 226744
<i>Syrhobodon prolifer</i>	AY 047012	Unpublished
<i>Mitthyridium constrictum</i>	AF 231143	Unpublished
<i>Octoblepharum albidum</i>	AF 231164	Unpublished
Family Dicranaceae		
<i>Paraleucobryum longifolium</i>	AF 231185	Unpublished
<i>Dicranum muhlenbeckii</i>	AF 231245	AF 231276
<i>Orthodicranum fulvum</i>	AF 231257	AF 231288
<i>Leucoloma serrulatum</i>	AF 231255	AF 231286
<i>Leucoloma rutenbergii</i>	AF 231254	AF 231285
<i>Dicranella heteromalla</i>	AF 231241	AF 231272
Family Wardiaceae		
<i>Wardia hygrometrica</i>	AF 023720	AF 023782
<i>Archidium donelli</i>	AF 229911	Unpublished

Table 1. Continued.

TAXON	GENBANK ACCESSION NUMBER	
	<i>TrnL-trnF</i>	<i>Rps4</i>
Family Rhabdoweisiaceae		
<i>Amphidium californicum</i>	AF 231169	AF 222896
<i>Kiaeria blyttii</i>	AF 231252	AF 231283
<i>Cynodontium jeneri</i>	AF 231240	AF 231271
<i>Oncophorus wahlenbergii</i>	AF 231256	Unpublished
<i>Rhabdoweisia crispata</i>	AF 231259	AF 222899
<i>Dicranoweisia cirrhata</i>	AF 231243	Unpublished
<i>Campylopus atrovirens</i>	AF 231239	AF 231270
<i>Dicranodontium denudatum</i>	AF 231242	AF 231273
Family Ditrichaceae		
<i>Pleuridium acuminatum</i>	AF 231262	Unpublished
<i>Ditrichum pallidum</i>	AF 231248	AF231279
Family Fissidentiaceae		
<i>Fissidens philonotulus</i>	AF 231167	Unpublished
<i>Fissidens subbasilaris</i>	AF 231250	Unpublished
Family Dicnemonaceae		
<i>Eucamptodon muelleri</i>	AF 231261	AF 231280
Family Erpodiaceae		
<i>Aulacopilum hodgkinsoniae</i>	AF 222897	AF222897
Family Drummondiaceae		
<i>Drummondia obtusifolia</i>	AF 229895	AF 223038
Family Scouleria		
<i>Scouleria aquatica</i>	AF 231179	AF 306984
Rhachithecaceae		
<i>Uleastrum paraguense</i>	AF 231264	AF 222898
Family Grimmiaceae		
<i>Grimmia pulvinata</i>	AF 231251	AF 222900
<i>Schistidium apocarpum</i>	AF 129577	Unpublished
Family Archidiaceae		
<i>Archidium donelli</i>	AF 229911	Unpublished

Phylogenetic Analysis

Maximum parsimony analysis was performed using PAUP version 4.0b4a (Swofford, 2000). The *trnL-trnF* data had some regions that could not be well aligned; these were excluded from the analysis. The analysed data matrix had 42 taxa and 1286 characters. The analysed data matrix is included on the attached CD. The analysis employed the heuristic search using the tree-bisection-reconnection (TBR) branch swapping algorithm with the steepest descent option turned off. Gaps were treated as missing data. Trees were collapsed to create polytomies if branch length was zero. Initial MAX trees was initially set at 200 (auto increased by 100). The most parsimonious trees were saved and the strict consensus tree computed. Successive character weighting was effected and the weighted strict consensus tree constructed. To asses nodal support for the recovered trees, jack-knife analysis was performed with 100 replicates and 33,7% character deletion.

Results

Of the 1286 characters included in the analysis, 622 were constant, 268 were variable but parsimony uninformative and 396 were parsimony-informative. The analysis of unweighted characters produced two trees. The only difference was in the relationship within a well-supported branch of Dicranaceae genera comprising *Paraleucobryum*, *Dicranum* and *Orthodicranum*. An expanded concept of Calymperaceae including *Octoblepharum* (La Farge et al. 2000) is supported by a jackknife value of 100%. This clade does not include *Hypodontium*. All Pottiaceae included in the analysis form a well-supported (jackknife 100%) clade and this also does not include *Hypodontium*. *Hypodontium* is sister to a group that contains Dicranaceae, *Wardia* (Wardiaceae) and *Eucamptodon* (Dicnemonaceae) and together they form a reasonably well-supported clade (jack-knife support value of 76%), which is sister to a clade that contains Pottiaceae. The Calymperaceae form their own clade with *Uleastrum* and *Amphidium*

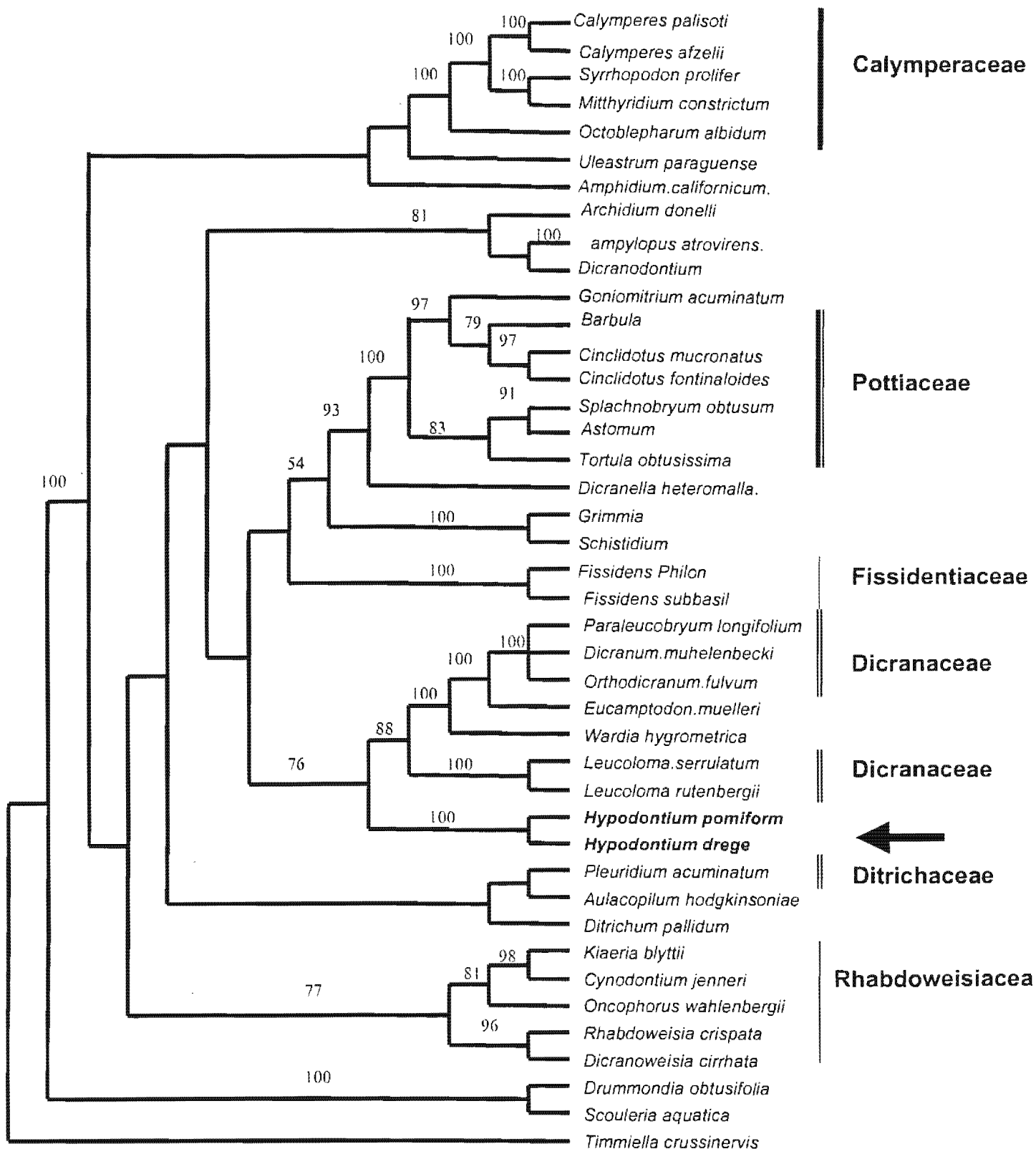


Figure 2. The unweighted strict consensus tree produced by maximum parsimony analysis

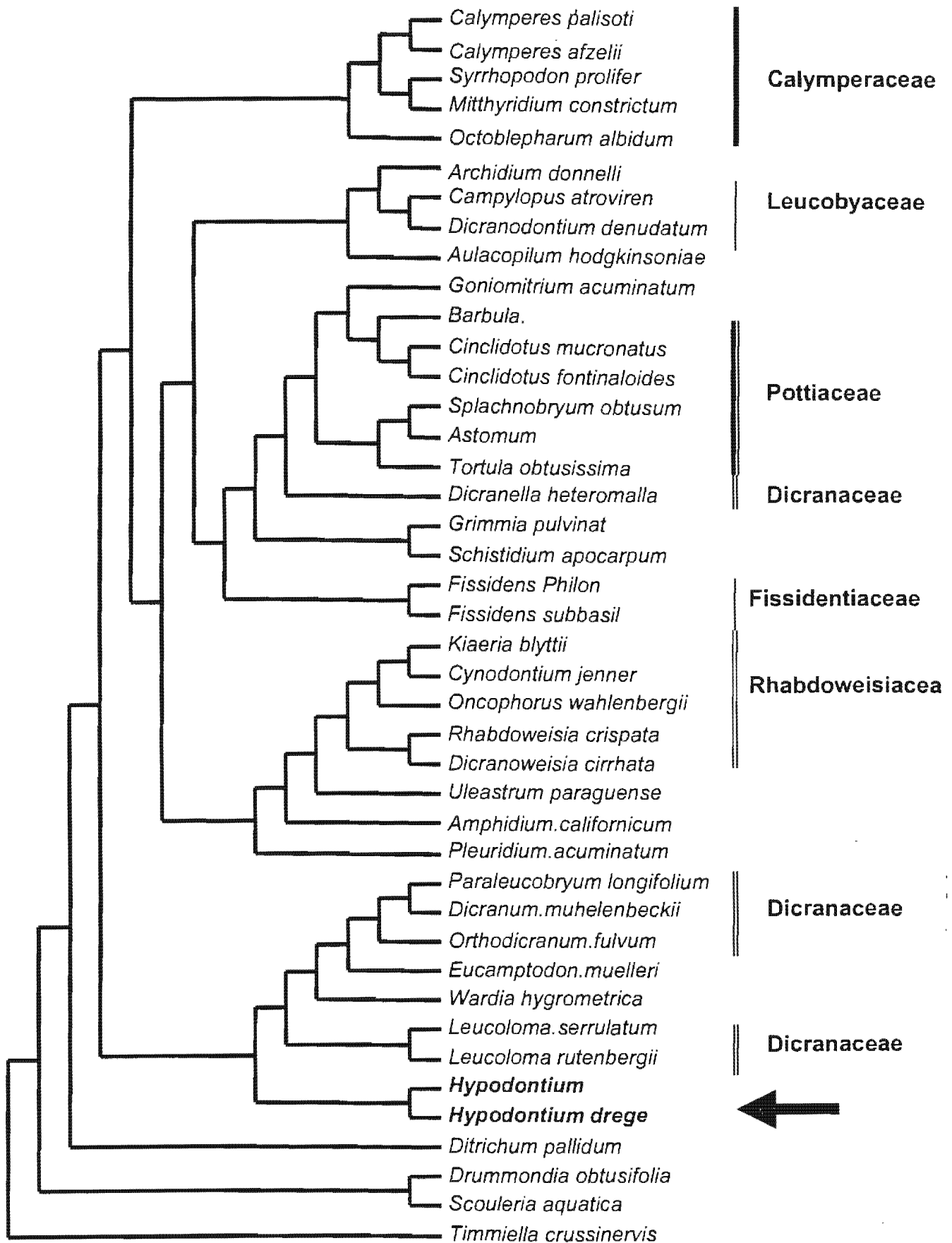


Figure 3. The weighted strict consensus Parsimony tree

(Rhabdoweisiaceae). This clade is sister group to everything else except *Drummondia*, *Scouleria* and the out-group.

