THE EVOLUTION AND EXPRESSION OF RBCL IN HOLOPARASITIC SISTER GENERA HARVEYA HOOK. AND HYOBANCHE L. (OROBANCHACEAE) AND SYSTEMATICS AND TAXONOMIC REVISION OF SOUTHERN AFRICAN SPECIES OF HARVEYA

DISSERTATION

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

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2004

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ABSTRACT

The evolution of holoparasitism in plants is often accompanied by reduction in structures and functions associated with photosynthesis and nutrient acquisition. This reduction in morphological and anatomical features occurs concomitantly with loss or truncation of photosynthesis-related genes freed from selective constraints. The chloroplast genome of many holoparasitic plants is drastically reduced in both the quantity and quality of genetic material. *Harveya* and *Hyobanche* (Orobanchaceae) are holoparasitic genera native to southern Africa. *Hyobanche* has undergone pseudogene formation at the chloroplast locus encoding the large subunit of the carbon-fixing holoenzyme Rubisco, *rbcL*, while *Harveya* maintains a seemingly functional copy of this gene, which is evolving under purifying selection. The regions flanking *rbcL*, the 5’- and 3’- Untranslated Regions, appear to be functional in both genera. However, Western blot analysis shows that the large subunit is present in both taxa, despite the presence of the pseudogene in *Hyobanche*. This may be explained by two hypotheses: 1) multiple copies of *rbcL* exist in *Hyobanche*, one of which codes for a functional protein, or 2) RNA transcripts are repaired by an as-yet unknown mechanism. Cloning and RNA experiments demonstrated that while there are indeed multiple copies of *rbcL* in *Hyobanche*, none of these codes for a functional protein. The hypothesis that Rubisco is obtained from host plants is discussed, as well as other possible functions of Rubisco in these putatively non-photosynthetic plants.
Of 53 species names attributed to the genus *Harveya*, 35 represent species endemic to southern Africa. However, the genus has never been treated as a whole taxonomically, and the southern African species have not been revised since Hiern’s treatment of 1904. Species delimitations were reassessed using material collected in the field as well as specimens borrowed from herbaria, resulting in the reduction of the genus to twelve species native to southern Africa. A diagnostic key for identification of species was presented, and host-plant records, habitat, and geographical distributions were summarized for each species circumscribed. Molecular sequence data from the chloroplast and nucleus were used to test hypothesized relationships among these species, and the evolution of floral characteristics, host-plant ranges, and geographical origins.
Dedicated to my favorite lepidopterist
ACKNOWLEDGMENTS

I would like to thank my adviser and committee for support, guidance and friendship. I thank my adviser, Andi Wolfe, for allowing me the freedom to pursue my intellectual goals no matter how fruitless or ludicrous they may have seemed. She has shared equally in my failures and successes, and has allowed me to see that the answers are most often in my mistakes. John Freudenstein has always been a willing mentor, and has provided most of the guidance needed for the completion of the taxonomic revision. I wish to thank John Wenzel for keeping things interesting, and especially for his genuine concern for my personal and professional well-being.

I would like to thank Dr. Tom Waite for help with statistical problems, and in driving away bike thieves, and Dr. Kim Steiner (at California Academy, Riverside) for providing plant material and advice in the field.

I would like to thank the botanists of South Africa, who have been helpful in my field work: Dee Snijman and all the staff at the Compton Herbarium, Trevor Edwards, Steve Johnson, and Christina Potgieter at the University of Natal, Mrs. Auriol Batten for hospitality and expertise, Terry Hederson at the University of Cape Town for saving me from a liquid nitrogen-failure disaster, Michele Pfab of Gauteng Nature Conservation, Charles Glass, and especially Cameron and Rhoda McMaster, for wonderful photographs and specimens of Harveya, for kindness and hospitality when it was most needed, for
warm meals and a comfortable bed, and for the most rewarding three days of fieldwork, ever.

The ability to keep passing the open windows been aided by those who struggle to complete their own work around me. Jenny Archibald, Dr. Nidia Arguedas, Shannon Datwyler, Jose Diaz, Shawnita Krosnick, Jefé Morawetz, Mark Mort, Kurt M. Pickett, Sarena Selboni, Mark “Puffy” Simmons, and Lisa “Virginia Slim” Wallace have been true homies through the last seven years and I hope that I know them for a long time.

I would like to thank my mother and father, Noël and William Randle for unparalleled selflessness, the best friendship that there is, and for my brothers and sisters who have always been my closest allies.

The greatest part of my gratitude goes to Sibyl Rae Bucheli who keeps me tethered to a life that I enjoy and is my best friend.

This work was supported through grants from the National Science Foundation, the Janice Carson Beatley Fund, the Graduate Student Alumni Research Award, the American Society of Plant Taxonomists Graduate Student Research Grant, the International Student Dissertation Travel Grant, and Sigma Xi.
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TABLE OF CONTENTS

Abstract ............................................................................................................................... ii
Dedication .......................................................................................................................... iv
Acknowledgments ............................................................................................................ v
Vita ..................................................................................................................................... vii
List of Tables .................................................................................................................... xi
List of Figures .................................................................................................................. xiii

Chapters

1. Introduction .................................................................................................................... 1

2. The evolution and expression of rbcL in holoparasitic sister-genera Harveya
   Hook. and Hyobanche L. (Orobanchaceae): the presence of Rubisco in the
   absence of a functional gene.......................................................................................... 14
   Introduction .................................................................................................................. 14
   Materials and Methods ............................................................................................... 18
   Results ........................................................................................................................... 22
   Discussion ..................................................................................................................... 29

3. The expression of rbcL in Hyobanche: Multiple gene copies or post-
   transcriptional modification? ......................................................................................... 43
   Introduction .................................................................................................................. 43
   Materials and Methods ............................................................................................... 49
   Results ........................................................................................................................... 52
   Discussion ..................................................................................................................... 56

   inferred using DNA sequence data from the rbcL operon and nuclear ITS rDNA
   ........................................................................................................................................ 70
   Introduction .................................................................................................................. 70
   Materials and Methods ............................................................................................... 74
   Results ........................................................................................................................... 77
   Discussion ..................................................................................................................... 79
5. Taxonomic Revision of *Harveya* Species of Southern Africa ................. 101

   Introduction ............................................................................ 101
   Materials and Methods ......................................................... 105
   Generic Taxonomy ................................................................. 107
   Key to the species of *Harveya* .............................................. 109
   The species of *Harveya* ....................................................... 110
      *H. hyobanchoides* .............................................................. 110
      *H. pumila* ................................................................... 115
      *H. scarlatina* ................................................................ 123
      *H. squamosa* ................................................................ 130
      *H. stenosiphon* ................................................................ 136
      *H. bodkini* .................................................................... 142
      *H. bolusii* .................................................................... 146
      *H. speciosa* .................................................................. 153
      *H. capensis* ................................................................... 164
      *H. purpurea* .................................................................. 176
      *H. coccinea* .................................................................. 189
      *H. huttonii* ................................................................... 198
      Uncertain names ............................................................... 210
      Excluded names ............................................................... 210

   List of References ................................................................ 212
### LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Primers used in the amplification and sequencing of the 5'-untranslated region (UTR), <em>rbcL</em>, and the 3'-UTR</td>
</tr>
<tr>
<td>2.2</td>
<td>Rates of synonymous (<em>d</em>&lt;sub&gt;S&lt;/sub&gt;) and non-synonymous substitution (<em>d</em>&lt;sub&gt;N&lt;/sub&gt;) for <em>rbcL</em> in <em>Harveya</em>+<em>Hyobanche</em>, <em>Harveya</em> and <em>Hyobanche</em>, calculated using the modified Nei and Gojobori method (Nei and Gojobori 1986; Kumar 2001) with Jukes-Cantor correction. Codons containing indels resulting in frameshift or mutations resulting in premature stop codons were removed from the data set. In <em>Harveya huttonii</em>, <em>rbcL</em> included a 341 base pair gap, and therefore, inclusion of <em>H. huttonii</em> resulted in a significant deletion of the data set. Therefore, <em>d</em>&lt;sub&gt;S&lt;/sub&gt; and <em>d</em>&lt;sub&gt;N&lt;/sub&gt; were calculated with <em>Harveya huttonii</em> included and excluded. The Wilcoxon signed ranks test was used to test the null hypothesis that the pattern of substitution within a lineage was random (<em>H</em>&lt;sub&gt;0&lt;/sub&gt;: <em>d</em>&lt;sub&gt;S&lt;/sub&gt; = <em>d</em>&lt;sub&gt;N&lt;/sub&gt;). ¹Tr/Ts = transition / transversion ratio, inferred from number of changes (Kumar 2001)</td>
</tr>
<tr>
<td>2.3</td>
<td>Summary of Rubisco oligomer banding patterns for calyx, corolla, leaf and stem tissue of <em>Harveya</em> and <em>Hyobanche</em>. Rubisco oligomers fell into six molecular weight categories identifiable by subunit composition. The holoenzyme is composed of eight large subunits and eight small subunits (8 LSU + 8 SSU). The holoenzyme and octamer were indistinguishable on membranes due to their large size (~300-600 kDa). Other bands detected in the analysis are labeled by size and probable composition</td>
</tr>
<tr>
<td>3.1</td>
<td>Fisher’s Exact Test (two-tailed) of substitution rates in cloned sequences from a single individual of each of four species of <em>Hyobanche</em>. If all substitutions in these matrices were the result of PCR and cloning error, these rates should be equal in all cloning experiments. <em>n</em> = the number of sequences gathered in one cloning experiment. <em>L</em> = the length of the shortest tree for each set of cloned sequences. Substitution rate (<em>µ</em>) is calculated as the number of observed changes divided by the number of possible changes, or <em>L</em>/(n-3)<em>x</em>, where (n-3) = the number of internal branches for <em>n</em> sequences, and <em>x</em> = the number of characters in the aligned matrix (constant of 1435 aligned nucleotides in all matrices). The test calculates the probability that the substitution rate of the</td>
</tr>
</tbody>
</table>
control matrix (20 cloned sequences from *Hyobanche glabrata* amplified from a set of UTR primers) could have produced observed \( \mu \) in the test matrices and a control matrix. The control matrix had a calculated \( \mu \) of 4.1069 E-05. The two columns on the right give the highest possible \( \mu \) of test matrices that would have yielded a non-significant p-value (<0.05)……………………………………………………………65

4.1 Record of specimens from which sequences were obtained………………89

4.2 Recorded hosts for species of *Harveya*……………………………………92
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Geographical distribution of <em>Harveya</em> and <em>Hyobanche</em></td>
<td>11</td>
</tr>
</tbody>
</table>
| 1.2    | A. *Harveya bolusii*  B. *Harveya capensis*  C. *Harveya scarlatina*  
D. *Harveya hyobanchoides*  E. *Harveya huttonii*  F. *Harveya speciosa*  
G. *Harveya squamosa*  H. *Harveya purpurea* subsp. *purpurea*  I. Two color morphs of *Harveya pumila*. Photo credits:  
Jenny Archibald (A,B,F,H); Andrea Wolfe (C,D,G); Cameron McMaster (E, I) | 12   |
| 2.1    | Phylogram of *Harveya* and *Hyobanche*, with outgroups *Alectra sessiliflora*, *Buchnera floridana*, *Cynnium racemosum*, and *Melasma scabrum* inferred from the DNA sequence of the *rbcL* operon composed of the 5′-UTR, *rbcL* (coding sequence) and the 3′-UTR. This is one of six most parsimonious trees (C.I.= 0.870; R.I.= 0.850; 315 steps). Support values from 500 jackknife replicates (10 random sequence additions each) appear above branches.  
* indicates branches which collapse in strict consensus. (Ψ) indicates an *rbcL* pseudogene evidenced by a deletion resulting in frameshift (slash), or the evolution of a premature stop codon (Δ). Deletions resulting in frameshift (base position of the aligned *rbcL* sequence):  
1) 45-386; 2) 1217-1220; 3) 524; 4) 923-927; 5)225; 6) 517-523.  
Substitutions resulting in the evolution of a premature stop codon (base position of the aligned *rbcL* sequence): a) 522; b) 610; c) 60;  
d)1066 | 39   |
| 2.2    | Strict consensus of six most parsimonious trees generated using the *rbcL* operon (only *Harveya* and *Hyobanche* lineages shown). Each branch shows the number of synonymous substitutions over the number of non-synonymous substitutions, inferred from aligned *rbcL* sequences.  
The number of each type of substitution was inferred by mapping individual characters onto most parsimonious trees, averaging over all optimizations | 40   |
| 2.3    | The 5′-UTR contains three sequences of known function in *rbcL* expression, the −35 and −10 transcription promoters and the ribosome-binding site, six bases upstream of the *rbcL* start codon. *Harveya* is polymorphic at two of these sites, substitutions evident in |
the –10 transcription promoter and the ribosome binding site. In both cases, the majority of Harveya species have identical nucleotide sequences as the hemiparasites and Hyobanche. At the fourth position of the –10 transcription promoter, the change from a C to a T is synapomorphic for the clade containing Harveya pulchra, H. huttonii, H. tubulosa, and H. silvatica (s 1 and 2). At the second position of the ribosome binding site, the substitution from G to A is autapomorphic in Harveya stenosiphon. Stem structures of the 3’UTR-IR (in gray) are nearly identical in all species examined. Sequences here vary mainly by terminal loop length. Harveya sequences fall into three groups varying by loop length: Harveya 1: clade H. huttonii, H. coccinea, H. pulchra, H. silvatica, H. speciosa and H. scarlatina; Harveya 2: clade H. capensis, H. purpurea, H. bolusii, H. squamosa and H. stenosiphon; Harveya 3: H. hyobanchoides …………………………………………………………………………41

2.4 Detection of Rubisco by means of Western Blot. Initially, a custom antibody raised against Spinacia purified Rubisco was used to detect Rubisco in tissues of Harveya and Hyobanche (left). Shown here are results from: A. Harveya capensis leaf extract, B. Hyobanche glabrata calyx extract, and C. Spinacia purified Rubisco (7.5 µg total protein). Both the large (LSU) and small subunit (SSU) were present in Harveya and Hyobanche tissues as evidenced by oligomer bands composed of both subunits (~160 kDa and ~70 kDa bands). The presence of a 112 kDa band and a 56 kDa band indicate the presence of the small subunit. A second antibody raised against a peptide conserved in all type I Rubisco confirmed this result (right)…………………………………………………………………………42

3.1 Schematic illustrating binding sites of primers in cloning experiments. PCR cloning utilizing rbcL 5’ and “rbcL 3’ resulted in little nucleotide variation among cloned sequences. However, when the internal primers were used, multiple copies of rbcL were obtained from each individual of four species of Hyobanche…………………66

3.2 Jackknife consensus of rbcL cloned sequences (all of which were pseudogenes) from four individuals, one of each species of Hyobanche. The aligned matrix consisted of 1435 characters, and resulted in one most parsimonious tree (length = 112, C.I, = 0.821, R.I. =0.931). Redundant sequences were excluded from the analysis, and therefore, each terminal represents a distinct haplotype. Sequences from Harveya species were used to infer the root of the tree. Sequences were moderately to strongly supported as monophyletic in regard to the individual from which
they were cloned, with the exception of sequences from a single individual of *Hyobanche atropurpurea*, which were resolved as polyphyletic……………………………………………………………………………………..67

3.3 Jackknife consensus of a matrix of *rbcL* clones (narrow terminals) combined with *rbcL* sequences obtained conventionally (thick terminals). The matrix consisted of 1435 aligned nucleotides and resulted in 43 most parsimonious trees (length = 119; C.I. = 0.807; R.I. = 0.949). Sequences from *Harveya* species were used to root the tree. In this tree, none of the sequences obtained from species are monophyletic………………………………………………………………..68

3.4 Jackknife trees inferred from *rbcL* sequences obtained from multiple individuals of each of four *Hyobanche* species (*Hy. atropurpurea* = A; *Hy. glabrata* = G; *Hy. rubra* = R; *Hy. sanguinea* = S). To demonstrate phylogenetic error that may be introduced by sampling one sequence from each taxon when multiple sequences exist, one sequence was chosen to represent each species in phylogenetic analysis. All trees were rooted with an *rbcL* sequence from *Harveya pulchra*. Of fifteen possible rooted topologies for four taxa, ten were recovered with strong jackknife support………………………………………………….…69

4.1 Map of southern Africa showing the range of *Harveya* (cross-hatched), major mountain systems including the Cape Fold Belt, the Drakensberg (darkened), the Central Plateau, and provincial boundaries………………….….95

4.2 Jackknife consensus of the *rbcL* operon matrix. * sensu* Hilliard and Burtt (1986). **Harveya purpurea** subsp. purpurea……………………………………………………………………….96

4.3 Jackknife consensus of the ITS matrix. * sensu* Hilliard and Burtt (1986). **Harveya purpurea** subsp. purpurea……………………………………………………………………….97

4.4 Jackknife consensus of combined data from the *rbcL* operon and ITS matrices * sensu* Hilliard and Burtt (1986). **Harveya purpurea** subsp. purpurea……………………………………………………………………….98

4.5 Optimization of pollinator syndrome characters on phylogeny inferred from combined data. * sensu* Hilliard and Burtt (1986)………………..99

4.6 Cladogram showing approximate geographical distributions of terminals……100

5.1 *Harveya hyobanchoides* A. Plant. B. Flowers. C. Longitudinal section of the corolla tube showing pistil, stamens, and densely villose constriction at the point of stamen insertion. D. Dissection
of the corolla showing the same as “C” E. Dissected calyx.
F. Distribution………………………………………………………………………114

5.2 Harveya pumila. A. Plant growing on the roots of Anthospermum pumilum (Rubiaceae). B. Flower. C. Dissected calyx. D. Dissected corolla and pistil……………………………………………………………………………………………………………………122

5.3 Harveya pumila distribution……………………………………………………124

5.4 Harveya scarlatina. A. Plant. B. Flowers. C. Dissected corolla and calyx with pistil…………………………………………………………………………………………………………….128

5.5 Harveya scarlatina distribution……………………………………………….129

5.6 Harveya squamosa. A. Plant with haustorium. B. Flower.
C. Dissected corolla showing stamens and pistil. D. Dissected calyx.
E. Haustorial tuber in cross-section demonstrating the host root.
F. Distribution…………………………………………………………………………134

5.7 Harveya stenosiphon. A. Flowers. B. Corolla, dissected with pistil. C. Harveya stenosiphon parasitizing the roots of a deciduous tree, most probably a species of Pterocelastrus (Celastraceae)
D. Dissected calyx. E. Anther. F. Distribution……………………………………140

5.8 Harveya bodkini. A. Flowering stem. B. Distribution.
C. Dissected corolla, showing stamens and pistil. E. Anthers.
F. Interior of dissected calyx………………………………………………………………145

5.9 Harveya bolusii. A. Dissected corolla. B. Flower. C. Pistil.
D. Dissected calyx. E. Anther. F. Plant and haustorium…………………151

5.10 Harveya bolusii distribution………………………………………………….152

E. Fruit in cross-section. F. Dissected calyx. G. Anthers showing extreme reduction of the sterile theca. H. Distribution………………………………………162

5.12 Harveya capensis. A. Parasitizing Centella sp. (Apiaceae).
H. Capsule, cross section, showing lateral compression.
I. Seed. J. Anther. K. Distribution……………………………………………………174
5.13  *Harveya purpurea*  
A. *Harveya purpurea* subsp. *purpurea* parasitizing a species of *Lightfootia* (Campanulaceae).  
B. Disected calyx.  
C. *H. purpurea* subsp. *purpurea* dissected corolla and pistil.  
D. *H. purpurea* subsp. *purpurea* flower.  
E. *Harveya purpurea* capsule in cross section.  
F. Capsule before and after dehiscence…………………………………………………………….186

5.14  *Harveya purpurea* distribution………………………………………………………….187

5.15  *Harveya coccinea*  
A. Plant.  
B. Flowers showing the narrowing of the corolla within the calyx.  
C. Dissected calyx.  
D. Dissected corolla and pistil………………………………………………………………...196

5.16  *Harveya coccinea* distribution…………………………………………………………197

5.17  *Harveya huttonii*.  
Flowers and dissected corollas exhibiting variation in shape and size of corolla tubes, lobes, and ovaries.  
From left to right, Randle 119, Randle 130, and Randle 136………………………………….207

5.18  *Harveya huttonii*.  
A. Plant.  
B. Dissected calyces exhibiting variation in shape and size.  
From top to bottom, Randle 119, Randle 130, Randle 136……………………………………………………….208

5.19  *Harveya huttonii* distribution………………………………………………………….209

xvii
CHAPTER 1

INTRODUCTION

Parasitism in flowering plants

Parasitism, as defined in plants, is the condition in which plants acquire nutrients from host plants directly by means of a specialized organ of transport, the haustorium (Kuijt 1969). The presence of a haustorium distinguishes parasitic plants from mycorrhizal heterotrophs, which also obtain nutrients from host plants, but indirectly, through a fungal intermediary. Parasitism has arisen at least nine times in the angiosperms, and more than 4,000 species of parasitic flowering plants are represented in 22 families and 265 genera (Nickrent et al. 1998). Parasitic plants are phylogenetically diverse, having arisen in a several major clades of angiosperms (see phylogenies of the Angiosperm Phylogeny Group 1998, 2003; Soltis et al. 2000).

Cassytha (Laurales; Lauraceae) and the family Hydnoraceae (Piperales) are members of the Magnoliid clade. Molecular data place Hydnoraceae in a clade with Lactoridaceae and Aristolochiaceae. While Hydnoraceae is almost certainly monophyletic, relationships with the remainder of the clade are as yet unresolved (Nickrent et al. 2002); these three families may later be supported as monophyletic, or conversely Aristolochiaceae may be expanded to include one or both of the others. The
order Santalales is composed entirely of parasitic families (Olaceae, Opiliaceae, Loranthaceae, Misodendraceae, Santalaceae, and Viscaceae); only Viscaceae and Opiliaceae have been supported strongly as monophyletic (Nickrent 1998), but analysis of multiple genes supports the monophyly of the order (APG, 1998). The precise phylogenetic placement of Santalales is unknown, but the order is strongly supported as a member of the core Eudicot clade (APG, 1998). Krameriaceae (Zygophyllales), a monogeneric family of shrubby parasites, is in the Eurosid I clade (APG, 1998; Soltis et al. 2000), as is the newly placed Rafflesiaceae (Barkman et al. 2004). Rafflesiaceae has long eluded phylogenetic placement, but mtDNA sequence data indicate that ordinal classification may require an expansion of Malphigiales to include it. Mitrastemonaceae has also long eluded phylogenetic placement, being most frequently allied with Rafflesiaceae. However, mtDNA data place this in the Asterid clade, sister to Ericales, which may also be expanded to include it (Barkman et al. 2004).

The Euasterid I clade exhibits three independent origins of parasitism: Lennoaceae, Cuscuta and Orobanche. The family Lennoaceae has traditionally been placed in Boraginales, an order not recognized by the Angiosperm Phylogeny Group. However, Lennoaceae has been shown to be closely related to Boragineae s.s., Cordiaceae, Ehretiaceae, Heliotropiaceae, and Hydrophyllaceae (a clade of families that has been referred to as Boraginales) in a phylogenetic analysis utilizing the nuclear rDNA ITS I locus (Gottschling et al. 2001). The genus Cuscuta, while at times placed in its own family Cuscutaceae, has been shown to be nested within in an otherwise autotrophic family, Convolvulaceae (Solanales) in analyses utilizing four chloroplast genes, rbcL, atpB, the psbE-J operon, and the trnL-trnF spacer (Stefanovic et al. 2002). All parasitic
members of Lamiales belong to Orobanchaceae (sensu Young et al. 1999). Many of these had been placed in Scrophulariaceae previously. Orobanchaceae may be the best known of the parasitic lineages, as it has several members that are economically important crop parasites, including species of the genera Orobanche (broomrape), Striga (witchweed), and Alectra (yellow witchweed).

Due to rapid evolution of the chloroplast genome and extreme morphological reduction (discussed below), several groups of parasitic plants are yet to be placed in the phylogeny of the angiosperms. In fact, of the sixteen unplaced taxa enumerated by the Angiosperm Phylogeny Group (excluding Rafflesiaceae and Mitrastemmonaceae; APG 2003), five are parasitic. Cynomorium is sufficiently reduced morphologically to have been initially categorized as a fungus, and its placement within the flowering plants was disputed until the mid 1800’s (Kuijt 1969). Other unplaced taxa include Bdallophyton, Cytinus, Balanophoraceae, and Apodanthaceae (APG 2003). Future studies may find that some of these taxa are allied with one another, an already placed group of parasites, or autotrophic taxa.

Parasitic plants exhibit a wide range of nutritional dependencies on host plants. Parasites that are not entirely dependent on host plants for water, minerals, and photosynthates have been termed hemiparasites. Nutritional acquisition may vary over the life span of a hemiparasitic plant, at times depending entirely on host plants for nutrients, and at other times being self-sufficient, and often exhibiting subtle gradations of dependence between these extremes. For example, the cotyledons of Tozzia (Orobanchaceae) do not emerge from the ground; instead, the plant spends the first years of its life as a subterranean parasite, entirely dependent on its host for all nutrients. In the
final life stage, a stem bearing chlorophyllous leaves emerges from the ground, the plant flowers, sets fruit and dies (Kuijt 1969). Some hemiparasites may survive free from a host plant for the entire life-cycle, albeit with decreased fitness. *Rhinanthus minor* demonstrates five times the rate of CO$_2$ assimilation when grown on *Trifolium repens* as opposed to on *Echium vulgare* or when free-living, probably due to low-levels of nitrogen uptake from the latter host (or when unattached) resulting in reduced allocation of resources to photosynthetic apparatus (Seel et al.1993). On the other hand, photosynthetically-capable hemiparasites may survive without undergoing photosynthesis; *Striga asiatica*, apparently photosynthetic, can also grow to maturity and reproduce if never exposed to light (Kuijt 1969). In any case, hemiparasites are photosynthetically capable to some extent and, other than the haustorium, appear in many ways indistinguishable from autotrophic plants.

Conversely, holoparasitic plants are entirely dependent on host plants for nutritional requirements. Holoparasitic plants are not capable of photosynthesis, and exhibit reduction in structures and functions associated with photosynthesis and nutrient acquisition. Leaves are reduced to scales or absent. Likewise, root systems and stems tend to be reduced or absent when not expressed in modified form as haustoria. Due to extreme reduction in vegetative structures, holoparasites often exist as nodes of undifferentiated tissue attached to the host plant through most of the life-cycle, appearing to be “plant-like” only when flowering. Those taxa that parasitize the roots of host plants may appear aboveground only when flowering, or in the case of *Hydnora triceps*, not at all (Musselman and Visser 1989; Nickrent et al. 2002). Holoparasites also frequently lack chlorophyll, giving vegetative structures a unique non-green appearance (de la Harpe
Plastids in holoparasites commonly do not reach full maturation and may function as amyloplasts (Dodge and Lawes 1974; Machado and Zetsche 1990). Additionally, holoparasites as well as photosynthetically functional hemiparasites may express fewer plastids per cell than non-parasitic plants (Dodge and Lawes 1974; de la Harpe et al. 1980). Often, plants have been categorized as holoparasites on the basis of these characteristics alone.

In addition to reduction in morphological and anatomical features associated with photosynthesis, parasitic lineages may also experience rapid genomic evolution as functional constraints on photosynthesis-related genes are relaxed (reviewed in Nickrent et al. 1998). It has been shown that many photosynthetic genes in holoparasitic lineages experience higher rates of substitution than in closely related photosynthetic plants resulting in pseudogene formation through the evolution of premature stop codons and frameshift mutations at coding loci, and gene deletion. As a result, many lineages of holoparasites have severely truncated plastid genomes. The particulars of this phenomenon will be discussed in detail in Chapter 2, but it will suffice here to say that the manner in which holoparasitic lineages undergo plastome reduction may be informative to the function of photosynthetic genes in fully autotrophic plants.

**Hypotheses of rbcL evolution in Harveya and Hyobanche**

In Orobanchaceae, *sensu* Young et al. (1999) hemiparasitism is apomorphic in all but one genus, *Lindenbergia* Lehm., which is sister to the rest of the clade. The phylogeny of this family, based on combined *matK* and *rps2* sequence data, requires at least five transitions from hemi- to holoparasitism. The genera *Harveya* Hook. and *Hyobanche* L. (Orobanchaceae) have been categorized as holoparasites due to vegetative reduction and
what appears to be a lack of chlorophyll in most plant organs. These genera have been supported as sister taxa in phylogenetic studies of Orobanchaceae (Young et al. 1999; Wolfe et al. unpublished), the ancestor of which represents one of the five putative transitional nodes from hemiparasitism to holoparasitism. In examining the sequence evolution of the plastid gene encoding the large subunit of Rubisco, \textit{rbcL}, Wolfe and dePamphilis (1998) found that pseudogene formation was evident in \textit{rbcL} sequences of \textit{Hyobanche}, whereas \textit{Harveya} maintained an open reading frame. There are two explanations for this phenomenon: 1) \textit{rbcL} is evolving free from functional constraint in both genera, and the presence of an open reading frame in \textit{Harveya} is due to an overall slower base-rate of evolution, or 2) the open reading frame is being maintained in \textit{Harveya} by selection. If the latter is true, the large subunit of Rubisco must be expressed and functional to some extent in \textit{Harveya}, which logically necessitates that one of the following hypotheses is true: 1) the large subunit of Rubisco functions in the fixation of carbon dioxide in \textit{Harveya}, in which case, \textit{Harveya} is not a holoparasite, but a cryptic hemiparasite, or 2) the large subunit of Rubisco has some as-yet-unknown function in \textit{Harveya}.

**Systematics of Harveya**

\textit{Harveya} and \textit{Hyobanche} are perennial herbs of Africa, about which little else is known. There are seven named species of \textit{Hyobanche}, but the genus has not been treated taxonomically and species delimitations are tenuous. Recent preliminary work indicates that there may be as many as 12-15 species, and that our understanding of the evolutionary history of this group is incomplete (Wolfe and Randle 2001; Wolfe et al. in prep).
Harveya was described by William Jackson Hooker (1837) based on the species Harveya capensis of the Western Cape of South Africa, named after the then Treasurer of the Cape Colony and later Professor of Botany at Trinity College, Dublin, William Henry Harvey. Species named in Aulaya Harv. (Harvey 1838) were later included in Harveya as were several species described as belonging to Orobanche L. by Thunberg (1794, 1823). There are 53 species combinations in Harveya, approximately 35 of which are attributed to species living in southern Africa.

Most species of Harveya are found in South Africa, but the range of this genus extends northward on the west to Angola and the Democratic Republic of the Congo, and to the east through Swaziland, Zambia, Burundi, Rwanda, Tanzania, Kenya, and Eritrea to Yemen on the Arabian Peninsula (Figure 1.1). Harveya exhibits a wide range of vegetative and floral morphology, specifically in color, shape and size of the corolla and calyx (Fig. 1.2). The corolla may be as long as 12 cm in the large, white flowers of Harveya speciosa Bernh. or scarcely as long as 2 cm in H. bolusii Kuntze, which also differs from H. speciosa in having a brilliantly scarlet corolla with a yellow throat.

Corolla tubes, limbs and throats are commonly different colors in a single flower, as in H. purpurea subsp. purpurea, which bears a white tube, a purple limb, and an ochre-yellow throat, or in H. hyobanchoides Hiern, of which the limb is chlorophyllous green, with a butter-yellow throat and greenish-yellow tube. Flowers and vegetative structures of Harveya squamosa (Thunb.) Steud. vary within and among populations from bright orange to sulphur yellow. Some flowers also exhibit variation in floral color within individuals, as in H. pumila, which may bear a white to rosy colored limb. Corolla tube shape ranges from the narrowly cylindrical H. stenosiphon Hiern to the expanded tube of
*H. huttonii* Hiern. *Harveya* includes dwarf species that appear as simple inflorescences emerging from the substrate, the stem having been reduced to be practically non-existent (as in *H. pumila* and *H. scarlatina* (Benth.) Steud.), as well as large plants such as *H. speciosa*, which may grow to more than a meter high.

Like other members of Orobanchaceae, *Harveya* obtains nutrients through haustoria attached to the roots of host plants. *Striga* and *Alectra*, close relatives of *Harveya*, are reported to bear only primary haustoria—those that arise from the radicular apex of the emergent seedling (Weber 1980). The development and morphology of the haustorium has only been examined closely for one species, *Harveya speciosa* (Young 1932). In this study, Young found that *H. speciosa* forms primary haustoria, but afterwards, minute lateral root branches may come into contact with host roots, and these form secondary haustoria. Secondary haustoria may be attached to the same host as the primary haustorium, or different host individuals. In one case, *H. speciosa* appeared to have formed a primary haustorium on *Haplocarpha scaposa* (Asteraceae) and secondary haustoria on an unidentified grass species. The primary haustorium develops into a tuberous mass, which functions in storage of starch as well as transfer of nutrients. While phloem development in the roots of the parasite appears to be normal, the haustorium itself lacks phloem, and contact with the host stele is accomplished primarily via tracheids, argued to be the main carbohydrate-transporting cells. Indeed, the tuberous primary haustorium may grow quite large before aerial stems of the parasite emerge from the soil surface. Secondary haustoria have not been reported in any other species of *Harveya*. However, when underground systems are excavated for study, the lateral roots connecting secondary haustoria to *Harveya* may be easily broken.
Little is known about the host range and specificity of *Harveya* species. While some authors have identified host plants, it is often unclear if these determinations were made by the excavation of underground haustoria or simply by identifying the closest possible host plant. I have observed that flowering stems of *Harveya* may be up to half a meter distant from host plants making haustorial excavation necessary for these determinations.

*Harveya* has not been taxonomically treated as a whole, though some excellent treatments exist for species restricted to given localities (Hiern 1904; Hilliard and Burtt 1986; Goldblatt and Manning 2000). However, in many cases, species delimitations are suspect, and many species have been collected only rarely. Complete assessments of species’ geographic distributions and host-ranges are lacking. Further, nothing is known about the phylogenetic affinities among species of *Harveya*.