Weighted parsimony also produced two trees. The weighted topology maintains the monophyletic grouping of *Hypodontium* and Dicranaceous genera, but it is sister to everything else except *Drummondia*, *Scouleria*, *Ditrichum* and the out-group.

Discussion

The weighted strict consensus tree (Figure 3) and the unweighted strict consensus tree (Figure 2) both show that the genera of each of the families included in the analysis are monophyletic. All calymperaceous genera used including *Octoblepharum* (La Farge et al.'s extended Calymperaceae concept) are monophyletic, with a 100% Jackknife support value. Most the Pottiaceae genera included in the analysis, including those where only *rps4* data was used, form a well-supported (100% jackknife value) monophyletic group. *Hypodontium* is not included in either of these groups. *Timmiella* was the only Pottiaceae not included. La Farge et al. (2000) and Hedderson et al. (in press) also produced similar position for *Timmiella* and suggest its exclusion from the family. The dicranaceous genera are all included in a monophyletic group that includes *Wardia* (Wardiaceae) and *Eucamptodon*. This group is supported by a jackknife value of 88%. *Hypodontium* is sister to this group and together they form a well-supported clade (jackknife value 76%). The Rhabdoweisiaceae, Fissidentiaceae and Archidiaceae all form their own well-supported monophyletic groups. The monophyly of these families indicates that the data used is phylogenetically informative at family level and can be relied upon in placement of genera in families.

The results support the morphological approach that was used in placement of most genera into families. However, they show that *Hypodontium* is genetically more closely related to Dicranaceae than it is to either other Pottiaceae or Calymperaceae. It is distant enough from both Pottiaceae and

Calymperaceae to be excluded from either of the families. The decision by Reese and Zander (1988) to move *Hypodontium* from Calymperaceae to Pottiaceae can be justified since it is genetically closer to Pottiaceae than Calymperaceae, but not close enough to be included in the Pottiaceae. The earlier suggestion (page 4) that *Hypodontium* could be closer to *Fissidens* is not supported here. The *Fissidens* here are monophyletic and sister to Pottiaceae.

Hypodontium shares quite a number of ecological and morphological characters with the Dicranaceae. These include occurrence in dense tufts and being terricolous or saxicolous. Morphological characters include an erect, tomentose stem, presence of central strand in the stem, presence of stereid bands, quadrate upper laminal cells, terminal perichaetia, a rostrate operculum, cucullate calyptra and possession of 16 peristomatic teeth. Papillose cells also occur in some Dicranaceae. Most of these it also shares with Pottiaceae and Calymperaceae (Magill, 1981).

Unlike in Calymperaceae and Pottiaceae (Zander, 1993), *Hypodontium*'s perichaetial leaves, as in most Dicranaceae, are sheathing below but narrowly subulate or awned apically. One of the characters used to place *Hypodontium* in Pottiaceae is the presence of external pores. These are also present in some Dicranaceae (Reese and Zander, 1988). Some of these similarities therefore might constitute synapomorphies for a *Hypodontium* plus Dicranaceae clade. Given the genetic distances from *Hypodontium* to members of the Dicranaceae, and its rather distinctive morphology compared to these, I would suggest that *Hypodontium* be placed in a family of its own.

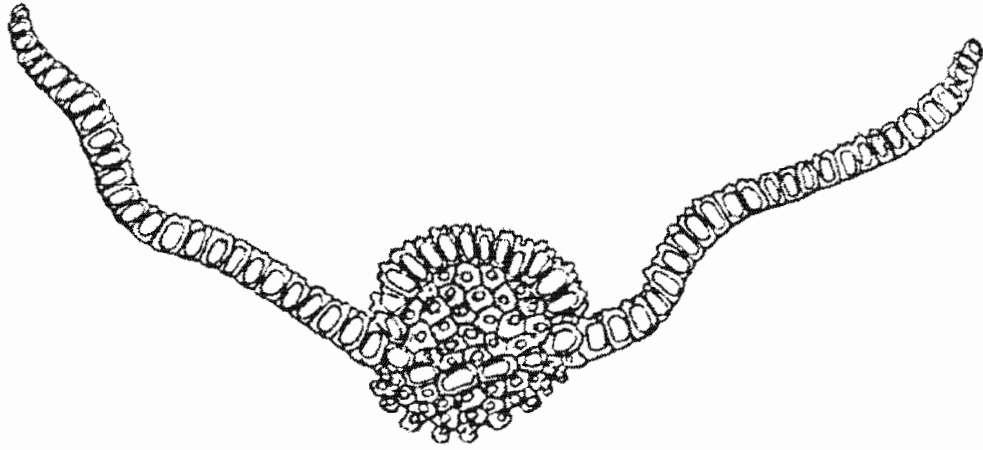
CHAPTER 3

MORPHOMETRIC ANALYSIS OF *HYPODONTIUM POMIFORME*

Introduction

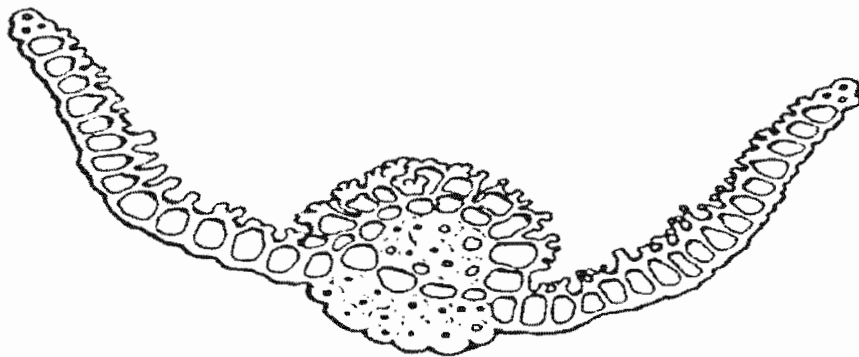
Hypodontium pomiforme occurs as two distinct morphological forms (Figure 4). One of the forms, which matches the typical descriptions of the species, has a more ventrally bulging leaf costa, few dorsal costa papillae and very short papillae on the ventral surface of laminal cells. In cross section each laminal cell has two or three short, small ventral papillae. The leaf margins are mainly unistratose. The other form has a ventrally inconspicuous leaf costa, numerous dorsal papillae and large tall ventral laminal papillae. In cross section each laminal cell has a single ventral papillae. The leaf margins are mainly multistratose and reflexed on one side. There are suggestions that the two forms of *H. pomiforme* might actually be two different species that are being considered as one. The aim of this chapter is to use morphological (morphometric measurements) data to test the hypothesis that the two forms of *Hypodontium pomiforme* are two species.

A morphological approach to systematics offers its own advantages such as its applicability to collections of preserved specimens in museums and herbaria. This makes data available from prohibitive situations such as rare species, inaccessible habitats, destroyed collection localities, legally protected habitats or species and high costs of procurement (Hillis, 1987). The approach is applicable to fossil species, which present a set of taxa that provides potential information about history. Morphological systematics is capable of making use of ontogenetic information to distinguish phylogenetically informative data (apomorphies) from phylogenetic noise (plesiomorphies) whereas molecular approaches can only use outgroups (Hillis, 1987). Traditional morphometrics, accounting for variation in size and shape have



a

(Magill 1981)



b

(Zander 1993)

Figure 2. Mid-leaf section of *H. pomiforme* (a) 'Typical' form and (b) 'Papillose' form.

successfully been used to identify and discriminate intraspecific groups (Cadrin and Friedland, 1999).

Materials and methods

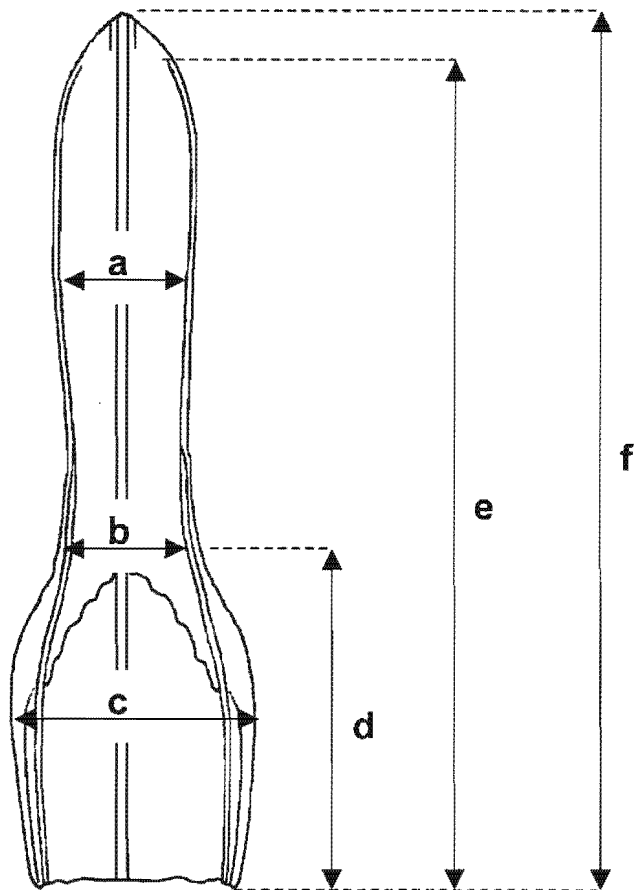
Sampling Methods

This study is based on Herbarium specimens from BOL and NBG (abbreviation as in Holmgren et al. 1990). A set of eighty-nine (89) *Hypodontium pomiforme* specimens was examined for this morphological analysis. The type specimen used is from BM. The list of specimens used is supplied in Appendix 1.

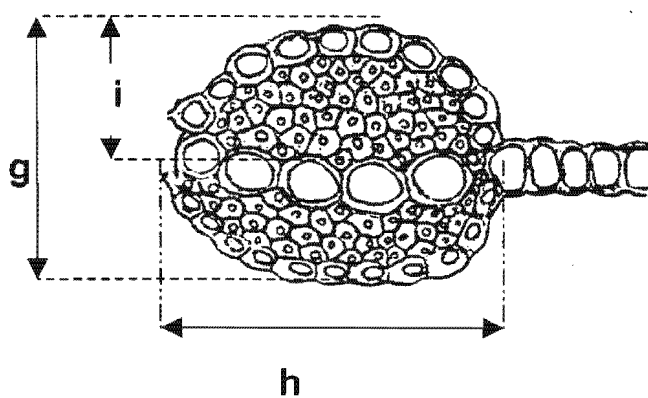
Preparation and Examination

Twenty (20) morphological characters, representing mainly leaf and stem measurements, were chosen for examination. Characters examined were: stem length and diameter, total leaf length, length of leaf base, width of leaf base, width of leaf at end of base and at middle, length of leaf border, width of leaf cells, costal width and thickness, distance from guide cells to ventral surface of costa, presence of papillae on ventral and dorsal sides of leaf costa, height of papillae on ventral and dorsal sides of leaf laminal cells and the number of papillae per cell. Figure 5 illustrates how the leaf measurements were taken. Colour reactions to KOH, tomentum and presence of a central strand in the stem were also investigated but were not used for analysis since they did not vary within the genus. Three individual plants from each specimen sample were measured and averaged. The averages for each specimen were then used in the analysis.

The plants were prepared for examination by placing them on a microscopic slide under a dissecting microscope, and then wetted with water containing



(Drawn not to scale)



(Drawn not to scale)

Figure 5. Illustrations depicting how the leaves were measured for the morphometric analysis.

a= mid leaf width, **b**= width of leaf near base, **c**= width of leaf base, **d**= length of leaf base, **e**= leaf border length, **f**= leaf length, **g**= costa thickness, **h**= costa width, **i**= distance from guide cell to ventral surface.

detergent as a surfactant. Leaves were then removed and placed on a separate slide and sections were cut using an industrial razor blade. A cover slip was then placed on the slide and the leaf sections as well as complete leaves examined under a compound microscope. Stem sections were prepared and examined in the same way. Measurements were made using a graduated eyepiece graticule, recorded and later converted to micrometers. Stem length was measured to the nearest 0.1 cm using a rule.

Multivariate Analysis

Data were entered into a computerised spreadsheet in STATISTICA 5.5. The spreadsheet was later transformed into a file format suitable for multivariate analysis. The data matrix used in morphological analysis is on the attached computer diskette. Measurements were log-transformed to eliminate the effect of different scales of measurement. Fourteen morphological character measurements were used in the analysis (Table 2). The number of papillae per cell was omitted because it was binary, and such variables tend to polarise multivariate analyses. Presence of papillae on ventral and dorsal sides of costa was also left out since they were not quantitative measurement records.

Statistical analyses were run using the STATISTICA 5.5 package. Three different techniques were used, namely Principal Component Analysis (PCA), Cluster Analysis and Canonical Discriminant Function Analysis (CDFA).

Principal Component Analysis (PCA) and Cluster Analysis

Patterns of correlation in the fourteen variables were summarized by PCA on the correlation matrix. PCA is a linear dimensionality reduction technique, which identifies orthogonal directions of maximum variance in the original data, and projects the data into a lower-dimensionality space formed of a subset of the highest-variance components (Ter Braak & Šmilauer, 1998). A scree plot was constructed to see which factors to use in cluster analysis.

Table 2. List of characters used in multivariate analysis.

Character	Abbreviated	Units
Stem Length	STEM_L	cm
Stem Diameter	STEM_D	µm
Length of leaf base	BASE_L	µm
Leaf Length	LEAF_L	µm
Width of leaf near base	BASE_W	µm
Mid-Leaf width	MID_W	µm
Width of leaf base	BBAS_W	µm
Leaf border length	BORD_L	µm
Cell width	CELL_W	µm
Costa width	COS_W	µm
Costa thickness	COS_TH	µm
Distance from guide cell To ventral surface	GC_VEN	µm
Dorsal papillae height	D_PAP_H	µm
Ventral papillae height	V_PAP_H	µm

Scores for the first five components were retained and used as variables in subsequent cluster analysis to identify groups of specimens exhibiting similar covariation in morphological characters. Ward's method and Euclidean distance were employed to construct cluster trees in order to determine whether groups correspond to the different forms of *Hypodontium pomiforme*.

Canonical Discriminant Function Analysis (CDFA)

CDFA was used to examine multivariate morphometric differences between the two forms of *H. pomiforme*. This technique derives linear combinations of variables, i.e. Canonical Variables (CVs), such that the correlations between the CVs and group membership, and the ratio of between- to within-groups variance are maximized (Krzanowski, 1990). Correlation between the original variables and the derived CVs, as well as the patterns of loading for the original measurements allow reification of the CVs in terms of shape and size differences among groups (Compton & Hedderson, 1997). This method tests the hypothesis of significant difference between the two morphological forms of the species i.e. it tests the hypothesis that the groups have the same multivariate mean.

Results

Principal Component Analysis

Table 3 shows the variance contributed by and the eigenvalues of the five main principal components extracted (decided based on scree plot) which account for almost 77% of the variance observed. Component 1 accounts for the greatest variance (29.9%), followed by component 2, which accounts for 17.8%. Components 3, 4 and 5 account for 13.8%, 9.1% and 6.2% respectively. The other ten components account for the remaining 23.1% of the variance. Table 3 shows the factor loadings of each of the characters on each of the 5 principal components extracted. Leaf width measurements have high factor loadings (above 0.7) for component 1. Costa measurements have high factor loadings for component 2 and leaf length measurements have high

Table 3.Component Loadings (unrooted) of the extracted first five Principal Components, their eigenvalues and variance contribution

	Component1	Component2	Component3	Component4	Component5
STEM_L	-0.070692	0.287803	-0.021126	-0.272558	0.759765*
STEM_D	0.528737	-0.013498	0.086130	0.287687	0.576212
BASE_L	-0.111558	0.120342	0.858491*	0.040830	0.166014
LEAF_L	0.314305	-0.045194	0.864843*	0.162709	-0.086419
BASE_W	0.875297*	0.216280	0.154497	-0.154014	-0.079443
MID_W	0.828326*	0.139937	0.116026	0.146085	-0.230073
BBAS_W	0.795353*	0.210251	0.324151	-0.211062	0.162172
BORD_L	0.244817	-0.069021	0.904947*	-0.022936	-0.050720
CELL_W	0.396872	0.143817	0.072361	0.575620	-0.106167
COS_W	0.088206	0.809142*	-0.027282	-0.043152	-0.028848
COS_TH	0.453918	0.749473*	0.011796	0.145924	0.205808
GC_VEN	0.160325	0.833357*	0.008676	0.157663	0.252473
D_PAP_H	-0.274525	0.157036	0.139708	0.796430*	0.040880
V_PAP_H	-0.304038	0.237516	0.005201	0.257417	0.663152
Expl.Var	3.105419	2.227529	2.479899	1.355243	1.592054
Prp.Totl	0.221816	0.159109	0.177136	0.096803	0.113718
Eigenvalue	4.191678	2.491574	1.930165	1.278842	0.867884
%Tot. Var.	29.94056	17.79695	13.78690	9.13459	6.19917
Cum E. val	4.19168	6.68325	8.61342	9.89226	10.76014
Cum % Var	29.94056	47.73751	61.52441	70.65900	76.85817

(* Loadings are > 0.700)

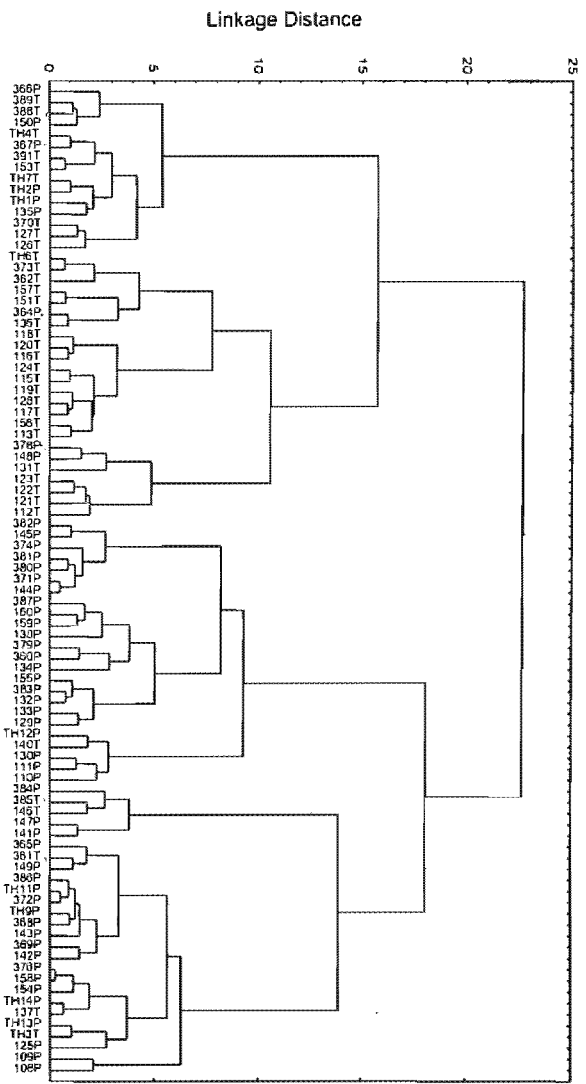


Figure 6. The phenogram showing clusters produced by cluster analysis of the two forms of *H. pomiforme*. (T = 'Typical'; P = 'Papillose'). Figures before P or T are arbitrary studied sample reference numbers.

factor loadings for component 3. Dorsal papillae height and stem length have high factor loadings for components 4 and 5, respectively.

Cluster Analysis

Figure 6 shows a phenogram produced by joining (tree clustering) using Ward's method and Euclidean distances. Six of the 36 'Typical' specimens are clustered within the 'Papillose' cluster and nine of the 53 'Papillose' specimens are clustered within the 'Typical' cluster. A linkage distance of 23 separates the two clusters. The type specimen was clustered with the 'Papillose' form.

Canonical Discriminant Function Analysis

A significant ($P < 0.001$) canonical variable accounting for the total variation between the two forms of *Hypodontium pomiforme* was extracted in the Discriminant Functions Analysis (Table 4). The analysis separated the specimens into two groups but with some overlap (Figure 5). Mahalanobis distance between the two forms in the 14 dimensional morphometric space is 11.11, which is significantly different from zero and the F statistic with 14 and 74 degrees of freedom is 13.99, which is also significant at $P < 0.0001$.

The combined histogram (not included) clearly shows a bimodal distribution, indicating two sets of normally distributed measurements. Separate histograms (Figure 7) also show normal distributions for both forms. The 'Papillose' form histogram ranges from -4.0 to 1.5 standardised score units and the 'Typical' form histogram ranges from 0 to 4.0. The two graphs overlap over the range from 0 to 1.5. *A Posteriori* probabilities and Mahalanobis distance calculations suggested that five specimens were misclassified. Three 'Papillose' specimens were classified as 'Typical' and two 'Typical' specimens were classified as 'Papillose'. However *a posteriori* probabilities for two of the misclassified specimens are near 0.5 and thus marginal.

Table 4. A summary of Canonical Discriminant Analysis for the two forms of *H. pomiforme*. Coefficients are standardised canonical coefficients of the Canonical variate derived from the analysis and *r* denotes the correlation between original variables and Canonical Variates.