In Chapter 2, the evolution and expression of *rbcL* are examined in *Hyobanche* and *Harveya*. Sequences were obtained for the entire *rbcL* operon—*rbcL* and the flanking untranslated regions, which have transcriptional and translational function—to determine if the open reading frame in *Harveya* has evolved under purifying selection and if the flanking regions are sufficiently intact to allow expression of the coding sequence. Western blot analysis was employed to detect the large subunit protein in tissues of *Hyobanche* and *Harveya*. In Chapter 3, patterns of *rbcL* evolution are further investigated by the detection of multiple copies of the gene in *Hyobanche* through PCR cloning, and through isolation and sequencing of RNA transcripts.

In Chapter 4, the phylogeny of *Harveya* is inferred from nucleotide sequence data, including the *rbcL* operon and the nuclear rDNA internal transcribed sequence (ITS)
locus. The phylogeny was used to investigate the evolution of diverse floral morphologies of *Harveya* species, shifts in host-ranges, and geographical origins of species. Chapter 5 is a taxonomic revision of *Harveya* species of southern Africa. It includes a diagnostic key to species and revised species delimitations with records of geographic distribution, host-ranges, and illustrations of morphological features of species.
Figure 1.1. Geographical distribution of *Harveya* and *Hyobanche*.
Figure 1.2 A. Harveya bolusii  B. Harveya capensis  C. Harveya scarlatina  D. Harveya hyobanchoides  E. Harveya huttonii  F. Harveya speciosa  G. Harveya squamosa  H. Harveya purpurea subsp. purpurea  I. Two color morphs of Harveya pumila. Photo credits: Jenny Archibald (A,B,F,H); Andrea Wolfe (C,D,G); Cameron McMaster (E, I).
Figure 1.2 continued
CHAPTER 2

THE EVOLUTION AND EXPRESSION OF RBCL IN HOLOPARASITIC SISTER-GENERA HARVEYA HOOK. AND HYOBANCHE L. (OROBANCHACEAE):
THE PRESENCE OF RUBISCO IN THE ABSENCE OF A FUNCTIONAL GENE.

INTRODUCTION

The endosymbiotic origins of the eukaryotes have necessitated the tight integration of organellar and nuclear gene expression. Comparative studies of the genomes of organelles and close relatives of presumed endosymbionts demonstrate genomic rearrangements, gene deletions, and organellar transfer events, many of which presumably increased fitness of eukaryotes by allowing more efficient control of gene expression in response to developmental cues and external stimuli. The result in the plant kingdom is the tight inter-regulation of chloroplast, mitochondrial, and nuclear gene expression with the vast majority of regulatory genes encoded in the nucleus (Martin and Herrmann 1998).

The expression of the holoenzyme Rubisco (ribulose 1,5-bisphosphate carboxylase oxygenase) involves nuclear-chloroplast intercompartmental regulation. Perhaps the single most influential enzyme in the development of the biosphere, Rubisco
fixes atmospheric CO$_2$ during the Calvin cycle of photosynthesis, providing virtually all chemical energy used by organisms, and producing the oxidizing conditions of the current atmosphere. Yearly, 40 million tons of Rubisco (~100 lbs. for every living person) convert 100 billion tons of carbon dioxide into glucose (Miziorko and Lorimer 1983). In green algae and land plants, Rubisco is a hexadecamer composed of eight large subunits and eight small subunits. The gene encoding the small subunit, $rbcS$, was transferred to the nucleus in the ancestor of this clade, while expression of the large subunit gene, $rbcL$, is maintained in the chloroplast. Cytoplasmic expression of the small subunit has been shown to regulate large subunit translation (Rodermel et al. 1996, 1999), an important example of intercompartmental gene regulation in eukaryotes. Given the importance of Rubisco, it is no surprise that its function in photosynthetic organisms has been well documented from the level of gross biochemistry to amino acid and nucleotide sequence (Kellogg and Juliano 1997). It is also not surprising that evolution of $rbcL$ is well conserved, providing phylogenetic signal for ancient clades of land plants and photosynthetic single-celled organisms (Chase et al. 1993; Pryer et al. 1995; Delwiche and Palmer 1996; McCourt et al. 2000; Nickrent et al. 2000; Zanis et al. 2002). The expression of Rubisco in several non-photosynthetic plants and algae indicates that this enzyme may play a role in non-photosynthetic life processes.

In holoparasitic plants, endogenous photosynthesis is not required, and therefore selection pressure on photosynthesis-related genes is lessened. The expected outcome of this loss of functional constraint is the rapid evolution of these genes resulting in increased rates of amino acid substitutions for protein-coding genes, pseudogene formation through the evolution of premature stop codons and indels resulting in
frameshift, and gene deletion. Studies of the chloroplast genomes of holoparasites reveal multiple losses of gene function. The chloroplast genome of the holoparasite *Epifagus virginiana* (Orobanchaceae) was one of the first sequenced and exhibits extreme reduction in genome size and gene content (dePamphilis and Palmer 1990; Morden et al. 1991; Wolfe et al. 1992a; Wolfe et al. 1992b). Similarly, plastome truncation is evident in holoparasites of Orobanchaceae, such as *Conopholis americana* (Wimpee et al. 1991; Colwell 1994), *Orobanche* spp. (Thalouarn et al. 1994; Lohan and Wolfe 1998), and *Lathraea clandestina* (Delavault et al. 1996), as well as *Cuscuta* spp. (Convolvulaceae; Bömmer, et al. 1993; Freyer et al. 1995), and *Cytinus* (Cytinaceae). In extreme cases, the plastome is yet to be detected, as in *Corynaea* (Balanophoraceae) and *Hydnora* (Hydnoraceae; Nickrent et al. 1997). In *Boschniakia, Hyobanche*, and *Orobanche* (all genera of Orobanchaceae), *rbcL* has undergone pseudogene formation (Wolfe and dePamphilis 1998). Although many holoparasitic plants have a reduced plastid genome, the plastid has retained function in several genera, primarily in gene expression apparatus. The plastome of *Epifagus virginiana* is by far the most well known of the above. Pseudogene formation or gene deletion has occurred at all photosynthetic and chemorespiratory loci of this plastid. Of the 42 intact genes in the *Epifagus* plastome, 38 encode ribosomal proteins, rRNA, and tRNA, while four others have unknown function (Wolfe et al. 1992). Correctly modified transcripts for a number of these genes have been detected *in vivo* indicating the retention of plastid function in *Epifagus* (Ems et al. 1995). The chloroplast functions in a number of non-photosynthetic biosynthetic pathways, and it is therefore reasonable to expect the plastid to retain minimal function even when photosynthesis is no longer necessary.
Studies have also shown the retention and expression of photosynthesis-related genes in holoparasitic plastids. In *Cuscuta reflexa, psbA* and *rbcL* are well conserved, despite the deletion of the expression-related genes *trnL, rpl2*, and *rpl23* (Bömer, et al. 1993). *RbcL* is expressed at low levels in the holoparasite *Lathraea clandestina* (Thalouarn, et al. 1989; Delavault et al. 1996) and in the heterotrophic euglenoid *Astasia longa*, which also exhibits a significantly reduced plastome (Siemeister and Hächtel 1990). A number of other holoparasites retain a functional copy of *rbcL* in the plastid. Examination of rates of synonymous and non-synonymous substitution indicates evolution under purifying selection for *Harveya purpurea, Striga gesneroides, Orobanche fasciculata* and *O. corymbosa* (Wolfe and dePamphilis 1997, 1998; Leebens-Mack and dePamphilis 2002).

*Harveya* and *Hyobanche* are recently derived holoparasitic sister-genera exhibiting alternative pathways of *rbcL* evolution; *Harveya* maintains an open reading frame (ORF) while multiple mutations have resulted in pseudogene formation in *Hyobanche* (Wolfe and dePamphilis 1998; Wolfe and Randle 2001). Both plants seem to lack chlorophyll or fully developed chloroplasts and are thus presumably incapable of photosynthesis (de la Harpe et al. 1980). It is logical to assume that the ancestor of these genera was also a holoparasite, and therefore it is curious that *Harveya* should maintain a seemingly functional form of *rbcL* while *Hyobanche* does not. In this study, the evolution of *rbcL* was examined in a phylogenetic context to determine the role of selection in the maintenance of an ORF in *Harveya* and the loss of function through pseudogene formation in *Hyobanche*. Patterns of *rbcL* expression in *Harveya* and *Hyobanche* were also examined using Western Blot.
MATERIALS AND METHODS

DNA sequencing

Tissues were collected in the field and desiccated on silica gel\(^1\). DNA was extracted from tissues using a modification of Doyle and Doyle’s CTAB protocol (1987) and further purified using the Eluquik system (Schleicher and Schull; Keene, NH). Amplifications of the entire region encompassing the 5’-untranslated region (UTR), \textit{rbcL}, and the 3’-UTR were carried out using the primers 766+970 and H3UTR (Table 2.1). Amplification reactions of 50 µl contained 0.5-4.0 µl purified DNA, 0.2 mM dNTPs, 0.15mM MgCl\(_2\), 0.64 µM each primer, 1U \textit{Taq} polymerase (Invitrogen; Carlsbad, CA), and 1X \textit{Taq} polymerase amplification buffer. Reactions were carried out under the following temperature conditions: 2 min. at 94°C ; 35 cycles of 1 min. at 94°C, 1 min. at 50°C, and 2 min. at 72°C; and a final extension of 10 min. at 72°C. Amplified DNA was purified on polyethylene glycol, followed by two ethanol precipitations (85% and 100% respectively) and resuspended in ddH\(_2\)O. Cycle sequencing was carried out using the standard ABI Big Dye 2.0 protocol and sequences were obtained using an ABI-3100 automated sequencer (Applied Biosystems; Foster City, CA).

DNA sequence analysis

Nucleotide sequences of \textit{rbcL} and inferred amino acid sequences were compared to sequences from \textit{Nicotiana tabacum} to investigate pseudogene formation, evidenced by the presence of premature stop codons and/or frameshift mutations. 5’-UTR, \textit{rbcL} and 3’-UTR sequences were aligned in Clustal X (Thompson et al. 1997) and manually

\(^1\) The text of this and the following chapter refer to the species \textit{Harveya pulchra}, \textit{H. leucopharynx}, and \textit{H. silvatica}. These three species have now been subsumed into \textit{Harveya huttonii} (see Chapter 5).
adjusted using the Se-Al data editor (Rambaut 1996). Closely related genera in Orobanchaceae included as outgroups were *Alectra sessiliflora*, *Buchnera floridiana*, *Cynium racemosum*, and *Melasma scabrum*. The entire region was used to infer the phylogeny of the group using PAUP*4.0b10 (Swofford 2002) with parsimony as the optimality criterion. A tree search was carried out with 500 random sequence additions, five trees held at each step, and TBR branch swapping. One thousand jackknife replicates using TBR branch swapping, 37% deletion, and the “emulate Jac” options were used to estimate clade support. The resulting topology was used in MacClade 3.08a (Maddison and Maddison 1992) to estimate the number of synonymous and non-synonymous substitutions occurring in *rbcL* on each branch averaged over all optimizations. To further investigate the effects of functional constraint on the evolution of *rbcL* in *Harveya* and *Hyobanche*, the number of synonymous substitutions per synonymous site (d_S) and non-synonymous substitutions per non-synonymous site (d_N) were calculated using MEGA 2.1 (Kumar et al. 2001). Codons for which mutations resulted in premature termination or frameshift mutations (via indels) were excluded from the analysis. D_S and d_N were calculated in a pairwise manner, using the modified Nei and Gojobori method (Nei and Gojobori 1986; Kumar et al. 2001) with Jukes-Cantor correction for the *Harveya + Hyobanche* lineage and for each genus separately. D_S and d_N values were compared in each clade (*Harveya + Hyobanche*, *Harveya*, and *Hyobanche*) using the Wilcoxon Signed Ranks test in SPSS (SPSS Inc. 1999) to test the null hypothesis of neutral evolution (H_0: d_S = d_N). Three regions of known function in the 5’-UTR were examined for mutations: the -35 and -10 transcription promoter sequences and the ribosome-binding site (Shinozaki and Sugiura 1982; Mullet 1988; Herrmann at
al. 1992; Gillham et al. 1994; Mayfield et al. 1995). The 3’-UTR ends in a terminal inverted repeat (IR) which functions in transcript processing and stability (Schuster and Gruissem 1991; Mayfield et al. 1995; Rott et al. 1998). RNA sequences were used to infer secondary structure of 3’-IRs on the M-fold server (Mathews et al. 1999; Zuker et al. 1999). The most thermodynamically stable configurations were chosen for comparison, although few differences were evident in less stable configurations.

**Western Blot Detection of Rubisco**

Tissues were collected in the field from nine species of *Harveya* (*H. capensis, H. pulchra, H. purpurea, H. scarlatina, H. silvatica, H. speciosa, H. squamosa, H. stenosiphon,* and *H. coccinea*), four species of *Hyobanche* (*Hy. atropurpurea, Hy. glabrata, Hy. rubra,* and *Hy. sanguinea*) and from five species of closely related hemiparasites (*Alectra capensis, Buchnera glabra, Cycnium racemosum, Melasma scabrum* and *Pedicularis lanceolata*), which served as positive controls. Rubisco may be differentially expressed in plant organs (Mayak et al. 1998; McCormac et al. 2001) and, therefore, scale leaves, stems, calyces, and corollas were collected from *Harveya* and *Hyobanche* populations when feasible (if more than fifteen flowering stems were evident in a population). Tissues were flash frozen in liquid nitrogen and transported to the lab on dry ice before storage at –80°C.

Tissues were ground in extraction buffer [100mM Tris, 0.5% polyethylene glycol, 1mM EDTA, 100mM 2-mercaptoethanol; pH 8.0] and centrifuged to remove non-soluble components. The volume of extraction buffer used was normalized according to dry tissue weight, 0.5g dry tissue per 1.0 ml extraction buffer. Extracts were combined with loading buffer [100mM Tris, 4.0% SDS, 2.0% (w/v) bromophenol blue, 20% glycerol,
and 200mM DTT] and were separated on 10% polyacrylamide gels containing 0.1% SDS. In one lane of each gel, 7.5µg of Spinacia-purified Rubisco (Sigma; St. Louis, MO) was used as a positive control. Full Range Rainbow Molecular Weight Marker (Amersham Pharmacia, Piscataway, NJ) was run in one lane on each gel to estimate band size.

Gels were electrophoretically blotted onto Hybond-P membranes (Amersham-Pharmacia, Piscataway, NJ). Binding sites on membranes were blocked for one hour in 5% non-fat dry milk (with 0.1% Tween) in phosphate buffered saline solution (Sambrook et al. 1989). Two anti-Rubisco primary antibodies were used to assay for the presence of Rubisco on membranes. Anti-Rubisco custom serum (Sigma; St. Louis, MO) was raised in rabbits against Spinacia-purified Rubisco. A second primary antibody, consisting of the IgY fraction of chicken serum raised against a peptide target conserved in all type I large subunits, was available commercially (Agrisera; Vännäs, Sweden). Primary antibodies were diluted in 5% non-fat dry milk/ phosphate-buffered saline solution, at dilutions of 1:1000 for rabbit anti-Rubisco serum and 1:5000 for the chicken anti-Rubisco IgY. Membranes were incubated in the diluted primary antibody for one hour at room temperature, and then probed with a horseradish peroxidase (HRP) conjugated secondary antibody, either 1:5000 goat anti-rabbit IgG-HRP (Amersham Pharmacia; Piscataway, NJ) or 1:5000 rabbit anti-chicken IgG HRP (Pierce; Rockford, IL). Banding patterns were visualized using the ECL Plus Western Blotting System (Amersham Pharmacia; Piscataway, NJ) and fluorescence images were captured on ECL Hyperfilm (Amersham Pharmacia, Piscataway; NJ).
RESULTS

The evolution of rbcL in Harveya and Hyobanche

The full rbcL operon (the 5’-UTR, rbcL, and the 3’-UTR) ranged in length from 1698 base pairs in Harveya huttonii to 2104 base pairs in Hyobanche rubra. All species of Hyobanche examined have undergone rbcL pseudogene formation, as evidenced by the evolution of premature stop codons and deletions resulting in frameshift (Figure 2.1). All species of Harveya examined maintain open reading frames at this locus, except for Harveya huttonii, which bears an autapomorphic 341 nucleotide deletion. As such, deletions may be PCR artifacts, rbcL of H. huttonii was re-amplified and re-sequenced with the same result. Alignment of the combined 5’-UTR, rbcL, and the 3’-UTR sequences for twenty taxa yielded a matrix of 2,193 characters, of which 99 were parsimony-informative. A parsimony search of combined sequences resulted in six most parsimonious trees (C.I.=0.870; R.I. = 0.850). Several of the mutations contributing to pseudogene formation are synapomorphies, supporting clades within the Hyobanche lineage, including two deletions uniting Hy. sanguinea, Hy. rubra, and Hy. glabrata, as well as two substitutions resulting in premature stop codons that unite Hy. rubra and Hy. glabrata (Figure 2.1). Hyobanche is strongly supported as monophyletic, with a jackknife of 100% (1,000 random sequence addition searches with TBR). Harveya, on the other hand, is less strongly supported (jackknife = 65%). However, lack of support for the Harveya clade is likely to be the result of short branches due to few parsimony-informative sites within this lineage (Figure 2.1). Both clades are strongly supported in a phylogenetic analysis utilizing ITS nrDNA (Wolfe et al. unpublished), and this gene in combination with matK, trnL and rps2 (Wolfe, et al. in preparation).
Two hypotheses may explain the difference in \textit{rbcL} evolution in \textit{Harveya} and \textit{Hyobanche}: a) pseudogene formation in \textit{Hyobanche} but not \textit{Harveya} is the result of stochastic events, and b) selection acting under functional constraint has prevented \textit{rbcL} pseudogene formation in \textit{Harveya}, while \textit{rbcL} in \textit{Hyobanche} has been evolving free from functional constraint. A comparison of synonymous and non-synonymous substitution rates is often used as an indicator of the type of selection operating on a protein-coding gene. Randomly evolving sequences are expected to have a rate of synonymous substitution ($d_s$) equal to non-synonymous substitutions ($d_N$); therefore, $d_s/d_N$ is approximately 1.0 for randomly evolving genes. A ratio significantly less than one is interpreted as an indication of positive selection (fixation of advantageous mutations), whereas a ratio greater than one may indicate purifying selection (selection against changes in amino acid sequence; Hughes and Nei 1988, 1989). To test competing hypotheses of the effect of selection on \textit{rbcL} in \textit{Harveya} and \textit{Hyobanche}, synonymous and non-synonymous substitutions were examined in a phylogenetic context and $d_s/d_N$ ratio was estimated for both the \textit{Harveya} and \textit{Hyobanche} clades.

The number of synonymous substitutions in the evolution of the \textit{rbcL} coding sequence was considerably greater than the number of non-synonymous substitutions for the \textit{Harveya} lineage (79.1\% synonymous; Figure 2.2). Within this lineage, 34\% of all substitutions occur as autapomorphies on the branch leading to \textit{Harveya huttonii}, the only species of \textit{Harveya} surveyed in which \textit{rbcL} pseudogene formation has occurred. On this branch, nine of 13 (69\%) substitutions are synonymous. The percentage of substitutions that are synonymous in the \textit{Harveya} lineage far exceeds that inferred for \textit{Hyobanche}, at
37.3%. In short, the rate of substitution appears to be greater in *Hyobanche* than *Harveya*, and substitutions have much less frequently resulted in amino acid change in the *Harveya* lineage.

This is supported by comparison of $d_s/d_N$ ratios for each clade. To calculate $d_s$ and $d_N$, stop codons or codons that contained part of an indel were excluded from the analysis. The inclusion of *Harveya huttonii* (which has a 341 base deletion) requires a significant portion of the matrix to be ignored, and therefore, calculation of $d_s$ and $d_N$ values was performed with and without *H. huttonii*. The inclusion or exclusion of *H. huttonii*, however, had little bearing on the results. In the *Harveya* lineage $d_s/d_N$ is significantly greater than 1.0 with or without the inclusion of *H. huttonii* in the analysis (with: $d_s/d_N = 11.0$, $p<0.001$; without: $d_s/d_N = 40.50$, $p<0.001$; Table 2.2). This indicates that *rbcL* has evolved under purifying selection in the *Harveya* lineage. In the *Hyobanche* lineage, *rbcL* appears to have evolved randomly (without functional constraint) as $d_s/d_N$ is not significantly different from 1.0 (with *H. huttonii*: $d_s/d_N = 0.90$, $p=0.686$; without: $d_s/d_N = 0.86$, $p=0.249$).

**5’- and 3’- untranslated regions**

The 5’- and 3’-UTRs are co-transcribed with *rbcL* and function in the expression of the large subunit of Rubisco. If selection has affected the evolution of *rbcL* in the *Harveya* lineage, one would expect that the 5’- and 3’-UTRs are evolving under some degree of selection also. Conversely, the evolution of pseudogenes in *Hyobanche* suggests that the 5’- and 3’-UTRs have been evolving free from functional constraint. The role of selection in the evolution of a non-coding gene is more difficult to determine than for a coding gene. However, the UTRs include several sequences and secondary
structures of known function. Therefore, these sequences and structures in Harveya and Hyobanche were compared with sequences from closely related hemiparasitic species with known photosynthetic function (Alectra sessiliflora, Buchnera floridiana, Cycnium racemosum, and Melasma scabrum).

The 5’-UTR contains three sequences of known function in rbcL expression: prokaryote-like –35 and –10 transcription promoter sequences, and a ribosome-binding site just upstream of the rbcL start codon (Shinozaki and Sugiura 1982). At two of the three sites of known function of the 5’-UTR, one may infer several changes in Harveya, compared to those of the hemiparasitic taxa and Hyobanche (Figure 2.3). However, the sites at which these changes occur, within the –10 promoter and the ribosome-binding site, are polymorphic in Harveya. The substitution at the fourth position of the –10 transcription promoter is a synapomorphy uniting the Harveya huttonii + H. coccinea +, H. pulchra + H. silvatica clade. The substitution at the fourth position of the ribosome binding is autapomorphic for H. stenosiphon. The majority of Harveya species have sequences identical to their photosynthetic relatives at these sites of known function. Therefore, in most species of Harveya, substitutions in the 5’-UTR are not sufficient to preclude rbcL expression. More curiously, substitutions in the 5’-UTR are also not sufficient to preclude rbcL gene expression in Hyobanche, even though pseudogene formation has been demonstrated in this lineage.

The 3’-UTR ends in an inverted repeat structure (IR), which functions in transcript stability and processing (Schuster and Gruissem 1991; Rott et al. 1998). Most substitutions occurred upstream or downstream of the IR in all taxa examined, and the secondary structure of the IR was strongly conserved, differing primarily by the length of
the terminal loop (Figure 2.3). *Hyobanche* exhibited secondary structure identical to that of the hemiparasitic outgroups, while *Harveya* demonstrated variation in the length of the stem and terminal loop. However, length variation was small for both structural characteristics. Loop length varied from three to eight nucleotides in *Harveya* (four in *Hyobanche* and hemiparasites). Stem length varied slightly in *Harveya* as well; all but one species have a stem of fourteen paired nucleotides (thirteen in *Hyobanche* and the hemiparasites). *Harveya hyobanchoides* exhibited an insertion in the terminal loop, resulting in a stem of eighteen paired bases. It is difficult to determine if these changes alter the function of the 3’-UTR. Low concentrations of *rbcL* mRNA were discovered in tissues of the holoparasitic species *Cuscuta reflexa* despite the deletion of the entire 3’-UTR-IR sequence, although large subunit polypeptides were not detectable (Haberhausen et al. 1992). This indicates that transcription in *H. hyobanchoides* may occur. *Hyobanche* and the hemiparasites have IR structures identical to that of *Nicotiana tabacum* indicating a high level of conservation.

**Western Blot Detection of Rubisco**

All but one species of *Harveya* surveyed maintain open reading frames at the *rbcL* locus, appear to have evolved under purifying selection at this locus, and bear no mutations that clearly prevent expression of *rbcL*. Thus, it was expected that the gene product of *rbcL*, the large subunit of Rubisco, would be present to some extent in the tissues of *Harveya*. It was also expected that *Hyobanche* tissues lack this holoenzyme, as *rbcL* pseudogene formation is apparent in all species assayed, and *rbcL* pseudogene evolution appears to have been effectively random in the *Hyobanche* lineage.
Initially, all Western Blotting was performed using a custom antibody raised in rabbits against *Spinacia*-purified Rubisco. Bands representing large and small subunits of Rubisco were present in tissues of both *Harveya* and *Hyobanche*, albeit with immunoreactivity 20-100 fold less than in an equivalent quantity of *Spinacia* leaf tissue. These results were anomalous, given the presence of pseudogenes at the *rbcL* locus in all specimens of *Hyobanche* examined. Therefore, a second antibody generated against a peptide fragment conserved in all type I large subunits (Agrisera; Vännäs, Sweden) was used in an attempt to replicate these data. This second probing confirmed previous findings (Figure 2.4).

Rubisco is a hexadecamer, composed of eight large (LSU) and eight small subunits (SSU). Dissociation of the holoenzyme during electrophoresis results in oligomers of varying molecular weight composed of combinations of large and small subunits. The small subunit is encoded by the nuclear gene family, *rbcS*, and varies in mass from 12-18 kDa (Berry-Lowe et al. 1982; Miziorko and Lorimer 1983; Rodermel 1999). The gene *rbcS* and the small subunit that it encodes have not been characterized in the Lamiales. The evolution of this gene family in other Asterid lineages is complex and varied (Dean et al. 1989). The small subunit may be expressed within individuals as isoforms of varying molecular weight and isoelectric properties. Molecular weight divergence of the small subunit in Asterid groups appears to be minimal, but amino acid substitutions may be important in determining electrophoretic properties (Ren et al. 1991), especially when low-denaturation conditions are utilized, as in the present study.
Several faint bands were present in experimental lanes that were not observed in purified *Spinacia* Rubisco. I infer that these bands represent oligomers of large and small subunits that include small subunit isoforms not present in *Spinacia*.

Bands detected were of five major molecular weight categories: a) the holoenzyme and octamer (8 LSU + 8 SSU) bands, which were difficult to distinguish due to their large sizes (~300-600 kDa); b) bands of approximately 140-160 kDa (2 LSU+2 SSU); c) a band of 112 kDa (2 LSU); d) bands of 70-75 kDa (1 LSU + 1 SSU); e) a band of 56 kDa (1 LSU); and f) bands less than 30kDa (1 SSU). The presence or absence of large and small subunits was inferred by the presence or absence of the oligomers above, when the single large subunit (e) or small subunit (f) band was not observed. The presence of an oligomer in a lane should not be taken as evidence of its presence in tissue, as Rubisco dissociates readily into oligomers under the conditions required by polyacrylamide electrophoresis such as denaturing conditions, temperatures over 25°C, and electric current (Ru et al. 2000; Li et al. 2002).

Although sampling varied between *Harveya* and *Hyobanche* and among tissue types, several qualitative patterns were observed in Rubisco immunoreactivity in these genera (Table 2.3). Both the large and small subunit were evident in all but one *Harveya* tissue sample (*Harveya silvatica*, calyx). Sampling of *Hyobanche* tissues was less robust than within *Harveya*; however, the pattern of Rubisco immunoreactivity was noticeably different. The large subunit was present in at least some calyx, corolla, and stem accessions of *Hyobanche* species, but was not detected in any of three leaf accessions (from three different species). The small subunit was detected in some accessions of all tissue types, including all three of the leaf accessions from which the large subunit was
absent. A separate blotting experiment was conducted to compare relative Rubisco immunoreactivity among tissues of single specimens, if all four tissue types were available. In *Harveya*, leaves displayed the greatest immunoreactivity, followed by the calyx, while the corolla and stem were markedly lower. Conversely, the calyces and corollas of *Hyobanche* demonstrated higher immunoreactivity than the stems, while only faint small subunit bands were evident in leaves.

**DISCUSSION**

The presence of rbcL pseudogenes in *Hyobanche* indicates the loss of functional constraint on this locus. This finding was supported by analysis of rates of synonymous and non-synonymous substitution (Table 2.1). Pseudogene formation and non-synonymous substitution rate increases may have occurred concomitantly with the loss of rbcL function. Conversely, rbcL has evolved under purifying selection in *Harveya*, as evidenced by open reading frames in all species except *Harveya huttonii*, and an overall high ratio of ds/dN. Using a novel power analysis of ds/dN ratios between *Harveya purpurea*, *H. capensis* and the closely related hemiparasite *Alectra sessiliflora*, Leebens-Mack and dePamphilis (2002) demonstrated purifying selection on rbcL in the *Harveya* lineage, with a low probability of failing to reject a false null hypothesis \([H_0: d_s = d_N]\). The rbcL pseudogene in *Harveya huttonii* appears to be the result of two recent deletion events. This species is nearly indistinguishable morphologically from the closely related species *H. pulchra* which bears an rbcL open reading frame, and is segregated from *H. pulchra* and *H. leucopharynx* on the basis of minor differences in corolla size and color (Hilliard and Burtt 1986). *Harveya huttonii* exhibits a greater overall substitution rate than its sister species, and a decrease in the percentage of synonymous substitutions
(69.2%) inferred for the rest of the Harveya lineage (90.2%). The number of substitutions on the branch leading to Harveya huttonii reported here is necessarily an underestimate, as 341 nucleotides have been deleted from this locus.

Changes in the 5’- and 3’-UTRs of Harveya and Hyobanche do not appear sufficient to preclude gene expression. The −35 and −10 promoters and the ribosome binding site of the 5’-UTR are identical in sequence and approximate position in all species of Hyobanche and most species of Harveya. Furthermore, these sites are strongly similar in sequence and position to those in hemiparasitic plants with known photosynthetic capability. The terminal inverted repeat of the 3’-UTR of these holoparasites also bears striking resemblance to that of hemiparasitic relatives and Nicotiana in sequence and inferred structure. This result is similar to that found by Wolfe and dePamphilis (1997) in parasitic plants from the genera Cuscuta and Orobanche, in that the parasites examined bore both rbcL open reading frames and pseudogenes. In their study, however, major deletions were detected in parasitic plants upstream of the terminal inverted repeat, a region not investigated here. In the present study, pseudogene formation in rbcL was not accompanied by major changes in the 5’- or 3’-flanking sequences in any instance. However, some aspects of flanking region function are not yet characterized, and other changes in sequence and structure not examined here may affect rbcL gene expression (Monde et al. 2000; Kuroda and Maliga 2001; McCormac et al. 2001; Whitney and Andrews 2001).

The most surprising result of this study is the apparent presence of Rubisco in tissues of holoparasites Harveya and Hyobanche. Expression of rbcL in Harveya is a reasonable outcome based on the maintenance of an open reading frame, evidence of
purifying selection, and apparently functional flanking sequences, but begs the question of \textit{rbcL} function in a non-photosynthetic plant. Evidence of Rubisco in \textit{Hyobanche} is anomalous given the presence of an \textit{rbcL} pseudogene in 30 specimens examined (exemplars were used in analyses presented here).

\textbf{Rubisco in \textit{Harveya}}

Ignoring the conspicuous presence of Rubisco in \textit{Hyobanche}, the simplest explanation for Rubisco in \textit{Harveya} is expression of \textit{rbcL} within the plastid. Of course, this raises the question of Rubisco function in a putative holoparasite. Several analogous conditions exist in organisms in which heterotrophy is a derived condition. \textit{Astasia longa}, a heterotrophic euglenoid, retains an \textit{rbcL} open reading frame despite the absence of photosynthetic pigments and an otherwise truncated plastid genome compared to that of photosynthetic euglenoids. Furthermore, \textit{rbcL} transcripts and the large subunit of Rubisco have been detected in \textit{Astasia longa} (Siemeister and Hächtel 1990). \textit{Cuscuta reflexa} is a stem holoparasite that exhibits low levels of light-induced $^{14}$CO$_2$ assimilation, presumably by the action of Rubisco (Machado and Zetsche 1990). \textit{RbcL} transcripts were detected at low concentrations despite the loss of the 3'-UTR-IR and point mutations in the promoters (Haberhausen et al. 1992). \textit{Lathraea clandestina}, another holoparasite, also demonstrates an \textit{rbcL} ORF (Delavault et al. 1995) accompanied by the presence and activity of Rubisco in leaf tissue (Bricaud et al. 1986; Thalouarn et al. 1989) despite significant divergence and reduction of the plastid genome and a lack of photosynthetically-competent chloroplasts (Delavault et al. 1996). The evolution of \textit{rbcL} in \textit{Lathraea} may differ from that in \textit{Harveya}, as retention of an \textit{rbcL} reading frame in \textit{Lathraea} was shown to be stochastic, rather than functionally constrained (Leebens-
Mack and dePamphilis 2002). However, one might expect negative selection to act quickly in terminating the expression of genes that do not contribute to fitness, as production of Rubisco may be costly to Lathraea, even at levels lower than photosynthetic plants. A recent loss of photosynthetic ability in Lathraea clandestina may explain this finding, or alternatively, the recent loss of Rubisco function following an older transition to holoparasitism. The same analysis inferred no loss in functional constraint on rbcL in holoparasitic lineages Striga gesneroides and Orobanche fasciculata / O. corymbosa, and had sufficient statistical power to reject a false null hypothesis (Leebens-Mack and dePamphilis 2002).

The maintenance of rbcL function in parasitic plants is puzzling. Harveya appears in most ways to be a holoparasite. All species bear strongly reduced leaves. Tissues of H. huttonii and H. squamosa were shown to contain few if any plastids (de la Harpe et al. 1980) and, furthermore, these two species have been reported to lack chlorophyll entirely (de la Harpe et al. 1981). However, plastids almost certainly exist in cells of Harveya species as they do in more ancient holoparasitic lineages, namely Conopholis, Epifagus, and Orobanche, and as evidenced in this study by the presence of rbcL in Harveya. One may also question the reported lack of chlorophyll, based on the presence of green pigmentation in the leaves and calyces of many live specimens. In this study, Rubisco immunoreactivity was greatest in these tissues of Harveya. The swollen stigma of H. capensis is also conspicuously green against the white background of the corolla tube, indicating that chlorophyll perhaps serves as a pollinator attractant. Further, the corolla limbs of H. hyobanchoides are also of a deep, chlorophyllous green color.
Therefore, one possibility is that *Harveya* is a cryptic hemiparasite, and may carry out photosynthesis at low levels. Conversely, Rubisco may play a non-photosynthetic role in *Harveya*.