CV		
Character	Coefficient	r
STEM_L	-0.185254	-0.220428
STEM_D	-0.190221	-0.015802
BASE_L	-0.253628	-0.145431
LEAF_L	0.831325	0.098301
BASE_W	0.516762	0.413521
MID_W	0.283035	0.369251
BBAS_W	-0.149073	0.218365
BORD_L	-0.749056	0.048895
CELL_W	-0.059859	0.073471
COS_W	0.148782	0.053275
COS_TH	0.195213	0.098317
GC_VEN	0.039484	-0.035194
D_PAP_H	-0.408001	-0.226435
V_PAP_H	-0.811848	-0.584853
Eigenval	2.651855	
Cum.Prop	1.000000	

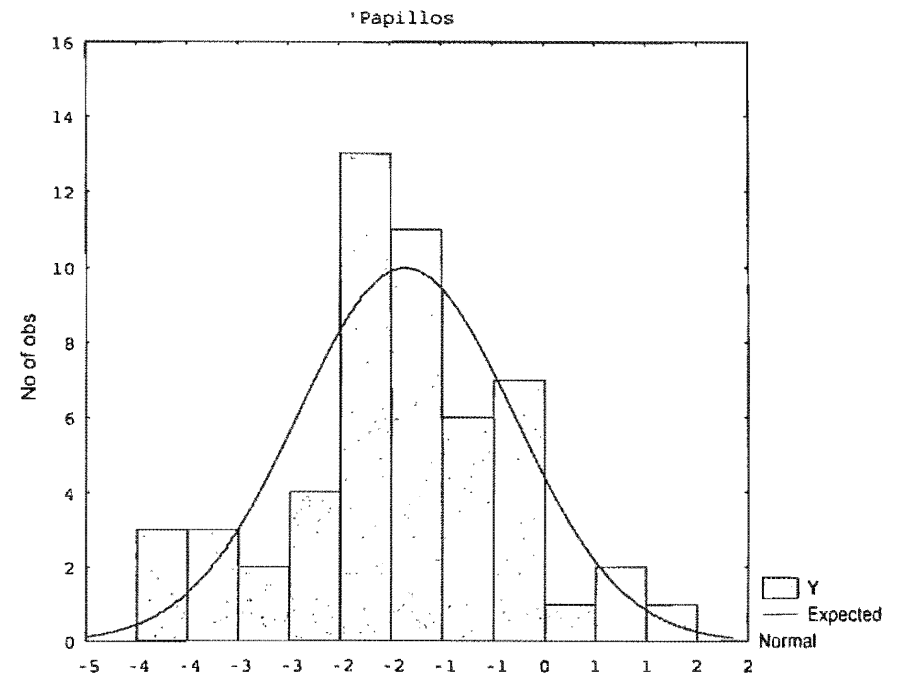
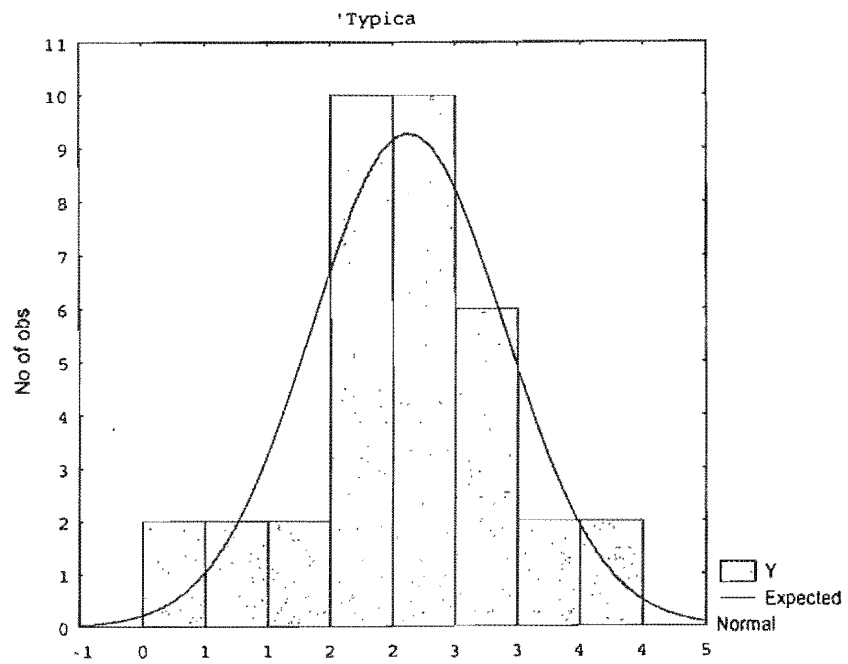


Figure 7. Separate histograms of the Discriminant Function Analysis of the two forms of *H. pomiforme*.

Discussion

The analyses described show substantial morphological differences between the two forms of *Hypodontium pomiforme*. Cluster analysis suggests clear difference between the two forms and canonical discriminant analysis shows significant differences although with some overlap. Component 1, largely a size component, contributes 29.9% of the total variation. Component 2 is a costa shape variable as shown by the importance of costa width, costa thickness and distance from guide cells to ventral surface. This is an important distinguishing feature in the genus. It distinguishes *H. pomiforme* from *H. dregei* and here it distinguishes the 'Typical' form, with a more ventrally bulging costa from the 'papillose' form with a less bulging costa. The distribution maps (Figures 11 and 12) show a similar distribution pattern for the two forms and this shows the morphological differences are not related to the geographical distribution. This suggests that the differences can be attributed to something else that is not the environment.

The C.V extracted (Table 4) accounts for total variation between the two forms. The loadings for leaf measurements are a mixture of positive and negative: thus leaf shape and not size is involved in the discrimination between the two forms. Leaf length, leaf base width and border length are important in separating the two forms as shown by the high coefficients. The 'Typical' form has a base width equal or wider than the mid-width while the 'Papillose' form has a base narrower than the mid-width. High loadings with opposite signs shown by ventral and dorsal papillae height reflect the contrast between the 'Typical' form with short or no papillae compared to 'Papillose' form with tall papillae. The goal of discriminant analysis is to determine which variables best discriminate between the observed groups and from the results above, papillae height, leaf length and base width seem to be most important in separating the two forms of *Hypodontium pomiforme*.

The efficiency of Canonical Discriminant Analysis depends on the univariate measurements on which it is based (Krzanowski 1990). The leaf

measurements used here are all taxonomically useful characters. In a phenetic approach, recognition of natural groups requires that the data be accepted as truly representative of the overall difference among the species (Williams and Humphries, 1992). The measurements used here are representative of the characters used to place species in this genus and other related genera.

Morphological differences have traditionally been the decisive criterion, but according to Mayr (2000), for two reasons it is inadequate for the study of species in nature. First, there are many good biological species that do not differ at all. This does not apply to the study case because there is a significant difference. Second, there are morphological types within a biological species, because of individual genetic variation or different life history categories (male, female or immature). There is no sexual dimorphism in *Hypodontium* and the plants used in the study were mature parts from mature plants. Genetic variation within the genus was examined in the following chapter. From a morphological species point of view there is strong evidence to consider the two forms of *H. pomiforme* separate species.

CHAPTER 4.

MOLECULAR VARIATION IN *HYPODONTIUM* *POMIFORME*

Introduction

In the previous chapter morphological analysis was used to assess the degree of difference between the two forms of *Hypodontium pomiforme*. This chapter utilises molecular data to further explore and test these differences. As with the morphological approach, the molecular approach offers its own distinct advantages in systematics. All heritable information of an organism is encoded in DNA. Molecular data therefore provides a large data set for phylogenetic studies. Since the size and diversity in the rates of change of different portions of the genome vary, molecular data can provide a phylogenetic record from very recent times to the origins of life. In molecular phylogenetics the characters under study represent heritable variation and environmental variation is eliminated. In some cases reproductively isolated species are morphologically indistinguishable and their reproductive isolation is made clear only after they are studied by molecular methods (Hillis, 1987).

Phylogeography

Phylogeography is defined by Avise, (2000), as 'the principles and processes governing the geographic distributions of genealogical lineages, especially within and among related species'. It encompasses processes operating on a wide range of temporal and spatial scales (Stone, 2000). Phylogeography increases understanding of underlying patterns in population structure. This helps in making meaningful analysis of other aspects of the biology of the organisms such as gene flow, the extent to which genetic diversity is affected by population size, the extent to which genetic subdivision is common to suites of species and the extent to which reproductive isolation precedes or follows ecomorphological divergence between lineages (Stone, 2000).

Phylogeography can help reveal possible causes of speciation. Enough variability can often be detected to build intraspecific phylogenies and to relate these phylogenies to geographic distribution of the haplotypes (El Mousadik and Petit, 1996). In phylogeography, individuals and not populations can be used as operational taxonomic units (OTUs) in population genetic studies, and phylogenetic concepts and approaches can be applied at the intraspecific level (Rieseberg, 2000).

The aims of this chapter are to use DNA (ITS region) data to test the hypothesis that the two forms of *H. pomiforme* constitute two species and to use the molecular data to examine the phylogeography of *H. pomiforme* in the South Western Cape.

Utility of the sequences used

The Internal Transcribed Spacer (ITS) region is part of the repeated unit of 18s-26s nuclear ribosomal DNA (nrDNA). The ITS region has three components: two spacers, ITS-1 and ITS-2, and the 5,8S subunit, which is an evolutionarily conserved sequence. The structure of the ITS region and the features that promote its use for phylogenetic analysis have been discussed by Baldwin, (1992) and Baldwin et al. (1995). These include its high repeat number in the plant nuclear genome. The high copy number promotes easy detection, amplification and sequencing. The region undergoes rapid concerted evolution, which promotes accurate reconstruction of relationships. Third, the small size of the region (<700 bp in angiosperms) and presence of highly conserved sequences flanking each of the two spacers makes the region easy to amplify using universal primers.

Materials and methods

The nuclear ribosomal (nrDNA) ITS region was sequenced for *H. pomiforme*. A total of seventeen (17) sequences were produced. Samples used were from Bolus Herbarium (BOL) or were freshly collected. Unfortunately, only one specimen from the large holdings at Pretoria Herbarium (NBG) yielded DNA that could be amplified by PCR. This could have been caused by destruction of DNA by chemical or other such form of treatment when the samples were being prepared for herbarium storage or due to degradation of DNA in stored specimens.

DNA extraction

The modified CTAB DNA extraction protocol from Gawel and Jarret (1991) was followed to extract DNA. Approximately 20mg of dried plant material was used for each specimen. This was mixed with sterile sand and ground to powder in 1.5 ml micro centrifuge tube and the extractions were carried out as outlined in the protocol.

DNA amplification, cleaning and sequencing

The gene regions were amplified by polymerase chain reaction (PCR) using primers 18KRC (forward) (Cox et al 2000) and ITS2 (reverse) (Baldwin, 1992). The thermocycling profile consisted of 30 cycles of 1 minute at 97°C, 1 minute at 52°C and 2 minutes 72°C, preceded by an initial melting step of 2 minutes at 97°C and followed by a final extension period of 7 minutes at 72°C to allow completion of unfinished DNA strands. Amplification was done in 50µl reaction volumes (29.7µl PCR H₂O, 5µl of 10 x NH₄ buffer, 5µl of 50mM MgCl₂, 2µl of 2.5mM dNTPs, 1.5µl of 10µM primers, 0.3µl of *Taq* polymerase (Perkin Elmer) at 5 units per micro litre and 5µl of unspecified concentration DNA template (DNA dilutions).

To check whether DNA amplification was successful, the PCR products were run on 1% agarose gel and visualised on ultra-violet (UV) transilluminators. The amplified DNA sequences were purified using QIAquick PCR purification (Qiagen) spin columns and eluted into 30µl of sterile water. The purified PCR products were sequenced using the amplification primers ITS2 and 18KRC, together with the ABI Prism™ Dye Terminator Cycle Sequencing Ready Reaction Kit (P. E. Applied Biosystems) in accordance with the manufacturers instructions. Twenty-five cycles of 30 seconds at 96°C, 15 seconds at 50°C and 4 minutes at 60°C were employed. Sequencing products were resolved on an ABI 3900 automated sequencing machine.

Sequence assembly, alignment and analysis

For each sequenced sample, forward (5' – 3') and reverse (3' – 5') sequences were assembled and checked for inaccurate base calling using SeqMan II (LaserGene System software DNA Star, inc.). The consensus sequences for all the regions were aligned manually in MegAlign (Lasergene System Software, DNA star, inc.) and alignments were saved in NEXUS format.

Phylogenetic Analysis

PAUP version 4.0b4a (Swofford, 2000) was used for phylogenetic analyses. The analysed data matrix is included on the attached CD. The distance analysis using neighbour joining was to investigate the phylogenetic relationship between members of the two forms. Bootstrap sampling with 100 replicates using heuristic search was employed to evaluate nodal support.