**The role of Rubisco in non-photosynthetic plants**

The expression of Rubisco in non-photosynthetic plants, as suggested by this study and others aforementioned (Bricaud et al. 1986; Machado and Zetsche 1990; Siemeister and Hächtel 1990; Wolfe and dePamphilis 1997), indicates the importance of this enzyme in a non-photosynthetic role. Rubisco may have a similar function in photosynthetic plants that has been overlooked due to the overwhelming importance of Rubisco in fixing carbon dioxide in photosynthesis. Several suggested functions of Rubisco other than photosynthetic carbon assimilation include the synthesis of serine and glycine through oxygenation in the glycolate pathway (Siemeister and Hächtel 1990; Wolfe and dePamphilis 1997), and the recycling of internal carbon (Thalouarn et al. 1989). In non-photosynthetic plants, *Cuscuta europaea* was shown to lack Rubisco activity while maintaining higher than average PEP-carboxylase activity, higher even than its photosynthetic relative *Ipomoea*, while *C. reflexa* maintained Rubisco activity but not PEP-carboxylase activity (Machado and Zetsche 1990), suggesting perhaps an overlap in function of these enzymes.

PEP-carboxylase fixes carbon dioxide non-photosynthetically, and may function in tricarboxylic acid intermediate synthesis needed for the synthesis of amino acids and chlorophyll, generation of NADPH, recapture and recycling of CO$_2$, carbon metabolism in aquatic plants, malate fermentation, cyanide-resistant respiration that prevents the build-up of a large number of sugars, nitrogen assimilation and amino acid synthesis,
maintenance of cytoplasmic pH, maintenance of electroneutrality, wave-length mediated light response and the amelioration of low temperature sensitivity (Latzko and Kelly 1983). PEP carboxylase activity in hemiparasites *Viscum album* (Viscaceae), *Thesium humifusum*, and *Osyris alba* (Santalaceae), and holoparasites *Orobanche hederae* and *Lathraea clandestina*, was shown to be higher than or comparable to the C4 plant *Sorghum bicolor* (Renaudin et al. 1982). This is significant in that C4 plants in general display higher PEP-C activity than C3 plants from which these parasites were presumably derived (Latzko and Kelly 1983). In *Platycerium coronarium* (staghorn fern), PEP-C and Rubisco activity are co-regulated by concentrations of atmospheric CO$_2$. Calluses cultured in high CO$_2$ environments showed an increase in PEP-C activity and a decrease in Rubisco activity (Kwa et al. 1997). In C3 plants, PEP-C is active in both the cytosol and within the chloroplasts, but curiously, in the holoparasite *Lathraea clandestina*, PEP-C expression is limited to the cytosol (Renaudin et al. 1984). This suggests that Rubisco (which is minimally expressed in *Lathraea clandestina*) may have taken over chloroplast PEP-C function. Perhaps this change in the expression pathway indicates a change in function. In holoparasites, some of these functions may be particularly important: a) in synthesis of necessary organic compounds not obtainable from host plants, b) in the recycling of carbon that may accumulate detrimentally in plants that respire but do not photosynthesize, and c) maintenance of pH and electroneutrality through oxidation or carboxylation. Alternatively, the products of Rubisco function may be important in maintaining an appropriate osmotic balance between parasite and host (Stewart and Press 1990). Recycling excess carbon dioxide may be especially important in root parasites, which spend most of the life cycle underground as nodules on host roots.
**Rubisco in Hyobanche**

The discovery of Rubisco in tissues of *Hyobanche* was unexpected in light of the presence of an *rbcL* pseudogene as documented here and in previous studies (Wolfe and dePamphilis 1998; Wolfe and Randle 2001). Many other parasitic plants have been shown to bear an *rbcL* pseudogene or deletion, including *Epifagus virginiana* (dePamphilis and Palmer 1990; Wolfe et al. 1992b), *Conopholis americana* (Colwell 1994), *Orobanche ramosa, O. cernua* (Wolfe and dePamphilis 1997, 1998), and *Buchnera floridiana, Boschniakia strobilacea,* and *B. hookeri* (Wolfe and dePamphilis 1998). Few studies have gone so far as to assay for the presence of *rbcL* transcripts or the large subunit in these plants. Of these, absence of expression has been reported only in *Epifagus virginiana,* which bears an *rbcL* pseudogene resulting from a deletion of a large portion of the gene (dePamphilis and Palmer 1990). Alternatively, *rbcL* expression was not detected in *Orobanche hederae* and *O. minor* holoparasites bearing an *rbcL* ORF (Thalouarn et al. 1994). The presence of bands on Western Blot films should always be interpreted with some caution, as contamination can occur. However, all materials used for preparing protein extracts in this study were cleaned thoroughly and in conditions that would have resulted in dissociation of the Rubisco holoenzyme, and so contamination would only have produced large and small subunit bands. Furthermore, unambiguous differences in banding patterns were observed for *Harveya* and *Hyobanche* specimens, which would be an unlikely result of contamination during the protein extraction process, as proteins were extracted from both plants simultaneously, and stored in the same conditions. *Hyobanche* extracts show greater holoenzyme dissociation than extracts from *Harveya,* as evidenced by decreased immunoreactivity of higher molecular-weight bands.
in the former. Finally, these results were repeated using two antibodies with different antigen specificities. There is always the possibility that the tissues themselves were contaminated with green algae (which express type I Rubisco). Plants of both genera are densely covered with glandular hairs, and algal colonies may be difficult to remove. However, there is no evidence that tissues were contaminated and therefore it must be concluded that these results are not artifactual, and that Rubisco evident on Western blots is an endogenous component of the tissues examined.

Two hypotheses may explain this phenomenon in *Hyobanche* and their justifications are discussed in detail in Chapter 3: 1) Multiple copies of *rbcL* exist in *Hyobanche*, at least one of which represents a functionally expressed open reading frame. 2) RNA transcripts undergo editing prior to transcription, resulting in RNA species with functional reading frames.

Holoparasitic plants provide a unique model system in which to study the function of genes related to photosynthesis. *RbcL* has followed two differing evolutionary paths in the recently derived sister-genera *Harveya* and *Hyobanche*. In *Harveya*, *rbcL* has been maintained by selection, and appears to be expressed in leaf and calyx tissues at levels comparable to that of closely related hemiparasites with known photosynthetic function. The presence of Rubisco in *Hyobanche* despite *rbcL* pseudogene formation is enigmatic. These results raise several questions. Is *rbcL* function maintained by a currently undocumented mechanism of post-transcriptional correction, or do these putative holoparasites undergo photosynthesis? By what mechanism is *rbcL* expressed in *Hyobanche*?
### Table 2.1. Primers used in the amplification and sequencing of the 5’untranslated region (UTR), rbcL, and the 3’-UTR. \(^1\) from Wolfe and dePamphilis (1997).

<table>
<thead>
<tr>
<th>Forward primers:</th>
<th>Reverse primers:</th>
</tr>
</thead>
<tbody>
<tr>
<td>766+970 GAACCGAAATCTATTAC</td>
<td>H3U TR CGAACGAAAAAGNCAATATAC AGGATGG</td>
</tr>
<tr>
<td>766+1080 GTATTTCAGCATATTSSTWW</td>
<td>(^{'}\text{rbcL} 3') CC GGAGCTCTTGA TAAGAGATT GGGCGAG</td>
</tr>
<tr>
<td>(^{'}\text{rbcL} 5') GGCCGTCAAGTCACCACCAAC AGARACTAAAGC</td>
<td>rbcL H-1020R (\text{AGARACTAAAGC})</td>
</tr>
<tr>
<td>rbcL H-430 GCTTATTTTTAATTTCCAAGG</td>
<td>rbcL H-674R (\text{GATTTCGCTTTCAGGCTGT}) G</td>
</tr>
<tr>
<td>rbcL H-674 TTTATAAGCAGGCTGAACCA (^{'}\text{rbcL} 350R)</td>
<td>(\text{TTTATAAGCAGGCTGAACCA})</td>
</tr>
<tr>
<td>(^{'}\text{rbcL} 1020) TGGGCTTTGTGATTTACTGC</td>
<td>(\text{TGGGCTTTGTGATTTACTGC})</td>
</tr>
<tr>
<td>H3-120 CTTGCTGCTGAGGGTAATG</td>
<td>rbcL 60R (\text{rbcL 60R})</td>
</tr>
<tr>
<td>H3-49 GCTGCCCCTGCTGAGGTATGG</td>
<td></td>
</tr>
</tbody>
</table>

### Table 2.2. Rates of synonymous (\(d_s\)) and non-synonymous substitution (\(d_N\)) for \(\text{rbcL}\) in \(\text{Harveya}+\text{Hyobanche}\), \(\text{Harveya}\) and \(\text{Hyobanche}\), calculated using the modified Nei and Gojobori method (Nei and Gojobori 1986; Kumar 2001) with Jukes-Cantor correction. Codons containing indels resulting in frameshift or mutations resulting in premature stop codons were removed from the data set. In \(\text{Harveya}+\text{Hyobanche}\), \(\text{rbcL}\) included a 341 base pair gap, and therefore, inclusion of \(\text{H. huttonii}\) resulted in a significant deletion of the data set. Therefore, \(d_s\) and \(d_N\) were calculated with \(\text{Harveya}+\text{Hyobanche}\) included and excluded. The Wilcoxon signed ranks test was used to test the null hypothesis that the pattern of substitution within a lineage was random (\(H_0: d_s = d_N\)). \(^1\text{Tr/Ts} = \text{transition / transversion ratio, inferred from number of changes (Kumar 2001)}\)

<table>
<thead>
<tr>
<th>Without (\text{H. huttonii})</th>
<th>(^1\text{Tr/Ts})</th>
<th>(d_s)</th>
<th>(d_N)</th>
<th>(d_s / d_N)</th>
<th>(z)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{Harveya}+\text{Hyobanche})</td>
<td>2.098</td>
<td>0.0194</td>
<td>0.0076</td>
<td>2.55</td>
<td>-8.470</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(\text{Harveya})</td>
<td>1.796</td>
<td>0.0081</td>
<td>0.0002</td>
<td>40.50</td>
<td>-6.468</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(\text{Hyobanche})</td>
<td>2.591</td>
<td>0.0194</td>
<td>0.0154</td>
<td>0.86</td>
<td>-1.153</td>
<td>0.249</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>With (\text{H. huttonii})</th>
<th>(^1\text{Tr/Ts})</th>
<th>(d_s)</th>
<th>(d_N)</th>
<th>(d_s / d_N)</th>
<th>(z)</th>
<th>(p)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(\text{Harveya}+\text{Hyobanche})</td>
<td>1.552</td>
<td>0.0240</td>
<td>0.0091</td>
<td>2.64</td>
<td>-9.124</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(\text{Harveya})</td>
<td>1.226</td>
<td>0.0132</td>
<td>0.0012</td>
<td>11.00</td>
<td>-6.915</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>(\text{Hyobanche})</td>
<td>1.577</td>
<td>0.0156</td>
<td>0.0173</td>
<td>0.90</td>
<td>-0.405</td>
<td>0.686</td>
</tr>
</tbody>
</table>
Table 2.3. Summary of Rubisco oligomer banding patterns for calyx, corolla, leaf and stem tissue of *Harveya* and *Hyobanche*. Rubisco oligomers fell into six molecular weight categories identifiable by subunit composition. The holoenzyme is composed of eight large subunits and eight small subunits (8 LSU + 8 SSU). The holoenzyme and octamer were indistinguishable on membranes due to their large size (~300-600 kDa). Other bands detected in the analysis are labeled by size and probable composition.
Figure 2.1. Phylogram of *Harveya* and *Hyobanche*, with outgroups *Alectra sessiliflora*, *Buchnera floridiana*, *Cynium racemosum*, and *Melasma scabrum* inferred from the DNA sequence of the *rbcL* operon composed of the 5’-UTR, *rbcL* (coding sequence) and the 3’-UTR. This is one of six most parsimonious trees (C.I. = 0.870; R.I. = 0.850; 315 steps). Support values from 500 jackknife replicates (10 random sequence additions each) appear above branches. * indicates branches that collapse in strict consensus. \( \Psi \) indicates an *rbcL* pseudogene evidenced by a deletion resulting in frameshift (slash), or the evolution of a premature stop codon (Δ). Deletions resulting in frameshift (base position of the aligned *rbcL* sequence): 1) 45-386; 2) 1217-1220; 3) 524; 4) 923-927; 5) 225; 6) 517-523. Substitutions resulting in the evolution of a premature stop codon (base position of the aligned *rbcL* sequence): a) 522; b) 610; c) 60; d) 1066.
Figure 2.2. Strict consensus of six most parsimonious trees generated using the rbcL operon (only *Harveya* and *Hyobanche* lineages shown). Each branch shows the number of synonymous substitutions over the number of non-synonymous substitutions, inferred from aligned *rbcL* sequences. The number of each type of substitution was inferred by mapping individual characters onto most parsimonious trees, averaging over all optimizations.
Figure 2.3. The 5’-UTR contains three sequences of known function in \textit{rbcL} expression, the –35 and –10 transcription promoters and the ribosome-binding site, six bases upstream of the \textit{rbcL} start codon. \textit{Harveya} is polymorphic at two of these sites, substitutions evident in the –10 transcription promoter and the ribosome binding site. In both cases, the majority of \textit{Harveya} species have identical nucleotide sequences as the hemiparasites and \textit{Hyobanche}. At the fourth position of the –10 transcription promoter, the change from a C to a T is synapomorphic for the clade containing \textit{Harveya pulchra}, \textit{H. huttonii}, \textit{H. coccinea}, and \textit{H. silvatica} (figures 1 and 2). At the second position of the ribosome-binding site, the substitution from G to A is autapomorphic in \textit{Harveya stenosiphon}.

Figure 2.4. Detection of Rubisco by means of Western Blot. Initially, a custom antibody raised against *Spinacia* purified Rubisco was used to detect Rubisco in tissues of *Harveya* and *Hyobanche* (left). Shown here are results from: A. *Harveya capensis* leaf extract, B. *Hyobanche glabrata* calyx extract, and C. *Spinacia* purified Rubisco (7.5 µg total protein). Both the large (LSU) and small subunit (SSU) were present in *Harveya* and *Hyobanche* tissues as evidenced by oligomer bands composed of both subunits (~160 kDa and ~70 kDa bands). The presence of a 112 kDa band and a 56 kDa band indicate the presence of the small subunit. A second antibody raised against a peptide conserved in all type I Rubisco confirmed this result (right).
CHAPTER 3

THE EXPRESSION OF \textit{RBCL} IN \textit{HYOBANCHE}: MULTIPLE GENE COPIES OR POST-TRANSCRIPTIONAL MODIFICATION?

INTRODUCTION

In the previous chapter, the evolution of \textit{rbcL} in the holoparasitic genera \textit{Harveya} and \textit{Hyobanche} was explored. All but one species of \textit{Harveya} examined maintain an open reading frame. The locus appears to be evolving under purifying selection and the flanking UTRs (untranslated regions), which have transcriptional and translational function, appear to be intact. Conversely, all specimens of \textit{Hyobanche} examined bear \textit{rbcL} pseudogenes and the locus appears to have evolved free from functional constraint. However, the flanking UTRs appear functional. The large subunit of Rubisco is expressed in \textit{Harveya} as expected, but also anomalously in \textit{Hyobanche}. Two hypotheses may explain the expression of \textit{rbcL} in \textit{Hyobanche}. First, it is possible that more than one copy of \textit{rbcL} exists in tissues of the plant. This may result from heteroplasmy (the condition of cells containing plastids of more than one haplotype) or duplication of \textit{rbcL} and subsequent transfer of a copy to a different genomic compartment, either the nucleus or mitochondrion. One of these copies may be maintained by selection as an open
reading frame while ancillary copies have fallen into disuse resulting in rapid evolution and pseudogene formation. Failure to recover functional sequences in previous sequencing experiments may have been due to a PCR dosage effect. Second, rbcL pseudogene transcripts may be repaired through RNA editing mechanisms in the plastids of Hyobanche, resulting in transcripts with open reading frames.

**Multiple copies of cpDNA; heteroplasm, haplotype polymorphism, or gene duplication**

Several events may result in the existence of multiple chloroplast haplotypes within an organism: heteroplasm, haplotype polymorphism, and gene duplication. Heteroplasm is a necessary condition in the evolution of organellar genomes. Before a plastid mutation can become fixed in a population of organisms, it must first become fixed within a population of organelles, within a cell or within an individual. Heteroplasm is normally a transient condition. Germ-line bottlenecks resulting from uniparental inheritance of organelles, selection against deleterious mutations, gene conversion through recombination (Dale et al. 2003; dealing with extant endosymbiotic prokaryotes), and vegetative segregation (Birky, 2001) shift cellular populations of organelles toward a condition of homoplasm. However, selection for mixed haplotypes, biparental and somatic inheritance of organelles, or the loss of recombination mechanisms may stabilize heteroplasmic lines when they occur. The distinction should be made at this point between heteroplasm and haplotype polymorphism, the condition wherein different tissues of an organism bear organelles of differing haplotype. For the purposes of this study, the outcome of these conditions is indistinguishable; either may characterize a plant bearing more than one plastid sequence for the rbcL locus.
Due to the typically transient nature of heteroplasmy and haplotype polymorphism, these conditions are not frequently invoked as important factors in expression studies. However, there are several documented incidents of stable heteroplasmy or haplotype polymorphism in the organellar genomes of plants. For example, a nucleotide substitution conferring triazine resistance in *Senecio vulgaris* was shown to exist alongside the wild type in leaf tissue for six generations (Frey 1998). Heteroplasmy or haplotype polymorphism of cpDNA has been discovered in *Silene* (McCauley 1998; McCauley and Olson. 2003; McCauley et al. 2003), *Medicago sativa* (Fitter et al. 1996), *Coreopsis grandiflora* (Mason et al. 1994), the holoparasite *Cynomorium* (Garcia et al. 2004), and in a conifer, *Chamaecyparis obtusa* (Shiraishi et al. 2001).

Duplication and transfer of genes from organelle to organelle, or from the organelle to the nucleus, is a well-documented phenomenon in plants (Ellis 1982; Martin and Herrmann 1998; McFadden 1999; Küchler and Soll 2001). Once duplicated and transferred, gene copies may experience positive, neutral, or purifying selection. If the transferred gene provides a selective advantage it is likely to become fixed, in which case the original copy may be freed from functional constraint and may evolve randomly, eventually becoming a pseudogene. If the transfer results in no change in fitness, fixation may occur stochastically. If the transferred gene decreases fitness, it will likely undergo pseudogene formation or be excised from the genome to which it was transferred. Alternatively, a transferred gene may be expressed in a different cellular compartment (the mitochondrion or the cytosol), where it may acquire a new function (Martin and Herrmann 1998).
The endosymbiotic ancestor of the chloroplast presumably had many more genes than the chloroplast has now. The cyanobacterium *Synechocystis* genome contains 3,168 protein-coding genes, while higher plant chloroplasts encode 60-80 (Martin and Herrmann 1998). Many endosymbiont genes were relocated to a different cellular compartment. The ribosomal subunit protein gene *rpl22* was duplicated and transferred to the nucleus in the ancestor of the angiosperms, where it acquired new function in the cytosol; thus, both gene copies were maintained as ORFs. In the legume lineage, the nuclear copy obtained a transit peptide, allowing its expression product to be imported into the chloroplast. This was followed by deletion of the chloroplast copy (Gantt et al. 1991). *RbcS* was transferred to the nucleus in the ancestor of green algae and land plants, and subsequently deleted from the plastome (Rodermel 1999). The maize mitochondrion bears several non-functional sequences of chloroplast origin, including *rpl2* (Hoch et al. 1991), a 12 kb fragment containing 16s rDNA, *trnL* and *trnV* (Stern and Lonsdale 1982), and a region homologous to *rbcL* that was successfully transfected into *E. coli* and expressed as the LSU of Rubisco (Lonsdale et al. 1983). Some tRNA genes have been transferred from the chloroplast to the mitochondrion in higher plants and retain function there (Palmer 1992). In *Epifagus* a photosynthetic gene, *psaA*, has been deleted from the chloroplast and survives in the mitochondrion (dePamphilis and Palmer 1990).

It is possible that *rbcL* has been transferred to either the nucleus or mitochondrion in the *Hyobanche* lineage. The pseudogenes sequenced in this study may be the remnants of the original chloroplast gene or of the transferred copy. It is unlikely that *rbcL*, if transferred to the mitochondrion, would maintain function in the absence of the small subunit. It is also unlikely that *rbcL* transferred to the nucleus would have cytosolic
function, in that the small subunit is imported into the chloroplast, and requires processing there before assembly of the Rubisco holoenzyme. If transfer has indeed occurred in this lineage, it seems most likely that \emph{rbcL} was transferred to the nucleus, is expressed in the cytosol and targeted to the chloroplast by means of a nuclear-acquired transit peptide, where it functions in some non-photosynthetic role. As each cell bears many more copies of the plastid than nuclear genome, pseudogenes sequenced here may represent the non-functional chloroplast copy, due to PCR dosage effect. If Rubisco has an important non-photosynthetic role in parasitic plants, a transfer to the nucleus may have allowed for more efficient gene regulation.

\textbf{Editing of \emph{rbcL} pseudogene transcripts.}

Post-transcriptional modification of mRNA in the chloroplast is more complex than was once believed. Regulatory mechanisms governing mRNA alteration are largely imported from the nucleus, and many nuclear-encoded factors present in the chloroplast are yet to be characterized (Schuster and Gruissem 1991; Herrman, Westhoff, and Link 1992; Gillham et al. 1994; Mayfield et al. 1995; Danon 1997). Such modifications include \emph{cis-} and \emph{trans}-splicing, intron splicing, and sequence-specific de-amination of cytosine residues resulting in uracil. The amination of uracil resulting in a cytosine has been reported for \textit{Anthoceros formosae} (a hornwort) and was shown to result in replacement of termination codons in \emph{rbcL} in this species (Yoshinaga et al. 1996). This type of modification has not been reported in the higher plants. RNA editing has been shown to function in restoration of conserved amino acids upon translation, the creation of appropriate initiator and termination codons, and the removal of stop codons by means of uracil amination (Bock 2000). Given the nature of the mutations resulting in
pseudogene formation in *Hyobanche* it is unlikely that documented post-transcriptional modification mechanisms are adequate to restore function to *rbcL* pseudogenes. If *Hyobanche* acquired a U → C editing factor, as in *Anthoceros formosae*, terminator codons could be replaced, restoring function to *rbcL* in *Hyobanche atropurpurea*. However, this is unlikely and would not restore function in the other three species of *Hyobanche* that bear deletions resulting in frameshift; in fact, there are no known post-transcriptional mechanisms for the restoration of reading frame following a frameshift mutation operating in the chloroplast.

To test these hypotheses, *rbcL* was cloned from an individual from each species of *Hyobanche* and sequences were examined to determine 1) if multiple haplotypes were present and 2) if any of the resultant haplotypes were open-reading frames. RNAs from *Hyobanche* species were then used to construct DNA libraries from which *rbcL* transcripts could be sequenced. These transcript sequences were examined to determine if post-transcriptional modification had taken place.

The two hypotheses listed above are not mutually exclusive; it is possible that both multiple copies of *rbcL* exist and that pseudogene copies are post-transcriptionally modified into open reading frames. Further, the hypotheses that multiple copies exist cannot be refuted by cloning experiments, but only corroborated. In any case, the finding of either an as-yet-unsequenced open reading frame for *rbcL* or an *rbcL* transcript with an open reading frame would provide a reasonable explanation for the presence of the large subunit of Rubisco in *Hyobanche*. An alternative hypothesis to these, not tested here, is that Rubisco is not expressed endogenously by *Hyobanche* but rather absorbed from
photosynthetic host plants. The haustoria of *Hyobanche* are unique among Orobancheaceae, and little is known about mechanisms of haustorial uptake in *Hyobanche*. Thus far, the uptake of macromolecules from hosts is unreported in any other taxon, and the differential deposition of host Rubisco in tissues of *Hyobanche* seems at least to be conceptually problematic.

**MATERIALS AND METHODS**

**PCR cloning of rbcL**

Fragments for cloning were amplified using standard PCR methods presented in Chapter 2. Amplifications were obtained from one individual of each of four *Hyobanche* species: *Hy. atropurpurea*, *Hy. glabrata*, *Hy. rubra* and *Hy. sanguinea*. In previous experiments, primers used to amplify *rbcL* were designed to anneal within the UTRs flanking the coding sequence. However, if *rbcL* open reading frames are transcribed in other genomic compartments, they are unlikely to be flanked by chloroplast control regions. Primers for PCR amplification were designed to anneal just within the *rbcL* coding sequence; these were designated “*rbcL* 5’ internal” (NATGTCACCACAAA-CAGACT) and “*rbcL* 3’ internal” (NTTACTTATCMAAAGTATCTACGCCG) (Figure 3.1). In a preliminary experiment, the UTR-annealing primers were used to clone *rbcL* in a single individual of *Hy. glabrata*. This experiment was rejected initially due to failure to find any sequence variation, but later proved useful as a control for determining the role of PCR error in producing observed variation.

PCR products were separated on 1% agarose, and bands were excised and extracted from the agarose matrix using the Sephaglass Band-Prep protocol (Amersham Pharmacia Biotech, Piscataway NJ). Purified fragments were inserted into a plasmid
vector and cloned using the TOPO TA Cloning Kit for Sequencing (Invitrogen, Carlsbad, CA). Colonies were cultured on master agar plates, so that each colony occupied a 1 cm by 1 cm square on a grid. Colonies were each dispersed in 20µl ddH$_2$O and incubated at 96°C for 10 minutes to denature bacterial cell membranes. PCR was used to screen colonies for transformation using vendor-supplied primers T3 and T7. Amplification reactions of 50 µl contained 4 µl of denatured colony preparations, 0.2 mM dNTPs, 0.15mM MgCl$_2$, 0.64 µM each primer, 1U Taq polymerase (Invitrogen; Carlsbad, CA), and 1X Taq polymerase amplification buffer. Reactions were carried out under the following temperature conditions: 7 min. at 94°C; 35 cycles of 1 min. at 94°C, 1 min. at 50°C, and 2 min. at 72°C; and a final extension of 10 min. at 72°C. Amplified DNA was purified on polyethylene glycol, followed by two ethanol precipitations (85% and 100% respectively) and resuspended in ddH$_2$O. Cycle-sequencing was carried out using the standard ABI Big Dye 3.0 protocol and primers in Table 2.1, and sequences were obtained using an ABI 3100 automated sequencer (Applied Biosystems, Foster City, CA). Sequence contigs were assembled using Sequencher (Applied Biosystems, Foster City CA). Thirteen to seventeen sequences were obtained from each individual.

Translations of sequences were examined to determine pseudogene status. Specifically, sequences with premature stop codons and indels resulting in frameshift were labeled pseudogenes. Sequences were aligned using Clustal X (Thompson et al. 1997) and MacClade 3.08a (Maddison and Maddison 1992) was used to determine how many distinct haplotypes were in each individual. Sequences that differed by a single base or indel were interpreted as representing different copies. However, in each case that a difference was identified, the chromatograms of the sequence contig were re-
evaluated to insure that differences did not arise from error in base calling. All redundant sequences were excluded from the matrix and phylogenetic analysis using parsimony was carried out in PAUP*4.0b10 (Swofford 2002), using 1000 random additions, two trees held at each step and TBR branch swapping. Sequences from Harveya were included to root resulting trees. Jackknife support was estimated using 1000 replications, 37% deletion, the “emulate Jac” option and TBR branch swapping.

**RNA extraction, RT-PCR, and transcript sequencing**

In the following protocol, all recommendations made regarding the handling of RNA in Sambrook et al. (1989) and vendors’ product guides were followed. Tissues were collected in the field and flash-frozen on liquid nitrogen. RNA was extracted from tissues demonstrating Rubisco immunoreactivity in Chapter 2. Corolla tissue was available for two individuals of *Hyobanche sanguinea* and one of *Hy. glabrata*. Additionally, RNA was extracted from calyx material from one individual of each of three species of *Harveya*: *H. capensis*, *H. coccinea* and *H. stenosiphon* for use as positive controls. RNA was also extracted from stems of species of both genera (*H. capensis*, *H. stenosiphon*, and *H. bolusii*\(^2\); *Hy. glabrata* and *Hy. sanguinea*) and served as negative controls. Tissues were ground in liquid nitrogen and RNA was purified using the RNeasy for Plant Tissue kit (Qiagen, Valencia CA). As an added precaution, DNA was

\(^1\)Stems of *Harveya bolusii* were not included in Western blot analysis in the previous chapter, and conversely, stems of *Harveya coccinea* were unavailable for RNA analysis in the current chapter. Both species have short slender stems, neither of which provided enough material for both protein and RNA experiments. This ostensibly marrs symmetry in experimental procedure. Nevertheless, I have reported results gleaned from the use of these materials in both chapters, not because the results from their inclusion are very important, but simply because results were obtained and should be reported.
degraded with DNase I (Qiagen, Valencia CA) following the “on-column” protocol option. RNA’s were separated on 1% agarose to estimate yield and purity.

RNAs were then used to construct cDNA libraries using reverse-transcriptase (RT) PCR. All reagents were purchased from Invitrogen (Carlsbad CA), and final concentrations are given in brackets. 5µl RNA extract were added to 1µl 250ng/ml Random Primers [25.9ng/ml], 1µl 10mM dNTP’s [0.5mM], and 6µl ddH₂O. The mixture was incubated at 65ºC for five minutes and immediately transferred to ice. 4µl of 5X First Strand buffer [1X], 1µl 0.1M DTT [5mM], 1µl Superscript III Reverse-Transcriptase [10U/µl], and 1µl RNase-Out Recombinant RNA Inhibitor [2U/µl] were added to the mixture, which was incubated at room temperature for five minutes, followed by incubation at 50ºC for 45 minutes. cDNAs were electrophoresed on 1% agarose to visualize RT-PCR products.

Transcripts of rbcL were then amplified from cDNA libraries in 50µl reactions, using the protocol above. As an additional negative control, for each cDNA reaction a reaction was carried out using an equivalent amount of purified RNA to insure that amplified fragments represented RNA transcripts rather than DNA contamination of RNA. Transcript sequences were obtained following the protocol in Chapter 2.

RESULTS

Multiple copies of rbcL in Hyobanche

In the preliminary experiments, primers were used that anneal within the 5’- and 3’- untranslated regions that flank rbcL. For the individual utilized, 20 sequences were obtained. Two haplotypes were recovered that differed by a single base pair. When fragments were amplified using primers annealing just within the coding sequence of
rbcL, however, differences were observed. Thirteen sequences were obtained from a single individual of *Hyobanche glabrata* and *Hy. sanguinea*, fourteen from *Hy. rubra*, and seventeen from *Hy. atropurpurea*. Of these, *Hy. glabrata* demonstrated five unique haplotypes, *Hy. sanguinea* and *Hy. atropurpurea* demonstrated six unique haplotypes, and *Hy. rubra* demonstrated eight unique haplotypes. However, each sequence obtained was a pseudogene, having undergone substitutions resulting in the evolution of premature stop codons or indels resulting in frameshift.

It may be argued that variation among cloned sequences is the result of PCR and cloning error. If this is true, one might expect the same degree of error from the parallel cloning experiment (amplifications utilizing UTR primers), resulting in the same level of variation. Therefore rates of substitution were compared between each set of clones and the control matrix of 20 sequences using Fisher’s Exact Test³, which calculates the probability of retaining the frequency of substitution in test matrices (matrices generated with primers inside the coding sequence) given the frequency of substitution required by the control matrix (primers in the UTRs). In each case, this probability is less than 0.01, indicating that variation in sequences within individuals cannot be attributed entirely to error (Table 3.1).

Phylogenetic analysis was carried out on the aligned matrix of 1435 characters of the rbcL coding sequence alone. Of these, 62 were parsimony-informative. One thousand random addition searches resulted in one most parsimonious tree of 112 steps;

³ The probability calculation of Fisher’s Exact Test is often approximated using the $\chi^2$ distribution, due to the laboriousness of calculation required by Fisher’s Exact Test for large samples (Zar 1996). However, advances in computing have made calculation of precise probabilities tractable, and online calculators exist, such as that found at [http://www.matforsk.no/ola/fisher.htm](http://www.matforsk.no/ola/fisher.htm).
C.I. = 0.821, R.I. = 0.931. The strict and jackknife consensus trees indicate that all sequences are monophyletic with respect to the individual from which they were obtained with the exception of *Hy. atropurpurea* sequences (Figure 3.2). All but one of the *Hy. atropurpurea* sequences formed a clade at the base of the tree, which was strongly supported with a jackknife of 99%. The remaining sequence (clone 97.130.1-16) was strongly supported as sister to the *Hy. sanguinea* clade, with a jackknife value of 91%.

To insure that this was not the result of contamination, the transformant colony was isolated from the original plate and re-sequenced, with the same result.

These results indicate that some *rbcL* gene coalescent events in *Hyobanche* may have preceded speciation events, as is clearly the case with *Hy. atropurpurea* sequence 130.1-16. In previous analyses, (Wolfe and Randle 2001) non-monophyly of *rbcL* sequences within a species was interpreted as an indication of hybridization. Indeed, some *Hyobanche* species demonstrate intermediate morphological traits that were interpreted by Wolfe and Randle (2001) as corroboration for hypotheses of reticulation. However, in light of these findings, it seems possible that non-monophyly within species was the result of sampling paralogous copies of *rbcL* in analyses. To investigate this possibility, it would be most appropriate to clone sequences from many individuals of *Hyobanche*, and include them in a single phylogenetic analysis to see how often gene coalescence is correlated with species divergence. However, this method is prohibited by time and expense. Rather, sequences obtained by conventional methods were included in the phylogenetic analysis with cloned sequences. Care was taken to include sequences from only individuals for which species diagnosis based on morphological characters was clear. The expanded matrix resulted in 43 most parsimonious trees (length=119; C.I. =
0.807; R.I. = 0.949). In this analysis, sequences from all species of *Hyobanche* were
resolved as polyphyletic (Figure 3.3) and this gene phylogeny contains at least one
strongly supported clade (jackknife = 94%) containing sequences from each of the four
species.

*rbcL* transcripts in *Hyobanche*

RNA extracted from stem tissue of three species of *Harveya* and two of
*Hyobanche* was used as a negative control, as these tissues showed no Rubisco
immunoreactivity on Western blots. When cDNA obtained from these accessions was
included in PCR reactions, no *rbcL* bands were produced. To control for possible DNA
contamination of purified RNA’s, RNA alone was included in PCR amplifications of
*rbcL*. All results were negative, indicating that DNA contamination was not sufficient to
result in artifactual amplification of genomic DNA rather than cDNA. cDNA libraries
from three species of *Harveya* were used as positive controls. Each resulted in a single
band in PCR reactions. Sequences from these bands were identical to *rbcL* sequences
obtained previously from these species. This confirms the findings of the previous
chapter; namely, that *rbcL* is expressed in *Harveya* calyces as the large subunit of
Rubisco, albeit at low levels. Bands were also obtained in PCR amplifications of each of
three cDNAs from *Hyobanche* species. Sequences of these bands were all pseudogenes,
including either premature stop codons or indels resulting in frameshift.

One sequence obtained from *Hyobanche glabrata* cDNA was identical to one of
the copies previously sequenced in *Hy. glabrata*. Likewise, the sequence obtained from
one individual of *Hy. sanguinea* (r03.03) was nearly identical to another sequence
previously obtained from this species, differing by a single autapomorphic base
substitution. However, the other sequence obtained from *Hy. sanguinea* differed strongly from any other haplotype yet sequenced (r03.08). When this sequence was included in phylogenetic analyses of the matrix of all *Hyobanche rbcL* sequences, it was resolved in a tritomy at the base of the clade containing all other sequences from *Hy. glabrata*, *Hy. sanguinea*, *Hy. rubra*, and *Hy. atropurpurea* 97.130.1-16 (Figure 3.3) with a jackknife of 99%. This placement did not affect other relationships in the tree. The tree required nine more steps with the inclusion of this cDNA sequence (the length increased from 119 to 128 steps), but the amount of homoplasy did not change drastically (C.I. = 0.812; R.I. = 0.947).

**DISCUSSION**

The outcome of these experiments is somewhat perplexing. While multiple *rbcL* haplotypes were evident in each individual of *Hyobanche* assayed, no open reading frame was discovered. It is possible that an open reading frame does in fact exist, but that the extent of cloning in this study has been insufficient to discover it. Multiple copies of *rbcL* in *Hyobanche* may be the result of duplication resulting in heteroplasmy or gene transfer, or both. The preliminary cloning experiment in which primers annealing to the *rbcL* flanking regions were used resulted in many fewer haplotypes being recovered than in the experiments utilizing primers annealing just within the *rbcL* coding sequence. This indicates that at least some copies are not flanked by chloroplast UTRs, and may have been transferred to other genomic compartments. In a related species, *Orobanche cumana*, Delavault and Thalouarn (2002) documented the existence of an extra-plastid copy of *rbcL*. Long PCR of the extra-plastid copy revealed flanking sequences most similar to nuclear transposons. The plastid copy is in fact severely truncated, while the
putatively nuclear copy is not, though it has undergone pseudogene formation through the
evolution of premature stop codons; these differences are most probably due the
difference in base rate of substitution between the chloroplast and nuclear genomes, with
the former being more rapid (Soltis and Soltis, 1998). However, unlike *Hyobanche*,
*Orobanche cumana* demonstrates no *rbcL* expression. On the other hand, *Lathraea
clandestina*, another closely related holoparasite, exhibits *rbcL* expression (Thalouarn et
al. 1989) and low-level Rubisco activity (Bricaud et al. 1986). Chloroplast RNA
polymerase genes are pseudogenes in this species, and the 5’- flanking region of *rbcL* is
more typical of that of a nuclear gene (Lusson et al. 1999). This was interpreted as
indicating that nuclear RNA polymerase was being imported into the chloroplast to
maintain function, but it seems possible that the expressed copy of *rbcL* in *Lathraea* is
located in the nucleus (and hence requires nuclear expression sequences). In any case,
we have no evidence that an open reading frame for this locus persists in *Hyobanche*.
Further, given the existence of multiple copies of *rbcL* in *Hyobanche*, phylogenies
generated previously must be re-examined.

**Paralogy and cpDNA-derived phylogenies**

That chloroplast gene trees may not be representative of species trees has long
been recognized (Doyle 1992). The processes of chloroplast capture through
introgression and lineage sorting have been cited as factors that may mislead inferences
about taxon relationships and result in incongruence between trees derived from nuclear
and plastid genomes (Doyle 1992; Soltis and Kuzoff 1995, and references therein; Sang
and Zhong 2000). Hybridization was cited specifically as the cause of discordance
between phylogenies generated with nuclear ITS and *rbcL* observed in a study of
*Hyobanche* (Wolfe and Randle 2001). However, in light of the finding of multiple *rbcL* loci presented here, the sampling of paralogous copies in phylogenetic analysis must also be cited as a possible misleading factor. Hybridization and lineage sorting aside, the chloroplast genome has often been treated by plant systematists as a non-recombining haplotype (Palmer et al. 1988), with each gene exhibiting a single copy within each organism (Soltis and Soltis 1998). However, a growing body of literature suggests that these assumptions are no longer tenable.

Plastome recombination does not appear to be a factor in this particular study. However, there is evidence of this in a number of plant taxa. Evidence of cpDNA recombination has been reported in *Pinus contorta* (Marshall et al., 2001) and *Cycas taitungensis* (Huang et al., 2001). It has also been induced by means of interspecific somatic cell fusion between *Nicotiana tabacum* and *N. plumbaginifolia* (Medgyesy et al., 1985), resulting in viable cytoplasmic hybrid lines or “cybrids.” Vijverberg et al. (1999) also concluded that rearrangements observed in the cpDNA of *Microseris* (Asteraceae) were best explained by homologous recombination with unequal crossing-over. Recombinant loci effectively share two or more phylogenetic histories, and homoplasy in phylogenetic inference using sequences from these loci may result from conflicting signal. However, the extent of this problem depends on whether recombination takes place between closely related or divergent haplotypes, and if the recombinant haplotype becomes fixed in populations prior to speciation. If the recombination event occurred between genomes of closely related individuals, the resulting phylogeny will most probably be similar to the recombinant fragment with the greatest phylogenetic signal. However, if recombination occurs between sequences of divergent taxa, inferred histories
may not match either of the histories of the recombinant fragments (Posada and Crandall 2002). As an extreme example, the *Sanguinaria canadensis* mtDNA gene *rps11* appears to be a chimera between genes from eudicots and monocots, best explained by a horizontal transfer event followed by recombination. Furthermore, it appears that this gene is functional and expressed (Bergthorsson et al. 2003).

When multiple copies of a chloroplast gene exist within an individual, through heteroplasmy, haplotype polymorphism, or through a duplication and transfer event, sampling of paralogous copies in phylogenetic analysis becomes a critical concern. Furthermore, uneven segregation of organelles in somatic and germ line cell division is conceptually the same as lineage sorting as applied to populations of endosymbionts rather than organisms. The transfer of genes to other genomic compartments complicates phylogenetic analysis in a unique way. The different genomic compartments frequently have drastically different rates of evolution, nucleotide compositional bias, and transformation probabilities. If sequences from different genomic compartments are included in an analysis, assumptions regarding models of evolution are likely to be violated. If paralogous genes are included in an analysis, phylogenetic inference will be misled (Olson and Yoder 2002).

In many phylogenetic studies, a single exemplar organism may be used to represent all members of a terminal, and at least for chloroplast loci, a single sequence may be used to represent all copies within that organism. In many cases this is an appropriate tactic, in that funding and human resources frequently do not permit extensive sampling of organisms or Southern blotting and cloning experiments that may reveal gene polymorphism within an organism, and that the presence of multiple copies
of chloroplast genes is not common. However, this sampling strategy may result in groundless inferences of species phylogeny, as in the case of *Hyobanche*.

To demonstrate the pitfalls of sampling one sequence from each taxon when many gene copies exist, matrices were constructed using one sequence from each species of *Hyobanche* and jackknife trees were obtained using the settings outlined above from each of these small matrices. Only sequences included in the analysis that resulted in the tree of Figure 3.3 were sampled. An *rbcL* sequence from *Harveya huttonii* was used to root each tree. For four taxa, there are fifteen bifurcating, rooted topologies. Using this inadequate one-sequence-per-taxon sampling strategy, ten of these fifteen topologies were obtained with strong jackknife support for each node (Figure 3.4).

It is clear that hybridization and lineage sorting may result in just such a pattern. However, it is also clear that the existence of multiple copies of each gene within individuals of *Hyobanche* species may also obscure phylogenetic relationships among taxa, if gene duplication occurs before speciation events and paralogous copies are maintained in resulting lineages. We have direct evidence that this is the case for at least one of the sequences obtained by cloning (*H. atropurpurea* 130.1-16; Figure 3.2).

**Implications of multiple *rbcL* pseudogenes and non-functional transcripts for endogenous expression in *Hyobanche*.**

While it remains possible that an *rbcL* open reading frame exists in species of *Hyobanche*, two experiments failed to corroborate this hypothesis. First, no such ORF was discovered in cloning experiments, and second, *rbcL* transcripts themselves were pseudogenes. This second point is perhaps more damning in that if an open reading frame existed, one would expect that this would at least be reflected in the pool of RNA
transcripts. Furthermore, these results do not support the second hypothesis, that \textit{rbcL} pseudogenes are repaired by means of post-transcriptional modification. In at least two cases, \textit{Hyobanche rbcL} transcripts were very similar to other sequences obtained from those species. However, in the third case (\textit{Hy. sanguinea} r03.08), the transcript sequence is sufficiently different from any other sequence to defy its placement within any inclusive clade of \textit{Hyobanche} sequences.

The case for endogenous expression of \textit{rbcL} in \textit{Harveya} seems clear-cut. The locus appears to be evolving under selective pressure, is transcribed, and the large subunit of Rubisco is present in at least some tissues. Only the function of Rubisco in \textit{Harveya} remains in question. On the contrary, nearly every aspect of the evolution and expression of \textit{rbcL} in \textit{Hyobanche} indicates a lineage in which holoparasitism is plesiomorphic; gene systems that support photosynthesis are in an early stage of degradation. This is contradicted only by the presence of the end product of expression, the large subunit of Rubisco. The presence of multiple copies of the gene and pseudogene transcripts, while bizarre and interesting, does not allow any insight into how the large subunit of Rubisco might be expressed. Therefore, I must offer an alternate hypothesis, which will not be tested in the current study; namely, that \textit{rbcL} is not expressed endogenously, but obtained from host plants.

**Exogenous acquisition of Rubisco from hosts of \textit{Hyobanche}**.

Rubisco may be taken up from the photosynthetic host of \textit{Hyobanche}, through haustoria. For holoparasites, the host serves as a source of photosynthates, water, and minerals. Haustorial function of other members of Orobanchaceae has been examined in some depth. The evolution of haustoria in Orobanchaceae is complex, and may include
convergence and reversal according to most recent phylogenetic hypotheses (Young et al. 1999). To further complicate matters, *Hyobanche* displays a unique type of leaf haustorium not found in any other species of parasitic plant, with the possible exception of *Orobanche teucrii* (Weber 1980), certainly an example of parallel evolution. Initially, *Hyobanche* seedlings form a primary haustorium at the base of the emerging radical from which rhizomes grow. In later stages, the primary haustorium disappears altogether, and all nutrients are obtained from the host by means of secondary haustoria, which arise from the scale leaves of rhizomes (Kuijt, et al. 1978; Weber and Visser 1980).

Conversely, *Harveya* species have been reported as exhibiting primary haustoria. If secondary haustoria are present, which has not been demonstrated thoroughly, they arise from lateral roots.

Several studies have shown the activity of cell wall and membrane degrading enzymes (cellulases, proteases, pectin methylesterases, and polygalacturonases) at the interface between metabolically active haustorial parenchyma and cells of the host root in Orobanchacea (Singh and Singh 1993; Losner-Goshen et al. 1998). Most of the transport of carbon from the host appears to be apoplastic, but *Striga hermonthica* may have xylem carbon concentrations five times greater than its host, *Sorghum bicolor*, although amino acid content in *Striga* leaf tissue mirrors that of its host (Stewart and Press 1990). Light microscopy studies of cellular markers in primary haustoria of *Orobanche crenata* and host *Vicia narbonensis* show phloem-phloem continuity that had not been demonstrated in previous microscopy studies or in secondary haustoria of *Orobanche* (Dörr and Kollmann 1995). Phloem-phloem continuity has also been demonstrated in the leaf-haustoria of *Hyobanche* (Weber 1980). All haustoria thus far
examined demonstrate the presence of cytoplasmically dense parenchyma at the interface between host and parasite cells, and these are thought to play an important role in transforming host nutrients into forms more easily utilized by the parasite. Of course, the same mode of Rubisco uptake could be hypothesized for \textit{Harveya} species (although this would be rather \textit{ad hoc} as \textit{rbcL} expression can be explained by conventional means). \textit{Harveya} haustoria exhibit specialized cambiform cells termed proteid bundles, which are hypothesized to be important in storing proteins taken up from the host (Young 1932). These findings about haustorial development in other members of Orobanchaceae indicate that uptake of complex molecules such as Rubisco may be possible. However, haustorial characteristics of other taxa should be interpreted with caution when hypothesizing the haustorial capability of \textit{Hyobanche}. It will be difficult to determine the role of leaf-haustoria in the uptake of Rubisco from host plants of \textit{Hyobanche} without more detailed anatomical and physiological information.

The hypothesis of uptake of host Rubisco is complicated by what would appear to be differential deposition of Rubisco in tissues of \textit{Hyobanche}. While \textit{Harveya} shows greatest immunoreactivity in leaf tissue, as would be expected in photosynthetic plants, \textit{Hyobanche} exhibits no large subunit immunoreactivity in leaves, but comparable concentrations in the calyx and corolla. Rubisco taken up from the host in \textit{Hyobanche} may simply be a product of host protein assimilation, and may have no function in the parasite. In this case, differential deposition in the calyx and corolla may simply be a byproduct of the increased flow of nutrients to reproductive structures during flowering. In \textit{Hyobanche}, flowers provide the greatest surface area for transpiration, perhaps resulting in an increased deposition of any solutes that may be in the xylem.
Alternatively, if Rubisco does play a role in carbon or oxygen fixation in *Hyobanche*, it is difficult to explain an increased need for such activity in reproductive structures.

This hypothesis may be even more difficult to test. Radiolabeling experiments may be inadequate. If host plants are grown in atmosphere containing labeled carbon dioxide, one may expect to see just such a transfer to a parasite grown in a normal atmosphere. However, if radiolabeled Rubisco were detected in *Hyobanche* it may be the result of endogenous assembly of radiolabeled amino acids from the host rather than the *in toto* transfer of the holoenzyme. Amino acid sequencing could be carried out on Rubisco obtained from the host and from *Hyobanche* to determine if sequences differed. To be effective this experiment would have to be carried out for a number of host plant-*Hyobanche* pairs, because the amino acid sequence of the large subunit is highly conserved in the flowering plants.
Table 3.1. Fisher’s Exact Test (two-tailed) of substitution rates in cloned sequences from a single individual of each of four species of *Hyobanche*. If all substitutions in these matrices were the result of PCR and cloning error, these rates should be equal in all cloning experiments. \( n \) = the number of sequences gathered in one cloning experiment. \( L \) = the length of the shortest tree for each set of cloned sequences. Substitution rate (\( \mu \)) is calculated as the number of observed changes divided by the number of possible changes, or \( L/(n-3)x \), where \( n-3 \) = the number of internal branches for \( n \) sequences, and \( x \) = the number of characters in the aligned matrix (constant of 1435 aligned nucleotides in all matrices). The test calculates the probability that the substitution rate of the control matrix (20 cloned sequences from *Hyobanche glabrata* amplified from a set of UTR primers) could have produced observed \( \mu \) in the test matrices and a control matrix. The control matrix had a calculated \( \mu \) of 4.1069 E-05. The two columns on the right give the highest possible \( \mu \) of test matrices that would have yielded a non-significant p-value (<0.05).

<table>
<thead>
<tr>
<th>Species</th>
<th>( n )</th>
<th>( L )</th>
<th>( \mu )</th>
<th>( p )</th>
<th>Highest possible n.s. observed ( \mu )</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Hy. atropurpurea</em></td>
<td>17</td>
<td>46</td>
<td>2.2995E-03</td>
<td>&lt;0.01</td>
<td>2.9866E-04</td>
</tr>
<tr>
<td><em>Hy. glabrata</em></td>
<td>13</td>
<td>7</td>
<td>4.8804E-04</td>
<td>&lt;0.01</td>
<td>2.7875E-04</td>
</tr>
<tr>
<td><em>Hy. rubra</em></td>
<td>14</td>
<td>10</td>
<td>6.3391E-04</td>
<td>&lt;0.01</td>
<td>3.1676E-04</td>
</tr>
<tr>
<td><em>Hy. sanguinea</em></td>
<td>13</td>
<td>8</td>
<td>5.5776E-04</td>
<td>&lt;0.01</td>
<td>2.7875E-04</td>
</tr>
</tbody>
</table>
Figure 3.1 Schematic illustrating binding sites of primers in cloning experiments. PCR cloning utilizing \textit{rbcL 5'} and \textit{rbcL 3'} resulted in little nucleotide variation among cloned sequences. However, when the internal primers were used, multiple copies of \textit{rbcL} were obtained from each individual of four species of \textit{Hyobanche}. 
Figure 3.2 Jackknife consensus of \textit{rbcL} cloned sequences (all of which were pseudogenes) from four individuals, one of each species of \textit{Hyobanche}. The aligned matrix consisted of 1435 characters, and resulted in one most parsimonious tree (length = 112, C.I. = 0.821, R.I. = 0.931). Redundant sequences were excluded from the analysis, and therefore, each terminal represents a distinct haplotype. Sequences from \textit{Harveya} species were used to infer the root of the tree. Sequences were moderately to strongly supported as monophyletic in regard to the individual from which they were cloned, with the exception of sequences from a single individual of \textit{Hyobanche atropurpurea}, which were resolved as polyphyletic.
Figure 3.3 Jackknife consensus of a matrix of *rbcL* clones (narrow terminals) combined with *rbcL* sequences obtained conventionally (thick terminals). The matrix consisted of 1435 aligned nucleotides and resulted in 43 most parsimonious trees (length = 119; C.I. = 0.807; R.I. = 0.949). Sequences from *Harveya* species were used to root the tree. In this tree, none of the sequences obtained from species are monophyletic.
Figure 3.4 Jackknife trees inferred from *rbcL* sequences obtained from multiple individuals of each of four *Hyobanche* species (*Hy. atropurpurea* = A; *Hy. glabrata* = G; *Hy. rubra* = R; *Hy. sanguinea* = S). To demonstrate phylogenetic error that may be introduced by sampling one sequence from each taxon when multiple sequences exist, one sequence was chosen to represent each species in phylogenetic analysis. All trees were rooted with an *rbcL* sequence from *Harveya pulchra*. Of fifteen possible rooted topologies for four taxa, ten were recovered with strong jackknife support.
CHAPTER 4

PHYLOGENETIC ANALYSIS OF SOUTHERN AFRICAN MEMBERS OF THE GENUS HARVEYA HOOK. INFERRED USING DNA SEQUENCE DATA FROM THE RBCL OPERON AND NUCLEAR ITS RDNA

INTRODUCTION

*Harveya* comprises 12 species (as treated in Chapter 5) in southern Africa including South Africa, Lesotho, and Swaziland. These species represent the majority of taxonomic and morphological diversity in the genus. Other species, not dealt with here, occur northwest in Angola and the Democratic Republic of the Congo, and to the northeast through Swaziland, Zambia, Burundi, Rwanda, Tanzania, Kenya, Eritrea and to Yemen on the Arabian Peninsula, and on the islands Madagascar and Comoros. While these are certainly more expansive with regard to geographical distribution, they probably include no more than eight separate species. *Harveya* has never been treated taxonomically as a whole, and the last revision of the South African species was by Hiern in Thistleton-Dyer’s “Flora Capensis” (1904). In this treatment nineteen species names appear, seven of which have been placed in synonymy in the following monograph (Chapter 5).
A shorter treatment by Hilliard and Burtt (1986), concentrated on the species of KwaZulu Natal, created three new species that had previously been included in *Harveya huttonii*: *Harveya pulchra*, *H. leucopharynx*, and *H. silvatica*. Hilliard and Burtt (1986) distinguished these species from one another on the basis of quantitative morphological features that have overlapping or nearly overlapping ranges, and the color of the corolla tube-- white in *H. leucopharynx* and *H. huttonii* but yellow in *H. pulchra* and *H. silvatica*.

However, specimens in the field frequently bear intermediate characteristics with tubes ranging from white to creamy white to very pale yellow to yellow. In Hilliard and Burtt’s treatment, *Harveya huttonii* is relegated to the Amatola range of the Eastern Cape. The distinction drawn between *H. pulchra* and *H. leucopharynx* is nebulous, based on overlapping calyx lengths (the former being slightly shorter), the color of the corolla tube (yellow in the former and white in the latter), and a non-overlapping length of the posterior corolla lobe. However, many specimens do not fall entirely within one species or another as currently circumscribed. Given that these specimens are morphologically quite similar to each other compared to other species of *Harveya*, it would be obfuscating to create a new species name for each specimen exhibiting novel combinations of these characters. *Harveya silvatica* was distinguished from these by only a single non-overlapping, qualitative characteristic, having a suborbicular rather than turbinate ovary. It is difficult to tell how much different from orbicular an ovary must be to be suborbicular without being simply turbinate. Given that these characters are scarcely diagnostic of many specimens in the field and that these species have overlapping geographical distributions, they have been tentatively re-subsumed under the umbrella-species *Harveya huttonii*, called hereafter the “*huttonii*” complex to distinguish it from
*Harveya huttonii sensu* Hilliard and Burtt. Throughout the remainder of this chapter, members of the “huttonii” complex will be referred to by the species names given by Hilliard and Burtt if it was found that the specimen fit entirely within a species circumscription. However, for those that did not, the name “huttonii” will appear in quotes, indicating that is an undiagnosable specimen of the larger complex. In any case, there is little doubt that the “huttonii” complex is closely related to both *H. pumila* and *H. coccinea* due to a high degree of morphological similarity.

Besides requiring taxonomic scrutiny, relationships among species of *Harveya* are thus far untested. Phylogenetic analysis based on chloroplast sequence data from *rps2* and *matK* place *Harveya* within Orobanchaceae, despite its traditional placement in Scrophulariaceae (Young et al. 1999). This result is born out by studies of the Lamiales and Orobanchaceae using sequence data from the ribosomal nuclear DNA Internal Transcribed Spacer (ITS) locus (Wolfe et al. in prep). Both sets of studies place *Harveya* as sister to *Hyobanche* with strong bootstrap support. However, these studies sample relatively few species of *Harveya* and little is known regarding the relationships among species in this genus. Phylogenetic analysis is necessary to test the monophyly of this diverse genus, the origins of the various pollination syndromes exhibited by species, geographical origins of the genus, and the evolution of host taxon preference.

*Harveya* may be distinguished from *Hyobanche* on the basis of several morphological characters: a) a five-parted rather than three-parted corolla, b) the presence of both sterile and fertile anthers, rather than complete loss of the sterile anther, and c) the absence of leaf haustoria. However, examination of the phylogeny of the family reveals
that these are all plesiomorphic conditions. It is therefore possible that Harveya represents a paraphyletic grade leading to a monophyletic Hyobanche given these three characters alone.

As previously stated, Harveya species vary in floral morphology, suggesting different pollinator syndromes⁴ (although pollinators have been identified in only two instances). Several species have morphologies suggesting pollination by birds, having orange to red flowers with long cylindrical corollas borne upright (H. bodkini, H. bolusii, H. scarlatina, H. stenosiphon, and H. squamosa). Two have morphologies suggesting moth pollination, bearing large, white, spicy-smelling flowers, with long corolla tubes and expansive limbs. One of these, H. capensis, has been observed undergoing pollination by a hawk moth (Sphingidae; Marloth 1932). Additionally, several other species, including all members of the “huttonii” complex, as well as H. coccinea, H. pumila, and H. purpurea, appear to be pollinated by bees or other insect generalists, bearing broadly funnelform corollas, borne perpendicular to the axis of the stem, and having a sweet, fruity smell. I observed one of these, Harveya “huttonii” undergoing pollination by a bee, although the capture of this swift insect for correct identification was not achieved. A final species, Harveya hyobanchoides, is truly bizarre and it is difficult to surmise what sort of pollinator it may be attracting. The corollas are erect and cylindrical, but the tube is short, and butter yellow with a chlorophyllous green limb.

⁴ Throughout, the use of the term “pollination syndrome” does not actually require pollination by a particular group of animals. Rather it refers to suites of morphological characters typical of flowers pollinated by those animals, an expedient in that pollinator data are lacking for most species.
Other questions surrounding *Harveya* center on its location of origin. In southern Africa, species are largely confined to the moister coastal regions and the mountains leading up to the dry central plateau, where the species cease to exist, with two notable exceptions, *H. speciosa* and *H. pumila* occurring as deeply inland as the Rand in Gauteng (Figure 4.1). This occurrence in relatively moist habitats is not surprising given the physiological requirements of parasitic plants, which often exhibit rapid rates of transpiration to maintain solute flow from the vasculature of its host (Press et al. 1988; Seel et al. 1993; Stewart and Press 1990). *Hyobanche* is entirely confined to South Africa, Namibia, Lesotho, and Swaziland, but the locality of its origin cannot be unambiguously inferred (A.D. Wolfe pers. comm.). While *Harveya* almost certainly arose in southern Africa, phylogenetic analysis will be employed to clarify the point of origin.

**MATERIALS AND METHODS**

**DNA sequencing**

Tissues were collected in the field and desiccated on silica gel. While morphological characteristics observed in Chapter 5 suggest that *Harveya pulchra*, *H. leucopharynx*, and *H. silvatica* should be subsumed in *H. huttonii*, specimens collected in the field were diagnosed as belonging to one of these species as delimited by Hilliard and Burtt if possible. DNA was extracted from tissues using a modification of Doyle and Doyle’s CTAB protocol (1987) and further purified using the Eluquik system (Schleicher and Schull; Keene, NH). Species of *Harveya* and outgroups *Hyobanche, Alectra, Buchnera, Cycnium*, and *Melasma* were included in this study (Table 4.1). In all analyses, trees were rooted using *Alectra, Buchnera, Cycnium* and *Melasma*, allowing the
monophyly of *Harveya* to be tested with *Hyobanche* sequences. The *rbcL* operon, including the 5'-UTR, the *rbcL* coding sequence, and the 3'-UTR, were amplified and sequenced following methods presented in Chapter 2. Additionally, the nuclear rDNA Internal Transcribed Spacer (ITS) was amplified and sequenced. The region containing ITS 1, 5.8S, and ITS 2 was amplified using universal primers (Wen and Zimmer 1996) NNC18S10 (AGGAGAAGTCGTAACAAG) and C26A (CTTTCTTTTCTCGCT). PCR amplifications of 50 µl contained 0.5-1 µl purified DNA, 0.2 mM dNTPs, 0.15 mM MgCl₂, 0.64 µM each primer, 0.5% DMSO, 1U *Taq* polymerase (Invitrogen; Carlsbad, CA), and 1X *Taq* polymerase amplification buffer. Reactions were carried out under the following temperature conditions: 2 min. at 94°C; 35 cycles of 1 min. at 94°C, 1 min. at 50°C, and 2 min. at 72°C; and a final extension of 10 min. at 72°C. Amplified DNA was purified on polyethylene glycol, followed by two ethanol precipitations (85% and 100% respectively) and resuspended in ddH₂O. Cycle sequencing was carried out using the standard ABI Big Dye 2.0 protocol and sequences were obtained using an ABI-3100 automated sequencer (Applied Biosystems; Foster City, CA), using external primers ITS 5 (GGAGGAGAAGTCGTAACAAG) and ITS 4 (TCCTCGCTTATTGATA-TGGC), and internal primers ITS 3 (GCATCGATGAAGAACGCAGC) documented in Sang et al. (1995) and ITS 2m (GCTGCGTTCTTCATCGATGC) of Wen and Zimmer (1996).

**Phylogenetic analyses**

Whenever possible, several accessions of each species of *Harveya* were included in analyses. In the ITS data set, multiple accessions were available from *H. speciosa* (5), *H. capensis* (3), *H. scarlatina* (2), *H. silvatica* (2) and *H. pulchra* (2). The entire *rbcL*
operon was only available for multiple accessions of *H. pulchra*. PCR amplification of the *rbcL* coding sequence was impossible for accessions of *H. pumila* and *H. leucopharynx* (*sensu* Hilliard and Burtt), but the 5’- and 3’-UTRs were obtained; as these regions contain the large majority of parsimony informative characters in *Harveya*, they were included in the matrix with the *rbcL* coding sequence treated as missing data for these two species.

Matrices were aligned separately in Clustal X (Thompson et al. 1997)) and manually adjusted using the Se-Al data editor (Rambaut 1996). Parsimony analysis was carried out using PAUP*4.0b10 (Swofford, 2002). For each matrix, tree searches were carried out with 1000 random sequence additions, two trees held at each step, and TBR branch swapping. The “condense trees” function was used to collapse all nodes that had minimum branch lengths of zero, eliminating duplicate topologies. One thousand jackknife replicates using TBR branch swapping, 37% deletion, and the “emulate Jac” options were used to estimate clade support.

The issue of whether matrices should be combined has been thoroughly debated in the literature (Barrett et al. 1991; Bull et al. 1993; Chippindale and Wiens 1994; Quieroz 1993). In the parsimony framework the Incongruence Length Difference test (Farris et al., 1995) has been used to determine if matrices are significantly incongruent to preclude their combination⁵, but this use has also been debated (Yoder et al. 2001). Before any analysis, it was decided that matrices would be analyzed both separately and in combination, with the realization that factors such as introgression might confound the

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⁵ Note however, that the ILD test was designed to determine the probability that two matrices were composed of characters from the same distribution, rather than to be used in the decision to combine matrices.
total evidence approach if plastid and nuclear trees were incongruent but correct. If the results of these analyses led to conflicting conclusions regarding character evolution, such a test may have been employed; however, this would not have clarified which matrix represented the best data for inferring character evolution. Despite the *a priori* decision to combine, the ILD was employed to determine the probability of the null hypothesis that sequence matrices obtained from *Harveya* species are congruent. One hundred “partition homogeneity test” permutations were conducted in PAUP*4.01b, using the same heuristics used in tree search above.

In any case, it has been shown that nuclear and plastid data are in conflict for the outgroup *Hyobanche* (Wolfe and Randle, 2001), and that *rbcL* cannot be used to infer the relationships among members of that clade reliably without thorough sampling of paralogues (Chapter 3). As the phylogeny of that group is not the focus of this study, the remaining issue is one of rooting. Different topologies inferred from either data set of the *Hyobanche* clade may conceivably imply different rootings for the *Harveya* clade. Therefore, combined analyses were run including and excluding *Hyobanche*.

When species were represented by multiple accessions in the ITS data matrix, but by a single sequence in the *rbcL* matrix, only one of the ITS sequences of that species was included in the combined analysis, preferentially chosen from the same individual represented in the *rbcL* matrix.

**RESULTS**

The *rbcL* operon matrix consisted of 2,227 aligned nucleotides for 23 taxa, and included 97 parsimony informative characters. Heuristic searching resulted in eight most parsimonious trees (Figure 4.2: L = 283; C.I. = 0.866; R. I. = 0.873). Jackknife analysis
supports the sister relationship of Harveya and Hyobanche, albeit rather weakly (77%), and provides somewhat stronger support for the monophyly of Harveya (84%). Members of the “huttonii” complex form an unresolved clade (jackknife = 74%) including Harveya coccinea, not surprising given their close morphological similarity.

The ITS matrix consisted of 631 characters for 33 taxa, including 195 parsimony informative characters. Heuristic searching resulted in three most parsimonious trees (Figure 4.3: L = 462; C.I. = 0.759; R.I. = 0.848). The consensus of these trees is largely congruent with the results of the rbcL operon matrix. Harveya and Harveya+Hyobanche are both well supported (jackknife = 100%). Again, the “huttonii” complex and H. coccinea form a well-supported clade (jackknife = 99%) with little internal resolution. Specimens that are diagnosable as Harveya pulchra are paraphyletic within this clade.

While the rbcL operon tree shows Harveya speciosa and H. scarlatina weakly supported as basal in the clade containing the “huttonii” complex, H. coccinea and H. pumila, these species are strongly supported (jackknife = 92%) as members of a clade containing all other species of Harveya by the ITS matrix.

The ILD resulted in a test statistic with p = 0.21, failing to reject the null hypothesis of congruence. Total evidence analysis resulted in a matrix of 23 taxa and 2,858 aligned nucleotide characters, of which 292 were parsimony informative. Heuristic searches resulted in two most parsimonious trees (Figure 4.4: L = 751, C.I. = 0.786, R.I. = 0.809). Again, the monophyly of Harveya, Hyobanche and Harveya+Hyobanche is strongly supported (jackknife = 100%). All species of Harveya fall into two mutually exclusive, well-supported clades. The first includes the “huttonii” complex, H. pumila
and *H. coccinea* (jackknife = 100%) and the second includes the rest of the species, with *H. scarlatina* basal (jackknife = 86%).

The exclusion of *Hyobanche* resulted in no change in the consensus topology within *Harveya* for the combined matrix, indicating that incongruence between *rbcL* and ITS data sets within *Hyobanche* does not result in repositioning of the root of *Harveya*.

**DISCUSSION**

**The “huttonii” complex**

Often, in delimiting species of *Harveya*, diagnosable morphological characters are clear. However, in the case of the species comprising the “*huttonii*” complex (*H. huttonii* sensu Hilliard and Burtt, *H. pulchra*, *H. leucopharynx* and *H. silvatica*), several morphological characters treated as diagnostic exhibit continuous variation, i.e., the length of the calyx and the color of the corolla tube. Calyx length varies continuously in specimens examined and the corolla color varies along the spectrum from snow-white to lemon yellow, including creamy-white, and pale yellow in between. For these species, there is no recognizable gap in these character traits that might allow unambiguous diagnosis of species. Furthermore, a combination of these characters provides no clear criteria for identification. For these reasons, these species have been re-subsumed into *Harveya huttonii* as described by Hiern. More in-depth study of other features not examined here (such as anatomical or developmental features) might certainly result in the dissolution of *Harveya huttonii* into less inclusive taxa. However, in the absence of these data, such a dissolution is unjustified.