Neighbour joining is a distance-based, stepwise clustering method. It is widely used in tree building because it combines computational speed with uniqueness of results since most computations give one tree. The algorithm of neighbour joining gives the minimum evolution tree most of the time. When the number of nucleotides used is large, neighbour joining, maximum likelihood and maximum parsimony show more or less the same efficiency in

tree construction, but maximum likelihood and maximum parsimony are exhaustive search methods and require longer computational time (Saiton and Nei, 1987, Saiton and Imanishi, 1989). Neighbour joining does not assume that all lineages have diverged equal amounts. The distance calculations involved have been corrected for multiple, undetected substitutions. The corrected distances are then estimates of the true evolutionary distance which reflects the actual mean number of changes per site that have occurred between a pair of sequences since their divergence from a common ancestor.

Phylogeography Analysis

The TCS version 1.13 (Clement et al. 2000) computer program was used to analyse the sequences and produce a phylogenetic network showing the different haplotypes and the samples that make each haplotype. Gaps were treated as a fifth state. The program collapses sequences into haplotypes and calculates the frequencies of the haplotypes in the sample. The frequencies are used to estimate haplotype outgroup probabilities, which correlate with haplotype age. TCS is essentially a parsimony method, connecting existing haplotypes in a minimum spanning tree and can infer network loops in them. It is more suitable for population level phylogenetics than the traditional methods such as neighbour joining because it considers existence of ancestral haplotypes in a population, in accordance with the Coalescence Theory. It assumes recombination, which is a reality at population level and it doesn't require a large number of variables. It has successfully been used with other traditional methods before (a number of publications cited in Clement et al. 2000).

Results

Phylogenetic Analysis

The tree produced by neighbour joining is shown in Figure 8 and is rooted along the longest branch. There is separation between the 'Papillose' and 'Typical' forms. The 'Papillose' form shows greater variation than the 'Typical' form.

Phylogeography Analysis

A total of 10 haplotypes was identified from the 17 specimens sampled. Of the ten haplotypes observed from all the five localities, seven were detected from the Table Mountain. Five of these occur exclusively in this population (Figure 9 and Table 4). Three haplotypes were detected from the Hottentot's Holland population, two of which also occur on Table Mountain and one at Cape point. The Jonkershoek population has two haplotypes, one of which it shares with both the Table Mountain and the Hottentot's Holland populations and the other exclusive to the population. The haplotype from Cape point population was also detected from Hottentot's Holland. The Transvaal haplotype is exclusive to this population.

Haplotypes A, B, C, D and F all contain exclusively the 'Papillose' form of *Hypodontium pomiforme*, while haplotypes E, G and H contain exclusively the 'Typical' form of *H. pomiforme*.

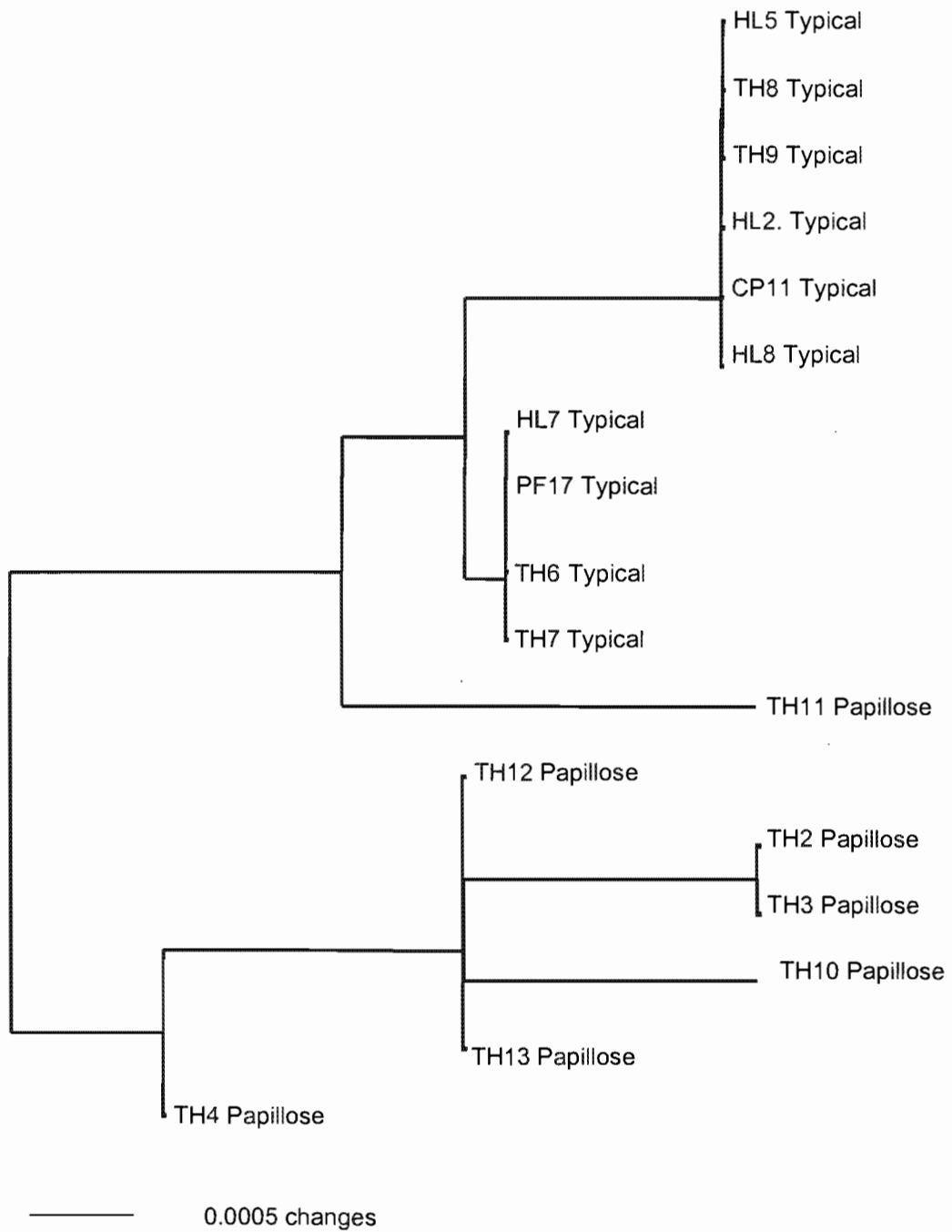


Figure 8. Neighbour joining tree produced in phylogenetic analysis of the two forms of *H. pomiforme* rooted at the longest branch.

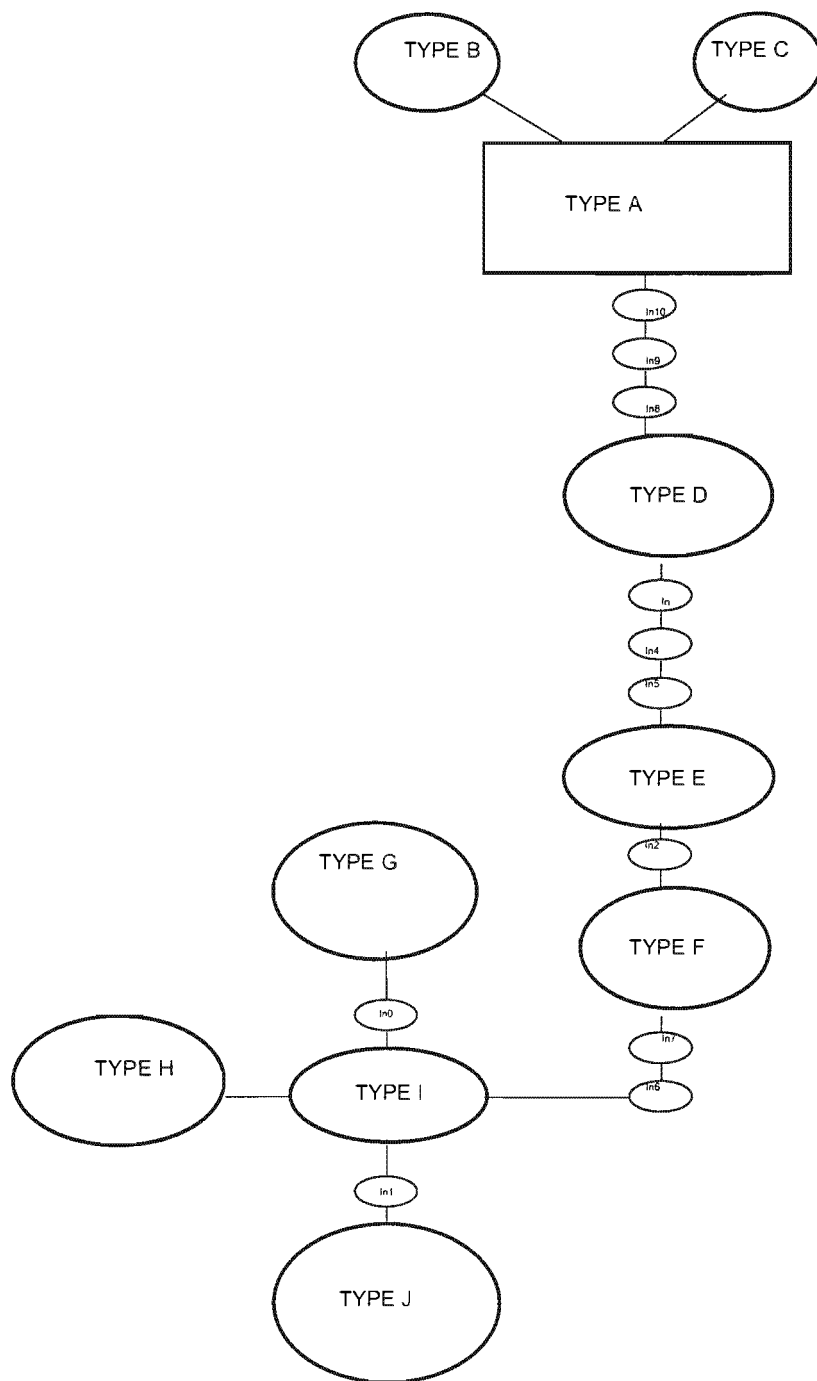


Figure 9. Phylogenetic network of the 10 haplotypes of *H. pomiforme* from the five areas studied.

Table 4. The genetic (Haplotype) composition of the five populations

Population	Haplotype									
	A	B	C	D	E	F	G	H	I	J
Table Mountain	2	1	2	1		1	1			1
Jonkershoek						1			1	
Hottentot's Holland						1	1	2		
Cape Point									1	
Transvaal					1					

Discussion

The haplotype network (Figure 9) shows too many missing haplotypes (eleven), which makes sensible interpretation of the relationship difficult. The unobserved haplotypes may be interpreted in three ways. They might have been missed during sampling, may be extinct, or may never have existed (if mutations did not accumulate in a single step) (Schaal and Olsen, 2000). In this case, all three could have contributed to the large number of missing haplotypes. Representation of the sampled areas is very poor with a lot of variation in sample number per locality. Nine of the seventeen samples used are from Table Mountain and these had seven haplotypes. Hottentots Holland had four samples, Jonkershoek had two and Cape Point and Transvaal had one each. The low sample numbers for most of the populations show poor representation of haplotypes from such places. Such uneven sampling contributed to the results having little meaning. The high number of haplotypes for the low number of samples indicates that there is great genetic differentiation, and the sample size would need to be much larger to evaluate the geographic component of this variation.

The observation of certain haplotypes (haplotypes A, B, C, D, E, I and J) being restricted to certain populations (Table 4) is expected when separated populations diverge and reach monophyly at which point all the alleles within each population are more closely related to each other than to those of other populations (Schaal and Olsen, 2000). The shared haplotypes (F, G and H) may be a reflection of inter-population gene flow, but they may also be ancestral haplotypes that persisted in populations after divergence. The later is more likely to explain the results here than the former because the populations are not sympatric. Haplotypes may also not correspond to population identity in recently diverged populations because the alleles are still either paraphyletic or polyphyletic with respect to populations (Schaal and Olsen, 2000).