Molecular phylogenetic analysis fails to support or reject the dissolution of *Harveya huttonii* into less inclusive species. However, terminal specimens diagnosable
as *Harveya huttonii* as circumscribed in Chapter 5 are closely related in plastid, nuclear and total evidence gene trees, and the inclusion of all these specimens under a unified circumscription is not unjustified by molecular analysis alone (Figure 4.3). One might argue however, given the paraphyletic status of *H. huttonii* in the broad sense, that *H. pumila* and *H. coccinea* should be included. This is in fact a viable option. However, as mentioned previously the latter two species are diagnosable on the grounds of distinct morphological characters from *H. huttonii* as circumscribed here. As a strictly morphological species concept is employed in this study, *H. pumila* and *H. coccinea* will remain separate.

**Evolution of pollination syndrome**

It has been argued that the evolution of the sizeable floral diversity of the Cape Floristic Kingdom of South Africa is the result of the heterogeneous nature of the terrain, a mosaic of strongly variable microhabitats allowing for large-scale diversification on a small geographical scale (Cowling and Lombard 2001; Goldblatt 1997; Goldblatt and Manning 2000; Richardson et al. 2001). The mountain systems of the Cape Fold Belt and Drakensberg (Figure 4.1) provide natural reproductive barriers between geographically close plant populations by concentrating moisture on the ocean-side of each range, and in drainages between peaks. There is an additional effect of varying soil types throughout the country, and pronounced climatic differences. The west has a Mediterranean climate, with cool wet winters and long dry summers. This is reversed in the east, where summers tend to be hot and damp and winters cool and dry. Along the southern coast, mild temperatures and heavy rainfall predominate year-round, producing
a narrow strip of subtropical coastal forest and thornveld. The Central Plateau is
dominated by karoo vegetation, and is generally warmer and dryer than any other part of
the region, bordered on the north by both the Namib and Kalahari deserts. Furthermore,
southern Africa has been unglaciated throughout recent geological history, as opposed to
Mediterranean climatic regions of North and South America and Europe, providing
ample time for the evolution of diversity. On the other hand, it has been argued that
when rapid floral evolution occurs without concomitant evolution of vegetative traits, as
is the case for many of the more diverse taxa of the Cape, this must be driven by
competition for pollinators (Johnson 1996). Unfortunately, Harveya does not provide a
good model to test between these competing (or cooperating) factors driving diversity. As
a parasite with highly reduced vegetative morphology, rapid diversification in any
structure other than flowers is unlikely to be observed.

In any case, some homoplasy is apparent in the evolution of floral morphology as
evidenced by pollinator syndrome (figure 4.5). The floral morphology of the ancestor of
Harveya is ambiguous. The generalist syndrome (broad, shallow corollas that are sweet-
smelling, and born at right angles to the stem) may have arisen once or twice. It is
possible that the ancestor of these species had this syndrome, in which case bird
pollination evolved convergently. On the other hand, the generalist syndrome may have
arisen twice, the ancestral condition in this case being bird pollinated: Once at the base
of the clade composed of the “huttonii” complex, H. coccinea and H. pumila and again in
H. purpurea. Harveya purpurea is frequently misidentified as H. pumila or H. huttonii
(or vice-versa) in the Eastern Cape where they co-occur. These all have pink limbs and white corolla tubes, but differ in other morphological characters, such as the shape of the calyx lobes and the shape of the throat. While the throat of *H. pumila* and *H. huttonii* may be yellow, *H. purpurea* bears a yellow spot on the palate of the lower lip only. The pink limb, yellow throat, and white tube are convergent in these clades.

Likewise, the bird pollination syndrome (red to orange cylindrical corollas borne erect) arose either once or twice, characterizing *H. scarlatina* and the clade uniting *H. bolusii*, *H. stenosiphon*, and *H. squamosa*. Further differentiation of floral morphologies in the latter clade may well have been driven by other ecological factors. *Harveya stenosiphon* occurs primarily in very damp soils, often surrounded by dense vegetation in south-facing ravines of the Langeberg and Swartberg ranges. The flowers have a rather expanded corolla limb, and stems tend to be quite tall, both traits that may increase visibility in these vegetatively dense environments. On the other hand, *Harveya squamosa* occurs primarily on well-drained soils of coastal dune systems, where decreased visibility is not an impediment to pollinator attraction. The stems are frequently rather reduced, and the limb of the flower is contracted so that the lobes are imbricate. However, the entire plant is often brightly orange-colored, including the expanded caudal leaves and floral bracts, which may serve collectively as a pollinator attractant. *Harveya bolusii* occurs in low vegetation typically at the crests of mountain ranges. It has a short stem as well, but due to the vibrantly scarlet corolla with small but expanded lobes, is also rather conspicuous.
The moth pollination syndrome (white corollas with long tubes, expanded limbs and spicy-smells) may have evolved once or twice, but may be ruled out as the ancestral condition. Given either bird or generalist pollinator syndromes as the ancestral condition, it is equally optimal that moth pollination arose once at the base of the clade containing *H. speciosa* as its least inclusive member, but was reversed with the evolution of the bird-pollination syndrome of the *H. bolusii + H. stenosiphon + H. squamosa* clade, or that it was derived convergently in *H. speciosa* and *H. capensis*. Interestingly, *H. capensis* and *H. speciosa* share very few other characteristics, and may be easily differentiated on the basis of vestiture (*H. capensis* is quite hirsute on all vegetative structures, while *H. speciosa* is glabrous), anther characters (the sterile anther of *H. capensis* is equal in length to the fertile anther, but is highly reduced in *H. speciosa*), and the shape of the corolla tube in cross section (laterally compressed in *H. capensis*, round in *H. speciosa*) among others.

**Biogeography**

Various methods have been proposed for determining ancestral area distributions, with diverse emphasis of the effects of vicariance, dispersal, and extinction (Bremer 1992; Ronquist 1997; Hausdorf 1998). The simplest means of determining ancestral areas is to treat geographical distributions as character states. If taxa occur in more than one area distribution, they may be treated as polymorphic. Optimization of the basal character state in a parsimony framework is at least conceptually straightforward, if not operationally so for a given set of taxa and distributions. This method assumes that the ancestral distribution is equivalent to one or more of the distributions in extant taxa, if
optimized as polymorphic. In this study, consideration of vicariance is trivial in that area distributions as described here have been at least geographically static throughout the evolution of the *Harveya* lineage, and therefore transformations of areas as characters may be interpreted as either dispersal and colonization events (gains) or extinction events (losses).

*Harveya* species largely exhibit one or two of four generalized distributions (Figure 4.6): 1) along the coast of the winter rainfall area (Coastal, west), 2) along the coast of the summer rainfall area (Coastal, east), 3) in the Cape Fold Belt, and 4) in the Drakensberg range. These distributions, as defined, are not in every case perfectly descriptive, in that some species may only inhabit a portion of the distribution or may occur occasionally in an area not defined in the study, nor are they exclusive, in that species may occur in more than one area. However, areas chosen here are ecologically discrete (based on altitude and climate), and are inclusive of the vast majority of specimens examined.

The distributions of species of *Hyobanche* cannot be used in the inference of the ancestral area of *Harveya*, given the uncertainty of the phylogenetic relationships among *Hyobanche* species due to paralogy and conflict between nuclear and plastid sequence data. Optimization of these characters on the consensus topology of the combined analysis indicates that the ancestral area of *Harveya* is the Drakensberg mountain system. Fortunately, this would be the case regardless of the topology of the *Hyobanche* clade, as the three basal-most nodes in the *Harveya* clade can be unambiguously optimized as such.

*Harveya* most likely arose in the Drakensberg range of the East Cape and KwaZulu
Natal. The diversification of Harveya and Hyobanche almost certainly occurred in the last 10 million years (Wolfe et al. in prep), and may have been driven, as in many other lineages of the Cape Floristic Kingdom, by the post-Miocene aridification caused by the establishment of the Benguela current (Richardson et al. 2001). At the base of the Harveya lineage, there are two well-supported clades. All members of the clade containing the “huttonii” complex + H. coccinea + H. pumila occur primarily in the Drakensberg range, or east of it, with the exception of Harveya coccinea, a coastal species occurring in KwaZulu Natal, the Eastern Cape and the Western Cape. The basal two members of the other main clade, H. scarlatina and H. speciosa, are also found primarily in the east—H. scarlatina exclusively in the East Cape and KwaZulu Natal Drakensberg, and H. speciosa throughout the eastern region. The species primarily living in the Western Cape, H. capensis, H. purpurea, H. squamosa, H. stenosiphon, and H. bolusii were derived later in this clade.

**Host preference**

Due to the difficulty in extracting host-parasite connections, the host record for many species of Harveya is sparse (Table 4.2). However, there is no evidence of co-evolution between Harveya species and host taxa, and many species of Harveya are capable of parasitizing phylogenetically distant hosts. In at least one case, that of Harveya speciosa, a single specimen was observed parasitizing both the thick tap-root of Berkheya purpurea (Asteraceae) and the fibrous roots of Themeda triandra (Poaceae) simultaneously. While phylogenetic trends are not apparent, some species demonstrate
somewhat restricted host preferences. For example, *H. scarlatina* has been observed parasitizing members of two genera of Asteraceae (*Euryops* and *Nestlera*). *Harveya capensis* has been most often observed parasitizing members of the *Apiaceae*. I observed it twice on species of *Centella*, some 200 km distant. *H. purpurea* has been observed parasitizing three genera of Campanulaceae: *Roella*, *Lightfootia*, and *Prismatocarpus*. In each instance that I excavated haustoria of *Harveya speciosa*, it was parasitic on a member of the Asteraceae, and usually a species of *Berkheya* (albeit one instance of simultaneous parasitism on the roots of a grass). It is clear that some species (like *H. speciosa*) are capable of parasitizing hosts with very different forms and physiologies. In a restricted locale, *H. stenosiphon* has been observed parasitizing the roots of *Osmitopsis osmitoides*, a perennial herb in Asteraceae and *Pterocelastrus* a forest tree of Celastraceae.

**Remaining questions**

While much of the diversity of southern African species of *Harveya* has been sampled, several species were not collected. *Harveya bodkini* is morphologically quite similar to *H. bolusii*, has flowers that suggest bird-pollination, and is quite restricted in its range, having only been collected in the Skurfedebeg and adjacent Cedarberg ranges of the Western Cape. It seems probable that this arose in the clade with the other Western Cape, bird-pollinated species. It has only rarely been collected, and in my own fieldwork, excursions over three years to its habitat have failed to produce a single specimen.
Regarding *Harveya purpurea*, only specimens of *H. purpurea* subsp. *purpurea* were available for use as exemplars in this study. In the following chapter, *H. purpurea* is expanded to include two new subspecies, *H. purpurea* subsp. *euryantha* and *H. purpurea* subsp. *sulphurea*, both of which are morphologically similar enough to indicate kinship, but were previously treated as separate species. *H. purpurea* subsp. *euryantha* is largely uncollected though its natural habitat is characterized by considerable human development. Schlechter, the author of the basionym *H. euryantha*, collected a large amount of type material and very probably depauperated the source population to some extent. It is possible that *H. purpurea* subsp. *euryantha* is endangered or extinct.

Likewise, *H. purpurea* subsp. *sulphurea* is extremely limited in its range, confined to mountain passes of the northern Cedarberg range, and collection efforts there have been unsuccessful. Though this habitat is by-and-large untouched by development, the infrequent nature of this plant is reflected in its extremely poor collection record.

It should also be noted that the analyses presented here are limited to those species endemic to southern Africa. Future studies in which more northerly species are included may alter our current understanding of the evolution of the group. *Harveya obtusifolia* (Benth.) Vatke occurs primarily in Madagascar. It is quite similar to other species of *Harveya* and inclusion in this genus is not in question, at this point. However, unlike other species, it bears large, chlorophyllous leaves-- clearly a hemiparasite. It is fascinating to speculate as to how this species relates to the others. On one hand, if it occurs at the base of the *Harveya* lineage, we may conclude that holoparasitism arose
independently in *Harveya* and in *Hyobanche*. We may also be forced to revise our
notions of the biogeography of the species. On the other hand, if *H. obtusifolia* is derived
in the lineage, it must be concluded that a reversal to photosynthetic capability of the
leaves has occurred, an awkward notion given current assumptions about the evolution of
parasitism.
<table>
<thead>
<tr>
<th>Taxon</th>
<th>Voucher</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Alectra capensis</em></td>
<td>Randle 112 (OS)</td>
<td>26 Jan 2001. Eastern Cape, Mt. Kubusie. S 32° 33.4'; E 27° 16.9'. 1440m</td>
</tr>
<tr>
<td><em>Alectra sessiliflora</em></td>
<td>Steiner s.n. (NBG)</td>
<td>No loc. data</td>
</tr>
<tr>
<td><em>Buchnera glabra</em></td>
<td>Randle 107 (OS)</td>
<td>26 Jan 2001. Eastern Cape, Mt. Kubusie. S 32° 33.4'; E 27° 16.9'. 1440m</td>
</tr>
<tr>
<td><em>Cynium racemosum</em></td>
<td>Randle 106a (OS)</td>
<td>26 Jan 2001. Eastern Cape, Mt. Kubusie. S 32° 33.4'; E 27° 16.9'. 1440m</td>
</tr>
<tr>
<td><em>Harveya bolusii</em></td>
<td>Steiner s.n. (NBG)</td>
<td>17 Jan 2000. Western Cape, Localized on ridge south of Somerset, Snoukop</td>
</tr>
<tr>
<td><em>Harveya bolusii</em></td>
<td>McMaster s.n. (OS, NBG)</td>
<td>20 Jan 2002. Eastern Cape, Gaika's Kop</td>
</tr>
<tr>
<td><em>Harveya capensis</em></td>
<td>Steiner s.n. (NBG)</td>
<td>18 Dec 1992. Western Cape</td>
</tr>
<tr>
<td><em>Harveya capensis</em></td>
<td>Randle 81 (OS)</td>
<td>21 Nov 1999. Randle 81. Western Cape, Route 45 near jct. with R321, across the road from a reservoir and within 15m. of the reservoir's edge, S 34° 01.467'; E 19° 12.668'; 490m.</td>
</tr>
<tr>
<td><em>Harveya capensis</em></td>
<td>Randle 82 (OS)</td>
<td>25 Nov 1999. Western Cape, Boskloof Farm, Clanwilliam, S 32° 56.351'; E 19° 11.537'</td>
</tr>
</tbody>
</table>

Table 4.1 Record of specimens from which sequences were obtained
Table 4.1 continued

<table>
<thead>
<tr>
<th>Harveya capensis</th>
<th>Randle 147 (OS)</th>
<th>24 Dec 2001. Western Cape, in a bowl-like depression on the mountain above Ceres. Head of trail is very close to RV park on Carson St., S 33° 22.583'; E 19° 15.318'. 903m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Harveya coccinea</td>
<td>Randle 144 (OS)</td>
<td>19 Dec 2001. Western Cape, Cape Town, Table Mountain. Window Gorge. S 33° 58.624'; E 18° 25.251'. 812m.</td>
</tr>
</tbody>
</table>

| Harveya huttonii ("leucopharynx" sensu Hilliard and Burtt 1986) | Steiner s.n. (NBG) | 1 Feb 2000. Kwazulu Natal, Mlambonja River Valley |

continued
<table>
<thead>
<tr>
<th>Species</th>
<th>Collector</th>
<th>Date</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Harveya hyobanchiodes</em></td>
<td>Matlock s.n. (OS)</td>
<td>30 Sep 2001.</td>
<td>Eastern Cape, Port Elizabeth, disturbed ground from land set aside for the building of a mosque between Godetia Ave, Westerling and Malabar.</td>
</tr>
<tr>
<td><em>Harveya purpurea</em></td>
<td>Randle 79 (OS)</td>
<td>20 Nov 1999.</td>
<td>Western Cape, Caledon. Along Highway M44. Rocky slopes across from &quot;Sunbird&quot; guest house at the mouth of the Steenbras River. 180m.</td>
</tr>
<tr>
<td><em>Harveya purpurea</em></td>
<td>Randle 84 (OS)</td>
<td>25 November 1999.</td>
<td>Boskloof Farm, Clanwilliam, S 32° 56.351'; E 19° 11.537'</td>
</tr>
<tr>
<td><em>Harveya scarlatina</em></td>
<td>Steiner s.n. (NBG)</td>
<td>1 Feb 2000.</td>
<td>Kwazulu Natal, Mlambonja River Valley, Garden Castle Reserve. 2300m</td>
</tr>
<tr>
<td><em>Harveya scarlatina</em></td>
<td>Randle 133 (OS)</td>
<td>7 Feb 2001.</td>
<td>Kwazulu Natal, Mlambonja River Valley. Garden Castle Reserve, on steep smooth rock on river bank on path to Rhino Peak</td>
</tr>
<tr>
<td><em>Harveya speciosa</em></td>
<td>Steiner 2463 (NBG)</td>
<td>7 Feb 1992.</td>
<td>Eastern Cape, Elliot Bastervoetpad. 2120m.</td>
</tr>
<tr>
<td><em>Harveya speciosa</em></td>
<td>Steiner s.n. (NBG)</td>
<td>26 Jan 2000.</td>
<td>Eastern Cape. Road to Carlisle</td>
</tr>
</tbody>
</table>

*Table 4.1 continued*
Table 4.1 continued

*Harveya speciosa*  
Johnson s.n. (NU)  
30 Dec 1999. Eastern Cape,  
Elliot, Bastervoetpad.

*Harveya speciosa*  
Randle 110 (OS)  
26 Jan 2001. Eastern Cape,  
Mt. Kubusie. S 32° 33.4'; E  
27° 16.9'. 1440m

*Harveya speciosa*  
Randle 117 (OS)  
28 Jan 2001. Barkly East,  
Maclear, long road from  
Naudes Nek, visible on the  
higher bank on the Maclear  
side. S 30° 44.598'; E 28°  
08.978'. 2222m.

*Harveya speciosa*  
Randle 123 (OS)  
30 Jan 2001. Eastern Cape,  
Elliot, Ben Wyvis Farm,  
along Bastervoetpad. S 31°  
10.535'; E 27° 57.194'.  
2060m.

*Harveya squamosa*  
Wolfe 710 (OS)  
31 Oct 1996. Rte. 364, 1km  
from entrance to Ysterfontein,  
S 32°09.978'; E 18°47.210'.  
300m

*Harveya stenosiphon*  
Randle 89 (OS)  
7 Dec 1999. Grootvadersbosch,  
Langebergen. In Farmkloof  
close to the ranger station.  
50-75m from mountain seep,  
at center of kloof.

*Hyobanche atropurpurea*  
Wolfe 716 (OS)  
1 Nov 1996. Ceres-Citrusdal  
Road. Farm Boskloof

*Hyobanche glabrata*  
Wolfe 702 (OS)  
29 Oct 1996. On road to  
Mientkiesplaas, 1km from  
Sutherland-Middelpos Rd.

*Hyobanche rubra*  
Wolfe 735 (OS)  
near Karoo National  
Botanical Garden. S  
33°36.809'; E 19°27.723'.  
270m

*Hyobanche sanguinea*  
Wolfe 704 (OS)  
30 Oct 1996. Road from  
Calvinia to Middelpos. Farm  
Bloemfontain on rocky  
outcropping near stream  
crossing.. 1371m
<table>
<thead>
<tr>
<th>Harveya species</th>
<th>Host taxa</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. bolusii</em></td>
<td>Stoebi sp.</td>
<td>Asteraceae</td>
</tr>
<tr>
<td></td>
<td>Disparago sp.</td>
<td>Asteraceae</td>
</tr>
<tr>
<td></td>
<td>Genus and species unknown</td>
<td>Cyperaceae</td>
</tr>
<tr>
<td></td>
<td>Cliffortia sp.</td>
<td>Rosaceae</td>
</tr>
<tr>
<td><em>H. capensis</em></td>
<td>Hydrocotyle sp.</td>
<td>Apiaceae</td>
</tr>
<tr>
<td></td>
<td>Anthospermum sp.</td>
<td>Rubiaceae</td>
</tr>
<tr>
<td></td>
<td>Centella sp.</td>
<td>Apiaceae</td>
</tr>
<tr>
<td><em>H. coccinea</em></td>
<td>Ficinia sp.</td>
<td>Cyperaceae</td>
</tr>
<tr>
<td><em>H. huttonii</em></td>
<td>Anthospermum sp.</td>
<td>Rubiaceae</td>
</tr>
<tr>
<td></td>
<td>Felicia sp.</td>
<td>Asteraceae</td>
</tr>
<tr>
<td></td>
<td>Euryops tysonii</td>
<td>Asteraceae</td>
</tr>
<tr>
<td></td>
<td>Sparrmannia ricinocarpa</td>
<td>Tiliaceae</td>
</tr>
<tr>
<td><em>H. hyobanchoides</em></td>
<td>Aspalathus laricifolia</td>
<td>Fabaceae</td>
</tr>
<tr>
<td><em>H. pumila</em></td>
<td>Selago sp.</td>
<td>Scrophulariaceae</td>
</tr>
<tr>
<td></td>
<td>Metalasia sp.</td>
<td>Asteraceae</td>
</tr>
<tr>
<td></td>
<td>Anthospermum rigidum</td>
<td>Rubiaceae</td>
</tr>
<tr>
<td><em>H. purpurea</em></td>
<td>Roella sp.</td>
<td>Campanulaceae</td>
</tr>
<tr>
<td></td>
<td>Prisamotocarpus diffusus</td>
<td>Campanulaceae</td>
</tr>
<tr>
<td></td>
<td>Lightfootia sp.</td>
<td>Campanulaceae</td>
</tr>
<tr>
<td></td>
<td>Myrsine sp.</td>
<td>Mrsinaceae</td>
</tr>
<tr>
<td></td>
<td>Erepsia ramosa</td>
<td>Aizoaceae</td>
</tr>
</tbody>
</table>

Table 4.2 Recorded hosts for species of *Harveya*
<table>
<thead>
<tr>
<th></th>
<th>Species</th>
<th>Family</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>H. scarlatina</strong></td>
<td><em>Euryops</em> sp.</td>
<td>Asteraceae</td>
</tr>
<tr>
<td></td>
<td><em>Nestlera</em> sp.</td>
<td>Asteraceae</td>
</tr>
<tr>
<td><strong>H. speciosa</strong></td>
<td><em>Berkheya</em> spp.</td>
<td>Asteraceae</td>
</tr>
<tr>
<td></td>
<td><em>Themeda triandra</em></td>
<td>Poaceae</td>
</tr>
<tr>
<td></td>
<td><em>Conyza pedocephala</em></td>
<td>Asteraceae</td>
</tr>
<tr>
<td><strong>H. squamosa</strong></td>
<td><em>Othonna</em> sp.</td>
<td>Asteraceae</td>
</tr>
<tr>
<td></td>
<td><em>Arctotis decurrens</em></td>
<td>Asteraceae</td>
</tr>
<tr>
<td></td>
<td><em>Cliffortia</em> sp.</td>
<td>Rosaceae</td>
</tr>
<tr>
<td><strong>H. stenosiphon</strong></td>
<td><em>Osmitopsis osmitoides</em></td>
<td>Asteraceae</td>
</tr>
<tr>
<td></td>
<td><em>Pterocelastrus</em> sp.</td>
<td>Celastraceae</td>
</tr>
</tbody>
</table>
Figure 4.1. Map of southern Africa showing the range of *Harveya* (cross-hatched), major mountain systems including the Cape Fold Belt, the Drakensberg (darkened), the Central Plateau, and provincial boundaries.
Figure 4.2. Jackknife consensus of the rbcL operon matrix. * sensu Hilliard and Burtt (1986). **Harveya purpurea subsp. purpurea.
Figure 4.3 Jackknife consensus of the ITS matrix. * sensu Hilliard and Burtt (1986).
**Harveya purpurea subsp. purpurea.
Figure 4.4. Jackknife consensus of combined data from the *rbcL* operon and ITS matrices *sensu* Hilliard and Burtt (1986).
Figure 4.5. Optimization of pollinator syndrome characters on phylogeny inferred from combined data. *sensu* Hilliard and Burtt (1986).
Figure 4.6. Cladogram showing approximate geographical distributions of terminals, and optimization of ancestral area.
CHAPTER 5

TAXONOMIC REVISION OF HARVEYA SPECIES OF SOUTHERN AFRICA

INTRODUCTION

*Harveya* Hook. (Orobanchaceae) is a genus of perennial root-parasites occurring in Africa, Madagascar, and the southern tip of the Arabian Peninsula. *Harveya* was originally placed in Scrophulariaceae, as were many parasitic lineages now placed in an expanded Orobanchaceae (Young et al. 1999). Bentham (1876) placed *Harveya* in tribe Gerardieae, and subtribe Hyobancheae with *Hyobanche* and *Campbellia* (since included in *Christisonia*), both genera of primarily holoparasitic species; *Hyobanche* occurs only in southern Africa, while *Christisonia* is East Asian. Phylogenetic analyses have never included members of all three genera, but have supported the sister-relationship between *Hyobanche* and *Harveya* (Chapter 4; Young et al. 1999; Wolfe et al. in prep).

Little has been written about the biology of *Harveya* aside from taxonomic descriptions of species. While most species of *Harveya* have showy, attractive flowers, these plants spend much of their life-cycle underground, and flowering is quite ephemeral. Vegetative structures are highly reduced in most plants, making species
identification impossible in non-flowering material. Due to the holoparasitic nature of the plant, cultivation has never been successful, though often suggested due to the potential of these plants to make attractive ornamentals. Furthermore, specimens of *Harveya* preserve poorly on herbarium sheets, all parts turning a deep black color shortly after dissociation from the host plant, a property recognized by early European settlers as being useful in the creation of an ink for writing—hence the Afrikaans name “Inkblom”.

The first species of *Harveya* on record were collected by Thunberg during his excursion to the Cape of Good Hope from 1772-1775, who intuitively recognized the kinship of this plant with *Orobanche*. He named two species, *Orobanche squamosa* and *O. capensis*, and Linnaeus (fils) named a third *Orobanche purpurea*, from these collections. The genus *Harveya* was created in 1838, by William Jackson Hooker. The name is in honor of the erstwhile Treasurer of the Cape Colony and professor of botany at Trinity College in Dublin, William Henry Harvey, who provided Hooker with original material of the first named species, *Harveya capensis*. In 1838, Harvey published a new genus, *Aulaya*, named for Mrs. M. McAulay, a friend and botanical illustrator in his *Genera of South African Plants*. By this time, he was cognizant of Hooker’s publication of *Harveya* as it appears just above *Aulaya* in that work. *Aulaya capensis* (Thunb.) Harv. appears in the subsequent edition of Hooker’s *Icones Plantarum* (1841), and it is clear that Hooker realized that these were quite similar, in his own words, “closely allied.”

Both genera appear in Bentham’s contribution to De Candolle’s *Prodromus* (1846), with seven species attributed to *Aulaya* and only one to *Harveya*. The last species attributed to *Aulaya* was *Aulaya coccinea* Harv. in his *Thesaurus Capensis* (1858), the protologue reading, “This species nearly unites the genera *Harveya* and *Aulaya* partaking of the
characters of each.” In the following year, the genus *Aulaya* was not included in Bentham and Hooker’s *Genera Plantarum*. The first definitive statement of the synonymy of these genera appeared in von Wettstein’s (1895) contribution to *Die natürlichen Pflanzenfamilien*, succinctly as “*Harveya* Hook. (*Aulaya* Harv.).” Over the next decade the species names of *Aulaya* were included in *Harveya*.

A good deal of disorder arose early in the taxonomic history of this group with the dissemination of the South African collections of Jean François Drège, Christian Friedrich Ecklon and his partner, Carl Ludwig Zeyher. These intrepid collectors brought large quantities of preserved material from South Africa for sale in exsiccatae throughout Europe. Herbaria purchasing these exsiccatae had little idea which other herbaria might have obtained them, and as a result, many names were attributed to Ecklon and Zeyher or Drège (as well as Ernst Meyer who handled Drège’s collections), although no descriptions or records of coloration or natural habit were provided. Most of the specimens were placed in *Orobanche* and given new names by the collectors (however, descriptions were not published). Various authors attempted to establish some sort of order from these collections, but were thwarted by the dearth of information surrounding them, which, until 1847, was only available in the form of a sales catalogue (compiled by Ernst Meyer). However, Ernst Steudel (1841) recognized the kinship of these specimens with Hooker’s *Harveya* only four short years after the publication of *Harveya capensis*, as did the Czech botanist Karl Bor von Presl shortly thereafter (1844)\(^6\). Presl’s use of Ecklon and Drège’s names was the first published account of some of them, and his

\(^6\) Steudel and Presl both used the spelling “*Harwaya*”, in their treatments. However, both attributed the genus to Hooker, and the misspelling is interpreted here as an orthographical error.
placement of some of these names into *Harveya* by the establishment of combined names in the same publication resulted in the unusual author citation “(Presl) Presl.” At the time that Steudel and Presl published, most of Ecklon and Drège’s names were without description, and neither Steudel nor Presl intended to describe, their works consisting of long lists of new combinations. Therefore, none of the names of Ecklon and Drège were validly published by Steudel or Presl. Only one of them is currently in use in the genus *Harveya, Orobanche scarlatina* E. Meyer (nom. nud.), made valid by Bentham’s publication of *Aulaya scarlatina* Benth, and at last placed in *Harveya* by William Phillip Hiern in his treatment of southern African species in 1904.

Fifty-three species names have been attributed to this genus since its creation in 1837, some of which have been placed in synonymy with other species of the genus and with other genera. Thirty-five of these names are attributed to species living primarily in southern Africa (South Africa, Swaziland, and Lesotho). *Harveya* has never been treated taxonomically as a whole. The most comprehensive treatment to date, by William Hiern in *Flora Capensis* (1904), included nineteen species, some of which were newly described. It is apparent that Hiern described several species that he did not see in living state, as color information is missing, and stems are described as “furrowed” (stems of living plants are terete or obtusely angular, becoming furrowed on drying). It is also apparent in some cases that he relied heavily on circumscriptions of these species as described by original authors. However, in Hiern’s defense, his key to the species of South Africa is the best means of identifying specimens to date, and examination and identification of poorly preserved specimens of *Harveya* is challenging, especially given the obscure history of many of the names. Since the publication of Hiern’s treatment,
five new species endemic to southern Africa have been described, notably three new species by Hilliard and Burtt (1986), who provided a key to the species of KwaZulu Natal. To my knowledge, only one species occurring in southern Africa occurs elsewhere; *Harveya pumila* Schlechter (= *Harveya randii* Hiern) has been collected in Zimbabwe and Mozambique (Launert and Pope 1990). Unfortunately, treatment of species occurring outside of southern Africa is beyond the scope of this study. The following is a taxonomic revision of the species of southern Africa, with notes on morphological characters, habitat and distribution, and parasitism, including a description of the genus *Harveya*. This description is not intended to characterize species not examined in this study, but rather to provide a summary of characters uniting species as delimited here.

**MATERIALS AND METHODS**

Approximately 800 specimens of *Harveya* species were borrowed from the following herbaria: BOL, BR, E, K, L, MO, NBG, NU, PRE, and TCD. The following herbaria were visited, and all specimens examined: BOL, GRA, MO, NBG, NU, NY, PRE. Because some of the defining characters of *Harveya* species, such as corolla color and three-dimensional features, are lost upon herbarium preservation, materials were also collected in the field, photographed and preserved in FAA. Whenever possible, haustorial connections were excavated to allow identification of host plants. Additionally, pressed and FAA preserved material and photographs were generously donated by many South African botanists.

The aboveground height of plants is difficult to obtain from herbarium specimens, especially when a large portion of the plant’s length is underground. Necessarily then,
plant heights included here were either obtained from collection labels, or may include a portion of the belowground height of the plant. Unless otherwise noted, measurements of calyx length represent the longer axis of bilabiate calyces, from the base of the calyx to the tip of the longest lobe. Similarly, corolla length represents the longest straight axis of a corolla, usually from the corolla base to the end of the tube along the ventral surface. The limb of the corolla was not included in this measurement. To conform with the measurement of corolla tube length, style length was measured as the longest straight axis of a curved or straight style, and filament length is the longest straight axis of the filament. Any curvature of structures is noted in the description. Most length measurements were those of pressed structures on herbarium sheets, but length ranges provided in species delimitations circumscribe all FAA preserved material. Dots on distribution maps represent actual sites at which specimens were collected. Capsules, seeds, and anther characteristics varied little among species, but illustrations were provided when material was adequate.

The overwhelming majority of specimens included in the study were collected before the advent of GPS technology, and many before the creation of accurate topographical maps of the country. A grid of latitude and longitude lines was overlaid on distribution maps, and each quadrant divided into sixteen sectors (approximately 27.5 x 23.7 km). If the collection data were inadequate to place a distribution point within a sector, no point was placed. Host plant identifications were recorded only when hosts were specified as such, rather than as a probable host or a nearby plant; in many cases, collectors included a host specimen with the *Harveya* specimen. However, this is not true in all cases and some of the hosts reported here may be anecdotal, due to either
failure to obtain the correct host, or failure to identify the host plant correctly. For specimens collected in the field, host identification was aided by specialists at South African herbaria.

Species were delimited using a morphological species concept. Under this concept, unique characters or character combinations are required to accord a group of specimens the status of species, and every attempt was made to insure that these characters were absolutely diagnostic. This admittedly may not be the best species concept, and many know that the debate of what constitutes species is heated. Nevertheless, the use of other species concepts requires sampling of specimens combined with experimentation far beyond the scope of this study. Future studies utilizing other concepts may well overturn taxonomic decisions made here, an eventuality that can only be illuminating.

**GENERIC TAXONOMY**

*Harveya* Hook. in Ic. Pl. t.118. 1837. TYPE: *H. capensis*.