Although each haplotype is made up of either the papillose or the typical form, there is no particular pattern. The results of the phylogeographic analysis do not show anything about either the phylogeographic or the phylogenetic relationship between the forms of *H. pomiforme*. A higher average number of samples from each location should have been collected to get more meaningful results. One sample per location was too little to say anything about the number of haplotypes and in this case there also was a need to compare the different forms. Such poor sampling could have been corrected with more time and resources.

Since this was a population level phylogenetic analysis, no outgroup could be considered close enough for meaningful comparison of characters. However the two forms of *Hypodontium pomiforme* are not very divergent and can be considered to have the same rate of evolutionary change. The root was therefore placed at the midpoint of the longest branch as suggested by Farris 1972 (cited in Kitching et al. 1998). The neighbour-joining tree produced (Figure 8) shows association between the phylogenetic relationship of specimens and the morphological forms of *H. pomiforme*. The 'Typical' specimens form a monophyletic clade separate from the 'Papillose' specimens. There is closer genetic relationship between specimens of the same form than between the forms. This shows a lack of gene flow between

the two forms even when the samples are from the same area. This supports the findings from the previous chapter on morphology and suggests that the two forms of *Hypodontium pomiforme* are separate species.

CHAPTER 5

GENERAL DISCUSSION

Hypodontium classification has been altered a considerable number of times. An especially contentious issue has been whether it must be included in Pottiaceae or Calymperaceae or in a family of its own, intermediate between the two. It has also been difficult to separate members of the two families based on morphology. Even with such a long history, there is very little literature on the genus. The genus has never been revised before. Recent molecular studies (La Farge et al. 2000; Goffinet et al. 2001) showed that Calymperaceae and Pottiaceae are not closely related, but unfortunately these studies never included *Hypodontium*. This therefore justifies the need for the current study.

The results obtained in this research are strong evidence in support of family status for *Hypodontium*. La Farge et al. (2000) included *Hypodontium* in their expanded concept of circumscription of the Calymperaceae. This is not supported by the analyses in this study. Both earlier results (La Farge et al., 2000; Goffinet et al., 2001) and this research show that Pottiaceae and Calymperaceae are easily separated by molecular studies. The results obtained here further show that *Hypodontium* is not closely related to either of the families. It is closer to the Dicranaceae. The results also do not support a position ancestral to *Syrrhopodon* for *Hypodontium*. *Hypodontium* does possess characters that are unlike both Pottiaceae and Calymperaceae, such as perichaetial leaves and capsule morphology (Zander, 1993). The *Hypodontium* clade always has a 100% jack-knife support value, making its members clearly monophyletic.

At present, the family would include only the genus *Hypodontium*. However, Zander (1993) notes some similarities between various other taxa and this genus, and it may be that continued sampling of the haplolepideous mosses would reveal that other genera should be assigned here.

Various criteria have been used to delimit species. Among such criteria are morphological discontinuity, interbreeding ability and reproduction isolation. There are a variety of species concepts, most of which I will not debate here. The phenetic species concept considers distinct phenetic clusters, produced by overall similarity in a multidimensional space, as species. The morphological results have shown that this condition obtains between the two forms of *H. pomiforme*. Under the phylogenetic species concept, the species should be distinguishable from each other and all others by unique characters that do not overlap. Phylogenetic analysis has shown that on the basis of ITS gene region the two forms can be separated without overlap. There is both molecular and morphological evidence suggesting a significant difference between the two forms of *Hypodontium pomiforme*. I suggest these two forms be treated as different species, thus making *Hypodontium* a three-species genus.

Unfortunately there is limited information from results of the phylogeography analysis. This could also have helped give more information on the pattern of breeding or history of the two forms, leading to a more informed conclusion. The amount of variation shown by the phylogeography analysis results, ten haplotypes from 17 sequences and 11 missing haplotypes, reflects existence of a lot of variation within *Hypodontium pomiforme*. Such variation clearly indicates that if wide well planned sampling is done, the phylogeography approach to the study of the species would yield very informative results.

TAXONOMIC TREATMENT

Hypodontium C. Müll. Original description in *Hedwigia* 38: 96 (1899); Broth. In *Natürl.* 10: 234 (1924). Type: lectotype species: *Hypodontium dregei* (Hornsch) C. Müll.

Plant large, grows in dense tuft, glaucous-green to yellow-green above, light tan to blackish brown below, terricolous or saxicolous. *Stem* erect, 12- 50 mm tall; central strand present, transverse section rounded-pentagonal. *Leaves* tubulose, incurled dry, spreading wet, linear to lingulate above clasping base, lanceolate, 3.5-6.0 mm in length, margins unistratose or bistratose, bordered to mid-leaf or near apex; involute above mid-leaf or at apex, apex rounded to acute, occasionally broadly mucronate. *Costa* percurrent or ending in a short, broad mucro, papillose to spinose, strong with dorsal and ventral stereid bands. Guide cells 4-6 in 1 layer, hydroid strand absent, Ventral surface cells strongly differentiated. *Upper laminal cells* rounded –quadrate, 8-11µm in width, superficially ventrally bulging, dorsally weakly convex, papillae thick, columnar, to 20 µm height.

Perichaetia terminal, leaves subulate, seta 5-8 mm in length, 1 per perichaetium, yellowish to reddish brown, twisted counter clock-wise. Capsule oval to short cylindrical; peristome teeth 16, flat, long triangular occasionally with weak properistomal thickening; operculum rostrate, calyptra cucullate, rough in upper half with forward-pointing papillae, 2.7-3.0 mm in length, spores large.

Key to the species in Hypodontium

1. Leaf margins involute from mid-leaf to apex, dorsal junction of lamina and costa with large spines above base, laminal cells with massive papillae, hyaline leaf border rarely reaching mid-leaf.....*H. dregei*.

- 1 Leaf margins plane or involute at apex, hyaline leaf border extending to near the apex, dorsal junction of lamina and costa lacks spines.....2

- 2 Leaf costa very prominent ventrally, ventral costal surface papillose and smooth dorsal costa surface, leaf laminal cells with low or no ventral papillae, If present in cross section 2-3 papillae per each laminal cell. Leaf margins unistratose.....*H. humilopapillosum*

- 2 Ventral costa inconspicuous, surface smooth, dorsal costa surface with numerous papillae, Laminal cells with tall ventral papillae that may or may not be crowned, in cross section a single papilla per each laminal cell. Leaf margins mainly multistratose.....*H. pomiforme*.

Hypodontium dregei (*Hornsh.*) C. Müll. Original description; *Hedwigia* 38: 97 (1899); Broth. in *Natürl. PflFum.* 10: 234 (1924). Type: Syntypes: Cape, Zwart-kei, Windvogelberg, Drège s.n., Table Mountain, Ecklon s.n. (BM).

Syrrhopodon dregei Hornsch. in *Linnaea* 15: 116 (1841); Sim (1926), *Bryo. S. Afr.* 2: 62

Syrrhipodon perichaetialis Bruch ex. Krauss (1846) in *Flora, Jena* 29: 132

Trichostomum cyanthiforme Dix. in *S. Afr. J. Sci.* 18 310 (1922).

Hyophila cyanthiforme (Dix.). Sim (1926) *Bryo. S. Afr.* 221.

Plants glaucous-green to olive green. Saxicolous or corticolous. Stems 10-30 mm tall, weakly tomentose, inner cortical cells 5-6 rows, outer cortical cells 1-2 rows. *Leaves* 4-5 mm long, contorted dry, margin entire, involute above mid-

leaf, plane below, hyaline border rarely reach mid leaf. *Costa* mucronate, ventral surface flat or weakly convex, very prominent dorsally, in section sub round, guide cells 4-6, ventral stereid band 2-3 cells thick, ventral surface cells large, incrassate, strongly papillose, dorsal stereid band 5-6 cells thick, cells near junction of costa and lamina spinose. *Upper laminal cells* quadrate, with massive papillae up to 18µm tall, dorsal surface with low blunt papillae, marginal cells in involute region smooth.

Examination of the type specimen of *H. pomiforme* var. *macowanianum* C. Müll. sensu Hedwigia 38: 97 (1899) from GRA showed that the specimen belongs to the species *dregei* and not *pomiforme* as prescribed earlier.

Ecology and distribution: It occurs in Zimbabwe and Southern Africa (Figure 10). It has been collected on rocks, on shallow soils over rocks and on bark of trees.

Hypodontium pomiforme (Hook) C. Müll. Original description; Hedwigia 38: 97 (1899); Broth. in Natürl. PflFum. 10: 234 (1924). Type: Holotype, Swellendam, Burchell s.n. (BM!).

Wessia pomiformis Hook. *Musc. Exot.* 131 (1819).

Syrhopodon pomiformis (Hook.) Hampe ex. C. Müll., *Syn. Musc.* 1: 531 (1849); Sim (1926). *Bryo. S. Afr.* 263.

Hypodontium pomiforme var. *macowanianum* C. Müll. in *Hedwigia* 38:97 (1899)

Plants green to yellow green. Saxicolous, or rarely corticolous. *Stems* 20-50 mm tall, often branched, often tomentose, with 5-6 rows of inner cortical cells and 1-2 rows outer cortical cells. *Leaves* 3-4 mm long, hyaline base, crisped above base dry, margin entire, involute at apex, multistratose border, hyaline border extend from base to near apex. *Costa* ventrally inconspicuous, guide cells 4-6, ventral stereid band 2-3 cells thick, ventral surface cells large with small papillae, cells near junction of costa and lamina have not spinose.

Upper laminal cells rounded, quadrate, incrassate ventrally with tall papillae up to 12µm tall which may be crowned, dorsal surface weakly papillose to smooth, basal cells rectangular to elongate-hexagonal.

Ecology and distribution: Endemic to Southern Africa. It has been collected in forests of South Western Cape and occasionally in Eastern Cape, Transkei, Kwazulu-Natal and Eastern Transvaal (Figure 12).

***Hypodontium humilopapillosum* Manyanga & Hedderson** Sp. nov. Type: Western Cape, Jonkershoek, *Hedderson* 13018 (BOL. holo.!) Selected here.

Diagnosis: *H. pomiforme* (Hook) C. Müll. similes sed papillis humilis et costis protuberantibus.

Plants in cushions, green to yellow-green. Saxicolous rarely corticolous. *Stems* 20-50 mm tall, often branched, often tomentose, in section no central strand, inner cortical cells 5-6 rows, outer cortical cells 1-2 rows. *Leaves* 3-4 mm long, contorted above base dry, erect spreading wet linear above obvate base, apex acute, margin entire and reflexed to one side, involute at apex, unistratose border, hyaline border extend from base to near apex. *Costa* percurrent, ventral surface strongly convex very prominent ventrally, in section sub round, guide cells 4-6, ventral stereid band 2-3 cells thick, ventral surface cells large with small papillae, dorsal surface with blunt papillae. *Upper laminal cells* rounded, quadrate, incrassate ventrally with 2-4 low papillae, ventral surface weakly papillose dorsal surface smooth.