Perennial, holoparasitic herbs, surface variously covered with jointed, glandular, hairs, often viscid or oily to touch, all parts becoming black upon dissociation from host-plants, largely achlorophyllous, or chlorophyll concentrated in certain structures, vegetative parts often reddish to purple, or yellow to light green with reddish or purplish markings. Connection to host roots by means of underground haustoria, often swollen and tuber-like, or appearing as smaller tubercles on the surface of host plant roots. Stems terete or obtusely angled in cross-section, erect, obsolete to long and flexuous, fleshy or at times woody below, simple or branching most often below ground. Caudal leaves reduced to scales, often imbricate at the base, opposite or alternate above, adpressed or
atrose, convex abaxially, sessile or subsessile, with entire margins, acute, subacute or obtuse at the apex. Inflorescence terminal, spicate, corymbose, or racemose, flowers arising from the axils of floral bracts. Floral bracts scale-like, often larger than caudal leaves, but similar in vestiture, shape, position and color. Flowers perfect, speciose, pedicellate or sessile, subtended by a pair of opposite bracteoles arising from the pedicel at the base of the calyx to the axil of the floral bract, bracteoles linear or lanceolate, sessile or subsessile with entire margins, acute to obtuse at the apex. Calyx tubular, campanulate to obconical or cuneate at the base, campanulate to cylindrical above, longer adaxially, five-lobed, bilabiate, with a posterior lip consisting of one or three lobes, and anterior lip consisting of two or four lobes, lips and lobes variously divided by shallow or deep sinuses, longitudinally 10-nerved, each nerve extending from the base of the calyx to the tip of a lobe or sinus. Corolla tube funnel-form, ampliate, or cylindrical, often curving, round or laterally compressed. Corolla limb bilabiate, posterior lip consisting of two lobes, anterior lip consisting of three lobes, lobes imbricate or expanding, orbicular, ovate or obovate, often wavy, entire, crenulate, or lobulate at the margin, often ciliate. Stamens four, didynamous or subdidynamous, inserted or exserted, filaments terete or flattened, anthers glabrous, transverse, bithecal, one theca polleniferous, crescent-shaped, falcate or mucronate at the apex, dehiscing by means of a longitudinal suture opening from the apex, the sterile anther highly reduced or subulate and as long as or slightly longer than the fertile theca, hooked or not at the apex. Pistil glabrous, ovary of two locules, orbicular, ovoid or flask shaped, with axile-apical placentation, placentae often splitting below forming two lobes, numerous ovules. Style barely inserted or exserted, incurved. Stigma entire or slightly lobed, capitate or not, globose, clavate or oblong.
Calyx persistent in fruit, capsule dehiscing septicidally. Seeds miniscule, less than half a millimeter long and less than a fifth of a millimeter wide, cylindrical with a central constriction or conical, seed wall with polygonal reticulate ridges dividing deep alveoli.

KEY TO THE SPECIES OF HARVEYA

1. Inflorescence spicate or corymbose.

2. Calyx divided nearly to the base, limb consisting of one posticous and four anticous lobes…………………………………………………………………………………1. H. hyobanchoides

2. Calyx shallowly divided, less than half of its length, limb consisting of three posticous and two anticous lobes.

3. Corolla limb rosy, pink or white. Corolla tube abruptly inflating, ampliate above. Stigma capitate, globose…………………………………………………………2. H. pumila

3. Corolla limb orange or scarlet. Corolla tube cylindrical, gradually inflating from the base, stigma not or only scarcely capitate, clavate-oblong.

4. Corolla lobes expanded, limb (16-) 21-31 mm broad…………3. H. scarlatina

4. Corolla lobes imbricate, limb 12-17 mm broad………………4. H. squamosa

1. Inflorescence racemose

5. Limb red or orange, corolla tube cylindrical.

6. Corolla limb broad, 29-50 (-60) mm in diameter, corolla lobes also broad, (10-) 12-22 mm in diameter……………………………………5. H. stenosiphon

6. Corolla limb narrow, 12-25 mm in diameter, corolla limbs smaller than above, 5-15 mm in diameter.

7. Corolla tube (42-) 47-52 (-63) mm in length…………………..6. H. bodkini

7. Corolla tube (24-) 27-35 (-39) mm in length…………………..7. H. bolusii
5. Limb white, yellow, pink, or rosy-purple, corolla tube inflated.

8. Stems, bracts, and calyces glabrous, sterile anther absent or less than 1mm in length

8. *H. speciosa*

8. Stems, bracts, and calyces hirsute, sterile anther as long as or longer than fertile anther.

8. *H. speciosa*

9. Corolla limb white, or slightly tinged with pink at the tips of the lobes, throat strongly compressed laterally, stigma chlorophyllous green

9. *H. capensis*

9. Corolla limb pink, purple, or sulphur-yellow, throat not strongly compressed, stigma white or yellow.

10. Calyx deeply divided, to at least half the length of the calyx, calyx lobes lanceolate

10. *H. purpurea*

10. Calyx divided to 1/3 of its length or less, calyx lobes deltoid.

11. Corolla tube 20-26 (-29) mm in length. Corolla limb 18-25 mm broad

11. *H. coccinea*

11. Corolla tube 28-44 (-50) mm in length. Corolla limb (27-) 30-45 (-55) mm broad

11. *H. huttonii*

SPECIES OF HARVEYA

Plant 18-34 cm in height, all parts scarlet except for the corolla and sexual organs of the flower, covered with viscid hairs, stem fleshy, villose, simple or rarely branching below, 8-13 mm in diameter. Caudal scales highly reduced, in imbricate whorls around the base of the stem, 8-9 x 5-6 mm, obovate, obtuse or acute, sessile, concave, adpressed, pubescent to villose, viscid, rarely above basal whorl resembling floral bracts, but not giving rise to flowers, nearly imbricate with maximum internode length of 18mm. Inflorescence a dense terminal spike of 12-36 sessile flowers, comprising 80-100% of the above ground height of the plant. Floral bracts concave, adpressed, (15-) 18-30 (-33) x 7-10 mm, elliptical to oblanceolate, villose to densely villose abaxially, pubescent adaxially, acute, sessile. Bracteoles arising from the anterior lip of the calyx, at the base, opposite, (13-) 21-23 mm, linear, villose or densely villose, acute, sessile. Calyx campanulate at the base, cylindrical above, (21-) 23-30 x (5-) 7-11 mm, villose or densely villose externally, pubescent-glabrescent internally, hairs becoming sparser and shorter toward the base, bilabiate, the anterior lip comprising four shallowly divided calyx lobes, 4-7 x 3-4 mm, deltoid-lanceolate, the posterior lip a single lobe, divided from the anterior lip by nearly the entire length of the calyx, linear, acute, 3-4 mm wide at the base. Corolla tube, yellow to yellow-green, cylindrical, 4-6 mm in diameter at the base, constricting 8-10 mm from the base, and then gradually inflating to 7-11 mm at the throat, longer dorsally than ventrally, at times somewhat curved, 30-34 (-37) mm ventrally, 35-37 (-42) mm ventrally, somewhat keeled dorsally, exterior pubescent to villose, with longer, denser hairs toward the apex, internally pubescent-glabrescent with the exception of a band of dense hairs encircling the tube at the base of the stamens at the point of corolla constriction. Limb of the corolla 10-14 (-17) x 10-14, bilabiate, lobes of
anterior and posterior lips olive-green to deep chlorophyllous green, fleshy, slightly reflexed, approximately equal, 4-6 (-9) x 3-5 mm, semiorbicular to deltoid, villose in and out, somewhat concave, entire. Stamens arising from ring of dense hairs in the corolla throat, filaments flattened, glabrescent or rarely puberulent, didynamous, the shorter, anterior pair 17-23 mm, the posterior pair exceeding the anterior pair by 2-4 mm, nearly or just exserted, fertile thecae of the anther crescent-shaped, 3x2 mm, somewhat hooked and mucronate at the apex, the sterile thecae 3-5 x 1mm (or narrower) subulate, straight or slightly hooked at the apex, both thecae glabrous. Ovary 4-6 (-8) x 3-6 mm, ovoid, glabrous, placentation axile, placentae divided in two lobes below, ovules numerous, style (21-) 31-35 mm, strongly curved at the apex, stigma exserted, clavate, scarcely or not capitate, 3-5 x 1-2 mm. Fruits not seen. Flowering July to October. (Figure 5.1)

**Taxonomy**

Hiern (1904) ascribed *Harveya hyobanchoides* to Schlechter Engl. Bot. Jahrb. xxvii, p. 184 (1899). In this work, Schlechter makes the combination utilizing a basionym ascribed to Harvey (without a citation of the publication), *Aulaya hyobanchoides*. However, this name does not appear in any of Harvey’s publications, nor was it described by Harvey, and therefore, Schlechter’s combination is a *nomen nudum*, validly published with description by Hiern; hence, the correct author citation reads “Schlechter ex Hiern”, as it appears in the International Plant Names Index. The type material must be chosen from original material used by Hiern. I have chosen *MacOwan 128* (K) because it appears to have been sent to Hiern as evidenced by an attached note, written by MacOwan, “Will you let Mr. Hiern see a sketch of my 128.”
Parasitism

Harveya hyobanchoides has been reported as parasitizing several species of Aspalathus (Fabaceae) including A. laricifolia. This may be the closest that any species of Harveya comes to parasitizing any economically important plants, as rooibos tea is derived from a closely related species, Aspalathus linearis.

Habitat and distribution

Harveya hyobanchoides is confined to sandy soils along the shore of the Eastern Cape from Humansdorp to Centani (Figure 5.1) It is quite rare and infrequently collected, perhaps due to the human development of much of its natural habitat.

Representative specimens examined

EASTERN CAPE. Albany: near Grahamstown, Aug 1867, MacOwan s.n. (K); 14 Aug 1942, 5 miles from Grahamstown on Fort Beaufort Rd., Bayliss 605 (NBG); 21 Sep Grahamstown, Collector(?) 535 (L). Centani: Near Qolora mouth between sand dunes, 8 Jul 1906, Pegler 748 (BOL). Humansdorp: Aug 1896, Wolley Dod 1516a (K).

Port Elizabeth: Bethelsdorp, Aug 1915, Paterson 3157 (BOL); 4 Aug 1938, Mirkin s.n. (NBG); Sep 1956, Bosman s.n. (PRE); between the extension of Godetia Ave., Westerling and Malabar, 30 Sep 2001, Matlock s.n. (OS).
Figure 5.1 *Harveya hyobanchoides* A. Plant. B. Flowers. C. Longitudinal-section of the corolla tube showing pistil, stamens, and densely villose constriction at the point of stamen insertion. D. Dissection of the corolla showing the same as “C” E. Dissected calyx. F. Distribution
Figure 5.1 continued


Plants dwarfed, stem nearly obsolete, appearing as a tuft of flowers arising from the surface of the ground. Plant height including inflorescences 7.4-10.0 cm, stems fleshy, 3-6 mm in diameter, rarely branching, but if so, at or above first flowering node, glabrous at ground level, hairs increasing in length and density toward the apex of the stem, ranging from puberulent below to villose above. Caudal leaves small, imbricate, or with internodes no longer than 7 mm, 4-6 x 2-6 mm, orbicular to elliptical to ovate or obovate, concave, adpressed, obtuse to acute at the apex, with varying vestiture from glabrous to sparsely pubescent, or glabrescent at scale base with hairs increasing in length toward the apex and margins. Inflorescence comprising nearly all of the aboveground biomass of the plant, spicate or somewhat corymbose with short pedicels (<10mm) on lower flowers, upper flowers sessile. Floral bracts concave, adpressed or atrorse, 9-14 x 4-6 mm, obovate to ovate, sessile, acute to obtuse at the apex, villose or grading from puberulent below to villose at the apex. Bracteoles sessile, inserted at the base of the calyx in sessile flowers or at the base to 1/2 the length of the pedicel in pedicellate flowers, linear, (9-) 14-21 x 1-2 mm, pubescent to villose in entirety or pubescent below grading to villose at the apex, acute. Calyx campanulate at base, campanulate to
cylindrical above, bilabiate, longer posticously, generally villose externally with hairs increasing in density and length toward the calyx lobes and at longitudinal nerves, glabrous internally except for the apex of the lobes, lobes villose, lanceolate, acute to obtuse, three posterior lobes (5-) 7-11 x 3-4 mm, anterior lobes 8-12 x 2-4 mm, posterior and anterior lobes divided by a sinus of 10-14 mm. Corolla tube white to pale yellow, 4-5 mm in diameter at the base, slightly constricting just above the ovary, and then inflating abruptly to 9-12 mm at the throat, curving, 32-39 mm long, not compressed laterally, externally more or less pubescent at the base, hairs increasing in length and density dorsally and apically to densely villose, internally glabrescent except for a narrow, ventral, longitudinal strip which is puberulous at the apex, and a dense ring of hairs at the insertion point of the anther filaments. Corolla limb white to pink to reddish violet, 25-32 mm in diameter, corolla lobes more or less equal, or posticous lobes slightly larger, patent, (5-) 6-10 x 7-13 mm, orbicular to obovate, crenulate or rarely lobulate, hairs decreasing in length and density toward the margin of the lobes, externally pubescent-villose, internally glabrescent- puberulent, often ciliate at the margin. Corolla throat yellow, puberulent to glabrescent, not compressed much laterally or vertically. Stamens inserted at the point of corolla inflation, didynamous, the shorter dorsal pair 2-4 mm shorter than the ventral pair, shorter pair 5-10 mm, the longer pair 7-14 mm in length, filaments puberulent at the apex, densely villose below, the fertile theca of the anther crescent shaped, falcate at the apex, 2-3 x 2 mm, the sterile theca subulate, 3-4 x 0.5-1 mm, not strongly hooked at the apex. Pistil glabrous, ovary 3-7 x 3-5 mm, ovoid, with
axile placentation, ovules numerous, style 21-27 mm, incurved at the apex, inserted, stigma somewhat 2-lobed, capitate, globose, 2-3 x 2 mm. Fruits not seen. Flowers August to December. (Figure 5.2)

**Taxonomy**

Schlechter’s types were deposited at Berlin-Dahlem (B), but the original material of *Harveya pumila* was destroyed during the second World War. The isotype at the Bolus Herbarium in Cape Town has been chosen to serve as a lectotype.

Given the large geographical distribution of *Harveya pumila* as circumscribed here, there is little surprise that several names have been applied to this species. It seems that specimens collected in the Eastern Cape have nearly always been identified as *H. pumila*, while specimens in KwaZulu Natal, the Free State, and Gauteng are nearly always identified as *Harveya randii*. Hilliard and Burtt included *Harveya randii* in their treatment of species of Kwazulu Natal (1986), but excluded *H. pumila*, presumably because they thought it exclusively a Cape species. Hiern’s choice to give status to the new species *Harveya randii* was probably based on lack of sufficient material of *Harveya pumila* to see that these are morphologically indistinct. In Thistleton-Dyer’s *Flora Capensis* Hiern differentiates these two species based on calyx indumentum, “glabrescent or on the nerves puberulous” for *H. pumila*, and “glandular-pubescent” for *H. randii*. The former is, in fact, how Schlechter described *H. pumila*. However, the type specimen appears to have a more hirsute calyx than this, and no differences can be detected between the indumentum of the two species. Another characteristic that Hiern used to distinguish between the two species is the shape of the apices of calyx lobes, subacute in
*H. pumila* and obtuse in *H. randii*. Again, these characteristics do not furnish a useful distinction, in that lobes may range from acute to obtuse in a single collection.

Hilliard and Burtt included *H. crispula* in synonymy with *H. randii* in their treatment, and examination of the type supports this; therefore, the name has been included as a synonym of *H. pumila* in this treatment.

It should be noted that the color of the corolla limb varies widely in *H. pumila* from white to pink to reddish-violet. However, subspecific taxa should not be based on this variation as plants with white and pink corollas may be found growing side-by-side within a population.

**Parasitism**

*Harveya pumila* is primarily parasitic on species of *Anthospermum* (Rubiaceae) throughout its range, including *A. aethiopicum*, *A. pumilum*, and *A. rigidum*. It has also been reported in more isolated cases as parasitizing species of *Metalasia* (Asteraceae) and *Selago* (Scrophulariaceae). Instances in which host connections have been excavated reveal few if any secondary haustoria. Primary haustoria do not appear to be major storage organs, as they are small and exist almost entirely within host root tissue, causing very little swelling.

**Habitat and distribution**

The habitat of *Harveya pumila* is rather varied, from moist grassveld to rocky slopes of the Drakensberg, and to the drier interior plateau of the western Free State and Gauteng (Figure 5.3). *Harveya pumila* is one of the few species to penetrate the central plateau, the other being *H. speciosa*. 
Representative specimens examined

EASTERN CAPE. **Albany:** between Assegaibosch and Raustenbachs drift (between Sidbury and Bushmens River), 28 Oct 1813, *Burchell 4195* (K); Grahamstown, Rocky Ridge near Hounslow, Oct 1888, *Galpin 238* (PRE); Grahamstown, beyond west hill, Nov 1926, *Dyer 665* (L); Grahamstown, Featherstone Kloof, 11 Sep 1932, *Rennie 394* (BOL); southwest of Grahamstown, border between Albany and Alexandria, between Narraway and Longford Farm, 23 Nov 1963, *Stauffer and Guillarmod 5202* (L).


**Humansdorp:** between Hankey and Loerie, Aug 1939, *Fourcade s.n.* (NBG). **Peddie:** Kaffir Drift near Peddie, 29 Nov 1945, *Compton 17742* (NBG). **Tarkastad:** boundary between Tarkastad and Bedford, Jan 20 1990, *Edwards s.n.* (NU).

**Gauteng. Florida:** grassy field north of Florida, 19 Sep 1896, *Moss s.n.* (MO).


**Utrecht:** Tweekloof, Altemoor, 1922, *Thode A203* (PRE); farm Naauwhoek, 6 Nov 1976, *Hilliard and Burtt 9174* (K, NU). **Vryheid:** Vryheid, Sep 1941, *van de Merwe 2425* (PRE); Hlobane, 10 Sep 1950, *Johnstone 449* (NU).

**Free State. Bloemfontein:** near Rhenosterspruit, 13 Oct 1917, *Potts 3320* (PRE); **Harrismith:** Rengsburgskop, Oct 1963, *Jacobsz 217* (K, PRE); Queens Hill.

Figure 5.2 *Harveya pumila*. A. Plant growing on the roots of *Anthospermum pumilum* (Rubiaceae). B. Flower. C. Dissected calyx. D. Dissected corolla and pistil.
Figure 5.3 *Harveya pumila* distribution.

Plant short, covered in red or crimson bracts, seeming to comprise little more than a dense spike of yellow-tubed flowers with bright orange limbs. Stem 11-18.5 cm in height, often branching below, branching frequently obscured by density of inflorescence, yellow to greenish yellow, at times tinged with red, viscid, puberulent or pubescent, or inclining to villose toward the apex, 5-6 mm in diameter. Caudal leaves highly reduced below, imbricate, concave, scale-like, 4-5 mm x 5-8 mm, obtuse to subacute, glabrescent to puberulent, those above sessile, consisting of no more to two or three pairs below the inflorescence, nodes 12-36 mm, 13-20 x 5-6 mm, oblong, concave, adpressed or atrorse, puberulous or pubescent below, with hairs increasing in length but not density above, apex obtuse or acute. Inflorescence a dense spike, comprising no less than 50% of the aboveground height of the plant, and frequently up to 100%. Floral bracts elliptical to lanceolate or oblong, concave, 18-24 x 6-10 mm, pubescent to sparsely or densely villose, acute at the apex, sessile. Bracteoles linear to lanceolate, 19-24 x 2-4 mm, sparsely or densely villose. Calyx obconical or cuneate below, broadly or narrowly cylindrical above, more than one-half the length of the corolla tube, (30-) 32-42 (-52) x 9-17 mm, sparsely or densely villose outside, pubescent within, somewhat bilabiate, the posterior lip only slightly longer than the anterior, lobes, deltoid to lanceolate, 8-12 x 3-7, the anterior two lobes more deeply divided that the posterior three. Corolla tube yellow, cylindrical, erect, only slightly

124
curved, scarcely inflating from the base, (41-) 46-56 (-63) mm in length, 8-12 mm in
diameter at the throat, pubescent outside, inside puberulent to glabrescent except for
denser, longer strip of hairs along the ventral axis, which increase in length and density
toward the throat. Corolla limb bright orange to orange-red, (16-) 21-31 mm in diameter,
posticous lobes reflexed, 8-12 (15) x 9-11 mm, anticous lobes more ore less concave, 8-
12 (15) x 8-13 mm in diameter, lobes orbicular to ovate, pubescent. Corolla throat
yellow, deltoid viewed from the front. Stamens inserted 14-20 mm from the base of the
corolla, didynamous, dorsal pair 15-37 mm long, ventral pair 21-42 mm long, nearly
exserted, puberulent, not especially flattened. Anthers glabrous, fertile anthers crescent
shaped, 3 x 2 mm, dehiscing by an apical suture, sterile anthers subulate, 3-4 x 1 mm.
Pistil glabrous, ovary orbicular-ovate, 5-7 x 4 mm, round in cross section, style somewhat
or strongly exserted, strongly incurved at the apex, stigma clavate, not capitate, 3-4 x 1-2
mm, ovules numerous, capsule not seen. Flowers November-March. (Figure 5.4)

**Parasitism**

*Harveya scarlatina* has been reported to parasitize the roots of *Euryops* sp. and
*Nestlera acerosa* (Asteraceae). However, no haustoria were included in the specimens
observed in this study, which may be difficult to excavate from the densely fibrous
grassland soil in which this species occurs. I have observed *H. scarlatina* growing from a
fissure in a vertical rock face of the Mlambonja River bank, at least a full meter below the
ledge. I was unable to obtain haustoria from this specimen, but it seems unlikely that it
was parasitizing any herbaceous plants given its distance from them, and the nearest
plants to the ledge above were small shrubs of a species of *Leucosidea* (Rosaceae).
Habitat and distribution

*Harveya scarlatina* occurs primarily in high alpine grassveld of the East Cape, Kwazulu-Natal, and Lesotho Drakensberg (Figure 5.5). Hiern (1904) reported two specimens from the “Kalahari” region, one from Doornkop in the Orange River Colony (*Burke s.n.: K!*) and at Johannesburg (*Galpin 6059: PRE!). Neither appear to be specimens of *H. scarlatina*. The former is poorly preserved and difficult to identify positively, while the latter is a specimen of *H. pumila* Schlechter.

Taxonomy

The International Plant Names Index incorrectly attributes *Harveya scarlatina* to “Hook. ex Steud.” Steudel (1841) recognized the name *Orobanche scarlatina* E. Mey., itself a *nomen nudum*, and included it in the new taxon *Harwaya scarlatina* Hook. However, there is no evidence that Hooker ever addressed this species in any of his works, and Steudel provided no description of the species, making the name invalid. However, Bentham included a citation of *O. scarlatina* E. Mey. in his description of *Aulaya scarlatina* (1846), validating the name, later cited as the basionym of *Harveya scarlatina* Hiern (1904). Therefore, Hilliard and Burtt’s citation “(Benth.) Hiern” (1986) is correct.

Representative specimens examined


**KWAZULU NATAL** **Underberg**: Garden Castle; Mlambonja Valley, 4 Jan 1982, *Hilliard and Burtt 14879* (NU); Mlambonja River, south bank of the southern-most tributary in fissure of vertical rock wall, 7 Feb 2001, *Randle 134* (OS).

Figure 5.4. *Harveya scarlatina* A. Plant. B. Flowers. C. Dissected corolla and calyx with pistil.
Figure 5.5. *Harveya scarlatina* distribution

TYPE: Thunberg 14477 (UPS, microfiche at NY!).

Aboveground portion of plants (20-) 30-40 cm in height, up to one-half of the entire body of the plant below ground. Stems, leaves, and all parts of the inflorescence often uniform in color, from sulphur-yellow (rare) to orange or red-orange (common), the aboveground portion of the plant covered with viscid hairs. Stems erect, simple with few or up to eight branches, each ending in a terminal spike. Stem terete, fleshy, 4-10 mm in diameter aboveground, 8-17 at the base, thicker specimens often appearing to be hollow at least in the dried state, glabrous below, pubescent to densely villose above. Secondary roots are frequently apparent at the base of stems beneath imbricate whorls of caudal leaf scales. Caudal leaf scales either absent or strongly reduced to series of imbricate whorls only at the base of the plant, when occurring above, similar to floral bracts, 4-12 x 3-7 mm, broadly ovate or ovate, adpressed, concave, subacute, sessile, glabrous to pubescent. Inflorescence one or more dense terminal spikes of 8-25 (-33) flowers each, comprising nearly the entire aboveground portion of the plant. Floral bracts atrorse or adpressed, somewhat concave, (14-) 20-30 (-34) x 5-9 (-13) mm, lanceolate-ovate, acute at the apex, sessile, villose to densely villose on both the adaxial and abaxial sides. Pedicels, either extremely short (to 5 mm) or absent. Bracteoles two, opposite, arising at the base of the calyx, (12-) 14-22 (-26) x 1-3 mm, linear or rarely oblanceolate, villose, acute, sessile. Calyx campanulate at the base, cylindrical above, (16-) 18-30 (-35) x 9-12 mm, villose or densely villose externally, pubescent internally, not notably bilabiate, calyx lobes more or less equal on an individual flower but varying within inflorescences and
among plants, (6-) 8-14 (-17) x 3-6 mm, lanceolate, sinuses between lobes varying from approximately one-quarter to one-half the length of the calyx. Corolla tube narrowly cylindrical, more or less curved, (32-) 35-45 (-48) mm long, 3-5 mm in diameter at the base expanding to 5-9 mm at the throat, circular in cross-section, uniformly pubescent to villose externally, or with a band of longer denser hairs circling the apex of the tube, internally puberulent at the throat becoming glabrescent at the base. Corolla limb scarcely bilabiate, 12-17 mm in diameter, lobes imbricate, concave, 4-7 x 6-10 (-13) mm, orbicular to semi-orbicular, pubescent- villose externally, puberulent-glabrescent internally. Corolla throat puberulent. Stamens inserted approximately half-way from the base of the corolla tube, filaments not flattened, didynamous, the longer pair inserted dorsally, 19 –24 mm long, nearly or just exserted, the shorter pair inserted ventrally, 15-19 mm in length, and due to the curvature of the corolla tube, also nearly or just exserted. Anthers glabrous, the fertile theca crescent-shaped, 2.5-3 x 1-1.5 mm, mucronate at the apex, dehiscing by apical suture, the sterile theca subulate, 3-4 x 1 mm. Ovary ovoid to nearly conical, 7-11 x 4-7 mm, glabrous, style 35-40 mm in length, exserted, incurved at the apex. Stigma oblong-clavate, 5-6 x 1-3 mm, scarcely capitate. In fruit, corolla and style often persistent calyx always persistent. Capsule ovoid to spherical, 9-16 x 11-12, glabrous, dehiscing septicidally, placentation axile, seeds numerous. Flowers September-November. (Figure 5.6)

**Taxonomy**

In living state *Harveya squamosa* may be easily distinguished from *Harveya bolusii* by the color of the corolla (uniformly yellow to orange-red in *H. squamosa* vs. crimson to orange, with a patch of yellow on the ventral side of the tube in *H. bolusii*)
and by the corolla lobes (imbricate in *H. squamosa* vs. expanded in *H. bolusii*).

However, preserved specimens have presented difficulty in that these characteristics are not apparent. Preserved specimens may be distinguished based on the length of pedicels (less than 5 mm if present in *H. squamosa*, and generally longer than that in *H. bolusii*), the size of the floral bracts (7-19 x 3-5 mm in *H. bolusii*, larger in *H. squamosa*) and by the generally thicker stem of *H. squamosa*.

**Parasitism**

*Harveya squamosa* has been reported parasitizing species of *Othonna* and *Arctotis decurrens* (Asteraceae), a species of *Cliffortia* (Rosaceae), and of *Willdenowia* (Restionaceae). *H. squamosa* often bears many secondary roots arising above the primary haustorium, and therefore it seems likely that an individual may have several hosts at once. However, these secondary roots are quite brittle, and secondary haustoria have not been positively identified.

**Habitat and distribution**

*Harveya squamosa* occurs primarily in dune habitats of the west coast, rarely far from the sea (Figure 5.6 F). The extensive underground rhizome of many specimens indicates that this species may be well adapted to loose, well-drained and shifting soils of the coastal plain.

**Representative specimens examined**

WESTERN CAPE. **Cape Town:** Blauwberg, near Cape Town, 5 Sep 1938, Lam and Meeuse 4265 (L); north of Blaauwbergstrand, 4 Oct 1956, Acocks 19064 (BOL).

**Clanwilliam:** Brackfontein, van Schoor 239 (K); Lambert's Bay, 6 Sep 1953, Compton 24156 (NBG); Farm Klein Kliphuis, Pakhuis Pass, 31 Oct 1963, Schlieben and van Breda
9911 (BR, K); 19 miles from Clanwilliam towards VanRhynsdorp, 6 Oct 1978, Visser s.n. (NBG); 17 km north of Clanwilliam on Main Road, Oct 1978, Visser 1232 (PRE).

**Hopefield:** Langebaan, Sep 1929, Grant 4705 (MO); Berg River Station, 30 Sep 1930, Galpin 11493 (K); Kotze: Berg River 2 Sep 1944, Compton 15946 (NBG); Saldanha Naval Base, 30 Oct 1989, Blake 132 (NBG). **Malmesbury:** Kleinpaternoster, 5 Oct 1930, Andreae 1364 (PRE); Melkbos Strand, 19 Sep 1935, Adamson s.n. (BOL), 8 Sep 1940, Pewfold s.n. (NBG), and 31 Oct 1948, Hall s.n. (NBG); Melkbosch Road, 3 Nov 1951, Maguire s.n. (NBG); Kalbaskraal, 13 Nov 1951, Meyer s.n. (NBG); Darling, near Oysterfontein [Yzerfontein?], halfway between Cape Town and Saldanha, 16 Oct 1959, Reusberg 156 (NBG); Melkbos Village, 13 Oct 1969, Axelson 85 (NBG); 0.5 miles from sea: Grotto Beach between Mamre and Darling, 7 Sep 1985, Littlewort mr/54/37a (BOL); Leerbaai, ~2 miles north of Bokbaai 20 Sep 1968, Rourke 1117 (NBG). **Piketberg:** Sauer, 1 Oct 1943, Barker 2682 (NBG). **Simon's Town:** Between Buffelsbay and Cape Point, Oct 1912, Glover s.n. (BOL); Near Vasco da Gama Peak, 13 Nov 1932, Salter and Barker 2851 (BOL, K); Kommietjiesberg, Good Hope Nature Reserve, 6 Nov 1968, Taylor 7370 (NBG). **Wellington:** Bain's Kloof, 30 Nov 1929, Grant 5026 (MO).

**Worcester:** Bergendal Farm, 8 Dec 1982, Rourke 1802 (NBG, PRE). **Unknown:** Between Oliphant's River and LangeValley, 1847, Drège 1315 (K); Harvey 239 (E); S. Afr., Sep, Krauss s.n. (MO).

**NORTHERN CAPE.** **Namaqualand:** Welkom, Khamiesberg near Garies, 16 Oct 1954, Esterhuysen 23,657 (BOL); Hondeklipbaai, between Hondeklipbaai and Wallekraal on Sand Dunes. 28 Sep 1976, Goldblatt 4230 (E, NBG); Farm Karootjie 316, 8 Oct 1986, LeRoux and Lloyd 602 (NBG).
Figure 5.6. *Harveya squamosa* A. Plant with haustorium. B. Flower. C. Dissected corolla showing stamens and pistil. D. Dissected calyx E. Haustorial tuber in cross-section of demonstrating the host root. F. Distribution.
Figure 5.6 continued

Tall, slender, herbs. Stem unbranched, or rarely branching at or below ground level, or in a single instance, branching at the apex resulting in three terminal racemes (rather than one as in most specimens), 30-50 (-70) cm in height, to 6 mm in diameter at the base, narrowing to 1-3 mm above, glabrescent at the base, gradually becoming villose at the apex. Stem, leaves, and calyces maroon or green with maroon markings. Leaf scales imbricate at the base only, otherwise small, and infrequent, separated by nodes of (25-) 44-120 (-205) mm, subopposite, elliptical or rarely obovate or oblanceolate, pubescent on the abaxial side, but glabrous-puberulent adaxially, atrorse to adpressed, concave, subsessile, acute at the apex, narrowing slightly at the base, subsessile. Inflorescence usually of a single terminal raceme, 57-190 mm in height comprising no more than one-third of the aboveground height of the plant, each raceme consisting of 2-6 flowers. Floral bracts opposite, subsessile, concave, villose, (7-) 9.5-11.5 (-13) mm in length and 3-5 mm broad, lanceolate to oblanceolate, and acute or subacute at the apex. Bracteoles opposite, place of insertion variable even within an inflorescence from the base of the pedicel to the base of the calyx, 7-11 x 1-2 mm, lanceolate, villose, sessile and acute at the apex. Pedicels of flowers villose, 9-23 (-30) mm in length. Calyx campanulate or slightly obconical below, with the tube campanulate to cylindrical, 13-20 (-23) x 7-11 (-14) mm, villose externally, pubescent internally at the tips of the lobes becoming glabrous in the base of the tube, not strongly veined but convex at the sinuses and concave at each lobe when viewed in cross section, not strongly bilabiate, but with a
slightly deeper sinus dividing the posticous and anticous limbs than between lobes of the same limb, although limbs never divided by as much as half the length of the calyx. Lobes of the calyx deltoid, acute or subacute at the apex, 5-8 x 3-5 (-7) mm wide. In rare cases, corolla entirely white, but otherwise as below. Corolla tube yellow below inclining to orange or scarlet at the limb, narrowly cylindrical, from 3-4 mm below, gently curving and expanding to 5-6 mm in the throat, pubescent below and ventrally becoming villose to densely villose above and dorsally, villose within. Limb quite broad, slightly oblique, 29-50 (-60) mm in diameter, lobes obovate nearly equal in size, (10-) 12-22 (-24) mm in diameter, orange to scarlet, at the margins, puberulent at the throat becoming glabrous toward the slightly crenate margins. Corolla throat yellow, puberulent, and nearly circular. Stamens inserted approximately halfway from the base of the corolla, not apparently didynamous, extending to within 5-10 mm of the corolla throat, filaments flattened and glabrous. Anthers glabrous, the fertile theca crescent-shaped, mucronate at the apex, 2-3 mm long by 1 mm wide, dehiscing by means of an apical suture, the sterile theca usually the same length as the fertile theca, subulate, and 1 mm or less in diameter. Pistil glabrous, ovary flask-shaped, 7-8 x 4-5 mm, style as long as the corolla tube, incurved at the apex, the stigma blocking the upper portion of the throat, slightly exserted, oblong, 5-6 x 2 mm. Calyx and corolla persistent in fruit, although frequently the style senescent, the mature capsule approximately twice the size of the ovary, 14-16 x 10-12 mm, dehiscing septicidally, with numerous seeds and axile placentation. Seeds 0.5 mm x 0.2 mm, cylindrical or slightly constricted in the center, seed coat reticulate resulting in numerous alveoli. Flowers November to March. (Figure 5.7)
Parasitism

Haustoria of *Harveya stenosiphon* are small. The base of the slender stems tends to be rather brittle making excavation difficult. It has been reported as parasitizing *Osmitopsis osmitoides* (Asteraceae). Specimens (*Randle 149; OS*) from the forest below Twelve O’Clock Peak in the Marloth Forest Reserve near Swellendam (Western Cape) appeared to be parasitizing the roots of a deciduous tree, and no forbs occurred in close enough proximity to allow parasitism. Most of the trees of this habitat were a species of *Pterocelastrus* (Celastraceae). However, precise identification of the host was not possible.

Habitat and distribution

*Harveya stenosiphon* occurs more or less continuously on the southern slopes of the Langeberg and Outeniquaberg (Figure 5.7 E). In the west, a disjunct population has been collected from Great Winterhoek near Tulbagh, and in the east, several specimens have been collected north of the small Karoo, in the Swartberg and Kammanasieberg ranges. *Harveya stenosiphon* occurs most frequently in damp areas, both open and shaded.

Representative specimens examined


**EASTERN CAPE. Humansdorp**: WitteElsBosch; mountain slopes, Dec 1921, *Fourcade 1927* (MO); Witte Els Bosch, *Fourcade 1927* (K).
Figure 5.7. *Harveya stenosiphon*. A. Flowers. B. Corolla, dissected with pistil. C. *Harveya stenosiphon* parasitizing the roots of a deciduous tree, most probably a species of *Pterocelastrus* (Celastraceae) D. Dissected calyx. E. Anther. F. Distribution.

Holotype: CGE; Isotypes: BOL!, K!

Plants usually 12-17 (-23) cm high. Stems simple or branching at the base, sometimes with many branches. Stems pubescent, more densely so toward the apex, terete below and somewhat obtusely angled in cross section above, from 3-8 (-15) mm in diameter, somewhat woody at the base. Caudal leaves scale-like, those at the base rather small and imbricate, to 5 x 5 mm, orbicular, obtuse at the apex, and sessile, those above opposite, obovate-elliptical, 8-14 mm long by 4-8 (-10) mm wide, obtuse or acute, pubescent, adpressed to atrorse, sessile, separated by internodes of 8-15 (-47) mm. Terminal racemes frequently comprising more than 75% of the aboveground portion of the plant, 4-10 (-18) flowers per inflorescence. Floral bracts 10-14 (-16) mm long and (2-) 3-5 (-7) mm wide, lanceolate to ovate or obovate, acute or subacute, sessile, and glandular pubescent. Bracteoles, two, opposite, 10-14 (-22) x 1-3 mm, linear-lanceolate, frequently inserted at the base of the calyx, rarely lower, pubescent to villose, acute, sessile. Pedicels 9-20 mm long, pubescent, erect. Calyces 10-nerved, pubescent below to pubescent or villose at the teeth, hairs frequently clustered at nerves, obconical-campanulate at the base, broadly cylindrical to campanulate above, longer dorsally, 14-19(-24) x 5-7 mm, bilabiate, three posterior teeth divided from anterior two by a sinus 6-8 mm deep, teeth lanceolate, 5-8 x 2-3 mm. Corolla orange to scarlet, corolla tubes sparsely pubescent externally, glabrous within, cylindrical, gradually widening toward the apex, slightly curved to erect, (42-) 47-52 (-63) mm long, 2-3mm at the base gradually widening to 8-10(-14) mm at the throat. Limb bilabiate, 20-25 mm broad, flat
to slightly oblique with the anterior lip jutting slightly forward, two posterior lobes
approximately the same size or slightly smaller than three anterior lobes, lobes orbicular,
(7-)10-15 mm in diameter, glabrescent inside and out, ciliate at margin, margin crenulate
to entire. Throat round, glabrescent to sparsely puberulent inside. Stamens inserted
halfway from the base of the corolla, didynamous, shorter pair ~30 mm long, inserted
dorsally, the longer pair 35-37 mm long, inserted ventrally, slightly exserted. Filaments
flattened, slightly pubescent. Sterile theca of anther subulate, 3 x 1 mm, slightly hooked
at the apex, fertile theca crescent-shaped, 3 mm x 2 mm, dehiscing by means of an apical
suture, anthers glabrous. Ovary glabrous, 4 x 4 mm, ovoid to spherical, two locules
divided by a septum, placentation axile, ovules numerous. Style glabrous. Stigma
exserted, oblong, 5 x 1-1.5 mm, strongly incurved, at maturity recurved far enough to
touch most exserted anther pair. Capsules not seen. Flowers December and January.
(Figure 5.8)

**Taxonomy**

The presence of the holotype at CGE has not been verified, and an investigation is
ongoing. However, this is the herbarium in which Hiern deposited types, and it seems
that in all probability the holotype is at this institution.

**Parasitism**

No hosts recorded.
Habitat and distribution

*Harveya bodkini* occurs primarily at the highest elevations at the junctions of the Skurweberg range (running north to south) and the Hexrivier range (running east to west) near the Ceres valley, but also extends to the northernmost mountains of the Cape Fold belt, to the eastern slopes of the Cedarberg range (Figure 5.8 E).

Representative specimens examined

Figure 5.8 *Harveya bodkini*  A. Flowering stem.  B. Distribution.  C. Dissected corolla, showing stamens and pistil.  E. Anthers.  F. Interior of dissected calyx.

TYPE: *Thunberg 14454* (UPS, microfiche at NY!).


Plants (7-) 12-20 (-29) cm high, frequently stems, bracts and calyces maroon to deep red. Stems simple or rarely branching at the base, glabrous-pubescent below to pubescent or villose above, hairs increasing in length and density from the base of the plant, 3-5 (-6) mm in diameter, terete or obtusely angled. Caudal leaves opposite-subopposite, few, reduced to scales, 6-9 x 3-4 mm, elliptical to ovate, puberulent or pubescent, sessile-subsessile, obtuse to acute at the apex, adpressed, usually less than 14 mm between leaf pairs if more than one pair is present, but internodes may be as long as 37 mm, scale-leaves at the base of the plant typically smaller, orbicular, obtuse and imbricate. Terminal racemes consisting of pairs of flowers arising in the axils of floral bracts, usually comprising 50-75% but as much as 100% of the above-ground portion of the stem, 4-10 (-22) flowers per inflorescence. Floral bracts pubescent to villose, 7-13 (-19) x 3-5 mm, elliptic-lanceolate, obtuse or subacute at the apex, and subsessile-sessile. Pedicels (7-) 10-18 (-25) mm long, villose. Bracteoles two, opposite, inserted at the base of the calyx, 9-11 x 1-2 (-3) mm, linear to narrowly elliptical, pubescent or villose, acute, sessile. Calyces campanulate or obconical at the base, broadly cylindrical or rarely urceolate, 12-25 x 6-7 mm, longer dorsally, bilabiate, lobes lanceolate to narrowly deltoid, 4-6 x 2-3 mm, posterior three divided from anterior pair by a deeper sinus of
approximately half to a little more than half the length of the calyx, 10-nerved, pubescent or villose externally, with longer and denser hairs at the nerves and on the calyx lobes, internally glabrous except for lobes, which are pubescent. Corolla cylindrical, scarlet, bright yellow on the ventral side of the tube and inside the throat. Corolla tube erect at the base, from 2-3 mm in diameter at the base gradually inflating to 7-8 (-11) mm at the throat, gently or strongly curving, (24-) 27-35 (-39) mm long, covered externally with glandular hairs, hairs increasing in length and density from the base to the apex and from the ventral to the dorsal sides, glabrous within. Limb bilabiate, flat, or if oblique with the anterior lip jutting slightly forward, 12-15 mm in diameter, lobes orbicular, 5-9 mm in diameter, the anterior and posterior lobes approximately equal or the anterior lobes slightly larger, lobes sparsely puberulent to glabrescent inside and out, margins minutely crenate or entire, ciliate. Stamens didynamous or subdidynamous, inserted (9-) 11-15 (-17) mm from the base of the corolla, nearly exserted. Filaments flattened and glabrous. Anthers glabrous, the fertile theca crescent-shaped, 2 mm x 1 mm, dehiscing by means of an apical suture, the fertile theca subulate, 2-3 mm x 0.5mm, somewhat hooked at the apex. Ovary glabrous, ovoid, 3-5 x 2-3 mm, placentation axile, placentae on either side of the septum reniform, not apparently lobed. Style glabrous, exserted, strongly incurving below the stigma which is oblong and not capitate, 3-4 x 1-2 mm. Corolla, calyx and style persistent in fruit. Capsule ovoid, 10 x 8 mm, dehiscing septicidally. Seeds numerous, approximately 1600 in a single capsule, approximately 0.6 x 0.2 mm, cylindrical and more or less constricted at the center, or more rarely irregular, embryo visible through the reticulate ridges and sunken alveoli of the seed coat. Flowers November -January. (Figure 5.9)
Taxonomy

According to article 7.3 of the International Botanical Code of Nomenclature, when a new epithet is created because a previously existing epithet is not available for use (as when Kuntze renamed *Aulaya capensis* (Thunb.) Harv. “*Harveya bolusii*”, because the name “*Harveya capensis*” was already in use), the type of the new name is the type of the previous name. Therefore, the type of *Harveya bolusii* is the type of *Aulaya capensis*, which is the type of its basionym, *Orobanche capensis*. This type must be chosen from Thunberg’s collection.

Schlechter’s types were deposited at Berlin-Dahlem (B), but the original material of *Harveya hirtiflora* was destroyed during the second World War. The specimen at the National Herbarium in Pretoria has been chosen to serve as a lectotype.

Parasitism

*Harveya bolusii* has been observed parasitizing the roots of *Disparago* and *Stoebe* species and *Euryops abrotanifolius* (all Asteraceae) and *Cliffortia* (Rosaceae). Some specimens appear to have secondary host connections arising directly from the stem or leaf axils above the primary haustorium.

Habitat and distribution

*Harveya bolusii* occurs primarily on south- or west-facing mountain slopes of Cape Peninsula, the Cape-fold belt of the Western Cape, and extends into the Amatole Mountains of the Eastern Cape where it occurs much more rarely (Figure 5.10).

Representative specimens examined

WESTERN CAPE. Cape Town: Table Mountain: 24 Jan 1811, *Burchell 631* (K); 1839, *Harvey s.n.* (K); Dec 1883, *Harvey s.n.* (K); Dec 1883, *Harvey 3999* (E);
Bolus 491 (BOL); Bolus 388 (BOL, K); Dec 1886, Thode s.n. (NBG); Dec 1897, Bolus 3897 (K); Dec 1908, Marloth 5981 (PRE); 24 Jan 1949, Gillet 3349 (NBG); 1880, Rehmann 870 (BR); Table Mountain summit, Dec 1859, H.M.S. Herald Mission s.n. (K); Nov, Pappe s.n. (K); above Skeleton Gorge, 26 Nov 1950, Esterhuysen 17825 (BOL); near Maclear’s Beacon, 30 Dec 1980, Visser 412 (NBG); slopes of Skeleton Gorge, 6 Dec 1945, Esterhuysen 12306 (BOL); sandy soil at the top of Nursery Ravine, 6 Dec 1999, Randle 86 (OS). Caledon: Onrust River, 1 Dec 1951, Esterhuysen 19267 (BOL); Route 2 East near Greyton, 30 Nov 1952, Esterhuysen 20805 (BOL), and 2 Jan 1953, Esterhuysen 21015 (BOL); Nooienskop above Greyton, 29 Nov 1992, Oliver 27 (NBG); Einde Mountains, Zonder River, 24 Oct 1940, Stokoe 7992 (BOL); Gagelberg, 15 Dec 1981, van Jaarsveld and Bean 6443 (NBG). Calitzdorp: Rooiberg, Baileys Peak, 4 Jan 1981, Vlok 171 (PRE). Ceres: lower slopes of Michells Peak, 10 Dec 1948, Esterhuysen 14798 (BOL, NBG). Heidelberg: Grootvadersbosch, 1 Jan 1951, Esterhuysen 18251 (BOL); Bosmansbos Wilderness Area, 1 Dec 1987, McDonald 1542 (NBG).

1118 (PRE); Stellenbosch: Bullerskop, 16 Dec 1939, Esterhuysen 1470 (BOL); southwest side of Guardian Peak, 13 Jan 1955, Esterhuysen 24128, (BOL); Hottentots Holland, 6 Jan 1944, Esterhuysen 9803 (BOL); Victoria Peak, 19 Jan 1948, Esterhuysen 14377 (BOL); southwest side of Victoria Peak, 2 Jan 1944, Esterhuysen 9785 (BOL); Jonkershoek, Panorama Peak above Bergrivier Nek, 3 Jan 1983, Goldblatt 6823 (MO).


Wynberg. Constantiaberg, north side of summit, 17 Dec 1939, Pillans s.n. (BOL).

Figure 5.10. *Harveya bolusii* distribution.


Plants (18-) 30-50 (>100) cm tall. Stems, leaves, and calyces greenish-yellow, sometimes tinged with red most frequently at the base of the stem. Stems erect, unbranched, glabrous, 4-20 mm in diameter, terete or obtusely 5-angled, sometimes woody below. Caudal leaves scale-like, glabrous on both sides, opposite to subopposite, or rarely alternate, sessile, acute or acuminate at the apex, elliptical to subovate, or more rarely spatulate, entire, usually (10-) 25-30 (-37) x 9-22 mm, adpressed to the stem and sparse, with internodes (2-) 3-7cm, at base smaller, obtuse, imbricate or nearly so. Inflorescences terminal, consisting of 4-12 flowers, subspicate to racemose, pedicels 10-15 (-53) mm, glabrous. Floral bracts opposite, frequently cupping calyces, sessile, elliptic-ovate or rarely spatulate, acute to acuminate at the apex, glabrous on both sides, (20-) 25-30 (-48) x 5-10 (-30) mm. Bracteoles two, opposite, inserted most frequently at the base of the pedicel, more rarely midway up the pedicel or at the base of the calyx,
subulate to linear, sessile, acute at the apex, glabrous, (12-) 20-24 (-36) x 1-3 (-5) mm. Calyx glabrous inside and out, campanulate-obconical at the base, cylindrical above, strongly 10-nerved, obtusely star-shaped in cross section, approximately the length of the corolla tube, (27-) 40-50 (-65) x 10-15(-17) mm. Calyx limb slightly oblique, longer posticously, and divided into five short lobes, the three posterior lobes scarcely divided, deltoid and acute, 2-3 x 2-5 mm, divided more deeply from the anterior lobes by sinuses 5-7 mm deep, two anterior lobes are more deeply divided than posterior lobes deltoid and acute, 3-5 mm x 5-7mm. Corolla tube creamy white to pale yellow, narrowly cylindrical inflating abruptly at the limb, strongly curved at the point of inflation, 5-10 mm below inflating to 9-17 mm at the throat, 41-80 (-126) mm long, externally more or less densely villose, hairs concentrated on longitudinal nerves extending from the base of the corolla to the limb. Limbs bilabiate, broad, creamy to snow white, sometimes tinged with pink at the margins, often marked with black patches in older flowers, anterior lip thrust forward, lobes orbicular (13-) 24-32 mm in diameter, two posterior lobes smaller and more connate than the anterior ones, more or less erect, (9-) 21-28 mm in diameter. Lobes glabrous internally and externally or furnished with very sparse, short hairs, wavy at the margins which may be entire or somewhat crenate, and reflexed. Corolla throat pale lemon-yellow, densely glandular villose, appearing obtusely triangular when viewed from the front, broader below and coming to an obtuse peak above. Stamens short, 3-10 (-15) mm, equal or subequal inserted in the corolla just below the point at which the corolla inflates, so that the anthers do not extend quite as far as the stigma. Filaments flattened, glabrous or with sparse, short glandular hairs. Anthers glabrous and essentially monotheceous, the sterile theca reduced to a small protrusion at the apex of the filament
or absent, the remaining theca fusiform, 4-5 x 2-3 mm, acute or acuminate at the apex, dehiscing by apical suture. Ovary glabrous, ovoid, 7-10 x 5-6 mm, inserted above two wedge-shaped nectaries, up to 3 mm in thickness at the septum of the ovary, slightly lobed externally. Placentation axile, placentae attached to a small column at the apex of the ovary, each placenta further divided into two lobes which may split from each other at the bottom, ovules very numerous. Style glabrous, incurved above, extending to the throat of the corolla. Stigma pale yellow, glabrous, capitate to spatulate, more or less two-lobed, 6-11 mm x 5-6mm, partially blocking the throat of the corolla. Calyx and corolla persistent in fruit, the capsule glabrous, ovoid, 18-26 x 10-16 mm, dehiscing septicidally, beaked above. Seeds very numerous (several thousand in each capsule) 0.6 mm x 0.2-0.3 mm wide, cylindrical or slightly constricted at the center, externally hexagonally-alveolate, and with a large central nucleus. Flowers October -March, most abundantly January-February. (Figure 5.11)

**Taxonomy**

Bernhardi’s collection was sold to the Missouri Botanical Garden in 1857 (D’Arcy 1971) and the specimen cited above is very probably that used in describing this species. *Harveya tubata* Reut. was included as a taxonomic synonym of *H. speciosa* Bernh. by Hiern (1904), but it may be argued that this name is illegitimate in that it is a later homonym of *Harwaya tubata* (E. Mey) Steud. In any case neither of these names is validly published in that the basionym, *Orobanche tubata* E. Mey\(^7\). was published as a nomen nudum, and neither was accompanied by a validating description or diagnosis. It

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\(^7\) Bentham validated Meyer’s *nomen nudum* by providing a description of *Cycnium tubatum*.
is unclear which of Ecklon and Zeyher’s specimens might be considered the type of any of these names, as the material amassed by these collectors was sold widely throughout Europe (See Harveya capensis). Orobanche tubata E. Mey. although itself invalid, was treated as the basionym of Cycnium tubatum E. Mey ex Benth. which was accompanied by a description and therefore is valid.

The type of Harveya cathcartensis Kuntze is certainly a specimen of Harveya speciosa. Harveya anisodonta C. A. Smith is also clearly a taxonomic synonym of Harveya speciosa, based on examination of the type material, collected by R. A. H. Flugge-De Smidt. Flugge-De Smidt revisited the population of the type in December of the same year. Several specimens were collected at this time, as well as a host plant, which were sent to Dr. R. Marloth for identification (Marloth 13643: PRE). Accompanying the specimens is a short, hand-written letter by Flugge-De Smidt on the status of the population; “In a small area there were 200 shoots of H. sp.”. It is not clear whether by “H. sp.” Flugge-De Smidt referred to Harveya "speciosa" or Harveya "species". In any case, Marloth identified the specimens as Harveya speciosa (which were changed to Harveya anisodonta in a later annotation by C. A. Smith). Thus, it is clear that initially these specimens were identified as H. speciosa. Unfortunately, very little of the original specimens remain and no flowers appear on the herbarium sheet.

In the diagnosis of Harveya anisodonta, Smith distinguished it from Harveya speciosa by the glabrous ovary of the former. This distinction is correct in that H. speciosa was described as having a “finely pilose-puberulous” ovary. However, this section of the H. speciosa description is in error. Other authors have noted that the ovary of H. speciosa is indeed glabrous, and the hairs described were actually fungal hyphae
present on the ovaries of the type specimen (Young 1932; Hilliard and Burtt 1986). C. E. Moss noted on sheet 18165 of his own herbarium that “The ovary in all the specimens [of H. speciosa] that I have seen (including the type specimen) is glabrous. Hiern’s description in the Flora Capensis iv, II, is erroneous…” and on sheet 18420, “Several mature flowers were examined, all had a glabrous ovary as in the type specimen, but contrary to the description in the Flora Capensis”. This error was also noted by the collector of the type of H. anisodonta, R.A.H. Flugge-De Smidt, in Flowers by the Roadside (1947). All specimens of Harveya speciosa examined in this study have a glabrous ovary as well. The stigma of Harveya speciosa was also described originally as partially covered in glandular-puberulous hairs, perhaps a related artifact, as I find them glabrous in all specimens examined thus far. It is clear that ovary pubescence alone is not sufficient to accord species status to H. anisodonta as delimited by Smith.

As in all other Harveya species, anthers consist of a fertile, crescent-shaped locule, and a thinner, curved, sterile locule. In H. anisodonta the sterile locule is described as being reduced to a spur, which is interpreted here as homologous to the short, blunt appendage of H. speciosa. In H. speciosa, this appendage is frequently small enough to be invisible except under magnification and as Hilliard and Burtt (1986) noted, H. speciosa is “effectively monothecous”.

Habitat and distribution

Harveya speciosa is one of the most widespread of the southern African species, occupying moist grasslands of both mountain slopes and coastal plain in six provinces of South Africa, Swaziland and Lesotho (Figure 5.11 H). Populations are most densely clustered along the escarpment of the central plateau, in the Amatole mountain range, the
Drakensberg, and throughout southern KwaZulu Natal. Specimens have been collected from as far west as the Eerste River mouth in the Eastern Cape. The two known populations in Gauteng (Dersley and Alberton) represent the northwestern extreme of the distribution, and are somewhat remote from the next nearest populations in the Free State and Mpumalanga. Only one specimen has been collected in the Northern Province, near Letaba, which represents the northern extreme of this species.

**Representative specimens examined**


1939, Galpin s.n. (BOL). **Underberg**: Cobham Forest Reserve; Sipongweni, 20 Feb 1981, Hilliard and Burtt 14036 (NU); Garden Castle, Dec 1999 T. Edwards 1796 (OS).


**FREE STATE. Ladybrand**: Appledore near Commissiepoort, 14 Jan 1944, Tylden s.n. (BOL, NBG). **Lindley**: Steynsrust, Feb 1934, Jones s.n. (BOL). **Unknown**: Orange River Colony, 1862, Cooper 984[?] (K).


**GAUTENG. Johannesburg**: Alberton: Rietvlei Zoo Farm, 13 Dec 2001, Pfab s.n. (OS).

**NORTHERN PROVINCE. Letaba**: Duiwelskloof, Piesangkop, 22 Jan 1960, Scheepers 867 (K).

SWAZILAND. Mbabane: 5 Feb 1940, Compton 23299 (NBG), Duiker’s bush, 6 Feb 1956, Compton 25534 (BOL); Duiker’s bush forest edge, 1 March 1956, Compton 25296 (NBG), Klambanyati Valley, 22 Feb 1955, Compton 24950 (NBG). Hlatsikulu, Stewart 74 (K).
Figure 5.11 continued


Plants 21-31 (-50) cm in height. Stems, leaves, and calyces dusky brown to greenish yellow, covered more or less densely in glandular hairs. Stems erect, simple or branching above or at the base, frequently with undeveloped branchlets emerging from the axils of caudal leaves. Stems, pubescent to villose, frequently with longer and denser hairs toward the apex, viscid, 2.4-4.6 mm in diameter, terete-obtusely angled in cross-section. Caudal leaves scale-like, varying in size, shape, and vestiture, sometimes on a
single specimen, ovate, elliptical or lanceolate, (4-) 9.5-11.2 (-18) x 3-4 (-8) mm, acute or obtuse at the apex, sessile to subsessile, adpressed or atrorse, frequently concave, pubescent to densely villose, subopposite to opposite or rarely alternate, imbricate below with internodes on upper leaves varying from 28-50 mm in length. Terminal raceme of (2-) 4-8 (-10) flowers, at times with branching occurring within inflorescences, less than half of the total height of the plant. Floral bracts opposite, ovate to lanceolate, 11-14 (-20) x 3-5 (-8) mm, villose, subsessile, and acute at the apex. Bracteoles in pairs arising from the base of the calyx, linear to narrowly lanceolate, 10-12 (-16) x 2-3 mm wide, acute, subsessile, villose. Pedicels considerably longer on basal flowers, from (12-) 33 (-60) mm on lower flowers, to (9-) 14 (-22) mm in length on upper flowers, densely villose. Calyces 17-22 x 10-13 mm, campanulate or somewhat obconical at the base, narrowly to broadly campanulate above, bilabiate, posticous limb consisting of three lobes, the anterior consisting of two lobes, lobes deltoid-lanceolate and acute, 7-9 x 4-6 mm, the two limbs separated by a sinus of 7-9 mm, the calyx slightly oblique, longer dorsally than ventrally, densely villose outside with long glandular hairs concentrated at the nerves and lobe margins, internally pubescent with shorter sparser hairs than outside. Corolla entirely white to cream colored, or with pale yellow corolla tube or slightly pink corolla lobe margins, frequently with irregular black markings on older or damaged flowers. Corolla tube (24-) 28-33(-40) mm long, cylindrical and erect below, abruptly inflating and curving 3-4 mm above the calyx, 3-5 mm in diameter at the base to 11-13 mm at the throat, the entire tube laterally compressed resulting in strong lateral compression at the throat (3-4 mm broad), externally pubescent-villose with hairs increasing in length from the base of the tube to the apex, and from ventrally to dorsally,
puberulent inside. Limb bilabiate, oblique, 19-37 mm in diameter, with the anterior lip forward, lobes obovate, the two posterior lobes smaller and more connate than the anterior three, more or less erect, (11-) 13-15 (-18) mm in diameter, the anterior lobes (13-) 15-17 (-26) mm in diameter and spreading, all lobes with wavy margins (appearing crenate when pressed), somewhat reflexed, often minutely ciliate at the margins and otherwise glabrescent to puberulent. Stamens didynamous, inserted 13-16 mm from the base of the corolla, the longer ventral pair of filaments 7-8 mm in length, the shorter dorsal pair 4-6 mm in length. Anthers included in the corolla, glabrous, the fertile theca crescent-shaped 2-2.5 x 1 mm, with a short spur at the apex, dehiscing by means of an apical suture, the sterile theca subulate, 3.5-4 x ca. 0.5 mm, apex acute, and slightly curved. Pistil green, ovary glabrous, ovoid, flattened somewhat in cross-section, along the same axis of lateral compression seen in the corolla, (4-) 7-9 mm long (3-) 5-7 mm in diameter on the broader axis, shallowly lobed perpendicular to the septum. Style glabrous, 21-27 mm in length, curving sharply downward just below the stigma, stigma capitate and globose, 2 x 2-3 mm, barely included in the corolla, appearing from the front of the flower to be suspended at the top of the compressed corolla throat, the bright green of the stigma standing in sharp contrast to the white corolla. Calyx, style, and frequently the blackened, shriveled corolla persistent in fruit. Capsule ovoid, approximately 11 x 7 mm, slightly flattened as is the ovary, dehiscing septicidally, with axile placentation, each placenta strongly lobed, sometimes appearing as two separate masses. Seeds numerous, cylindrical, 0.4-0.5 x 0.2-0.3 mm, embryo visible through reticulate ridges and sunken alveoli of the seed coat. Flowers November through January. (Figure 5.12)
Taxonomy

*Harveya capensis* is the type species of the genus. Hooker did not include collection numbers in his protologue, but mentions collectors Harvey, Sieber, Thom, and Villets, and includes as a location “On a dry hill between Wynberg and the Campground... found in various sandy places on ‘the Flats’, *Hon. W. H. Harvey.*” No specimens from Hooker’s collections at Kew bear this specific information and neither do any of the specimens from TCD, where Harvey’s types were deposited. However, Hooker’s collections at Kew are available for lectotypification, and one of these has been chosen as the lectotype for this species as well as the genus.

All of the names appearing in synonymy above (other than *Harwaya*\(^8\) *tulbaghensis* (Presl) Presl\(^9\) and *Orobanche tulbaghensis* Ecklon et Zeyher ex Presl) appear as synonyms in Hiern’s treatment (1904). Much of the confusion regarding synonymy as apparent in Hiern’s list, resulted from the broad dissemination of collected materials from Jean François Drège (1794-1881), Christian Friedrich Ecklon (1795-1868) and Carl Ludwig Phillip Zeyher (1799-1858), plant collectors who amassed and sold large quantities of preserved material. Many of the taxon names attributed to them (several of which are here considered synonyms of *Harveya capensis* Hook.) were published in several catalogues in which collections from all three botanists appear, most

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\(^8\) The misspelling seems most likely an error copied by Presl from Steudel, who used this spelling as well. It is reasonable to assume that these authors were in fact referring to “Harveya” and that this is simply an orthographical error.

\(^9\) This author citation is strange but correct. The first appearance of both the new combination and the epithet on which it is based, albeit as a *nomen nudum*, are in the same publication (also true for several of Steudels new combinations). In this particular instance, Ecklon and Zeyher probably labeled a specimen on a sheet with the new name *Orobanche tulbaghensis*, but this name was not itself published prior to Presl’s use of it.
notably Drège’s *Catalogus Plantarum Exsiccatarum Africae Australis* (1837-1840), and a reprinting of the catalogue in 1847 in the journal *Linnaea*, which may include new materials and collections. Unfortunately, Stafleau and Cowan (1976) report no libraries in which copies of the former may be found. The latter was used with caution in interpreting the origins of these names. In any case, these collections were used by Steudel and Presl in making new combinations that resulted in several new species of *Harveya*, but no types have been discovered for any of these names, many of which were placed in synonymy with *Harveya capensis* Hook. by other authors, most comprehensively by Hiern.

Steudel’s *Nomenclator Botanicus* created new combinations using a unique but valid approach as explained in the *International Code of Botanical Nomenclature*, article 33.1 (2000). These consist of the new combination with the basionym indented beneath it. However, in at least several cases, the indented, older name was clearly intended to be interpreted as a taxonomic synonym rather than a basionym. In one such case, *Harwaya [sic] capensis* Hook. appears above the indented *Orobanche capensis* Thunb. This is in error, in that *Orobanche capensis* Thunb. is not a taxonomic synonym of *Harveya capensis* Hook., nor is it a basionym of that name. Rather, *Orobanche capensis* Thunb. is the basionym of *Aulaya capensis* Harv., which when transferred to *Harveya*, was given the nomen novum, *Harveya bolusii* Kuntze (1898), because the epithet “capensis” was already in use (for more than 60 years!). It can be hypothesized that this is a result of the unusual format in which Drège enumerated his species (at least as viewed in the reprint of his catalogue). The epithet “capensis Harv.” unaccompanied by a generic name appears beneath *Aulaya grandiflora*, where it is placed in synonymy with *Orobanche*.
capensis Thunb., and directly below, appears the name Harveya capensis Hook. In this case the unwritten generic name should be interpreted as Aulaya rather than Harveya. All the new combinations authored by Steudel in that work (except for one, curiously) were assigned the author citation “Hook.” This is confusing in that Hooker was not responsible for the creation of any of these names in any other printed matter. One possible explanation is that Steudel felt sufficiently indebted to Hooker for some contribution to the new combination. Some of these names are included as synonyms of Harveya capensis Hook. by Hiern in Flora Capensis (1904) including Harwaya lutea (Steud.) Steud. and Harwaya spectabilis (Steud.) Hook. ex Steud. However, it appears that Steudel based these names on the basionyms Orobanche lutea Ecklon et Zeyher ex Steud. and Orobanche spectabilis E. Meyer ex Steud., which themselves are nomina nuda, having been published without description or diagnosis and are invalid. The new combinations are also thus invalid.

Although many of these names are not valid, attempts have been made to determine original material, albeit with little success. Presl notes in his introduction of Botanische Bemerkungen (1944) that the broad dissemination of materials from collectors abroad was resulting in taxonomic confusion:

“Rapid classification, which has caused such disorder in the herbaria of Sieber, Pöppig, and collections of the Travel Society, has resulted in broad and yet meaningless rankings not attributable to Ecklon and Zeyher, or Professor E. Meyer who arranged Drège’s collections. Other no less unfortunate circumstances surround namely the various treatments of the works of Ecklon and

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10 Orobanche spectabilis Benth. ex. Mey. in Drege’s “Zwei Pflanzen Documente” 1844, as cited in IPNI. However, this citation was published three years after the name Orobanche spectabilis appeared in Steudel’s Nomenclator. The first use of this name regarding this taxon may have occurred earlier in either the Meyer or Ecklon and Zeyher’s enumeration of species collected in South Africa.
Drège…as compiled by other botanists, resulting in incredible disorder, so much so that sometimes with only the greatest difficulty may I gain insight regarding original exemplars, and sometimes, I lose hope altogether.” [My translation from the original German].

However, Presl’s treatment fails to identify original material for any species, repeating the errors that caused him such ennui. Furthermore, his combinations *Harveya lactea*, and *Harwaya (Harwayastrum) tulbaghensis* were also based on *nomina nuda* and are thus invalid.

Of the names shown above, only *Harwaya tulbaghensis* (Presl) Presl, though invalid, is newly placed into synonymy in the present study. It is possible that the material observed in this study is not that observed by Presl, but is clearly material observed by Ecklon and Zeyher, the authors of the basionym, and it is also rather clearly a specimen of *Harveya capensis* Hook.

**Parasitism**

*Harveya capensis* was described by Hooker as parasitizing *Blaeria muscosa = Erica muscosa* (Ericaceae) but has been observed primarily parasitizing genera of Apiaceae: *Hydrocotyle, Centella* and one unnamed genus. Excavation of haustoria of *H. capensis* revealed a species of *Centella* as host at two locations some 200 km distant. Haustoria of *Harveya capensis* may be obscured by anastomosing root masses, some of which are clearly connected to the haustorium itself.