Ecology and distribution: Also endemic to Southern Africa. It has been collected in forests of South Western Cape and occasionally in Eastern Cape, and Eastern Transvaal. (Figure 11)

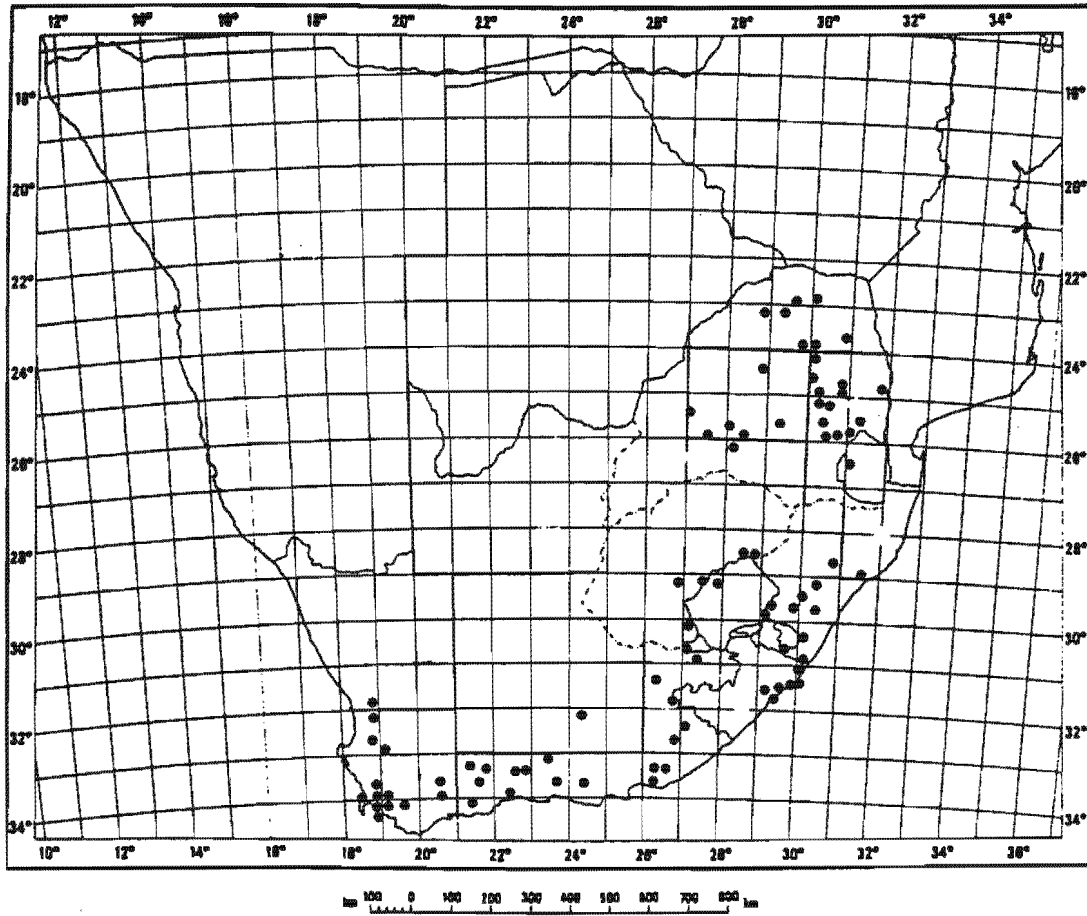


Figure 10. Distribution of *Hypodontium dregei*

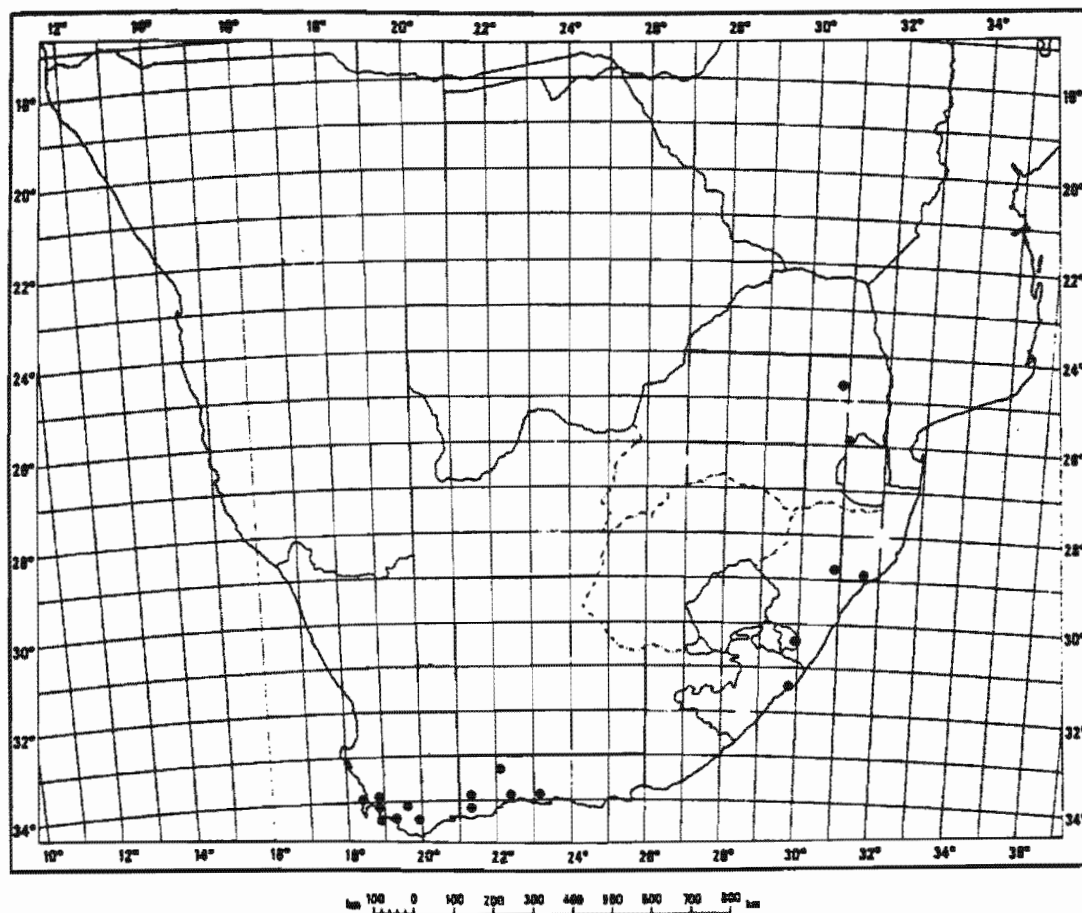


Figure 11. Distribution of *Hypodontium humilopapillosum*.

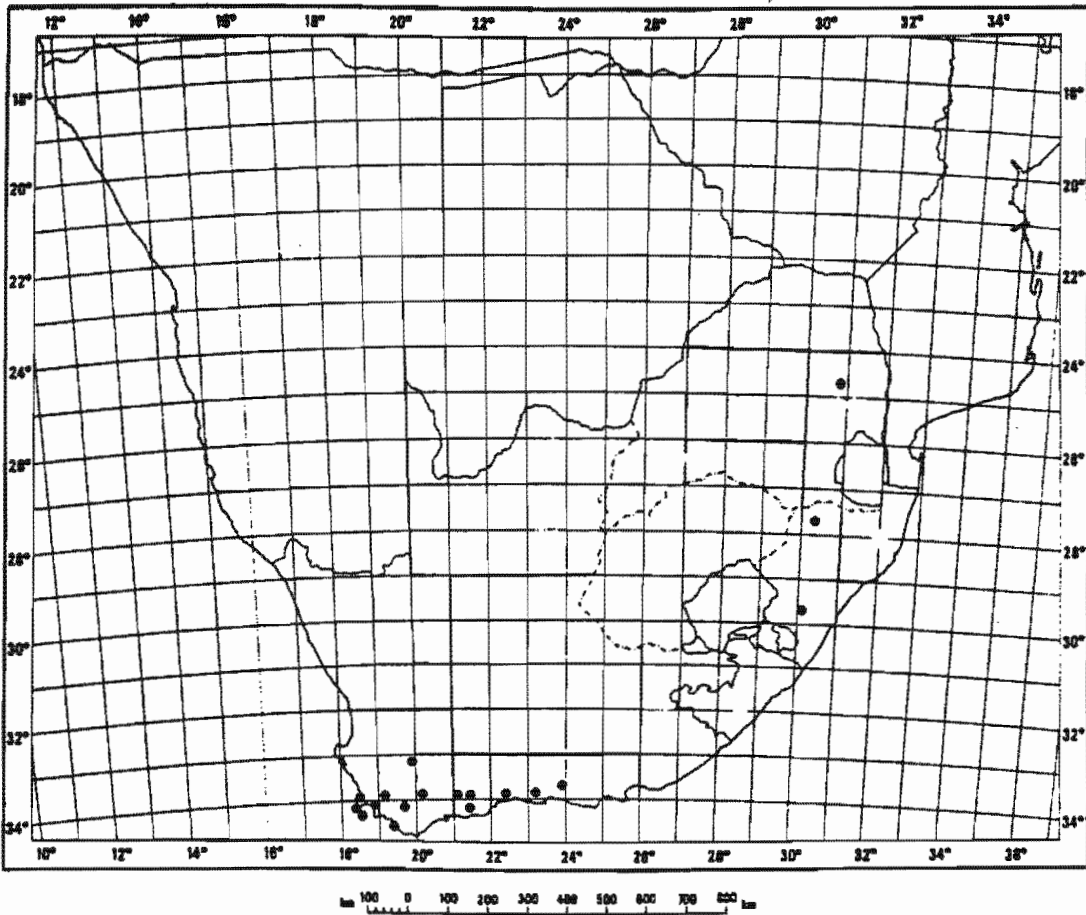


Figure 12. Distribution of *Hypodontium pomiforme*

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APPENDIX 1. Specimens studied

1. Specimens studied for morphological analysis

Hypodontium dregei

- CAPE- 3027 Witterberg Mountains, (CA), *Van Rooyen* 2647 (NBG); Lady Grey road, (CD), *Van Rooyen* 2647 (NBG);
- 3118 Namaqualand (Matsikama), (DD), *Smook* 3584 (NBG);
- 3126** Molteno, (AD), *Perold & Koekem* 3017 (NBG); Bathcart, (DD), *Rehmann* 487 (NBG); Queenstown, (DD), *Sim* 10311. (NBG)
- 3218** Mount Synnott, (BB), *Magill & Schelp* 2761 (PRE); Pakhuis Pass, (BB), *Cholknoky* 607, 610, 640a (NBG); Simon's town, (BD), *Brenan* 2761 (NBG); Pakhuis Pass, (BB), *Stirton* 9353, 9357, 9358 (NBG); Farm Morester, (DC), *Stirton* 9393 (NBG);
- 3219** Tarakamma, (CC), *Van Jaarsveld* 6358a (NBG);
- 3224** Curve Mountain, (BA), *Sim* 8144 (NBG);
- 3227** Queenstown, (AC), *Rahmann* 9154 (NBG);
- 3318** Camps bay, (CD), *Rehmann* 125 (PRE), Table Mountain (CD); Modderkloof (Perdeberg), (DB), *Hugo* 3034 (NBG); Paarl Rock, (DD), *Sim* 9630 (NBG);
- 3320** Anysberg, (DA), *Meyer* 177 (NBG);
- 3321** Seven weeks poort, (AD) *Magill* 6150 (PRE); Kangoberg, (BD), *Oliver* 7471 (NBG); Rooiberg, (DA), *Thompson* 3571 (NBG);
- 3322** Swartberg pass, (AC), *Stirton* 9533 (NBG), *Oliver* 7487 (NBG); Schoemans Poort, (AD), *Magill* 6114, 6124 (PRE); Outeniqua pass, (CD), *Magill* 1980 (NBG);
- 3323** Quartzite ridge, (AB), *Magill* 5917 (NBG); Boskloof cliff, (DA), *Stirton* 9558 (NBG);
- 3324.** Karooberge, (CB), *Stirton* 9573 (NBG);
- 3326** Witterberg quartzite ridge, (BC), *Goldblatt* 2120d (NBG); Graham's town road, (CB), *Bayliss* 8291, 8372 (NBG);
- 3332** Quartzite ridge, (AB), *Magill* 5919 (NBG);
- 3418** Palmietrivier valley, (AA), *Strokoe* 9172 (NBG); Helderberg, (BB), *Pillans* CH13219 (NBG);

3419 Galgeberg summit, (BA), *Bean & Van Jaar 6384a* (NBG);
3421 Hesmanshoek Rivarsdale, (AB), *Muir 4184* (NBG);
 FREE STATES-**2828** Hoogland National Park, (DA), *Liebenberg 7650* (NBG);
2926 Thaba 'Nchu, (BB), *Roberts 2277* (NBG);
2927 Ladybrand (Leliehoek), (AB), *Magill 7609663a* (NBG);
3027 Stellenbosch, (AA), *Van Rooyen 2470* (NBG);
 LESOTHO-**2927** Mamathe's (Berea District), (BB) *Jacot-Guillarmod 6328*
 (NBG);
2929 Sehlabathebe National Park, (CC), *Magill 4308a, 4349* (NBG);
 NATAL-**2828** Royal National Park, (DB), *Roberts 6738* (NBG);
2830 Qudeni Forest, (DB), *Magill 5142* (NBG);
2831 Ngoya Forest, (DC), *Sim CH 7620* (NBG);
2929 Natal Drakensberg, (CB), *Van Rooyen 1362* (NBG); Ngwangwane
 River, (CC), *Van Rooyen 1479* (NBG); Rosetta, (DB), *Sim CH 7207* (NBG);
2930 Karkloof, (AC), *Sim CH 7211* (NBG); Maritzberg, (CB), *Sim CH9191a*
 (NBG); Bhukwaber, (AB), *Sim 10095* (NBG);
3029 Weza Forest Reserve, (DA), *Von Breitenbach 195* (NBG);
3030 Mtwalume Falls, (AC), *Van Rooyen 1037* (NBG); Umtamvuna Nature
 reserve, (CC), *Abbott 1869* (NBG);
 3129 Port St. John's, (DA), *Russell 2645a* (NBG);
3130 Umtamvuna Nature reserve, (AA), *Abbott 761* (NBG);
 TRANSKEI-**3129** Pondoland, (AD), *Van Rooyen 1687* (NBG); Fraser Falls
 Gorge, (BC), *Van Rooyen 1877* (NBG); Wild Coast, (BD) *Van Rooyen 760*
 (NBG); Port St. John's, (DA) *Van Rooyen 1957* (NBG);
3130 Mkambati Nature reserve, (AC) *Van Rooyen 1772, 1742, 1785* (NBG);
 TRANSVAAL-**2229** Peover, Sout pansberg, (DC), *Magill 3816* (NBG);
2230 Messina, (CC), *Von Breitenbach 192b* (NBG);
2328 Blouberg Nature Reserve, (BB), *Glen 2192* (NBG);
2329 Mount Lejuma (sout pansberg), (AB), *Magill 3704, 3712, 3713, 3719,*
3744, 3750, 3767, 3787 (NBG); Zoutpansberg, (AB), *Glen 2193* (NBG);
 Woodbush Forest Reserve, (DD), *Magill 6561* (NBG);
2330 Ndebezeni Falls (Tzaneen District), (CC), *Schelppe CH12637* (NBG);
 Marieskop PLantation Nature reserve, (DB), *Marshall & Cros 7582, 7649*
 (NBG);

2430 Wolkberg (Serela Mountain), (AA), *Von Brèitenbach* 137 (NBG); Sekhukhune land, (CA), *Magill* 3211, 3318 (NBG); Natuurreseervaat, (CD), *Crosby & Crosb* 13416 (NBG); Mariepskop South, (DB), *Vorster*, 1103, 1138, 1356, 1551 (NBG); Fairyland, (DD) *Glen* 2977 (NBG);

2431 Mac Falls, (DD), *Smook & Phelan* 841a (NBG);

2527 Bariaanskranz Farm (Magaliesberg), (CA), *Magill* 3816 (NBG); Magaliesberg, (DC), *Smook* 1036 (NBG);

2528 Willows, (Pretoria district), (CA), *Lansdell* CH10529 (NBG); Tiger Poort, (CD), *Perold* 152 (NBG).

2529 Farm Blankfontein (Middelburg), (CB), *Van Rooyen & Perol* 662 (NBG);

2530 Drakensberg, (AB), *Rankin* 70 (NBG); Long tom pass, (BA), *Perold* 417 (NBG); Starvation Nature reserve, (DA), *Kluge* 1032 (NBG); Waterval-bo (Farm Skoonwater), (CB), *Smook* 879 (NBG); Farm uitkomst, (DC), *Perold* 1011 (NBG); Graskop, (DD), *Univ. Wits* CH 12852 (NBG);

2531 Mananga/ Lebombo, (CB), *Hardy* CH12697 (NBG); Barberton District, (CC), *Kluge* 1049 (NBG).

2628 Johannesburg, (AA), *Sim* CH 10709 (NBG);

SWAZILAND-**2631** Nkwalini (Hlohho District), (AC), *Magill* 3525 (NBG), *Braun* 1459 (NBG).

Hypodontium pomiforme

TRANSVAAL- **2430** Mariepskop, (-DB), *Vorster* 504 (NBG), 1030 (NBG), 1231 (NBG); *Van Der Schijff* 6209 (NBG);

NATAL- **2930** Maritzberg, (-CB) *Sim* 7528 (NBG).

CAPE- **3318** Table Mountain (-CD), *Pillans* 3535 (NBG); *Marshall & Crosby* 8170 (NBG), *Soini* CH13420 (NBG), Hedderson 13553 (BOL), 13490, (BOL) 13571. (BOL), 13490 (BOL), 13480 (BOL), 13498 (BOL) 13557 (BOL) 13361 (BOL), Disa Ravine, (-CD), *Sim* 9135 (NBG), 9153 (NBG); Nursary Gorge, (-CD), *Esterhuysen* 19181; St John's Peak, (-CD) *Pillans* 4050 (NBG); Orange Kloof (-CD) *Barnard* SAM46145 (NBG); Ferny Gully, (-CD) *Howes* 18 (NBG); Kasteels Poort Stream (-CD) *Howes* 13 (NBG), Fir Tree Ravine, (-CD), *Howes* 3 (NBG); Blinkwater, (-CD), *Sim* 9268 (NBG), Platteklip Ravine, (-CD),

Sim 9290 (NBG); The Saddle, (-CD), *Sim* 9404 (NBG); Valku Ravine, (-CD) *Bews*.8463 (NBG); Jonkershoek Hedderson, 13022, (BOL).

3319 Oudebos ZonderHaalkop, (-BB), *Thorne* M50507 (NBG), (-CC), *Stokoe* 9175 (NBG); (Zachariashoek- Oukraalkoppie) La Motte Forest Station, (-CC) *Viviers* 105 (NBG),

3320 (Swellendam) Koloniesbos, (-CD), *Magill* 6194 (NBG).

3321 (Gracias pass) Riversdale, (-CC), *Muir* 3644 (NBG), 3701 (NBG), CH5660 (NBG), *Thorne* SAM38808 (NBG); (Kampscheberg) Riversdale, (-CD), *Muir* 3684 (NBG).

3322 Montagu pass, (-CD), *Rehmann* 123 (NBG), 124 (NBG), 123.6 (NBG), NH 124 (NBG); Outeniekwa pass, (-CD), *Magill* 6057 (NBG), *Van Zanten* 7609422 (NBG).

3323 Prince Alfred's pass (-CC), *Magill* 5947; (Uniondale Div.) Kauga Mountains, (-DB), *Esterhuysen* 16564 (NBG).

3418 Silvermine plateau, (-AB), *Goldblatt* 1498 (NBG), Kogelberg State Forest, (-BB), *Boucher* 3662 (NBG), Hottentots Holland, (-BB), *Stephens* CH5641 (NBG); Platteklip Gorge, (-CD), *Esterhuysen*. 20109 (NBG), Betty's Bay Porter Nature Reserve, (-CD), *Magill* 6303 (NBG),

3419 Riviersonderend Mountains, (-BA), *Wilman* 518 (NBG); Bredasdorp Mountain, (-DB), *Thompson* 3795 (NBG)

3421 (Langerbergen) Riversdale, (-AB), *Muir* 1552 (NBG), 3682 (NBG);

Hypodontium humilopapillosum

TRANSVAAL- **2430** Kowyns pass (-DB), *Striton* 9888 (NBG); Paradise camp, (-DB), *Brenan* M3322 (NBG); Mariepskop, (-DB), *Vorster* 1118 (NBG), 476 (NBG), 1022 (NBG), 1713 (NBG), 746 (NBG), 682 (NBG), 733 (NBG); *Marshall & Crosby* 7738 (NBG).

2531 Kangwane (-CC), *Smook* 8097 (NBG).

NATAL- **2830** Qudeni Forest, (-DB), *Magill* 5136 (NBG)

2831 (Ngoya) Zululand, (-DC), *Sim* CH7522 (NBG)

3029 (Weza Forest Reserve) Kokstad, (-DA), *Von Brietenbach* 410 (NBG).

TRANSKEI- **3129** Mkambati Nature Reserve, (-BD), *Van Rooyen* 1725 (NBG), Ponderland, *Burt-Davy* CH6128 (NBG).

CAPE- **3318** Table Mountain, (-CD), *Howes* 29 (NBG), *Van Rooyen* 800 (NBG); Hedderson, 13524 (BOL), 13356 (BOL), 13524 (BOL), Devils Peak, (-CD), *Sim* 9244 (NBG); Grootdrakensteinberge, (-DD) *Taylor* 9626 (NBG); Jonkershoek Hedderson 13018.

3321 Monti Jabulani, (-CD), *Sim* CH6115; (Kampscheberg) Riversdale, (-CD), *Muir* 3689A.

3322 Swartberg Pass, (-AC), *Magill* 6129 (NBG); (George Tonnelbos) Saasveld, (-CD), *De Kock* 31 (NBG); Outeriqua Pass, (-CD), *Van Zanten, Groen & Ringenaldus* 7609415 (NBG).

3323 Deep walls-Big tree, (-CC), *Jacot- Guillarmod* sn. (NBG),

3418 Hottentots Holland, (-BB), *Brenan* M2766 (NBG), Simonstown, (-BD), *Brenan* M2759 (NBG),

3419 (Caledon) Vogelgat, (-AD), *Williams* 3215 (NBG); Riviersonderend Mountains, (-BA), *Schelppe* 7906 (NBG); Kogelberg Caledon Dist., (-BD), *Stokoe* CH12928 (NBG).

3421 Riversdale, (-AB), *Muir* 3689 (NBG),

2. Specimens studied for Molecular Analysis

Hypodontium dregei

CAPE **3318** Kristebosch Botanic Garden, (CD), *Hedderson* 13269 (BOL).

Hypodontium Pomiforme

CAPE **3318** Table Mountain, (CD), *Hedderson* 13361, 13480, 13490, 13498, 13553, 13557, 13571 (BOL); Jonkershoek *Hedderson* 13022 (BOL).

Hypodontium humilopapillosum

CAPE **3318** Table Mountain, (CD) *Hedderson* 13356, 13524 (BOL); Jonkershoek Hedderson 13018 (BOL).

3418 Hottantots Holland, (BB), *Manyanga*, HL5 HL7, HL8 (BOL)

TRANSVAAL **2531** Kangwane (-CC), *Smook* 8097 (NBG).