**Habitat and distribution**

*Harveya capensis* occurs on wet mountain slopes of the Cape Fold belt and the Cape Peninsula, usually in proximity to seepages and mountain springs. It is almost
entirely confined to the Western Cape province, but several specimens have been collected from the far southwestern mountains of the Eastern Cape (Figure 5.12 K).

**Representative specimens examined**

**NORTHERN CAPE.** *Namaqualand. Morris s.n. (BOL).*


**Simonstown**: Between Tygersberg and Simonsberg, *Drège s.n.* (K); Tygerberg; sand downs, Nov 1941, *Name s.n* (K); Klaasjagersberg, 16 Jan 1896, *Woley Dod 579* (BOL).


Wellington: Bain's Kloof, March 1926, Grant 2236 (MO). **Worcester:** Dutoits Kloof; Drège s.n. (K).

EASTERN CAPE. **Humansdorp:** Hills near Storms River, 13 Nov 1894, Schlechter 5957 (BR,E,MO) and 14 Nov 1933, Long 1120 (K); WitteElsBosch, flats, Nov 1920, Fourcade 1003 (BOL); WitteElsBosch, 11 Nov 1928, Gillet 2263 (BOL) and 16 Nov 1941, Esterhuysen 6800 (BOL); Coldstream; 8 Nov 1935, Laughton s.n. (PRE) and 29 Nov 1993, Steiner 2785 (NBG); Keurboom River; 28 Nov 1947, Taylor 2980 (NBG); Tsitsikama, Nov 1965, Bockelman pl56n1 (PRE).

Plants (8-) 13-16 (-34) cm in height. Stems, leaves and calyces frequently red, at times tinged green or yellow. Stems erect, fleshy, seldom branching, glabrescent to pubescent below inclining toward villose above, viscid, 2-4 mm in diameter, terete to obtusely angled in cross section. Caudal leaves opposite, 5-13 (-21) x 2-5 mm, erect or atrorse, concave, ovate to lanceolate, more or less acute at the apex, sessile or subsessile below, imbricate and obtuse at stem base, internodes above 20-30 (-80) mm, vestiture similar to that on adjacent stem. Inflorescence a terminal raceme of 2-10 (-12) flowers, comprising 40-60% of the length of the above ground portion of the plant. Floral bracts opposite, elliptical-lanceolate, 10-13 x 2-4 mm, acute or rarely subacute at the apex, sessile or subsessile, pubescent to villose. Pedicels (5-) 15-30 (-50) mm in length, villose. Bracteoles opposite, linear, 10-16 x 1-2 mm wide, villose, acute at the apex, sessile, inserted approximately at the center of the pedicel, rarely at the base of the pedicel or the base of the calyx. Calyces villose, strongly 10-nerved, with nerves extending to tips of lobes and sinuses, (16-) 18-24 (-27) mm long (2-3 mm longer dorsally than ventrally) and 7-13 (-14) mm wide, campanulate to obconical at the base, broadly campanulate above, strongly bilabiate, calyx lobes lanceolate, the posticous three 8-12 x 2-3 mm, the anticous lobes 8-12 x 2-4 mm, the posticous and anticous limbs separated usually by a sinus deeper than half the length (12-16 mm) of the calyx, although occasionally by slightly less. Corolla tube white to yellow with either a sulphur yellow or pink to purple limb (coloration distinguishes subspecies, q.v.). Pink flowers
typically with an ochre-sulphur yellow spot on the palate, lower-most corolla lobe.

Corolla tube ampliate, inflating abruptly 3-5 mm from the base, gently curved, dorsally keeled resulting in slight lateral compression of the corolla mouth, (23-) 24-36 (-38) mm in length and 9-15 (-22) mm in diameter at the throat (again, a character distinguishing subspecies, q.v.), puberulent-pubescent below, with hairs becoming longer and denser dorsally and above. Corolla limb bilabiate, not strongly oblique, 40-50 mm in diameter, lobes ovate-ovate, glabrescent to puberulent, crenate or occasionally lacerate and ciliate at the margins, three anticous lobes 11-17 (-21) x 11-19 (-27) mm, posticous lobes 11-17 (-21) x 11-19 (-24) mm, frequently somewhat reflexed, the lowermost anticous limb with a slightly raised pallet, which may partially block the throat of the corolla. Corolla throat glabrescent. Stamens only slightly didynamous if at all, inserted on the corolla tube at the point of inflation (3-8 mm from the base), filaments 20-24 mm in length, puberulent-pubescent. Anthers glabrous, included in the corolla, fertile theca crescent-shaped, 3-4 mm x 2 mm, with a short, hooked spur at the apex, dehiscing by means of an apical suture, sterile theca subulate, acute at the apex, 4-6 (-8) x 1 mm, frequently splitting along its entire length into two equal halves when dried. Ovary glabrous, 5-9 x 5-6 mm, spherical-broadly elliptical, style glabrous, 23-29 mm in length extending along the dorsal keel of the corolla, strongly down-curved just below the stigma so that the stigma is just included within and partially blocks the mouth of the corolla, capitate, most frequently globose (2-3 mm in diameter) and occasionally oblong, to 6 x 3 mm. Capsule glabrous, 9-10 x 8 mm, spherical, dehiscing septicidally, with axile placentation, each placenta strongly lobed perpendicular to the plane of dehiscence, seeds
numerous, 0.5 mm in length and 0.2 mm in diameter, varying in shape within a fruit, from conical to cylindrical, the seed coat deeply reticulate. (Figure 5.13)

**Taxonomy**

*Harveya laxiflora* appears to be a synonym, and is differentiated by Hiern in Fl. Capensis from *Harveya purpurea* by calyx lobes that are no more deeply divided than one-half the length of the calyx (as opposed to *Harveya purpurea*, which is more deeply divided). However, from inspection of an isosyntype of *H. laxiflora* (Pappe s.n.; K) this is clearly not the case as some calyces are more deeply divided than this. Similarly, Hiern distinguishes *H. euryantha* Schlechter from *H. purpurea* by the same criterion, but isotypes of Schlechter 9633 have calyces more deeply divided (though again, not all). *Harveya euryantha* may also be distinguished from *purpurea* by the presence of an oblong stigma (rather than orbicular) but again, in isotypes, this character is variable; while several stigmata are longer than broad, they are not much so (3x1 mm) in the Kew specimen, and 6x3 mm in the specimen of the Bolus herbarium (rather than largely orbicular as seen in most specimens of *H. purpurea*). Specimens of *H. euryantha* also have generally broader calyx tubes than *H. purpurea* as the epithet may indicate, (to 15 mm in *H. purpurea* and to 22 mm in *H. euryantha*) but the length is similar. This difference seems to hold, in so far as many specimens diagnosed as *H. purpurea* (but that have broad corollas like *H. euryantha*) and all the specimens of *H. euryantha* are confined to southwestern Caledon and Bredasdorp; these specimens may be all considered to belong to a single taxon, a geographically confined morphotype of *H. purpurea*. Unfortunately, no specimens have been collected from the Kouerivier population since Schlechter's initial collections (which were extensive and may have
depauperated the population). Furthermore, (and incomprehensibly) Schlechter did not include any information on the color of the corolla in his description of *Harveya euryantha*, an important marker of *Harveya purpurea*. However, other herbarium specimens that will be included in this taxon have been clearly characterized as having purple to pink corollas with some description of yellow coloration of the throat. Another named species, *Harveya sulphurea* Hiern, appears to differ from *Harveya purpurea* as described by Hiern (1904) only in the color of the corolla, sulphur yellow throughout, rather than pink with a yellow throat.

Therefore, I have chosen to include *Harveya euryantha* and *H. sulphurea* specimens in the circumscription of *H. purpurea*. These will be represented as two new subspecies, using the species names of either as a basionym. *Harveya laxiflora* is treated as a synonym of the autonym *Harveya purpurea* subsp. *purpurea*.

**KEY TO SUBSPECIES OF HARVEYA PURPUREA**

1. Corolla pink to purple
   
   2. Corolla tube less than 15 mm in diameter at the throat. Stigma orbicular……………………………………………………….10a subsp. *purpurea*

   2. Corolla tube 16-22 mm in diameter at the throat. Stigma orbicular to oblong……………………………………………………….10b subsp. *euryantha*

1. Entire corolla sulphur yellow………………………………………10c subsp. *sulphurea*
10a. *Harveya purpurea subsp. purpurea*.

*Orobanche uitenhagensis* Ecklon ex Hook. Ic. Pl. t351. 1841. nom. nud.


*Aulaya grandiflora* Benth. DC Prodr. x p.523 1846. TYPE: S. Afr., Drège 7874 (Isosyntype: L!), and Uitenhage, Zwartkop River, Jan, Zeyher 638. (Isosyntype: E!).


Corolla tube white to pale yellow, limbs purple to pink, with a characteristic ochre-yellow spot on the palate lower lip of anticous limb. Corolla tube (23-) 24-36 (-38) mm in length and 9-15 mm in diameter at the throat. Corolla lobes 11-16 x 12-17 mm. Otherwise as above. Flowers September to January.

**Taxonomy**

*Harwaya (Pseudoharwaya) pratensis* (Presl) Presl, *Orobanche uitenhagensis* Ecklon ex Hook and their nomenclatural synonyms are based on unknown types (see explanation under the “taxonomy” section of *Harveya capensis*). Both basionyms are *nomina nuda* and therefore invalid, as are new combinations based on them.

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11 An investigation into Hiern’s material at CGE is pending. If an isotype of Pappe s.n. is discovered there, it should serve as the lectotype.
Parasitism

*Harveya purpurea* subsp. *purpurea* parasitizes primarily species in Campanulaceae: species of *Wahlenbergia* (= *Lightfootia*), *Prismatocarpus*, and *Roella*. Additional hosts may include *Erepsia ramosa* (Aizoaceae), as reported on specimen *Liede 16002* (MO), *Myrsine* sp. (Myrsinaceae) specimen *Kerfoot 5574* (NBG), or species of *Chrysopogon* (Poaceae), *Anthospermum* (Rubiaceae), and *Stoebe* (Asteraceae) (Visser, 1981). However, I believe that the latter two hosts, *Anthospermum* and *Stoebe*, were included due to misidentification of herbarium material; specimens initially identified as *H. purpurea* and associated with these two hosts have been re-examined and identified as other species in this study.

Habitat and distribution

*Harveya purpurea* subsp. *purpurea* occurs throughout the mountains of the Cape Fold belt in both fynbos and rhenosterveld habitats, as well as the coastal plains near Bredasdorp and Agulhas, and as far east as Grahamstown in the Eastern Cape (Figure 5.14).

Representative specimens examined


10b. *Harveya purpurea subsp. euryantha* (Schlechter) Randle. comb. nov.

*Harveya euryantha* Schlechter Engl. Jahrb. xxvii. 184 1898. TYPE: In saxosis montis Kouderivierberg prope Elim, solo arenoso (in ditione Bredasdorp) alt. 1,000 ped., 4 Dec. 1896, *Schlechter 9633*. Schlechter's types were deposited in Berlin-Dahlem. The type for this species was reportedly destroyed during the
second World War, but fortunately many isotypes exist. Lectotype (here chosen):

BOL! Isolectotypes: BR!, E!, K!, L!, MO!

Corolla limbs pink to purple, the throat yellow. Corolla tube 24-37 long and 15-22 mm in diameter. Stigma orbicular or oblong, up to 6mm in length. Otherwise as above. Flowers September to December.

Parasitism No hosts have been identified.

Habitat and distribution

_Harveya purpurea_ subsp. _euryantha_ occurs in the mountainous habitats of southwest Caledon and Bredasdorp districts of the Western Cape Province (Figure 5.14).

Representative specimens examined

WESTERN CAPE. **Caledon:** Hottentots Holland, 22 Sep 1940, _Prior s.n._ (K); Houwhoek, 18 Nov 1951, _Maguire 1234_, (NBG); Botrivier, Hermanus Rd., 6 Oct 1955, _Nickerk 658_ (BOL); Lebanon St. Forest: Jackaalsrivier catchment, 21 Nov 1972, _Kruger 1589_ (NBG). **Bredasdorp:** between the Poort and Vogelvlei, 25 Sep 1933, _Leighton 21137_ (BOL); Southwest of Bredasdorp, 25 Sep 1949, _Sidey 1816_ (MO); Potteberg, 13 Nov 1954, _Barker 8464_ (NBG); Perderberg; Farm Lucerne, 6 Oct 1981, _Stirton 9734_ (PRE); between Mierkral and Vogelvlei, 7 Sep 1995, _Patterson-Jones 616_ (NBG).

Corolla sulphur yellow in its entirety, corolla tube 23-32 mm long and 12-13 mm in diameter, corolla lobes 13-15 x 11-13 mm. Otherwise, as above. Flowers September to October.

**Parasitism** No hosts have been identified.

**Habitat and distribution**

*Harveya purpurea* subsp. *sulphurea* is primarily confined to the northwest portion of the Cape Fold belt, in the Cedarberg mountains and the low hills to the north and east of them. The “Middleburg-Westaway” specimen (*Acocks 23442*) was most probably collected at “Middleberg”, 20 km east of Pakhuis Pass, rather than “Middleburg” more than 800 km away in the Eastern Cape. This subspecies is rarely collected, perhaps due to its limited distribution (Figure 5.14).

**Representative specimens examined**


Figure 5.13 continued
Figure 5.14 *Harveya purpurea* distribution.

*Harveya pauciflora* (Benth.) Hiern in Dyer Fl. Cap. II. 408. 1904. Lectotype (here chosen): **Uitenhage**: VanStaadensberg. 1000-2000’. *Drège* 964b. (K!)

*Harveya tubulosa* Harv. ex Hiern in Dyer Fl. Cap. II. 408. 1904. TYPE: **Cape Town**: Table Mountain. 24 Jan 1811. *Burchell* 643. Lectotype (here chosen): K!

Plant slender, 20-64 cm tall, stem, scales, bracts and calyxes dull red to dusky purple. Stem rarely branching, and if so, below ground, glabrous-puberulent below becoming villose toward the apex, 3-5 mm in diameter, terete or obtusely angled in cross-section. Caudal leaves opposite, subopposite, or alternate, highly reduced below, 4 x 2 mm, glabrescent, above 6-11 x 2-4 mm, elliptical, pubescent to villose, concave, atrorse or adpressed, sessile, acute at the apex, separated by internodes (7-) 13-50 (-77) mm. Inflorescence a loose raceme of 4-10 alternate or opposite flowers, usually occupying less than 50% of the above ground height of the plant. Floral bracts opposite or alternate at the base of the inflorescence and appearing subopposite or opposite at the apex, elliptical to oblanceolate, often concave, 9-15 (19) x 3-6 mm, villose, subsessile or sessile, acute or rarely obtuse at the apex. Pedicels of flowers villose, 7-20 (-24) mm. Bracteoles arising from half-way up the pedicel to the base of the calyx, linear or lanceolate, 6-14 x 1-3 mm,

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12 Two such specimens exist at Kew. The specimen cited is the one with the label “Herbarium Hookerianum 1867” rather than the specimen sharing a sheet with *Drège* 964 (probably 964a) labeled “Herbarium Hookerianum 1854”.

189
villose, sessile or subsessile, acute at the apex. Calyx campanulate at the base and above, in cross section convex at sinuses between teeth, pubescent to villose externally with glandular hairs often concentrated at longitudinal nerves running to lobe tips and sinuses, and often hairs longer and denser above, internally glabrescent, 9-14 (-17) x 7-13 mm, bilabiate, 2-3 mm shorter ventrally than dorsally, three posticus lobes 2-4 x 2-4 mm, deltoid, two antiacus lobes 4-6 x 4-5 mm, deltoid. Corolla tube creamy white, pale pink or yellow, narrow below, inflating abruptly just below or beyond the calyx, curved, 20-26 (-29) mm long dorsally, (6-) 8-12 mm broad at the throat, dorsally keeled, externally pubescent or villose with hairs increasing in length and density on the dorsal keel, internally glabrous except for throat which is puberulent. Corolla limb bilabiate, lobes of the posticus lip often angled forward, concave, lobes of antiacus lip reflexed, limb 18-25 mm in diameter, usually in living state appearing to be slightly broader than long due to forward angle of posticus lip, lobes of posticus and antiacus lips approximately equal. 8-11 x 7-11 mm, orbicular, ovate or obovate, externally pubescent or puberulent becoming glabrous toward the margins, internally glabrous, crenulate or rarely lobulate, somewhat wavy, often minutely ciliate at the margin. Stamens inserted at the point of corolla inflation (6-8 mm from the base of the corolla), didynamous, the longer ventral pair 8-12 mm long, shorter ventral pair 6-7 mm long, filaments puberulent-pubescent, not flattened. Anthers inserted, glabrous, fertile theca crescent-shaped, falcate at the apex, 2-3 x 1-2 mm, the sterile thecae 3-4 x 0.5-1 mm, subulate. Pistil glabrous, ovary ovoid, narrowing or scarcely narrowing at the base, 5-7 x 4-5 mm, style curving above, incurved at the apex, 14-24 mm, stigma inserted, sometimes scarcely so, blocking the corolla.
throat from above, capitate, globose, 2-4 x 2-3 mm. Placentation axile, locules two.

Capsule not seen. Flowers in most locations from October through January. (Figure 5.15)

**Taxonomy**

The name *Harveya coccinea* is Schlechter’s combination based on the basionym *Aulaya coccinea* Harv. It is probable that Schlechter’s experience with the species was limited to collections made in the Western Cape, although the type for the species was collected far afield, on the east coast in Kwazulu Natal. In *Genera of South African Plants* (1838), Harvey introduced the name *Harveya tubulosa* in a footnote, but did not again use that name in any of his subsequent publications. Only later, in 1904 was the name validated by Hiern in *Flora Capensis*, in which the name *Harveya coccinea* also appears. In his key to the species of *Harveya*, Hiern differentiates the two species by the characteristics “corolla tube sub-cylindrical, 1/6-1/4 inch in diameter” for *H. tubulosa*, and “corolla tube funnel-shaped 1/4-1/3 inch in diameter about the middle” for *H. coccinea*. His circumscription includes cited specimens from the west and east coasts for both species. Hilliard and Burtt (1986) distinguish between these two species anecdotally, characterizing the corolla tube of *H. coccinea* as strongly narrowing below, as opposed to that of *H. tubulosa*, which does not become significantly narrower below.

In this study, specimens were observed from a broad geographical distribution, and the distinctions drawn by Hiern and Hilliard and Burtt were found to be inadequate. In all specimens examined, the corolla becomes abruptly narrower toward its base, sometimes above the lip of the calyx and at other times below it, giving the superficial appearance of having a sub-cylindrical calyx in the latter. Nonetheless, this characteristic is more a function of the length of the calyx than any actual attribute of the corolla tube.
In many specimens, the ratio of corolla to calyx length is greater in the older flowers than the younger, giving the impression that the older flowers are *H. coccinea* and that the younger ones are *H. tubulosa sensu* Hilliard and Burtt. Further, it is difficult to interpret Hiern’s key-characteristics relating to corolla tube diameter; in this study, no corolla tubes were observed that were as narrow as 1/6\(^{th}\) of an inch above the point of constriction. The diameter “about the middle” is also somewhat difficult to interpret. Given that *Harveya coccinea*, as circumscribed here, has a broad geographical distribution, an attempt was made to distinguish between specimens found in the Cape Fold Belt, and Kwazulu Natal and no diagnostic morphological characters were found. Therefore, *H. tubulosa* has been included here as a synonym of *H. coccinea*.

Finally, *H. coccinea* as circumscribed above must include *H. pauciflora*. Hiern distinguished this from the key-bracket containing the former two species, by the depth at which the calyx is cleft: “about half way down” for *H. pauciflora* and “shortly five-toothed” for the bracket containing *H. tubulosa* and *H. coccinea*. Examination of the type material indicates that Hiern was incorrect on this character, of which the calyx is much more shallowly divided.

**Parasitism**

In only one instance has a host been reported for *Harveya coccinea*, a species of *Ficinia* (Cyperaceae). My experience with the plant in the field is that the haustoria are exceedingly small and the host plant connection brittle and tenuous. I did not observe the co-occurrence of *H. coccinea* and any member of Cyperaceae, but several specimens from other herbaria exhibit very fine fibrous roots attached to the base of the parasite, which seems to corroborate a Cyperaceous host.
Habitat and distribution

*Harveya coccinea* is widely distributed along the coast of South Africa from the Western Cape through Kwazulu Natal. (Figure 5.16) One specimen examined (*Peeters, Gericke, Burelli 381, MO*) was collected from Thaba N’chu mountain in the Free State, at some distance from the nearest collection of this species. The habitat of *H. coccinea* is as broad as its distribution. In most cases *H. coccinea* occurs in damp microhabitats on the ocean side of the Cape Fold Belt, and the Drakensberg range.

**Representative specimens examined**


Figure 5.15. Harveya coccinea  A. Plant.  B. Flowers showing the narrowing of the corolla within the calyx.  C. Dissected calyx.  D. Dissected corolla and pistil.
Figure 5.16. *Harveya coccinea* distribution.


Isotype: NU!

Plants 15-40 (-50) cm high, stems, scales, and calyces greenish yellow, or dull red to crimson, or greenish yellow, with dull-red or crimson markings. Above ground, stems simple, or scarcely branching, below ground simple to heavily branching with up to 20 flowering stems arising from a single rhizome, stems terete or obtusely angled, glabrous to villose, with a general trend of increasing hair length and density from base to apex. Caudal leaves imbricate at the base, with internodes of 14-43 mm above, opposite or rarely alternate, elliptical, 7-13 (-23) x 3-5 mm, concave, atrorse, pubescent or villose, acute or rarely subacute or obtuse at the apex, sessile at the base. Inflorescence a loose raceme of 6-20 flowers, usually equal in length to 1/2 the aboveground height of the plant. Floral bracts opposite or rarely alternate, in which case, flowers arise solitarily.
from bract axils, 14-28 x 3-7 mm, elliptical or oblanceolate, concave, pubescent to villose, acute at the apex, sessile or subsessile at the base. Bracteoles arising from the base of the pedicel to the base of the calyx, 9-17 (-26) x 1-3 mm, linear to lanceolate, pubescent to villose, acute at the apex, sessile at the base. Pedicel 14-32 mm, villose. Calyx campanulate or obconical at the base, campanulate to cylindrical above, 14-24 (-30) x 7-11 (-15) mm, more or less densely villose externally with hairs concentrated on longitudinal nerves to the lobe apices and sinuses, glabrous within, bilabiate, posticous lobes deltoid, 3-6 x 3-7 mm, anticus lobes deltoid-lanceolate, 6-8 (-11) x 3-5 (-7) mm. Corolla tube 28-44 (-50) mm in length, narrow below, ample above, inflating above or below the apex of the calyx to 9-16 (-20) mm in diameter at the throat, white or pale yellow, somewhat keeled dorsally, externally pubescent or villose with longest, densest hairs concentrated at dorsal keel, internally glabrescent except for narrow strip on ventral surface which is puberulent. Corolla limb pale or bright pink to mauve or lilac, (27-) 30-45 (-55) mm in diameter, corolla lobes orbicular-ovate, more or less wavy, crenulate, lobulate, or lacerate, and minutely to heavily ciliate at the margins, puberulent or pubescent, with decreasing hair length and density toward the margins internally and externally, posticous lobes variously erect, reflexed or angled forward, convex or concave, 10-17 (-27) x 9-14 (-20) mm, anticus lobes reflexed slightly, 9-15 (-25) x 9-15 (-18) mm. Corolla throat variously white, pale yellow, or bright yellow, puberulent. Stamens inserted 5-13 mm from the base of the corolla, corolla tube glabrous-puberulent at point of insertion, didynamous, the longer ventral pair 11-14 mm, the shorter dorsal pair 4-11 mm, filaments round in cross-section, puberulent-pubescent. Anthers glabrous, well inserted, the fertile theca crescent-shaped, falcate, 2-3 x 1-2 mm, the sterile theca 3-4.
x 0.5-1 mm, subulate. Pistil glabrous, ovary 5-8 x 3-5, orbicular, ovoid, or flask-shaped (ovoid, but truncated below), style 19-30 (-37) mm, incurved at the apex, stigma partially blocking throat from above, capitate, globose-subglobose, barely inserted, 2-3 x 1-2mm. Capsules not seen. Flowers November-February. (Figures 5.17-18)

**Taxonomy**

In a treatment of *Harveya* species of Kwazulu Natal, Hilliard and Burtt (1986) recognized three new species that had previously been sheltered under the name *Harveya huttonii* Hiern: *H. leucopharynx*, *H. pulchra*, and *H. silvatica*. Given the morphological variation present among specimens identified as *Harveya huttonii*, a taxonomic investigation of the group was certainly warranted. Hilliard and Burtt distinguished the new species from *H. huttonii* and each other based on calyx length, posticous corolla-lobe length, the color of the corolla throat, and the shape of the ovary, although *H. huttonii* was not included in their key, having been relegated to an Eastern Cape distribution in this treatment. However, these characters are not entirely diagnostic; in many instances they overlap, and often, specimens which would have clearly been recorded as *H. huttonii sensu* Hiern, cannot be identified as belonging to any one of Hillliard and Burtt’s new species, or *Harveya huttonii sensu* Hilliard and Burtt.

For example, *Harveya pulchra* and *H. leucopharynx* are distinguished by the length of the calyx (*H. leucopharynx*: (17-) 20-31 mm vs. *H. pulchra*: 17-23 mm), the size of posticous corolla lobes (*H. leucopharynx*: 14-17 mm vs. *H. pulchra*: 8-12 mm) and the color of the corolla throat (*H. leucopharynx*: white vs. *H. pulchra*: yellow), and the length of the anticous calyx lobes (*H. leucopharynx*: (4-) 5-10 mm vs. *H. pulchra*: 4-5 mm). Two of the quantitative characteristics (calyx length and calyx lobe length) have
overlapping ranges, and therefore cannot be used to identify specimens without other characters. The size of the posticous corolla lobes does not overlap between species. However, upon examination of a number of specimens identified as either *H. pulchra* or *H. leucopharynx*, this character appears to vary continuously between both ranges given. If flowers with larger lobes were all white-throated, and flowers with smaller lobes were all yellow-throated, the combination of these characteristics would allow unambiguous identification of any specimen belonging to either one of these species. This is not the case, though. Almost all of the large-lobed specimens are white-throated, and none of the yellow-throated specimens are that large. However, many of the smaller-lobed specimens are also white-throated. These have often been identified as *Harveya huttonii* on this basis; however, many of the smaller-lobed, white-throated specimens have calyces far too long to be identified as *H. huttonii* as circumscribed by Hilliard and Burtt. In fact, these specimens are not unambiguously identifiable under this system. Considering the use of throat color in diagnosis, many specimens in the field have throats ranging from snow-white, to creamy-white, to pale yellow, to bright yellow, and variation is apparent between newer and younger flowers on the same plant.

*Harveya silvatica* was distinguished from *H. pulchra* and *H. leucopharynx* on the basis of the number of flowers in a raceme (*H. silvatica*: 7-20 vs. 1-8 (-10) in *H. pulchra* and *H. leucopharynx*), the length of the calyx (*H. silvatica*: (11-)13-19 mm vs. 17-31 mm in *H. pulchra* and *H. leucopharynx*), the length of the corolla tube (*H. silvatica*: 28-34 (-40) mm vs. 33-50 mm in *H. pulchra* and *H. leucopharynx*) and finally the size and shape of the ovary (*H. silvatica*: 4-5 x 3.5-4 mm and suborbicular vs. 5-8 x 3-5 mm and turbinate in *H. pulchra* and *H. leucopharynx*). The quantitative characters have
overlapping ranges, and at least for calyx and corolla tube length, specimens of *H. pulchra* and *H. silvatica* overlap extensively. Further, under this system, *H. pulchra* and *H. silvatica* are indistinguishable on the basis of throat-color. Ovary shape in living and formalin-preserved specimens varies continuously as well, from orbicular to ovoid to flask-shaped (although I have identified none that looked turbinate—this may be an artifact of pressing).

Phylogenetic analysis of the previous chapter indicates that specimens diagnosable as these species are quite closely related (although not all specimens could be so identified). Given the degree of morphological diversity *Harveya huttonii* (as delimited here), it may be beneficial to find diagnostic characters that allow for the description of new specific or subspecific taxa. However, no such characteristics were discovered in this study. The diagnostic characters chosen by Hilliard and Burtt vary continuously and for the most part independently in the specimens observed, and therefore, I have chosen to re-subsume these species under the aegis of *Harveya huttonii* until more discrete characters can be found.

**Parasitism**

*Harveya huttonii* has been recorded parasitizing species from diverse taxa: *Euryops tysonii* and *Felicia* sp. (Asteraceae), *Sparrmannia ricinocarpa*. (Tiliaceae), and *Anthospermum* sp. (Rubiaceae). The haustoria tend to be small (pea-sized or smaller), and connections fragile.

**Habitat and distribution**

*Harveya huttonii* occurs from the Amatola mountains in the Eastern Cape through the entire Drakensberg range of the Eastern Cape, Kwazulu Natal, Lesotho, the northeast
Free State, Swaziland, Mpumalanga, and the Northern Province (Figure 5.19). While extending nearly to the Limpopo at the northeast political boundary of South Africa, no collections from Mozambique or Zimbabwe have been recorded to my knowledge. The habitat of *H. huttonii* is typically montane grassveld, but many specimens have also been reported to live on forest margins (*Harveya silvatica sensu* Hilliard and Burtt, 1986), particularly in northern KwaZulu Natal and neighboring Swaziland.

**Representative specimens examined**

**EASTERN CAPE.** Kaffrarious Mountains, Sep 1867, Barber 26 (K, PRE).

Queenstown: Hangklip mountain near Queenstown, 1894, Galpin 1767 (PRE).

Sterkstroom: Andriesberg near Queenstown, 17 Jan 1897, Galpin 5719 (K,PRE).

Stutterheim: Evelyn Valley, 13 Jan 1947, Compton 19158 (NBG); Mt. Kemp, 14 Dec 1977, Hilliard and Burtt 11033 (E, NU). Xalanga: [Cala distr.] Kalanga, Jan 1896, Bolus 8751 (BOL); Cala, 8 Feb 1910, Pegler and Kolbe 1612 (K).


KWAZULU NATAL. Bergville: Cathedral Peak, 1 Dec 1950, Killick 1179 (K, PRE); Drakensberg, valley below Ship's Prow Pass, 7 Dec 1983, Balkwill, Manning, and Meyer 1044 (NU). Estcourt: Giant's Castle, 17 Jan 1973, Wright 1347 (NU); Giant's Castle, Valley beyond forester’s house, 26 Dec 1976, Hilliard and Burtt 9578 (NU).

Hlabisa: Hlabisa, east shore of lake St. Lucia, 14 Nov 1978, Pooley 2207 (E, K, NU).

Mahlabitini: Ceza Forest, 17 Dec 1965, Burtt and Hilliard 3322 (NU). Mpendle: Impendele, between Maledhlana and Loteni, 2 Dec 1972, Wright 1321 (NU).

T. Edwards 1752 (OS); 2 Feb 2001, Randle 130 (OS). Garden Castle and Valley forester’s house, 26 Dec 1976, Hilliard and Burtt 9578 (K); 5-7 m NNW of Castleview Farm, headwaters of Mlahlangubo River, 26 Nov 1980, Hilliard and Burtt 13706 (E); Garden Castle, stream beyond forester’s house, 4 Dec 1980, Hilliard and Burtt 13780 (E); Garden Castle, Mlambonja valley, 9 Jan 1982, Hilliard and Burtt 14876 (E); Cobham Forest Reserve, Lakes Cove area, 13 Dec 1982, Manning, Hilliard and Burtt 16025 (K).

NORTHERN PROVINCE. Letaba: 9 Jan 1960, Scheepers 841 (MO);
Magoebaskloof, 16 Dec 1964, Burtt 2915 (E); Metz Mission Hospital 15 Nov 1976,
Venter 1146 (K) and 16 Nov 1976, Venter 1158 (K). Louis Trichardt: Zoutpansberg,
near Louis Trichardt, 16 Dec 1928 Hutchinson 2024 (BOL). Pietersburg: Blouberg
[Blauwberg] Mohlakeng Plateau, 11 Jan 1955, Codd and Dyer 9006 (K); Blouberg
Mountains, middle buttress, 20 Feb 1990, Stirton, Venter, and T. Edwards 12644 (NU);
Blouberg Nature Reserve, 4 Dec 1990, Vos 192 (NU). Sibasa: ~20m from Sebasa on
1928, Hutchinson 2274 (BOL, K).

LESOTHO. Mamalapi, 26 Dec 1948, Compton 21303 (NBG). Dikolsberg, 28
Between Ongeluk’s Nek and Qacha's Nek, between HaRamoroba and Hasekaka, 14 Jan
1983, Matthews 934 (K).

SWAZILAND. Jan 1911, Stewart 9518 (PRE). Mbabane: Ukutulu, 28 Nov
1954, Compton 24750 (NBG, PRE); hill NE of Mbabane, 21 Nov 1957, Compton 27253
(NBG). Hlatsikulu: forest shade, 8 Jan 1957, Compton 26357 (NBG, PRE). Horo:
mine, 24 Dec 1890, Galpin 1263 (BOL).
Figure 5.17. *Harveya huttonii*. Flowers and dissected corollas exhibiting variation in shape and size of corolla tubes, lobes, and ovaries. From left to right, *Randle 119*, *Randle 130*, and *Randle 136*. 
Figure 5.18 Harveya huttonii. A. Plant. B. Dissected calyces exhibiting variation in shape and size. From top to bottom, Randle 119, Randle 130, Randle 136.
Figure 5.19. *Harveya huttonii* distribution.
UNCERTAIN AND EXCLUDED NAMES


To my knowledge the type specimen is the only reported collection of *Harveya vestita*, and its whereabouts are unknown. From its description, it seems to differ from *H. purpurea* primarily on the indumentum of the anthers: hirsute in *H. vestita* and glabrous in *H. purpurea* (as in all other species of *Harveya*). In several specimens of other species examined here, hairs were observed on anthers, but these differed from the hairs typically found on *Harveya* specimens, in that the latter are jointed and glandular, while the former were fine, smooth, and not apparently glandular. I interpreted these hairs as being fungal hyphae.

EXCLUDED NAMES


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